FINAL REPORT

Demonstration and Certification of Amphibian Ecological Risk Assessment Protocol

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DEMONSTRATION AND CERTIFICATION OF AMPHIBIAN ECOLOGICAL RISK ASSESSMENT PROTOCOL FINAL REPORT
ESTCP Project Number ER-0514

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14. ABSTRACT
This ESTCP project was designed to demonstrate and validate an innovative technique for the evaluation of potential risks to amphibians in palustrine wetland environments. This technique builds on previous DoD research which resulted in development of a tiered amphibian ecological risk assessment (ERA) protocol as well as laboratory toxicity tests for evaluating potential risks to amphibians due to exposure to contaminated soils and sediments.
The field demonstration described in this technical report was conducted to achieve the following objectives:
Demonstrate and validate use of the soil and sediment exposure protocols at two DoD sites with potential amphibian risk assessment concerns; Apply an amphibian ERA framework at a DoD field site to evaluate whether or not it provides valuable risk management information; and Evaluate the use of lead and copper screening values designed to be protective of amphibians developed during the laboratory validation phase of this project.

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Abbreviations and Acronyms

AFB	Air Force Base
AFCEE	Air Force Center for Engineering and the Environment
ANOVA	Analysis of Variance
APG	Aberdeen Proving Ground
ARMI	Amphibian Research and Monitoring Initiative
ASTM	American Society of Testing and Materials
AVS	Acid Volatile Sulfides
CEC	Cation Exchange Capacity
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
COPC	Constituent of Potential Concern
COTS	Commercially-Off-The-Shelf
CWA	Clean Water Act
DNT	2,4-Dinitrotoluene
DOC	Dissolved Organic Carbon
DoD	Department of Defense
DOE	Department of Energy
DOI	Department of the Interior
E	Energy
Eco-SSL	Ecological Soil Screening Level
ERA	Ecological Risk Assessment
ESTCP	Environmental Security Technology Certification Program
FCETL	Fort Collins Environmental Toxicology Laboratory
GPS	Global Positioning System
I	Intensity
IACUC	Institutional Animal Care and Use Committee
IDW	Investigation Derived Wastes
IPR	In-Progress Review
IR	Installation Restoration
LCS	Laboratory Control Spike
LOAEC	Low Observed Adverse Effect Concentration
mg/kg	milligram per kilogram

mg/L	milligram per liter
MS/MSD	Matrix Spike/Matrix Spike Duplicate
NAVFAC	Naval Facilities Engineering Command
NAVFAC LANT	Naval Facilities Engineering Command Atlantic
NOAEC	No Observed Adverse Effect Concentration
NAVFAC ESC	Naval Facilities Engineering Service Center
PAH	Polycyclic Aromatic Hydrocarbon
PCB	Polychlorinated Biphenyl
PEC	Probable Effect Concentration
QA/QC	Quality Assurance/Quality Control
QAPP	Quality Assurance Project Plan
RCRA	Resource Conservation and Recovery Act
RDX	Cyclotrimethylenetrinitramine
RPD	Relative Percent Difference
SEM	Simultaneously Extracted Metals
SEM	Standard Error on the Mean
SETAC	Society of Environmental Toxicology and Chemistry
SOP	Standard Operating Procedure
TBD	To Be Determined
TEC	Threshold Effect Concentration
TNT	2,4,6-Trinitrotoluene
TOC	Total Organic Carbon
USACHPPM	United States Army Center for Health Promotion and Preventive Medicine
USEPA	United States Environmental Protection Agency
USFWS	United States Fish and Wildlife Service
USGS	United States Geological Survey
UXO	Unexploded Ordnance
λ	Wavelength
XRF	X-ray Fluorescence

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Executive Summary

This ESTCP project, *Demonstration and Certification of Amphibian Ecological Risk Assessment Protocol, CU-0514*, was designed to demonstrate and validate an innovative technique for the evaluation of potential risks to amphibians in palustrine wetland environments. This technique builds on previous DoD research which resulted in development of a tiered amphibian ecological risk assessment (ERA) protocol as well as laboratory toxicity tests for evaluating potential risks to amphibians due to exposure to contaminated soils and sediments (referred to herein as the soil protocol and sediment protocol, respectively).

The field demonstration described in this technical report was conducted to achieve the following objectives:

- Demonstrate and validate use of the soil and sediment exposure protocols at two DoD sites with potential amphibian risk assessment concerns;
- Apply an amphibian ERA framework at a DoD field site to evaluate whether or not it provides valuable risk management information; and
- Evaluate the use of lead and copper screening values designed to be protective of amphibians developed during the laboratory validation phase of this project (NAVFAC, 2007b).

The soil exposure protocol focused on evaluation of potential risks to terrestrial salamanders, with the red-backed salamander (*Plethodon cinereus*) selected as the model organism. The sediment protocol focused on evaluation of potential risks to early life stage frogs -- the northern leopard frog (*Rana pipiens*) was selected as the test organism for the sediment protocol. Travis Air Force Base (AFB) in California and the Aberdeen Proving Ground (APG) in Maryland were selected as the demonstration sites since both sites have amphibian habitat co-located with contamination associated with firing ranges. Copper and lead concentrations were measured at both demonstration sites; however, copper concentrations in most samples were lower than anticipated and were not present at levels high enough to result in significant adverse impacts to amphibians. Therefore, evaluation of potential impacts due to lead exposure was the primary focus of the field demonstration.

Soil exposure tests were conducted with six samples, including three samples from each of the two demonstration sites. Copper concentrations in all samples were below concentrations associated with effects during the laboratory validation phase of testing so the field demonstration testing focused on evaluation of potential effects on salamanders due to lead exposure. Lead concentrations ranged from 10.8 to approximately 17000 mg/kg in the six tested samples. Survival was not impacted in any of the field demonstration samples, while a concentration of 9,167 mg/kg lead in the laboratory validation phase of testing caused 80% mortality. A reduction in growth was observed over 28 days of exposure to the highest field-collected lead concentration. These results demonstrate that the field-collected soils were substantially less toxic than similar levels of lead in the laboratory spiked soils tested in the laboratory validation phase of testing. Therefore, for the range of conditions encountered at the two demonstration sites, the ecological screening levels derived based on the laboratory validation testing with spiked soils would be sufficiently conservative for use in assessing risks to salamanders exposed to lead under field conditions.

The sediment exposure testing showed similar results with greater toxicity observed in the laboratory validation testing than in the field demonstration testing. A total of 16 sediment samples (eight from each demonstration site) were tested using the 10-day early life stage frog sediment exposure protocol. Lead concentrations ranged from 15 to 17000 mg/kg in the 16 tested samples. Although lead was the primary focus of the demonstration, copper levels in some samples collected from the APG study area may have contributed to observed effects. Impacts on survival and growth of tadpoles were observed in samples collected from both demonstration sites. At both demonstration sites, lead concentrations resulting in adverse impacts were higher than predicted by the sediment screening values developed during laboratory validation testing. These results indicate that, at least for the range of conditions encountered at the two demonstration sites, the screening levels derived using spiked sediments would be sufficiently conservative for use in assessing potential risks to larval amphibians exposed to lead under field conditions.

The tiered amphibian ERA protocol was determined to be useful for conducting both screening level and more sophisticated ERA analyses. The application of the soil and sediment exposure protocols resulted in a more appropriate site-specific assessment of potential risks to amphibians than would have been accomplished using more traditional methods (e.g., comparison to non-amphibian literature-based screening levels, application of alternative soil or sediment toxicity tests using inappropriate receptors like benthic invertebrates or terrestrial worms).

The performance objectives for the field demonstration effort were met. The soil and sediment toxicity testing protocols were appropriate for use at both demonstration sites and were sensitive enough to detect lethal and sub-lethal impacts due to exposure to firing range contaminants. The ERA protocol was also applicable at both demonstration sites. Although results of this testing program have not been submitted to regulatory agencies, the sediment exposure protocol has been applied at several sites under federal and state regulatory review. Technology transfer efforts are on-going and an ASTM standard containing the sediment exposure protocol was approved by ASTM International in November 2007 is currently available on the ASTM website as ASTM E2591-07 Standard Guide for Conducting Whole Sediment Toxicity Tests with Amphibians.

The costs of conducting the tiered amphibian ERA protocol are expected to vary significantly from site to site depending upon the spatial scale of the area under investigation and the number of samples required to meet the data quality objectives. It is anticipated that the cost to conduct the sediment exposure protocol in accordance with the ASTM standard will be within ± 20% of the costs to conduct 10 day sediment toxicity tests with benthic invertebrates. Although the costs to conduct the amphibian sediment toxicity test could be slightly higher than historically used benthic invertebrate sediment toxicity tests, this additional cost is returned when the use of appropriate test species is used to establish remedial goals and avoid unnecessary wetland remediation and restoration.

The sediment exposure protocol and the amphibian ERA framework are both applicable for investigating potential impacts to amphibians at wetland sites under investigation by the DoD or other entities. Although the soil exposure protocol is a valid approach to investigating toxicity from chemicals in soil to a terrestrial salamander, ethical and financial obstacles preclude its regular application as part of site characterization efforts. However, this method may be appropriate for controlled toxicological investigations designed to derive safe soil levels for particular compounds.

1.0 Introduction

The Department of Defense (DoD) Environmental Security Technology Certification Program (ESTCP) has funded the Naval Facilities Engineering Service Center (NAVFAC ESC) and its DoD partners Naval Facilities Engineering Command (NAVFAC), Naval Facilities Engineering Command Atlantic (NAVFAC LANT), U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM) and Air Force Center for Engineering and the Environment (AFCEE), as well as their contractor ENSR Corporation (ENSR), to demonstrate and validate an innovative technique for the evaluation of potential risks to amphibians in palustrine wetland environments.

This report presents the results of the ESTCP amphibian risk assessment project (*Demonstration and Certification of Amphibian Ecological Risk Assessment Protocol, ER-0514*), with a primary focus on the field demonstration effort. The following reports were previously prepared under the ER-0514 program:

- NAVFAC, 2005. Laboratory Validation Plan for ESTCP Project CU-0514 Demonstration and Certification of Amphibian Ecological Risk Assessment Protocol. December 2005.
- NAVFAC, 2006. Site Selection Memorandum for ESTCP Project CU-0514 Demonstration and Certification of Amphibian Ecological Risk Assessment Protocol. February 2006.
- NAVFAC, 2007a. Field Demonstration Plan for ESTCP Project CU-0514 Demonstration and Certification of Amphibian Ecological Risk Assessment Protocol. May 2007.
- NAVFAC, 2007b. Test Refinement Interim Report for ESTCP Project CU-0514 Demonstration and Certification of Amphibian Ecological Risk Assessment Protocol. June 2007.

1.1 Background

The ESTCP amphibian risk assessment demonstration program is timely and topical, in that nationwide declining amphibian populations and losses of wetland acreage are viewed as significant environmental concerns, and regulatory agencies are increasingly requesting amphibian ERA at DoD sites. In fact, in 2000 the President and Congress directed Department of the Interior (DOI) agencies to undertake a national amphibian research and monitoring initiative (ARMI). This multi-agency effort is coordinated by the U.S. Geological Survey (USGS) and involves numerous state and federal agencies, other organizations, and academic scientists.

The evaluation and remediation of DoD sites requires the careful selection of ERA-based remedial goals. When ERA-based remedial goals are concise, quantitative, and well-supported, the DoD benefits by minimizing costs and expediting response action schedules. Some of the most challenging impediments to regulatory consensus relate to balancing the trade-offs between destructive and costly wetland remediation and leaving residual contamination in place. When selecting remedial goals for addressing wetland contamination, risk professionals are entrusted to balance the objectives of remediation (ecological receptor and habitat protection) with the potential financial costs and short-term and long-term ecological impacts from the disruption caused during remediation. This risk of remedy concern was recently articulated by the United States Environmental Protection Agency (USEPA) in their sediment remediation guidance (USEPA,

2005), which clearly states that relative or net risk analysis is an integral part of the recommended strategy for risk-based closure of sites with sediment contamination concerns.

When selecting appropriate receptors to derive ERA-based remedial goals, amphibians must increasingly be considered. Amphibians play a key ecological role in wetlands, and are an important link in ecological food chains, serving both as predators and prey items. Moreover, public concern regarding recent declines in amphibian populations and additions of many amphibian species to threatened or endangered status suggest that amphibians are important sentinel species in stressed environments. However, because limited ecotoxicity data are available for amphibians, decisions regarding wetland remediation are often inappropriately based on data from aquatic (e.g., fish) or terrestrial (e.g., earthworm) species that are not typical of wetlands, and may be more or less sensitive to chemical stressors than amphibians.

Considerable uncertainty is associated with the application of these terrestrial and aquatic clean-up standards to wetlands. For instance, mineral-based upland soils typically contain low concentrations of organic carbon, whereas wetland hydric soils typically contain much higher organic contents (especially histosols), which have the potential to render many contaminants biologically unavailable as they are bound up in the organic fraction of the soil. Likewise, considerable uncertainty is associated with the application of benthic sediment clean-up standards to wetland systems. Benthic standards are often developed from large lacustrine (i.e., Great Lakes) databases and have little relevance in palustrine wetlands. Although two-phase partitioning (biota and hydric soil) in seasonally flooded wetland soils may influence bioavailability, dynamic three-phase equilibrium partitioning of constituents between water, sediment, and biota, which results in reduced constituent bioavailability in true aquatic sediments, may not always occur in seasonally inundated or saturated palustrine wetland systems.

The DoD is the country's third largest federal land manager, with over 25 million acres at more than 425 military installations. Wetland habitats often comprise a significant portion of open space at DoD facilities, and are prime habitat for various amphibian species, which play a key ecological role in wetlands. This phenomenon is illustrated at the former Naval Air Station in Weymouth, MA where wetlands comprise approximately 40% of the property, and where an amphibian-based ERA was a critical component of the Navy's Installation Restoration (IR) program and ERA-based remedial decisions. Although wetlands comprise a larger percentage of open space at East Coast DoD installations, wetlands containing amphibians are present at installations across the U.S. For instance, at Camp Pendleton, CA, wetland endangered species concerns have played a significant role in the IR program (over 5,700 acres of wetlands and 17 federally listed species occur at this activity), and at least two sites with endangered Arroyo toads (*Bufo microscaphus californicus*) have been remediated.

Wetland remediation can be environmentally destructive, and wetland restoration is publicly sensitive, technically challenging, and financially costly. When inappropriate receptors and methods are used to derive ERA-based remediation goals, the result is often an overestimation of site risks resulting in the unnecessary excavation and destruction of wetlands. In addition to the financial burdens associated with unnecessary wetland remediation, preventable losses of valuable wetland resources may occur if risk-based decisions are based on inappropriate wetland risk assessment techniques. Wetland restoration is still an evolving science, and the short-term and long-term impacts to wetland communities are tangible – wetlands represent successional habitats

that can take decades to mature and many restoration efforts have been unsuccessful. As a result, achieving long-term restoration objectives can be extremely challenging, and decisions relative to wetland alteration should be made with the best available and most current scientific information.

In addition to the direct financial and ecological costs associated with unnecessary wetland remediation and subsequent restoration, many other indirect financial and temporal impacts can arise when inappropriate ERA methods are applied. Potential cost impacts include onsite handling, transport, and disposal of wetland material, which is high in organic content. Saturated organic soils can pose technically challenging problems that have significant cost implications when it comes to treatment and disposal. For instance, excavation of saturated soils can be costly, thermal desorption of wetland soils requires that most of the water be removed, and many hazardous waste landfills will not take a saturated or high organic content soil. Other potential costs include the technical and management efforts associated with reproducing project documents and negotiating with regulatory agencies. One of the more significant overall impacts is the additional time necessary to proceed with remedial design and implementation, and the subsequent delay in site closure and property transfer.

1.2 ER-0514 Work Conducted to Date

This ESTCP project (*Demonstration and Certification of Amphibian Ecological Risk Assessment Protocol, CU-0514*) has been established to build on two previous DoD innovative technology programs:

- NAVFAC recently developed a laboratory toxicity test for evaluating potential risks to early life stage frogs and toads from exposure to sub-aqueous sediments. This technology, referred to herein as the "sediment exposure protocol", evaluates effects on amphibian growth and survival following exposure to contaminated sediments. The final technical report (NAVFAC, 2004) can be downloaded from the Navy's Ecological Risk Assessment homepage (http://web.ead.anl.gov/ecorisk/index.cfm), which is hosted by Argonne National Laboratory. This document also presents the framework for a tiered amphibian ecological risk assessment (ERA) protocol that can be used to assess potential risks to amphibians as part of site evaluations.
- USACHPPM recently completed a similar innovative technology effort resulting in development of a toxicity test focusing on adult salamander exposures to mesic soils. This technology, referred to as the "soil exposure protocol", evaluates effects on salamander growth, survival, and target organ effects based on histopathological evaluations following contaminant exposure (Johnson, et al., 2004).

1.2.1 Protocol Test Refinement

The initial phase of this ESTCP project was focused on validating and refining these existing toxicity test protocols in the laboratory with lead- and copper-spiked sediment and soil prior to the field demonstration. These two constituents were selected for technology validation because they are commonly co-located and are found at military sites and ranges. A primary goal of test validation was to ensure that the amphibian test protocols consider site-specific conditions that

influence exposure (e.g., bioavailability) and yield results that are protective of various life stages of amphibians.

As described in more detail in Section 3.4 (Pre-Demonstration Testing and Analysis), and in previous project deliverables (NAVFAC, 2005; NAVFAC, 2007b), the laboratory validation of the sediment exposure protocol evaluated the impact of a number of bioavailability factors (i.e., pH, total organic carbon (TOC), cation exchange capacity (CEC), grain size) on the observed toxicity of lead and copper. The laboratory validation testing also evaluated the duration of the test by conducting the sediment exposure protocol through frog metamorphosis. The no observed adverse effect concentrations (NOAECs) and low observed adverse effect concentrations (LOAECs) identified during the sediment exposure validation testing can be used as preliminary sediment screening levels.

The soil exposure protocol was evaluated in the laboratory validation phase of the project to assess whether the protocol, originally developed to evaluate energetic compounds, could be used to evaluate inorganic compounds. The laboratory validation testing finalized the test protocol itself relative to the endpoints evaluated and developed dose-response relationships that could be used as soil screening levels (i.e., NOAECs and LOAECs).

The laboratory validation phase of testing finalized the two protocols that were used in the field demonstration effort. The validation testing also developed preliminary sediment and soil screening levels that that could be used to evaluate potential impacts to amphibians due to exposure to copper or lead in the field. The remainder of this document describes the 2006 field demonstration of these two protocols, as well as the preliminary screening levels, at the Travis Air Force Base (AFB) in California and the Aberdeen Proving Ground (APG) in Maryland.

1.3 Objectives of the ESTCP Field Demonstration

The field demonstration effort builds on the technology refinement phase of work, which was described in the December 2005 *Laboratory Validation Plan* (NAVFAC, 2005) and in the June 2007 *Test Refinement Interim Report* (NAVFAC, 2007b). The *Field Demonstration Plan* (NAVFAC, 2007a) described the proposed tasks for the demonstration effort. Appendices A and B from the approved *Field Demonstration Plan* have been re-presented in this document; these appendices respectively present Analytical Methods Supporting the Experimental Design (Appendix A) and the Quality Assurance Project Plan (Appendix B). The site-specific Health and Safety Plan presented in the *Field Demonstration Plan* (NAVFAC, 2007a) is on file at ENSR and available upon request. Appendix C presents a summary of the results from the analytical laboratories and the toxicity testing laboratories.

The specific objective of the Field Demonstration Program is to validate and demonstrate use of the soil and sediment exposure protocols at two existing DoD sites with potential amphibian risk assessment concerns. This validation and demonstration effort was also designed to validate the previously developed amphibian ERA framework and the ecological screening values developed during the laboratory validation phase of this project (NAVFAC, 2007b).

Two demonstration sites were selected where both the soil and sediment exposure protocols could be applied. These two sites have co-located amphibian habitat with lead and copper contamination

associated with firing ranges. Lead, copper, and other metals like antimony and zinc are expected contaminants in firing-range soils due to their presence in bullets and shell casings (Thorbjornsen, and Myers, 2007).

The primary objective of the field demonstration was to evaluate the potential impact of the lead and copper contamination on existing or future amphibian populations. The amphibian ERA field demonstration included conducting laboratory toxicity tests at select stations co-located with chemical analysis sampling stations, performing field surveys to evaluate habitat and amphibian populations, and evaluating site-specific media concentrations relative to screening values developed during the laboratory validation testing (see Table 1-1). Although the May 2007 *Field Demonstration Plan* (NAVFAC, 2007a) proposed field collection of amphibians in order to conduct tissue and histological evaluations, this task did not prove to be feasible due to concerns about the local extirpation of resident amphibian communities at the demonstration sites.

In addition to conducting the field demonstration, a secondary objective of this project was to achieve American Society of Testing and Materials (ASTM) certification for the testing protocols. Following the field demonstration effort it was determined that the focus of the ASTM certification effort would be on the sediment protocol. As discussed in the *Test Refinement Interim Report* (NAVFAC, 2007b), ethical and financial obstacles involved in the soil protocol likely discourage its use for the identification of risks at individual sites. Therefore, the sediment protocol was submitted for review by the ASTM Sediment Assessment and Toxicology sub-committee in January 2007. This draft ASTM protocol was revised in response to comments from sub-committee members and submitted to the full Biological Effects and Environmental Fate committee in August 2007. It was accepted by the committee in November 2007 and was published in December 2007. The guide is presented in Appendix D and is currently available on the ASTM website as ASTM E2591-07 Standard Guide for Conducting Whole Sediment Toxicity Tests with Amphibians.

Following the November 2006 In-Progress Review (IPR) for this project, ESTCP funded an additional set of studies to investigate the differential sensitivities of larval amphibians to copper and lead. This set of studies was designed to assess, compare, and contrast the responses of multiple amphibian species to exposure to two chemical stressors (lead and copper) in hydric soils. The results of the species sensitivity testing are presented and discussed in Appendix E.

1.4 Regulatory Drivers

As a component of site investigation activities, regulatory agencies are increasingly requesting amphibian ERA at DoD sites, as well as at other government and industry-led state and federal environmental sites. Since limited ecotoxicity data are available for amphibians, it can be difficult to effectively evaluate potential impacts to these receptors. Assessments and remedial decisions may be inappropriately based on data from surrogate receptors (e.g., fish or earthworms) that are not typical of wetlands. Application of the soil and sediment exposure protocols at DoD sites should more successfully address requests from regulatory agencies to assess potential impacts to amphibians.

In addition, the use of the soil and sediment exposure protocols to evaluate the potential for adverse impacts to amphibians is consistent with the requirements of the Comprehensive Environmental

Response, Compensation, and Liability Act (CERCLA), the Resource Conservation and Recovery Act (RCRA), and the Clean Water Act (CWA), as well as state-led programs and DoD standards.

1.5 Stakeholder/End-User Issues

ERAs are often conducted in palustrine wetland systems where traditional risk assessment methods (e.g., screening values, toxicity tests) based on non-wetland receptors may not be the most appropriate way to address the potential for risk to amphibians inhabiting the wetland. The field demonstrations conducted for this project provide additional information to stakeholders on the costs, level of effort, and benefits associated with applying the recently developed toxicity testing methods and the amphibian ERA protocol at a site under investigation.

2.0 Technology Description

Two laboratory toxicity testing protocols have been developed during recent DoD projects. Both protocols evaluate potential impacts to amphibians from exposure to sediment or soil. The soil and sediment exposure protocols have recently completed the final stages of laboratory validation and refinement (NAVFAC, 2007b).

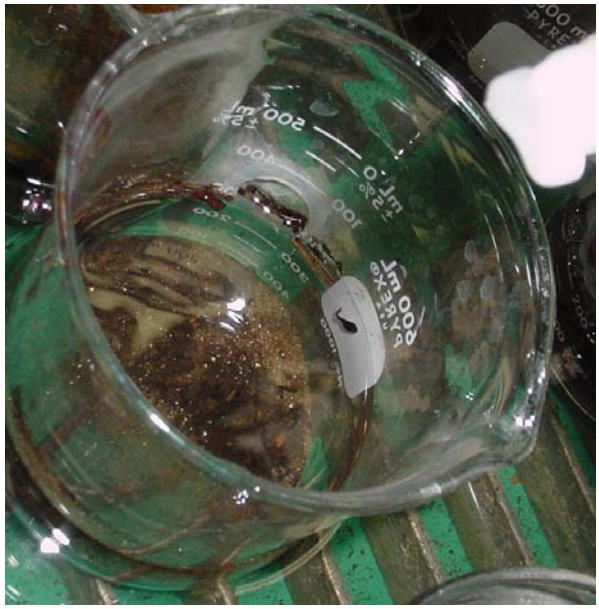
2.1 Technology Development and Application

The sediment and the soil exposure protocols are laboratory bioassays developed to represent model systems for evaluation of amphibian risks on a nationwide basis. Each methodology is summarized briefly below.

The sediment exposure protocol is a 10-day laboratory toxicity test for evaluating potential risks to early life stage frogs and toads from exposure to sediments. This bioassay evaluates effects on amphibian survival and growth following exposure to contaminated sediments. The sediment exposure protocol was developed with a focus on inorganic constituents, and was peer-reviewed and updated to incorporate input from national experts, including DoD, USEPA, USGS, Department of Energy (DOE), and United States Fish and Wildlife Service (USFWS) representatives.

Sediment tests are conducted with recently hatched tadpoles (*Rana pipiens*; Gosner Stages 17-20). Young tadpoles are placed in beakers containing sediment and overlying water (Figure 2-1). The overlying water in each beaker is replaced continuously via a flow-through delivery system. At test termination all living organisms are counted and removed for sub-lethal (width and body length) measurements. Additional endpoints may also be measured at test termination: weight, head-to-vent length, eye width, the occurrence of supernumerary limbs, spinal curvatures, behavioral impairments (e.g., feeding, swimming, orientation), eye displacement. Depending upon project-specific objectives, longer duration studies (i.e., 28 days or until complete metamorphosis) may also be conducted to evaluate potential impacts on tadpole development.

Figure 2-1 *R. pipiens* in Sediment Exposure Protocol Test Chamber



The soil exposure protocol (also presented in Appendix A) assesses adult red-backed salamander (*Plethodon cinereus*) exposure to mesic soils by evaluating effects on salamander growth, survival, and target organs following 28-days of test exposure.

In this protocol, each test organism is placed into an individual Petri dish containing treatment-specific soil (Figure 2-2). Animals are observed at least daily for signs of overt toxicity (e.g., lethargy, sensitivity to touch, abnormal behavior) and body weights are measured weekly. At test termination, surviving salamanders are weighed, anesthetized, and euthanized. Growth, mortality, and health criteria (blood parameters, histological organ evaluation including quantification of liver melanomacrophages) are evaluated as the endpoints for this assay. The liver histopathological biomarkers (melanomacrophages) used in the soil exposure protocol are a non-specific indicator of stress, and show potential as biomarkers for a wide variety of chemical stressors.

Figure 2-2 *P. cinereus* in Soil Exposure Protocol Test Chamber

2.2 Previous Testing of the Technology

Both the soil and sediment exposure protocols are mature technologies, with little remaining development or refinement warranted. Extensive laboratory and data analysis efforts have been conducted during the past four years as part of the research and development of these technologies. Final refinement of both protocols was described in the December 2005 *Laboratory Validation Plan* (NAVFAC, 2005). A final report describing the results of the laboratory validation effort was submitted to ESTCP in June 2007 (*Test Refinement Interim Report*; NAVFAC, 2007b).

The sediment exposure protocol was originally developed under the Navy's YO817 program and presented in the March 2004 *Development of a Standardized Approach for Assessing Potential Risks to Amphibians Exposed to Sediment and Hydric Soils* (NAVFAC, 2004). This document was developed as a guidance manual for risk assessment staff and state/federal regulators involved in the review and approval of risk assessment work plans and reports, and included a standard operating procedure (SOP) for conducting the sediment exposure toxicity test as well as recommendations for field survey methodologies. An initial phase of this ESTCP project included a number of laboratory assays designed to validate the sediment exposure protocol with lead and copper prior to the field demonstration. This laboratory validation phase included the evaluation of several bioavailability factors that could affect the results of the assays. The duration of the test was also assessed. The results of the laboratory validation phase testing were incorporated into the SOP presented in Appendix A.

Since the sediment exposure protocol was developed, it has been used operationally at several state and federal environmental sites, including at the Naval Air Station, Cherry Point, North Carolina, at the Massachusetts Military Reservation, Cape Cod, Massachusetts, at the Naval Weapons Station (NWS) Yorktown, York County, Virginia, at a lead-contaminated state-led site operated by the Massachusetts Highway Department, and at a cadmium-contaminated site led by USEPA Region 4.

The soil exposure protocol methodology was initially established to generate toxicity data for the development of soil screening levels for 2,4,6-trinitrotoluene (TNT), 2,4-dinitrotoluene (DNT), and cyclotrimethylenetrinitramine (RDX). As part of the laboratory validation phase of this ESTCP project, the assay was conducted with copper- and lead-spiked soils to assess how the protocol could be applied to inorganic contaminants. This testing finalized the protocol itself relative to endpoints evaluated for the test metals and developed dose-response relationships that were further evaluated using field-collected mesic soils in the field demonstration.

The refinement testing of both protocols is discussed further in Section 3.4 (Pre-Demonstration Testing and Analysis).

2.3 Factors Affecting Cost and Performance

Implementation of the two toxicity testing protocols according to the established SOPs should be relatively consistent between various laboratories. As with other similar laboratory bioassays, the cost of the assays will be primarily dependent on the number of individual samples tested (i.e., the cost per test may decrease as the number of samples increases) and the level of sub-lethal endpoint assessed. For the sediment assay, measurements on a variety of sub-lethal endpoints (e.g., eye width, behavioral impairments) will be more costly than simply assessing width and length. Extending the duration of the 10 day sediment assay to consider potential impacts on development will also increase costs, but may identify more subtle sub-lethal effects. For the soil assay, extensive histological assessments may increase costs over a more simple evaluation of growth or blood chemistry parameters.

Performance of the assays will be primarily dependent on the supply of test organisms (i.e., laboratory or field-collected) and is seasonally influenced. During the duration of this ESTCP project, the project team continually encountered challenges relative to native test organism

acquisition. Field-collected organisms are generally available from commercial suppliers, but only during the spring months. However, in certain springs (e.g., 2007), factors such as regional droughts adversely affected amphibian populations throughout the southeastern United States, and neither field-collected nor pond-raised amphibian eggs were widely available. Likewise, the availability of laboratory-induced eggs is also seasonally influenced – such eggs are generally available from commercial suppliers from November through April.

Although the SOP and ASTM guide employ standard methods, laboratories with prior amphibian toxicity testing experience should generally be considered for these assays over laboratories that are less familiar with the culturing and testing of amphibians.

2.4 Advantages and Limitations of the Technology

The use of the sediment and soil exposure protocols to assess potential impacts to amphibians in wetlands is often more appropriate than using existing toxicity tests with alternative species. Using aquatic species (e.g., fish), benthic species (e.g., amphipods), or terrestrial species (e.g., earthworms) does not address the unique interaction between amphibians and the sediment or hydric soil milieu. Use of these alternative species may over- or under-estimate potential impacts to amphibians, and may result in less informed risk-management decision-making process.

Due to the potential seasonal availability of amphibians, the use of these protocols may be limited to times of year when the test organisms are available. For the salamander (soil) assay, red-backed salamanders are generally available for testing in the late winter and spring months (February through May). Frog eggs are generally available from commercial vendors during the spring months (field-collected), as well as during the late fall and winter months (reproduction artificially induced in the laboratory). During the refinement stage of this ESTCP program, the project team experienced significant shipment-related mortality during the winter months (possibly due to frog eggs being exposed to extreme winter weather conditions during shipment). During the field demonstration effort and the supplemental species sensitivity testing, delays were incurred due to the availability of frog eggs, as well as other larval amphibians under investigation.

Lastly, the salamander protocol uses significant numbers of field-collected adult organisms. Although the Maryland populations used in the current ESTCP program are robust and do not appear to be substantially affected by the field collection activities in support of this ESTCP program, field collection of adult organisms should only be conducted if local amphibian population and meta-population dynamics are robust enough to support the loss of several dozen adult salamanders.

Proposing and thus promoting the use of this assay to investigate toxicity of mixtures at individual sites risks local and possibly wide-scale extirpation of the species. Additionally, there is circumstantial evidence that these species are relatively long-lived (~ 20 years), adding to the ethical concerns from harvesting these species for site-specific toxicological investigations. Moreover, the test methods used are quite expensive, and likely not feasible for site-specific analysis. Altogether, current constraints suggest that these methods may be appropriate for controlled toxicological investigations designed to derive safe soil levels, but are not feasible for the wide-scale use in determining toxicity from mixtures at individual sites in support of environmental restoration.

These limitations have excluded the soil exposure protocol from being considered as a testing procedure in the development of an ASTM guide to conduct whole sediment toxicity tests with amphibians.

3.0 Demonstration Design

3.1 Performance Objectives

Performance objectives are a critical component of the overall demonstration plan since they provide a measurable basis for evaluating the performance and costs of the technology. Meeting these performance objectives is essential for successful demonstration and validation of the technology.

In general, the quantitative performance objectives for typical remediation-related ESTCP projects (e.g., end-point criteria, remediation time, and analytical sensitivity) are indirectly associated with the performance objectives of this project (e.g., ecological risk and toxicity based performance objectives). Table 3-1 presents the performance objectives for evaluating the field demonstration effort. The evaluation of these objectives will be discussed in Section 4.

Table 3–1 Performance Objectives

Type of Performance Objective	Primary Performance Criteria	Expected Performance Metric
Qualitative	Sediment protocol is applicable to evaluating copper and lead in palustrine wetlands	Correlation between sediment concentrations and lethal or sub-lethal results
	Soil protocol is applicable to evaluating copper and lead in forested uplands	Correlation between mesic soil concentrations and lethal or sub-lethal results
	Collection and biological evaluation of native salamanders is applicable for evaluating potential impacts due to metals	Correlation between mesic soil concentrations and histopathological evaluation
	Regulatory acceptance of toxicity test protocols	Results are accepted by agency as component of ERA
	Versatility of the overall ERA protocol	Application of the ERA protocol at both field demonstration sites
	Technology transferred to other potential end-users	Presentation at conference or in journal; ASTM certification

Table 3–1 Performance Objectives (continued)

Type of Performance Objective	Primary Performance Criteria	Expected Performance Metric
Quantitative – Sediment Exposure Protocol	Sediment toxicity test is valid and acceptable	Mean survival in laboratory control is >80%
	Lethal endpoint indicates toxicity or lack of toxicity	Statistical difference between survival in control or reference samples and site samples
	Sub-lethal endpoints indicate toxicity or lack of toxicity	Statistical difference between sub-lethal endpoints in control or reference samples and site samples (may include growth, abnormalities, behavior, metamorphic stage, or other measurements)
Quantitative – Soil Exposure Protocol	Soil toxicity test is valid and acceptable	Mean survival in laboratory control is >80%
	Lethal endpoint indicates toxicity or lack of toxicity	Statistical difference between survival in control and site samples
	Growth endpoints indicate toxicity or lack of toxicity	Statistical difference between growth endpoints in control or reference samples and site samples
	Blood parameters indicate toxicity or lack of toxicity	Statistical difference between blood parameters measured in control or reference samples and site samples

3.2 Selecting Test Sites

Two potential demonstration sites were selected primarily based on the known presence of amphibian habitats overlapping with copper and lead contamination. Copper and lead were selected as the constituents for technology refinement because they are commonly co-located and are often found at military sites and ranges.

Eleven potential demonstration sites were initially considered in the site selection process. The list of demonstration sites was finally narrowed to two locations based on the likely presence of amphibian habitat with copper and lead contamination, as well as conversations with site personnel on the feasibility of performing the demonstration tasks at the particular locations. The selection criteria used to identify the two sites are briefly described below, and were presented in more detail in the February 2006 *Site Selection Memorandum* (NAVFAC, 2006).

3.2.1 Chemical Parameters

Based on the testing data and screening values generated from the test refinement phase of work (NAVFAC, 2007b), the preferred range of copper levels in mesic soil and palustrine hydric soil was determined to be from 150 milligrams per kilogram (mg/kg) to 6000 mg/kg. The preferred

range of lead levels in mesic soil and palustrine hydric soil is from 100 mg/kg to 3000 mg/kg. These values represent lead and copper concentrations that are often found at DoD sites with firing ranges, and they bracket the upper and lower limits of amphibian mortality and sub-lethal effects, based on the environmental toxicity data generated through this and other amphibian assessment projects. These levels were selected to be high enough to result in observable impacts to the test subjects, but not so high as to result in complete mortality.

The site selection process also considered the presence of other chemical stressors (e.g, pesticides used in grounds maintenance or polycyclic aromatic hydrocarbons (PAHs) from parking areas). Sites with mixed contamination that could make interpretation of the results difficult were eliminated from consideration. Chemical analysis of sediment/soil selected for toxicity testing included other potential stressors in at least a sub-set of the tested samples to assess the potential for interferences.

3.2.2 Ecological Parameters

The presence of suitable habitat for either Plethodontid salamanders or Anurans (frogs or toads) was critical to the site selection process since the protocol focused on evaluating wetland habitats for potential impacts to amphibians and assessing the need for potential wetland remediation to improve existing habitats. However, it was not critical that the selected sites have a reported presence of species from either group, since it is possible an amphibian survey has not been conducted, that the native population may contain related con-generic or con-specific representatives, or that current site conditions have impacted historic populations.

The site selection process eliminated sites with non-chemical stressors in the vicinity of the study area that could make interpretation of the results difficult. Non-chemical stressors included roadways, bridges, drainage ditches, or other physical stressors that might impact the wetlands or the amphibian populations.

Preference was given to sites that did not have known occurrences of federal or state-listed threatened or endangered species in order to reduce the need for federal or state resource agency coordination prior to on-site amphibian collection. However, the presence of a threatened or endangered species did not eliminate a site from consideration.

3.2.3 Site Historical and Logistical Parameters

For the field demonstration to be most applicable and of use to site managers, preference was given to sites currently under investigation to determine whether remedial response actions are required to address potential risks to amphibians. However, otherwise acceptable sites were not eliminated from the process if amphibian impacts had not previously been investigated.

However, some level of previous investigation was important in order to identify levels of lead and copper which may overlap with acceptable amphibian habitat and to identify other stressors (chemical or non-chemical) that might confound the interpretation of the test results.

Safety, accessibility, and the geographic location of the site were also considerations in the site selection process. Safe access to the study area (i.e., wetland habitat) within the DoD facility was

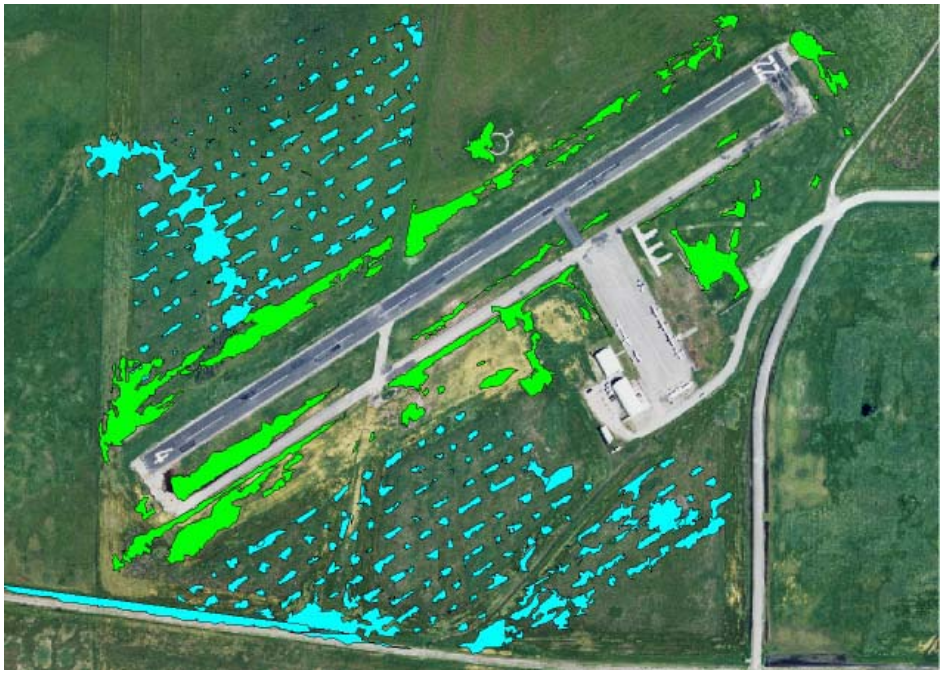
critical. Possible hazards included the potential presence of unexploded ordnance (UXO), active firing ranges, or exposure to on-site hazardous wastes, as well as more general health and safety concerns (e.g., trip hazards, ticks). It was also preferred that the selected sites be representative of the regional diversity of DoD sites, and not both be located in the same geographic region of the country.

3.3 Test Site Description

Two sites were selected for the implementation of the field demonstration phase of work for this project. A brief synopsis of information about these sites and the rationale behind the selection are discussed below.

Site 1: Travis Air Force Base (AFB), Fairfield, California is located midway between Sacramento and San Francisco in northern California. Travis AFB contains several well documented palustrine wetland complexes and vernal pools in close proximity to firing ranges. In addition, Travis AFB has a documented vernal pool complex in close proximity to an active skeet range. Previous vernal pool work at the site has focused on preserving and restoring the natural vernal pool ecosystem, in part to prevent the extinction of a federally endangered plant, the Contra Costa goldfields (*Lasthenia conjugens*). Over 250 vernal pools have been constructed at the AFB to supplement the existing natural vernal pools (see Figure 3-1).

Figure 3-1 Constructed and Natural Vernal Pools at Travis Air Force Base Aero Club Site[1]



[1] Blue represents constructed vernal pools and green represents natural vernal pools. Figure downloaded from website of Sharon Collinge at the University of Colorado – Boulder http://spot.colorado.edu/~sharonc/vernalpools.html

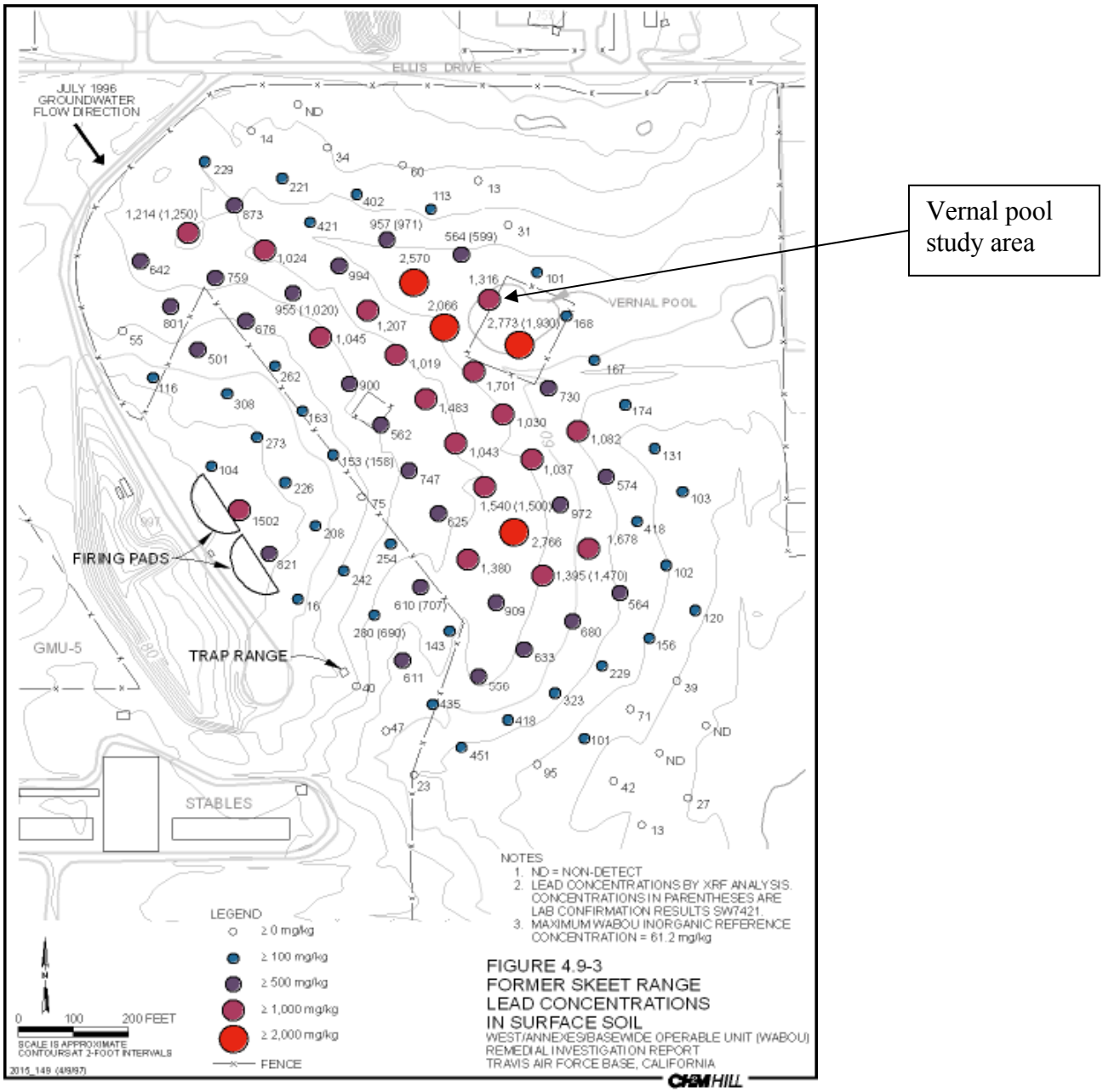
Travis AFB was selected as a preferred location for the demonstration of the amphibian risk assessment protocol based on the variety of vernal pools (both constructed and natural) and palustrine wetlands, the level of interest from the environmental manager, and the likelihood of contamination due to firing ranges.

A brief site reconnaissance visit at Travis AFB was conducted in February 2006 by Navy and contractor personnel with oversight from Air Force personnel. Sites surveyed included the decommissioned small arms firing range and the skeet shooting range, both known to contain elevated levels of lead. Each site was surveyed to determine the presence or absence of potential amphibian breeding habitat. Suitable habitat for amphibians was not present within the area of the firing range that was surveyed for lead and copper soil concentrations. Many small vernal pools exist within the site but none hold enough water for a long enough period of time to be considered suitable amphibian breeding habitat.

A vernal pool located approximately 800 feet northeast of the skeet shooting range was surveyed during the site reconnaissance visit. This vernal pool is down range, down wind, and down slope of the skeet shooting range and is know to contain lead-contaminated soils (pers. com. Glenn R. Anderson, Base Hydrologist, Travis AFB, February 8, 2006) (Figure 3-2). The site is also close enough to the skeet shooting range that lead shot could potentially reach the site. The vernal pool is located within the Travis AFB Equestrian Center horse pasture and the deepest portions of the vernal pool are fenced in to keep the livestock out. The vernal pool, which was inundated at the time of the survey, provides potential breeding habitat for amphibians. Average pool depth was approximately 50 cm with deeper areas over 80 cm. The surface area of the pool was approximately 1,200-square meters. Plant species within the pool were similar to those in the vernal pool southwest of the firing range. A Pacific tree frog (*Hyla regilla)* egg mass and several Pacific tree frog tadpoles were observed within the pool, and the calls of at least one adult tree frog were also heard.

The vernal pool associated with the skeet shooting range was selected as the primary study area at Travis AFB.

Figure 3-2 Vernal Pool Study Area at Travis Air Force Base



Site 2: Aberdeen Proving Ground (APG), Maryland was also selected as a location for the field demonstration of the amphibian risk assessment protocol. One of the ESTCP team partners (Dr. Mark Johnson) is stationed at the proving ground and is intimately familiar with the overlap between amphibian habitat and lead contaminated ranges at this facility.

The facility occupies more than 72,500 acres in Harford County, Maryland and is bounded by the Susquehanna and Gunpowder Rivers, the Chesapeake Bay, and the Amtrak Railroad. APG comprises two principal areas, separated by the Bush River: the northern area known as the Aberdeen Area; and the southern area, formerly the Edgewood Arsenal, known as the Edgewood Area. Activities at the APG have included environmental and chemical research, as well as testing of field artillery, weapons, and ammunition. Numerous exterior and interior firing ranges, automotive courses, and underwater explosive test ponds are located on-site. Due to the active and classified nature of the APG, aerial images of the proposed study area are not included in this report.

The APG also provides large areas of natural habitat for many species. Excluding wetlands within the open water areas, the wetlands at APG total about 13,600 acres or about 35 percent of the land surface area. Non-tidal wetlands total over 6,000 acres with approximately 1,770 acres of emergent wetlands, 4,350 acres of forested wetlands and 134 acres of scrub/shrub wetlands.

More than 40 species of reptiles and amphibians occur within the streams, ponds, wetlands, and forests of the APG. The most abundant amphibian species include bullfrog (*R. catesbeiana*), green frog (*R. clamitans*), northern cricket frog (*Acris crepitans*), northern spring peeper *Pseudacris crucifer crucifer*), southern leopard frog (*R. sphenocephala*), Fowlers toad (*B. fowleri*), and red-backed salamander (*P. cinereus*).[2]

Based primarily on the wide range of wetlands present on-site, the observations of amphibian populations, and the likelihood of contamination due to firing ranges, the APG was selected as a preferred location for the demonstration of the amphibian risk assessment protocol. Army personnel identified an on-site small arms range adjacent to a palustrine wetland as the specific area of study. Although data and mapping are available for the lead contamination in the palustrine wetland complex at the small arms range and the data can be used to select sampling locations, these figures are not currently cleared for public distribution.

3.4 Pre-Demonstration Testing and Analysis

As described in Section 2.2, laboratory validation of the soil and sediment exposure protocol technologies was conducted using soils and sediments spiked with known concentrations of copper and lead. The validation testing conducted for the sediment exposure protocol also evaluated different test durations and several bioavailability factors that could potentially affect the results of the assays. The results of the soil and sediment testing were presented in the *Test Refinement Interim Report* (NAVFAC, 2007b) and were used to finalize the SOPs for each test protocol. The

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[2] Information obtained from Aberdeen Proving Ground Ecology website http://www.apg.army.mil/apghome/sites/directorates/restor/ecology.html

results of the field demonstration testing described herein have been compared against the laboratory validation results to determine whether site-specific factors (e.g., organic carbon) or the aging of the metals in the environment has an effect on the toxicity of lead to amphibians.

3.4.1 Soil Exposure Protocol

Table 3-2 presents a summary of the survival and growth of salamanders exposed to copper- or lead-spiked soils during the laboratory validation phase of testing. The 28-day copper exposure assay indicated no survival effects at 803 mg/kg; however, reduced survival was observed at 1,333 mg/kg and above. Eleven of twenty salamanders in the 1,333 mg/kg group were found dead or were euthanized prior to the scheduled test termination due to humane considerations; death or euthanasia occurred from 1 to 3 days post-exposure. All twenty of the salamanders in the 2,700 mg/kg group were found dead within 24 hours of initial exposure. All other salamanders survived to test termination at Day 28. There were no significant differences among treatments compared to controls for body weight, or for white and red blood cell counts (leukocytes and erythrocytes, respectively). There were no copper-related histopathologic findings.

Table 3–2 Salamander Survival and Growth Following 28 Days of Exposure to Copper- or Lead-Spiked Soil

	Concentration (mg/kg) [1]	Mean Survival (%)	Mean Body Weight (g)
Copper	18	100	0.7847
	283	100	0.8050
	803	100	0.8287
	1333	45	0.7174
	2700 [2]	0	NA
Lead	14	100	0.8977
	553	100	0.8988
	1700	100	0.9151
	4700	85	0.9026
	9167	20	0.7668

Survival LOAEC is indicated in boldface text
1 – Concentrations based on the average measured value from three sampling events (Day 0, Day 14, and Day 28).
2 – Concentration is based on one sampling event due to complete mortality prior to second sampling event.

The 28 day lead exposure assay indicated no survival effects at 1,700 mg/kg, but reduced survival in the 4,700 mg/kg and 9,167 mg/kg treatments. Three of twenty salamanders in the 4,700 mg/kg group and sixteen of the twenty salamanders in the 9,167 mg/kg group were found dead or were euthanized prior to the scheduled test termination due to humane considerations; death or euthanasia occurred from 1 to 5 days post-exposure. All other salamanders survived to test termination at Day 28. Although the mean body weight of the salamanders exposed to the 9,167

mg/kg treatment is lower than in the control treatment; this difference was not reported to be statistically different. No significant differences among treatments compared to controls were observed for erythrocyte counts and hemoglobin levels. However, the 4,700 mg/kg treatment had a low leukocyte count compared to controls (p=0.007). There were no lead-related histopathologic findings.

Although only a relatively small dataset was available for the salamander assay, the data provide an important preliminary estimate of potentially toxic levels of copper and lead. The no observed adverse effect concentrations (NOAECs) and low observed adverse effect concentrations (LOAECs) for lethal and sublethal endpoints were used to generate preliminary soil screening levels that could be used to evaluate potential impacts to amphibians due to exposure to copper or lead in the field (Table 3-3).

Table 3–3 Summary of Soil Screening Values Developed During Laboratory Validation Testing

Compound (mg/kg)	Survival		Sub-Lethal Endpoint	
	NOAEC	LOAEC	NOAEC	LOAEC
Copper	803	1333	2700 [1]	>2700 [1]
Lead	1700	4700	1700 [2]	4700 [2]
1 - No sub-lethal effects were observed at the highest tested concentration. 2 - Reduced leukocyte count observed at 4700 mg/kg lead.				

The laboratory validation testing conducted for the soil exposure protocol indicated that adverse lethal or sub-lethal effects would not be expected at approximately 800 mg/kg copper or 1,700 mg/kg lead (the survival NOAEC). Sites with soil concentrations at or below these levels would not warrant additional evaluation for impacts to amphibians. Soil concentrations of 1,333 mg/kg copper and 4,700 mg/kg lead (the survival LOAEC) may result in reduced amphibians survival and sites with concentrations at and above these levels likely warrant a more detailed assessment of metals bioavailability and toxicity. The sub-lethal NOAECs and LOAECs were also used to derive more conservative screening levels; however, few sub-lethal impacts were observed in the testing so these levels may be overly conservative.

These preliminary screening values are likely to be overly protective since the metals used in the laboratory testing were selected to represent a maximum exposure and may be more bioavailable than metals found within more natural soils where organic carbon and the aging process may limit bioavailability. In addition, it is difficult to know the significance of the impact that a reduced leukocyte count would have on the health of an individual salamander or a salamander population exposed to lead in the soil. Basing a sub-lethal screening level on this endpoint may be overly protective of population-level effects.

3.4.2 Sediment Exposure Protocol

The laboratory validation testing for the sediment exposure protocol was conducted through a sufficient range of copper and lead concentrations and test durations to result in a range of lethal

and sub-lethal responses. The evaluation of bioavailability variables such as TOC, pH, grain size and CEC, provided valuable information on the important impact these chemical and physical factors may have on modifying the bioavailability, and therefore toxicity, of a contaminant in the field.

The NOAECs and LOAECs derived during the laboratory validation testing may be appropriate preliminary screening values for evaluating potential risks to amphibians due to exposure to copper or lead. Table 3-4 presents preliminary screening values based on the NOAECs and LOAECs derived from 10 day tests with copper- or lead-spiked sediments. The bioavailability variables were not modified in these tests so these screening values would be most applicable to sediments that are similar to the base sediment used by the laboratory (sediment with low TOC [0.066%] and pH of approximately 7.5).

Table 3–4 Summary of Sediment Screening Values Developed During Laboratory Validation Testing

Compound (mg/kg)	Survival		Sub-Lethal Endpoint [1]	
	NOAEC	LOAEC	NOAEC	LOAEC
Copper	230	>230	21	87
Lead	1200	>1200	100	260

Values were derived from 10-day tests conducted using laboratory control sediment spiked with either copper or lead. No bioavailability factors were modified. The TOC level in the base sediment was 0.066%.

Concentrations based on measured values at test initiation.

1 – Based on lower of values for body width and body length endpoints.

Sediment concentrations above the laboratory–derived LOAECs may require additional investigation and may present a potential ecological risk to amphibian receptors. Concentrations below the NOAECs are unlikely to cause harm to the local amphibian population and these sites may require no further investigation.

The validation testing indicated that the copper and lead dilution series studies, as well as several of the studies where TOC and pH were modified, were most consistently related to the observed effects. No apparent relationships were observed between toxicity and copper or lead concentrations when grain size and CEC were included in the regression models. Table 3-5 presents the survival data extracted from several studies with varying levels of TOC in sediments spiked with either copper or lead (mixture data are not considered in this table). The general trend is that when metals concentrations are elevated, more toxicity (i.e., lower survival) is observed in sediments with lower TOC levels, particularly below 1%. For example, in the copper tests a concentration of approximately 250 mg/kg results in 60% survival in sediment with <1% TOC but survival increases to 100% at approximately 4% TOC. A similar pattern occurs in the lead tests where 780 mg/kg lead results in only 65% survival in sediment with <1% TOC; however, when the TOC is increased to 10%, concentrations as high as 1367 mg/kg do not adversely impact survival.

Table 3–5 Influence of Total Organic Carbon Content on Tadpole Survival During Laboratory Validation Testing

% TOC Range	% TOC	Copper-Spiked Sediment Tests		Lead-Spiked Sediment Tests	
		Copper [1] (mg/kg)	Mean Survival (%)	Lead [1] (mg/kg)	Mean Survival (%)
<1% TOC	0.06	7.2	100	4.5	97.5
	0.06	7.2	100	4.5	97.5
	0.06	15	100	63	100
	0.06	21	97.5	100	100
	0.06	87	97.5	260	100
	0.06	140	95	680	100
	0.06	230	77.5	1200	70
	0.64	39 [2]	0	780	65
	0.64	250	60	1500	35
1 - 4% TOC	1.4	80	100	800	95
	1.4	140	90	1200	80
	3.9	143	100	933	100
	3.9	250	100	1400	95
>10% TOC	10.2	142	85	911	100
	10.2	248	90	1367	95

1 - Concentrations based on measured values at test initiation.

2 - Copper concentration in some replicates of this treatment may have been higher than the reported value of 39 mg/kg from a sample collected prior to the start of the test. The target concentration was 350 mg/kg and copper levels in other sediments using the same spiked sediment ranged from 140 to 248 mg/kg (average was 169 mg/kg). Total mortality at this copper concentration is unusual.

It is clear that the same level of a contaminant may be more or less toxic depending on the environmental conditions (e.g., TOC) of the sampling location.

3.5 Testing and Evaluation Plan

The following sub-sections describe the details of the on-site activities conducted during the field demonstrations at Travis AFB and the small arms range at APG. Since lead was the primary contaminant present at these firing ranges, the focus of the field sampling was to identify and collect samples that would represent a range of lead concentrations. Copper was also measured, but historic information and initial reconnaissance samplings indicated that copper levels were not likely to be present at levels high enough to result in significant adverse impacts to amphibians. Therefore, potential impacts due to lead exposure became the focus of the field demonstration.

3.5.1 Demonstration Set-Up and Start-Up

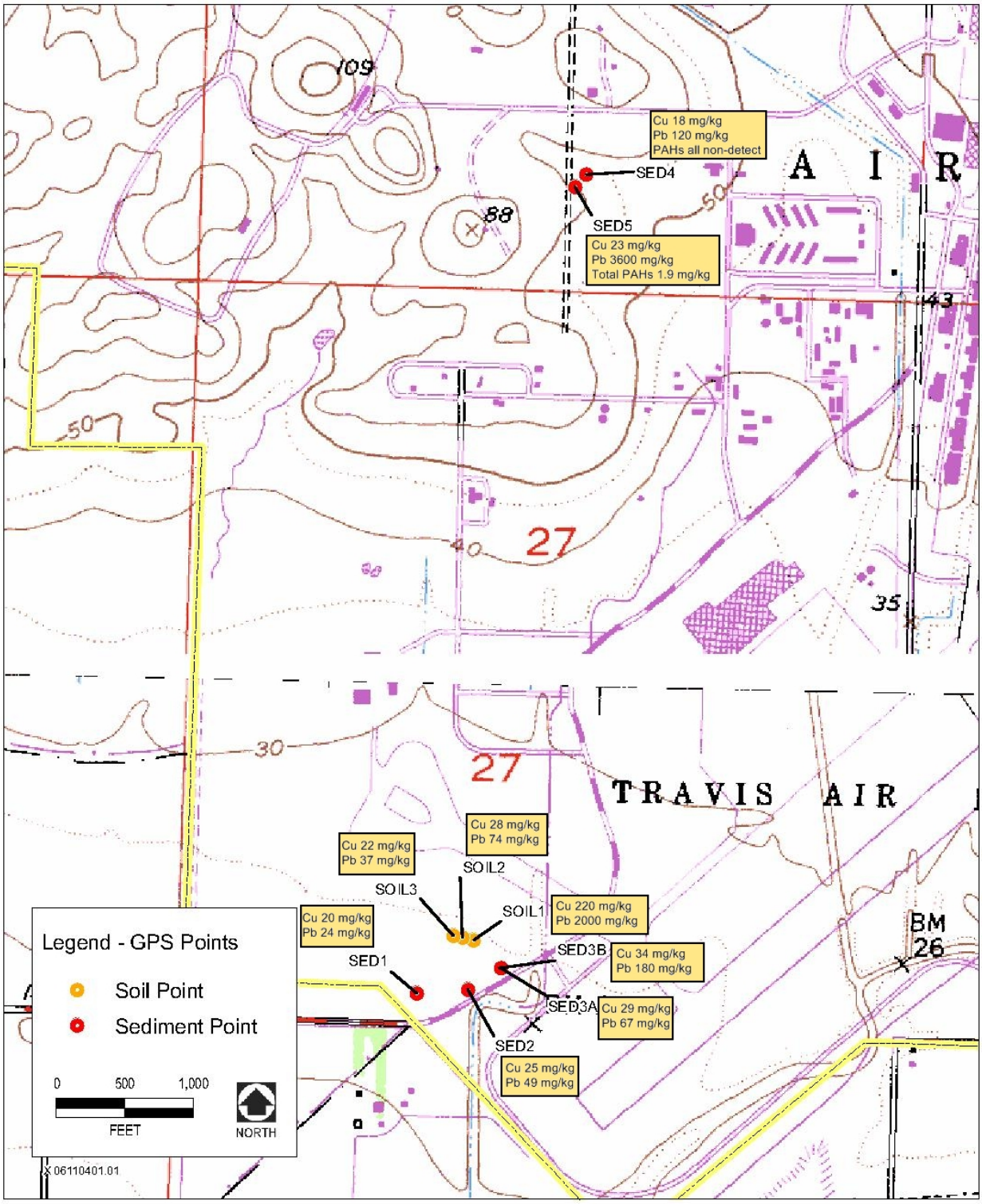
Field reconnaissance visits were conducted at both of the selected sites prior to the field demonstration. Initial field reconnaissance site visits by members of the project team were conducted in February 2006 at Travis AFB and in April 2006 at APG. These field reconnaissance visits helped to determine approximate sampling locations, access, and other logistics.

Field instruments used at each site included a global positioning system (GPS) unit, echosounder, and YSI multi-parameter meter. Calibration procedures for laboratory instruments consisted of initial calibrations, initial calibration verifications, and continuing calibration verifications.

Prior to collecting soil or sediment samples, an X-ray fluorescence (XRF) survey was conducted by USACHPPM personnel at each demonstration site to identify appropriate sampling locations. XRF is a non-destructive qualitative and quantitative analytical technique used to determine the chemical composition of a sample. An element is identified by its characteristic X-ray emission wavelength (λ) or energy (E). The amount of an element present is quantified by measuring the intensity (I) of its characteristic emission. The XRF survey information was used to select soil/sediment sampling locations such that they represented a concentration gradient bracketing and containing the concentrations suspected to result in lethal and sub-lethal responses in amphibians, based on the previously conducted work (including the laboratory refinement phase of this ESTCP project).

Because few lead samples were previously analyzed in the vicinity of the Travis AFB vernal pools, the project team collected nine surficial soil and sediment samples during the February 8, 2006 site reconnaissance (Figure 3-3). All sampling locations were marked in the field with surveyors flagging and GPS data were collected. Samples were packaged and shipped to Paragon Analytics of Fort Collins, Colorado for copper, lead, and PAH analyses (PAHs were evaluated to ensure that there was no residual organic contamination associated with an adjacent railroad track). The analytical data from the field reconnaissance effort are presented in Figure 3-3.

Figure 3-3 Travis Air Force Base Site Reconnaissance Sampling Locations and Analytical Data



The Travis AFB reconnaissance also included an ecological survey of the decommissioned small arms firing range and the skeet shooting range to determine the presence or absence of potential amphibian breeding habitat. The surveys indicated that, assuming that the vernal pools observed on site persist through the breeding season and long enough for the larvae to metamorphose, suitable amphibian breeding habitat exists in the vicinity of both the small arms firing range and skeet shooting range sites. However, no suitable breeding habitat was identified within the area of the small arms firing range that had been surveyed for copper or lead contamination. Based on the results of the analytical chemistry sampling effort and the habitat survey, the Travis AFB demonstration effort focused on the vernal pool in the vicinity of the skeet shooting range. An XRF survey was conducted by USACHPPM personnel one day before the field demonstration sampling in order to focus the sediment and soil sampling in the vicinity of the vernal pool itself. Due to the active nature of the skeet shooting range, communication with skeet range personnel was critical. No other safety issues were noted during the field reconnaissance effort.

The lead contamination in the APG palustrine wetland at the small arms range is well documented; therefore, the project team determined that no additional field sampling was warranted as part of the reconnaissance. On April 10, 2006, just prior to the field demonstration effort, an XRF survey was conducted by USACHPPM personnel in order to identify soil and sediment sampling locations. The area was also cleared with a magnetometer to allow for safe digging. The XRF survey identified a range of lead concentrations in soil and sediment from 33 mg/kg in a reference location to 12,387 mg/kg in a soil sample. These data were used to select sampling locations for the field demonstration effort. The sampling area was limited to the area cleared of UXO by Army personnel. No other safety issues were noted during the field reconnaissance effort.

3.5.2 Period of Operation

A brief site reconnaissance was conducted at Travis AFB in February 2006 by Navy and contractor personnel with oversight from Air Force personnel. The field sampling effort was conducted on March 27 and 28, 2006. An XRF survey was conducted on March 27, 2006 in order to focus the sediment and soil sampling planned for the next day. Surface water samples were collected from the vernal pool and the reference location on March 27, 2006 and were submitted for chemical analyses. Sampling on March 28, 2006 involved the collection of soil and sediment samples for chemical analyses and toxicity testing.

An XRF survey of the APG study area was conducted by USACHPPM personnel on April 10, 2006 in order to identify soil and sediment sampling locations. The APG field sampling effort was conducted on April 12, 2006 and involved the collection of soil and sediment samples for chemical analyses and toxicity testing and surface water samples for chemical analyses.

Due to a lack of available frog eggs, there was a delay in the initiation of the sediment toxicity testing program. The sediment testing was conducted by ENSR's Fort Collins, Colorado Environmental Toxicology Laboratory (FCETL) from December 18 to 28, 2006 for the APG samples and from January 13 to 23, 2007 for the Travis AFB samples. It is recognized that it is desirable to initiate sediment tests as soon as possible following field collection of sediments. However, due to the lack of eggs this was not possible. It was also not possible to conduct additional field efforts to collect fresh sediments once eggs became available. All samples were stored in the dark at 4°C to minimize any changes to the sediment. One concern with longer

holding times is the loss of some labile chemicals such as ammonia and volatile organic compounds that can degrade or volatize during storage. However, more stable sediments can be stored for much longer periods of time with little change in toxicity (USEPA, 2001). Since the compounds of interest at these two sites were metals, the concentrations were not expected to change significantly over time.

This was confirmed by re-analyzing the copper and lead levels in the sediments prior to the sediment toxicity tests. Copper concentrations at the start of the test ranged from 68% to 124% of the concentrations measured just after sampling with the largest variation noted in samples with low copper concentrations (i.e., 17 mg/kg after field collection to 21 mg/kg at test initiation). Less variability was observed for the lead concentrations with concentrations at the start of the test ranging from 76% to 113% of the concentrations measured just after sampling. For both metals, the change in concentrations over time was not consistently higher or lower than that observed initially. The sediments at the time of test initiation were determined to still be appropriate to use for testing; however, it is possible that unmeasured changes occurred during the extended holding time that may have had an impact on the outcome of the tests.

The soil exposure testing was conducted by USACHPPM's Aberdeen, Maryland laboratory between May 24 and June 23, 2006 with tests starts staggered such that each test would run for 28 days.

A protocol deviation in the soil testing resulted in half of the salamanders being exposed for 29 days instead of 28 days. The necropsies were originally planned over the course of two days with the beginning exposures staggered accordingly; all were necropsied on one day. Therefore, half the animals were exposed for an extra day.

Sediment, soil, and surface water analyses (e.g., TOC, grain size, metals) and analytical data review were conducted between March and October 2006.

3.5.3 Amount/Treatment Rate of Material to be Treated

This section is not applicable to this field demonstration project.

3.5.4 Residuals Handling

Following the completion of sampling, residual soils and sediments were discarded adjacent to the sampling location. Any investigation derived wastes (IDW) generated during the decontamination of equipment were containerized and disposed of according to the DoD facility's instructions.

3.5.5 Operating Parameters for the Technology

This section is not applicable to this field demonstration project.

3.5.6 Experimental Design

The primary objective of the field demonstration was to evaluate whether or not the sediment and soil exposure protocols and the associated lead and copper screening values developed during the

laboratory validation phase of this project, were readily validated in the field. Naturally aged soils and sediments were collected from each site and tested in the laboratory with naive salamanders and frogs to determine if site-specific conditions ameliorate toxicity. The naturally aged soils and sediments from each site have been exposed to the elements for months and years which may modify the bioavailability of certain compounds over time. At some sites where the chemicals have weathered for decades, the chemicals are held tightly by the soil and are unavailable for transport through the soil or into biological organisms (Loehr, 1996). The bioavailability of metals in soil will be influenced by the species present, particle size, and whether the metals have been encapsulated or coated by other mineral phases (Chaney, et al., 1989). The naïve test organisms have not previously been exposed to copper or lead so they are not expected to exhibit any natural resistance to the potential toxicity of these compounds. The test species used in the toxicity tests were the tadpoles of the Northern leopard frog (*R. pipiens*) for the sediment exposure assay and the adult red-backed salamander (*P. cinereus*) for the soil exposure assay.

The soil and sediments collected from the two demonstration sites were expected to contain a range of copper and lead concentrations as a result of current (Travis AFB) and historic (APG) firing range activities.

Three samples from each site (two impacted samples and one reference sample) were tested with the soil exposure protocol. Four impacted samples and a reference sample from each site were tested with the sediment exposure protocol. To provide a better distribution of lead concentrations in the tested sediments, three dilutions of the highest tested concentration were generated and included in the sediment exposure testing program. The sediment exposure tests also included a laboratory control sediment treatment with each set of tests.

Data from toxicity studies were statistically evaluated by comparing responses of individual soils/sediments to the laboratory control (standard, clean sediment that will not produce adverse effects) and/or through comparison to a reference soil/sediment collected concurrently with other samples.

Both lethal (mortality) and sub-lethal (growth) endpoints were measured in each toxicity study. Each data type was analyzed independently. Because sub-lethal measurements of surviving organisms can be skewed by a significant reduction in sample size, treatments that demonstrated significant mortality were excluded from sub-lethal analyses of growth endpoints.

Before conducting tests to identify statistical differences, suitability of the data for parametric analyses was evaluated through normality and equality of variance tests. Based on the results of the tests for normality of distribution and homogeneity of sample variances, data sets were evaluated using the appropriate parametric or non-parametric Analysis of Variance (ANOVA) statistic. Pair-wise comparisons were conducted to determine statistical differences between tested samples and reference or control results. Statistical difference was evaluated at $\alpha=0.05$. The alpha level represents the probability (5%) of committing a Type I statistical error, that is, finding a significant difference when, in fact, one does not exist. Reducing the alpha level will reduce the probability of committing a Type I error, but will also increase the probability of committing a Type II error, or not finding a statistical difference when, in fact, one does exist. For that reason, 0.05 is generally accepted as a reasonable level for most analyses.

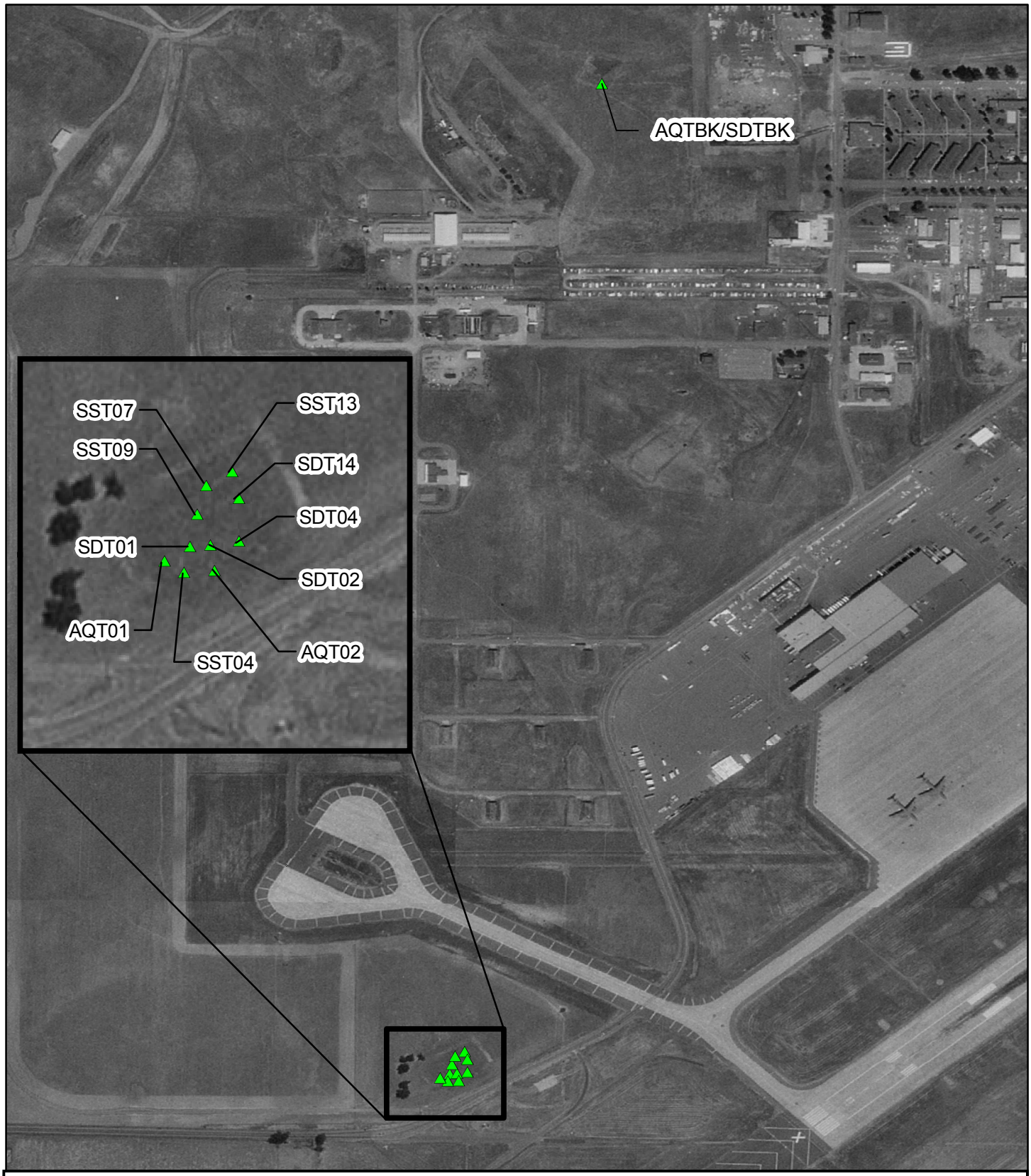
3.5.7 Sampling Plan

The sampling plan for both demonstration sites used the results of XRF surveys to identify appropriate sampling locations. Sampling was conducted in accordance with the Quality Assurance Project Plan presented in Appendix C of the *Field Demonstration Plan* (NAVFAC, 2007a) and the completed document is on file at ENSR and available upon request. Additional detail for each demonstration site is provided in the following subsections.

3.5.7.1 Travis Air Force Base (Travis AFB)

As described in Section 3.5.1, the results of the analytical samples collected during the field reconnaissance effort (Figure 3-3) were used to direct the XRF survey conducted as part of the field sampling event on March 27, 2006. The XRF survey included analysis of 26 samples for 22 metals.

Based on the results of the XRF survey, soil and sediment samples were collected from nine locations within the vernal pool study area and one reference location (Figure 3-4). Based on the moisture content of the material, five samples, including the reference, were identified as sediment and the remaining four were identified as soils. Table 3-6 presents the copper and lead data from the XRF survey and the confirmation analyses conducted at Paragon Analytical, Inc (Fort Collins, CO) for the nine samples collected as part of the Travis AFB field demonstration.



CA Digital Orthophoto Quads, 2002
Elmira, CA
Approximate Sampling Locations,
3/28/06

N

0 445 890 1,780 Feet

Travis Air Force Base Sampling Locations

SCALE	DATE	PROJECT NO.
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Figure Number

3-4

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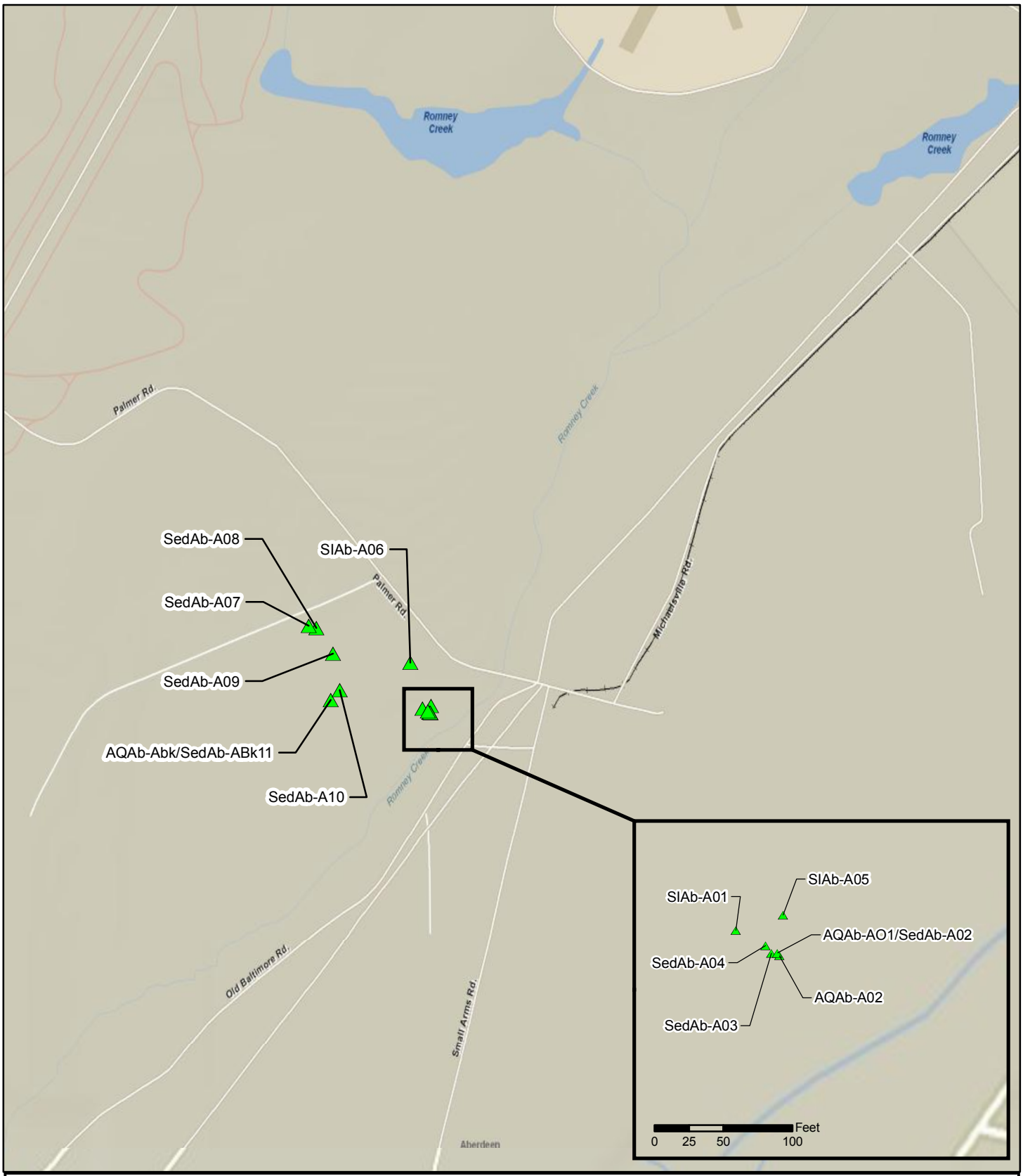
All sediment and soil samples were analyzed for copper, lead, TOC, grain size, CEC, simultaneously extracted metals (SEM), and acid volatile sulfides (AVS) (Table 3-6). Three samples were also analyzed for a full suite of 23 metals, 21 pesticides, and polychlorinated biphenyls (PCB) as Aroclors. The background sample was also analyzed for 17 PAHs. PAHs, pesticides, and PCBs were not detected in any samples. Metals, other than lead, were present at low levels and concentrations were similar between vernal pool samples and the reference location.

Surface water samples were collected from 2 locations within the vernal pool (center of pool and at the outlet) and at the reference location. Samples were analyzed for 23 total recoverable metals, total hardness, TOC, dissolved organic carbon (DOC), and dissolved phase copper, lead, and hardness. Fourteen metals were not detected in any of the vernal pool surface water samples. The detected metals (barium, calcium, iron, lead, magnesium, manganese, potassium, selenium, and sodium) were detected at low levels that would not be expected to be acutely toxic to aquatic receptors. Copper, lead, hardness, and organic carbon results are presented in Table 3-7.

3.5.7.2 Aberdeen Proving Ground (APG)

The APG study area has been the site of historic XRF data collection efforts as part of previous site investigation activities. These existing data were used to focus the XRF survey conducted just prior to the field sampling effort on specific areas of interest with lead concentration gradients desired for conducting the toxicity tests (see Section 3.5.1 for discussion of the XRF survey). The APG study area was also limited to the area cleared of UXO by Army personnel.

Based on the results of the XRF survey, samples were collected from ten locations within the palustrine wetland study area and one reference location (Figure 3-5). Based on the moisture content of the material, eight samples, including the reference, were identified as sediment and the remaining three were identified as soils. Table 3-8 presents the copper and lead data from the XRF survey and the confirmation analyses.



ArcGIS Online Imagery Service 2008
Aberdeen, MD
GPS Coordinates of Sampling Locations, 4/12/06

N

Feet
0 1,000 2,000 4,000

Aberdeen Proving Ground Sampling Locations

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Figure Number

3-5

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Table 3–6 Analytical Results for Sediment and Soil Collected from Travis Air Force Base

Location ID	Sample Matrix	Chemical Concentration (mg/kg)				TOC (%)	[Sum SEM - AVS]/f_{oc} (umol/g_{oc})	Bulk Density (lb/ft3)	Cation Exchange Capacity (meq/100g)	Grain Size (%)		
		XRF Survey [1]		Analytical Laboratory [2]								
		Copper	Lead	Copper	Lead					Gravel	Sand	Silt & Clay
Travis Air Force Base												
SDTBK	sediment	<16	20	15	14	1.5	-65.4	110	9.2	0	47.3	52.7
SST04	soil	<17	464	16 [3]	935 [3]	1.75 [3]	***325*** [3]	112	1.5	0.8	46.7	52.5
SDT04	sediment	no data	no data	21	1500	1.8	NC	106	13.4	0.4	45	54.6
SST09	soil	<18	1802	16	1600	1.6	***409***	116	1.3	0.9	48.9	50.2
SST07	soil	<20	4025	17	2000	1.6	***466***	110	8.0	0.3	48.1	51.6
SDT01	sediment	<15	910	20	2100	2	***452***	100	2.1	0	47.5	52.5
SDT02	sediment	<18	1268	20	2100	1.6	NC	114	1.5	0	47.4	52.6
SDT14	sediment	<134	1459	17	2500	1.9	***987***	110	11.9	0	45.3	54.7
SST13	soil	<120	2928	19	4200	2.3	***726***	106	1.8	0.5	47.8	51.7

BK in location ID identifies background reference location; all others are site locations.

1 - Samples analyzed by XRF survey prior to sediment and soil sampling. Values are not corrected for percent moisture (corrected values would be approximately 25% lower; assuming percent solid is approximately 75%).

2 - Samples analyzed by analytical laboratory directly following field sampling effort. Values reported on a dry weight basis.

3 - Average of parent and duplicate sample results.

One-half detection limit used in calculation for non-detect SEM analytes.

Shading and bold text indicates [Sum SEM-AVS]/f_{oc} > 130 umol/g_{oc}

- Values < 130 umol/g_{oc} are presumed to be "not likely" to be toxic (USEPA, 2005a).

f_{oc} - fraction organic carbon

g_{oc} - gram organic carbon

NC - Not calculated; Calculations not completed if AVS was not detected.

Table 3–7 Analytical Results for Surface Water Collected from Travis Air Force Base

	Chemical Concentration (mg/L)							
	Copper		Lead		Hardness		Organic Carbon	
Location ID	Dissolved	Total	Dissolved	Total	Dissolved	Total	Dissolved	Total
Travis Air Force Base								
AQT01 [1]	0.01 U	0.01 U	0.012	0.017	47	46.5	11	12.5
AQT02	0.01 U	0.01 U	0.0078	0.016	50	50	12	13
AQTBK	0.01 U	0.01 U	0.003 U	0.003 U	17	22	15	22

BK in location ID identifies background reference location; all others are site locations.
1 - Average of parent and duplicate sample results for total copper, lead, hardness, and organic carbon.
U – Not detected at or above the stated detection limit.

All sediment and soil samples were analyzed for copper, lead, TOC, grain size, CEC, SEM, and AVS (Table 3-8). Two samples and a field duplicate were also analyzed for a full suite of 23 metals, 21 pesticides, and PCB Aroclors. Pesticides and PCBs were not detected in any samples, with one exception. 4,4-DDE was detected in one sample at 6.2 micrograms per kilogram (ug/kg). This level is between the threshold effect concentration (TEC; 3.2 ug/kg) and the probable effect concentration (PEC; 31.3 ug/kg) discussed in MacDonald et al. (2000), indicating that the possibility of toxicity to benthic receptors could not be excluded in this sample. Most metals, other than lead, were present at low levels. Copper was present at higher concentrations than were observed at Travis AFB. Copper concentrations increased with increasing lead concentrations to a maximum of 1,150 mg/kg copper.

Surface water samples were collected from two locations and a field duplicate within the wetland and at the reference location. Samples were analyzed for 23 total recoverable and dissolved phase metals, total and dissolved hardness, TOC, and DOC. Fourteen metals were not detected in any of the surface water samples. The detected metals (calcium, copper, iron, lead, magnesium, manganese, potassium, sodium, and zinc) were detected at low levels that would not be expected to be acutely toxic to aquatic receptors. Copper, lead, hardness and organic carbon results are presented in Table 3-9.

3.5.7.3 Sample Collection

As described in Section 3.5.7, surface water, sediment, and hydric soil samples were collected from both Travis AFB and APG for chemical and toxicological analyses. During sample handling it was critical that cross-contamination between samples, or contamination from extraneous sources, not occur. Sample handling tools were constructed of inert materials wherever possible (stainless steel, polypropylene, or Teflon, as appropriate) and were decontaminated between sampling locations.

Decontamination consisted of a tap water rinse to remove gross contamination (if needed), followed by a non-phosphate detergent (e.g., Alconox) water rinse, a rinse with deionized water, and followed by another deionized water rinse. If equipment was to be stored or transported, it was wrapped in aluminum foil after air-drying. Water generated during decontamination of sampling equipment was containerized and disposed of according to DoD facility instructions.

Surface water samples were collected from mid-depth at selected sediment sampling locations prior to the collection of the sediment sample. All sediment samples were collected from relatively shallow locations so a boat was not required and samplers could wade in to the stations. Sediment was generally collected using stainless steel trowels and spoons. The sample was collected from the top 6 inches of sediment, with as little disturbance as possible. Soil samples were collected from the surficial 6 inches also using stainless steel trowels and spoons.

Soil and sediment samples from each sampling location were composited in a large stainless steel bowl prior to sub-sampling for chemical and toxicological analyses. To allow for accidental loss, spillage, analytical chemistry, or test reruns, a minimum of two gallons of each sediment and soil sample was collected from each location. Samples were cooled to 4°C before shipping and when not being used.

Table 3–8 Analytical Results for Sediment and Soil Collected from Aberdeen Proving Ground

Location ID	Sample Matrix	Chemical Concentration (mg/kg)			TOC (%)	[Sum SEM - AVS]/f_{oc} (umol/g_{oc})	Bulk Density (lb/ft3)	Cation Exchange Capacity (meq/100g)	Grain Size (%)		
		XRF Survey [1]	Analytical Laboratory [2]								
		Lead	Copper	Lead					Gravel	Sand	Silt & Clay
Aberdeen Proving Ground											
SedAb-ABk11	sediment	33	8.4	34	0.46	NC	123	7.3	0.2	46.2	53.6
SlAb-A06	soil	38	17	35	1.3	NC	119	9	0	26.1	73.9
SedAb-A08	sediment	343	21	200	0.36	-40.3	101	3.1	3.3	87.7	9
SlAb-A05	soil	1542	55	260	0.41 [3]	***371*** [4]	102	5.1	0	45.2	54.8
SedAb-A10	sediment	168	27	310	0.62	NC	112	13.8	0.3	14.2	85.5
SedAb-A07	sediment	229	34	460	1.9	49	106	10.9	3.9	36.4	59.7
SedAb-02B	sediment	1200	120 [3]	1275 [3]	0.55	***581***	128	1.6	2.3	59.5	38.2
SedAb-A04	sediment	4001	120	850	0.57	NC	117	4.3	2.9	47.7	49.4
SedAb-A09	sediment	741	73	870	0.11	***3336***	121	0.6	1.6	82.9	15.5
SlAb-A01	soil	12387	700	9900	0.088	NC	125	0.9	3.9	76.2	19.9
SedAb-A3A	sediment	2000	1150 [3]	17500 [3]	2.0 [3]	NC [3]	100	5.8	0	23.3	76.7

Bk in location ID identifies background reference location; all others are site locations.
1 - Samples analyzed by XRF survey prior to sediment and soil sampling. Values are not corrected for percent moisture (corrected values would be approximately 25% lower; assuming percent solid is approximately 75%).
2 - Samples analyzed by analytical laboratory directly following field sampling effort. Values reported on a dry weight basis.
3 - Average of parent and duplicate sample results.
4 -AVS was not detected in duplicate sample so only results of parent sample are presented.
Shading and bold text indicates [Sum SEM-AVS]/f_{oc} > 130 umol/g_{oc}
- Values < 130 umol/g_{oc} are presumed to be "not likely" to be toxic (USEPA, 2005a).

f_{oc} - fraction organic carbon
g_{oc} - gram organic carbon
NC - Not calculated; Calculations not completed if AVS was not detected.
NS - Not sampled.
One-half detection limit used in calculation for non-detect SEM analytes.

Table 3–9 Analytical Results for Surface Water Collected from Aberdeen Proving Ground

	Chemical Concentration (mg/L)							
	Copper		Lead		Hardness		Organic Carbon	
Location ID	Dissolved	Total	Dissolved	Total	Dissolved	Total	Dissolved	Total
Aberdeen Proving Ground								
AQAb-Abk	0.01 U	0.01 U	0.003 U	0.004	13	13	12	14
AQAb-A01	0.04	0.045	0.027	0.063	15	15	13	16
AQAb-A02 [1]	0.038	0.0555	0.0395	0.14	14	15.5	12	15.5

Bk in location ID identifies background reference location; all others are site locations.

1 - Average of parent and duplicate sample results for total copper, lead, hardness, and organic carbon.

U – Not detected at or above the stated detection limit.

Samples were accompanied by a properly completed chain-of-custody form. This record documents the transfer of custody of samples from the sampler, to another person, and to the permanent laboratory. Shipping containers were secured with strapping tape and sealed with custody seals. Samples were shipped daily from the field to the laboratory using an overnight courier.

Holding times are listed in Table 3-10. Holding times were met for metal, PCB, pesticide, and PAH analyses in soil and sediment and for all surface water analytes. Holding times were not met for the TOC or SEM and AVS analyses. Impacts to the data due to exceeded holding times are discussed in Section 3.5.7.6.

Table 3–10 Sample Holding Time Requirements

Parameters	**Holding Time[1]**
Sediment/Soil	
Metals	28 days for Hg; 6 months for others
TOC	14 days
Pesticides	14 days to extraction; 40 days from extraction to analysis
PCBs	14 days to extraction; 40 days from extraction to analysis
PAHs	14 days to extraction; 40 days from extraction to analysis
SEM/AVS	14 days
Grain size	None
Cation exchange capacity	None
Bulk density	None
Surface Water	
Metals (dissolved)	28 days for Hg; 6 months for others
Metals (total recoverable) and hardness	28 days for Hg; 6 months for others
DOC	28 days
TOC	28 days
1 - Holding time begins from date of sample collection.	

QC samples collected during the field sampling effort at each site included an equipment rinsate blank, a field duplicate for each medium, and MS/MSDs as appropriate for the parameter and media sampled.

Figure 3-6 Sediment Collection at Travis Air Force

Figure 3-7 Sediment Sampling Location at Travis Air Force Base

3.5.7.4 Sample Analysis

Sediment and soil samples were shipped on ice under chain-of-custody directly to the chemistry and toxicity testing laboratories. All sediment and soil samples were analyzed for copper, lead, TOC, grain size, CEC, SEM, and AVS following the field sampling effort. A subset of sediment and soil samples were also analyzed for a full suite of 23 metals, 21 pesticides, 17 PAHs, and 7 PCB Aroclors. Surface water samples were analyzed for 23 total recoverable and dissolved phase metals, total and dissolved hardness, TOC, and DOC.

Copper and lead were considered to be the primary chemicals of concern in the sediment and soil. Detection limits ranged from 1.0 to 1.8 mg/kg for copper and 0.31 to 47 mg/kg for lead in sediment and soil. The elevated detection limits for lead were reported for samples requiring dilutions due to concentrations that exceeded the calibration range.

Samples selected for toxicity testing are presented in Table 3-11. Copper and lead concentrations in soil and sediment were reviewed in order to select a wide range of concentrations. The sediment toxicity testing was conducted at ENSR's Fort Collins Environmental Toxicology Laboratory (FCETL) in Fort Collins, Colorado and the soil toxicity testing was conducted at USACHPPM's APG , Maryland laboratory. Toxicity testing was conducted according to the quality assurance/quality control (QA/QC) plans in place at the ENSR and the USACHPPM toxicity laboratories and the protocols presented in Appendix A.

Table 3–11 Samples Selected For Toxicity Testing

Location ID	Sample Matrix	Chemical Concentration [1] (mg/kg)		Samples Selected For Toxicity Testing	
		Copper	Lead	Salamander [Soil Exposure]	Tadpole [Sediment Exposure]
Travis Air Force Base					
SDTBK	sediment	15	14	x	x
SST04 [3]	soil	16	935		
SDT04	sediment	21	1500		x [2]
SST09	soil	16	1600	x	
SST07	soil	17	2000		x
SDT01	sediment	20	2100		
SDT02	sediment	20	2100		
SDT14	sediment	17	2500		x
SST13	soil	19	4200	x	x
Aberdeen Proving Ground					
SedAb-ABk11	sediment	8	34		x
SlAb-A06	soil	17	35	x	
SedAb-A08	sediment	21	200		x
SlAb-A05	soil	55	260	x	
SedAb-A10	sediment	27	310		
SedAb-A07	sediment	34	460		x
SedAb-02B [3]	sediment	120	1275		
SedAb-A04	sediment	120	850		x
SedAb-A09	sediment	73	870		
SlAb-A01	soil	700	9900	x	
SedAb-A3A [3]	sediment	1150	17500		x [2]

BK or Bk in location ID identifies background reference location; all others are site locations.

1 - Samples analyzed by analytical laboratory following field sampling effort.

2 - Three dilutions from this sample were generated and tested in order to achieve a better distribution of lead concentrations.

3 - Average of parent sample and duplicate results for copper and lead.

Due to delays in starting the sediment toxicity tests associated with test organism availability, samples selected for the sediment toxicity testing were submitted for an additional set of copper and lead analyses prior to test set-up. Lead in soil was analyzed by an in-house USACHPPM laboratory on Days 0, 14, and 28 of the soil exposure test. These additional sets of analytical data are presented in Section 4 with the results of the sediment and soil toxicity tests.

3.5.7.5 Experimental Controls

At least one negative control treatment was included in the sediment and soil tests. Negative controls represent sediment and soil without significant levels of copper or lead. This may be accomplished through the use of a "clean" sample provided by the laboratory or through the collection of a reference control soil or sediment that represents a similar habitat type to the study area, but does not contain significant levels of copper or lead.

Reference samples were collected in both soil and sediment at each of the demonstration sites. Reference locations with similar physical characteristics (e.g., organic carbon, grain size) to the tested samples were selected to avoid the impact of these characteristics on the interpretation of the test results. The reference soil samples served as the negative controls in the soil tests. The sediment tests included the sediment reference samples as well as laboratory control samples as the negative controls.

3.5.7.6 Data Quality Parameters

The *Field Demonstration Plan* (NAVFAC, 2007a) specified collection and handling procedures designed to ensure the representativeness and integrity of the samples. The analytical program was designed to generate definitive data of sufficient quality and sensitivity to meet the project objectives.

Laboratory QA/QC measures were performed by the analytical laboratories to ensure that all environmental efforts to produce the data are technically sound and legally defensible. The quality assurance measures include standard operating procedures, applicable certifications, training programs, internal audits, and internal QC checks. Internal QC checks differ slightly for each individual procedure but in general include the following:

- Method blanks – used to define the level of laboratory background and reagent contamination,
- Laboratory control spikes (LCSs) – provide information on method accuracy and laboratory performance,
- Matrix spikes – determine accuracy of the method for the matrix,
- Duplicate samples – used to demonstrate acceptable method precision by the laboratory at the time of analysis,
- Surrogate spikes – used to detect problems in sample preparation procedures.

The laboratory SOPs for each analysis define the type, frequency, and corrective action for the applicable QC checks.

For the sediment toxicity testing laboratory, laboratory controls were tested with each set of samples and reference toxicant tests were completed within the acceptable results range.

Measures to ensure representativeness, completeness, comparability, accuracy and precision of the data are discussed below and in the Quality Assurance Project Plan (QAPP; Appendix B).

Representativeness – Representativeness expresses the degree to which data accurately and precisely represent a characteristic of a population, parameter variations at a sampling point, a process condition, or an environmental condition within a defined spatial and/or temporal boundary. Therefore, representativeness of the field data is highly dependent upon the proper design of the sampling program, and was achieved through strict adherence to the Field Demonstration Plan (NAVFAC, 2007a). Representativeness of the laboratory data was achieved by strict adherence to analytical SOPs and conformance to the majority of sample holding times. Other than holding time nonconformances for TOC, SEM, and AVS analyses, no significant deviations were noted. Exceeding the holding time for TOC analysis would not be expected to significantly impact the sample results. Exceeding the holding time for AVS analyses may result in an under-estimation of the sulfide levels as they dissipate over time. It is possible that the SEM mercury results are biased low due to the exceeded holding time.

Completeness – Completeness is a measure of the amount of valid data obtained from a measurement system compared to the amount that was expected to be obtained under normal conditions. "Normal conditions" are defined as the conditions expected if the sampling plan was implemented as planned.

Field sampling completeness was 100%. Samples from all proposed stations were collected and submitted for the analyses in the QAPP.

Laboratory completeness is a measure of the amount of valid measurements obtained from all the measurements taken in the project. All analyses were successfully performed by the laboratory for the requested parameters. However, soil and sediment pH measurements were inadvertently not performed.

Comparability – Comparability expresses the confidence with which one data set can be compared to another. Analytical data from this program are considered to be comparable since similar sampling and analytical methods were used at each demonstration site as documented in the Field Demonstration Plan (NAVFAC, 2007a).

Accuracy – Accuracy is the degree of agreement between the observed value and an accepted reference or true value. Accuracy in the field was assessed through the use of equipment rinsate blanks and through the adherence to sample handling, preservation, and holding time requirements. The objective for equipment rinsate blanks is that no target analytes are present above the reporting limit. This objective was achieved for the equipment rinsate blanks collected at each demonstration site except for one equipment rinsate blank associated with the soils and sediments collected at APG (see Appendix C). Zinc was detected in the equipment rinsate blank at a concentration of 0.040 mg/L (2x the detection limit). This level of zinc contamination is insignificant compared to the zinc concentrations in the samples and does not impact sample results.

Laboratory method blanks were free of contamination for all parameters except SEM zinc and mercury. Low level concentrations of these analytes reported in the samples analyzed for SEM are likely to be biased high.

The impacts to sample data due to exceeded holding times are presented above as part of the discussion on representativeness.

Laboratory accuracy was assessed through the analysis of matrix spike/matrix spike duplicates (MS/MSDs), LCSs, and surrogate compounds, and the subsequent determination of the recoveries of the spiked analytes.

Due to a laboratory oversight, MS/MSD analyses were not performed for TOC. However, LCSs were analyzed with each batch of samples. Acceptable recoveries were obtained for the LCSs demonstrating acceptable laboratory performance of the method.

In general, MS/MSD, LCS, and surrogate recoveries fell within the laboratory control limits. MS recoveries for AVS were very low, most likely due to difficulties with the sample matrix. AVS results should be considered to have a very low bias.

Precision - Precision is a measure of the degree to which two or more measurements are in agreement. Precision was measured through the calculation of relative percent difference (RPD). The objectives for field precision RPDs are 30% RPD for aqueous samples and 50% RPD for solid samples. Overall, the objectives for field precision were met with only a few exceptions. The RPDs for total lead in the surface water field duplicate pair collected at APG and for total manganese in the surface water field duplicate pair collected at Travis AFB exceeded the RPD criterion. The RPDs for antimony and lead exceeded the criterion for the sediment field duplicate pair collected at APG. The RPD for SEM lead exceeded the criterion in the soil field duplicate pair collected at APG. High RPDs in solid samples are most likely due to sample non-homogeneity.

Precision in the laboratory is assessed through the calculation of RPD for duplicate samples, either as MS/MSDs or as laboratory duplicates, depending on the method. The laboratory utilized current in-house control limits at the time of analysis for assessing precision. In general, RPDs for laboratory duplicates and MS/MSDs met the acceptance criteria. Sample nonhomogeneity was likely the source of the high RPDs observed for selected metals in the solid samples.

3.5.7.7 Data Quality Indicators

Statistical analyses were used to identify significant differences in the soil and sediment toxicity tests. Organism responses for both lethal and sub-lethal endpoints were compared against the associated laboratory control or reference station results.

Equations used to evaluate analytical data quality are presented in the QAPP (Appendix B).

3.5.7.8 Calibration Procedures, Quality Control Checks, and Corrective Action

Field instruments used during the sampling events included a GPS unit, an XRF unit, and YSI multi-parameter meter. All field instruments were free from obvious defects, damage, and contamination and were properly functioning during the field events. Daily operational checks and calibrations were conducted. In general, field instruments were calibrated prior to daily use, and were checked after every 15 samples and at the end of the day. Calibration procedures were consistent with the manufacturer's recommendations.

Routine testing and preventive maintenance is performed by the analytical laboratories as part of their in-house QA programs. Calibration procedures for laboratory instruments generally consisted of initial calibrations, initial calibration verifications, and continuing calibration verification in accordance with the SOP for each analysis.

For the toxicity laboratories, the performance of test organisms in the laboratory control and/or the reference samples is used to determine test acceptability. As described in Section 3.5.7.5, these negative controls represent sediment and soil without significant levels of copper or lead and these samples may be used to evaluate the health of the test organisms and the test conditions (e.g., lighting, temperature). If survival in the control treatment (or the reference sample in the soil exposure assay) is less than 80%, then the test data should be carefully examined to determine if it is acceptable. Survival in the negative controls was >80% in the soil and sediment tests; therefore all tests were considered to be acceptable

Reference toxicant tests were also conducted to evaluate the health of the tadpoles used in the sediment exposure test. The response of test organisms in the reference toxicant tests and the negative controls indicated that the health of the test organisms was not impaired prior to exposure to the test sediments.

3.5.8 Demobilization

Following the completion of field surveys and sample collection at the selected sites, samples were shipped to the appropriate analytical laboratory or toxicity testing laboratory for analysis. Sampling and homogenization equipment was washed and decontaminated between samples and prior to demobilization. Sampling equipment was shipped back to the appropriate point of origin.

3.6 Selection of Analytical/Testing Methods

Protocols for conducting the sediment and soil bioassays are provided in Appendix A. These protocols were developed under previous DoD-funded projects. The laboratory validation phase of this project was designed to finalize the protocols and the associated protocols (NAVFAC, 2007b). Analytical methods are described in the QAPP presented in Appendix B.

3.7 Selection of Analytical/Testing Laboratory

The sediment and soil toxicity testing was conducted at the FCETL and the USACHPPM toxicity laboratories, respectively. The sediment and soil exposure protocols were developed and validated at these laboratories and both facilities have been involved in conducting similar types of tests for many years.

Chemical analyses were conducted by the following laboratories:

- Paragon Analytics of Fort Collins, Colorado – metals, pesticides, PCBs, PAHs in soil, sediment and water
- Mitkem Corporation of Warwick, Rhode Island – TOC
- STL-Burlington of Colchester, Vermont – SEM and AVS

- GeoTesting Express of Boxborough, Massachusetts – grain size, bulk density, and CEC analyses

The soil toxicity testing protocol requires some special expertise in animal handling (e.g., for test termination) and in tissue processing and analysis (e.g., for histopathological and blood chemistry endpoints). The sediment testing protocol should not require expertise beyond what would be typical for a laboratory conducting sediment toxicity tests with invertebrates or fish. The chemical analyses conducted to characterize the soil and sediment are typical for site investigations and do not require any special processing or expertise.

4.0 Performance Assessment

4.1 Performance Criteria

Performance criteria for the field demonstration are presented in Table 4-1 and are based upon the performance objectives presented in Table 3-1. These criteria were originally presented in the *Field Demonstration Plan* (NAVFAC, 2007a). The types of performance objectives and criteria established for typical remediation-related ESTCP projects (e.g., end-point criteria, remediation time, and analytical sensitivity) are indirectly associated with the ecological risk and toxicity based performance objectives and criteria developed for this project. The success of the performance of the innovative technology was determined based on whether or not the soil and sediment exposure protocols were able to correlate an amphibian response with contaminant concentrations and whether the protocols could be broadly applied at sites requiring risk assessment characterization for amphibians.

Table 4–1 Performance Criteria

Performance Criteria	Description	Primary or Secondary
Sediment protocol is applicable to evaluating copper and lead in palustrine wetlands	Describe whether or not there is a statistical relationship between contaminant concentrations and the results of the assay.	Primary
Soil protocol is applicable to evaluating copper and lead in forested uplands	Describe whether or not there is a statistical relationship between contaminant concentrations and the results of the assay.	Primary
Collection and biological evaluation of native salamanders is applicable for evaluating potential impacts due to metals	Describe whether or not there is a relationship between contaminant concentrations and the blood and/or histological evaluation.	Secondary
Regulatory acceptance of toxicity test protocols	Describe interaction with regulatory agencies regarding amphibian ERA results	Primary
Versatility of the overall ERA protocol	Describe whether or not ERA protocol was applicable at both field demonstration sites	Primary
Technology transferred to other potential end-users	Identify and describe presentations of the technology at conferences or in journals.	Secondary
Sediment Exposure Protocol - Sediment toxicity test is valid and acceptable	Describe whether test acceptability criteria were met	Primary
Sediment Exposure Protocol - Lethal endpoint indicates toxicity or lack of toxicity	Describe whether statistical differences were observed between tested samples and the control or reference stations	Primary

Table 4–1 Performance Criteria (continued)

Performance Criteria	Description	Primary or Secondary
Sediment Exposure Protocol - Sub-lethal endpoints indicate toxicity or lack of toxicity	Describe whether statistical differences were observed between tested samples and the control or reference stations	Primary
Soil Exposure Protocol - Soil toxicity test is valid and acceptable	Describe whether test acceptability criteria were met	Primary
Soil Exposure Protocol - Lethal endpoint indicates toxicity or lack of toxicity	Describe whether statistical differences were observed between tested samples and the control or reference stations	Primary
Soil Exposure Protocol - Growth endpoints indicate toxicity or lack of toxicity	Describe whether statistical differences were observed between tested samples and the control or reference stations	Primary
Soil Exposure Protocol - Blood parameters indicate toxicity or lack of toxicity	Describe whether statistical differences were observed between tested samples and the control or reference stations	Primary

This study was conducted consistent with the standards found in Title 40 Code of Federal Regulations (CFR), Part 792, Good Laboratory Practices and through an approved protocol with the Institutional Animal Care and Use Committee (IACUC). The investigators and technicians adhered to the following guidelines: the Public Health Service Policy on Humane Care and Use of Laboratory Animals, "U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training," and the Animal Welfare Act.

Although not identified in the *Field Demonstration Plan* (NAVFAC, 2007a) as a formal performance criteria, in order to comply with DoD requirements the two laboratories conducting the toxicity tests were required to have appropriate Animal Use Protocols in-place. This Protocol ensures that all vertebrate animals are treated humanely and do not endure any unnecessary pain.

The CHPPM laboratory conducting the soil exposure testing had established the appropriate Animal Use Protocols prior to the laboratory validation phase of testing. The FCETL did not already have an existing Animal Use Protocol so a project-specific IACUC was convened to oversee and evaluate the animal care program and ensure that treatment of animals at the laboratory was in compliance with applicable regulations and laws (e.g., animal welfare regulations).

The project-specific IACUC toured the laboratory facility, reviewed documentation, questioned researchers, and provided conditional approval of the testing protocol in April 2006. To achieve final approval from the IACUC, the laboratory modified their animal care standard operating procedure (SOP) and generated a project-specific Animal Care and Use Questionnaire for conducting the sediment protocol and submitted both documents for review in August 2006. Final approval from the IACUC was received in September 2006. The final approval memo, the SOP

and the questionnaire are provided in Appendix A of the *Test Refinement Interim Report* (NAVFAC, 2007b).

4.2 Performance Confirmation Methods

Adherence to the data collection methods and analyses presented in the *Field Demonstration Plan* (NAVFAC, 2007a) ensured that reliable data was collected. Data quality was assessed through the use of duplicate analytical samples, MS/MSD analyses, and the use of negative controls in the toxicity tests. Sufficient data were collected to evaluate the performance criteria listed in Table 4-1. Table 4-2 presents the expected performance metric for each of the performance criteria, the method that was used to confirm performance, and the actual performance noted during the demonstration.

The primary measurement for determining the effectiveness of the demonstration was whether or not there was a relationship between the concentrations of copper and/or lead in the soil and sediment and the results of the associated toxicity tests. Statistical methods were used to determine whether or not test organism responses in the tested samples containing elevated levels of lead were different from responses in laboratory controls or reference sample containing much lower levels of lead.

4.3 Data Analysis, Interpretation and Evaluation

As described in Section 3.5.6, statistics were used to evaluate whether or not toxic responses in tested soil or sediment at each demonstration site were significantly different from the laboratory control or reference stations. Following the statistical evaluation the analytical chemistry data were reviewed in order to identify media concentrations that may correlate with a toxic response. A lead concentration gradient was tested at both sites, allowing the development of LOAECs and NOAECs for both survival and sub-lethal endpoints. To derive these values, the survival or sub-lethal data for all stations at a site were ranked by the associated lead concentration with an indication of which samples were statistically toxic compared to the reference locations.

Some tested samples were identified as toxic compared to the reference while others were consistent with the reference results, indicating a non-toxic response. LOAECs and NOAECs were estimated by identifying the concentration of each analyte at the demarcation between toxic and non-toxic samples, as indicated by the statistical evaluation. The NOAEC represents the tested sample with the highest concentration of a constituent of potential concern (COPC) that was not significantly different from the control or reference station, whereas the LOAEC is the tested sample above which all concentrations were significantly different from the control or reference.

The results of the field demonstration tests were also evaluated relative to the screening values developed during the laboratory validation phase of testing (presented in Section 3.4) with lead-spiked soil and sediment.

Finally, the use of the amphibian ERA framework was evaluated at each site to determine whether it would be applicable for characterizing potential risks to amphibians at the two demonstration sites.

Table 4–2 Expected Performance and Performance Confirmation Methods

Performance Criteria	Expected Performance Metric	Performance Confirmation Method	Actual
Primary Criteria (Qualitative)			
Sediment protocol is applicable to evaluating copper and lead in palustrine wetlands	Correlation between sediment concentrations and lethal or sub-lethal results	Statistical evaluation to be conducted	Several samples with higher concentrations of lead from each demonstration site were statistically different from the reference samples
Soil protocol is applicable to evaluating copper and lead in forested uplands	Correlation between mesic soil concentrations and lethal or sub-lethal results	Statistical evaluation to be conducted	Several samples with higher concentrations of lead from each demonstration site were statistically different from the reference samples
Regulatory acceptance of toxicity test protocols	Results are accepted by agency as component of ERA	Study results submitted to regulatory agency as part of site assessment	Study results have not been submitted to agencies; no on-going investigations are being conducted at either demonstration site; however ASTM approval of the sediment protocol has been achieved
Versatility of the overall ERA protocol	ERA protocol applicable for various sites	Application of ERA protocol at both field demonstration sites	Tiered ERA protocol is appropriate for use at various sites

Table 4-2 Expected Performance and Performance Confirmation Methods (continued)

Performance Criteria	Expected Performance Metric	Performance Confirmation Method	Actual
Primary Criteria (Quantitative)			
Sediment Exposure Protocol - Sediment toxicity test is valid and acceptable	Mean survival in laboratory control is >80%	Laboratory controls evaluated at test termination	Laboratory control results met acceptability criteria
Sediment Exposure Protocol - Lethal endpoint indicates toxicity or lack of toxicity	Statistical difference between survival in control or reference samples and site samples	Statistical evaluation to be conducted	Statistical evaluation indicated significant mortality in some samples
Sediment Exposure Protocol - Sub-lethal endpoints indicate toxicity or lack of toxicity	Statistical difference between sub-lethal endpoints in control or reference samples and site samples[3]	Statistical evaluation to be conducted	Statistical evaluation indicated significant growth reduction (i.e., body width and length) in some samples
Soil Exposure Protocol - Soil toxicity test is valid and acceptable	Mean survival in laboratory control is >80%	Laboratory controls evaluated at test termination	Tests did not include laboratory control; survival was acceptable in reference samples
Soil Exposure Protocol - Lethal endpoint indicates toxicity or lack of toxicity	Statistical difference between survival in control and site samples	Statistical evaluation to be conducted	No lethal toxicity observed in any sample

[3] Sub-lethal endpoints may include growth, abnormalities, behavior, metamorphic stage, or other measurements.

Table 4-2 Expected Performance and Performance Confirmation Methods (continued)

Performance Criteria	Expected Performance Metric	Performance Confirmation Method	Actual
Primary Criteria (Quantitative)			
Soil Exposure Protocol - Growth endpoints indicate toxicity or lack of toxicity	Statistical difference between growth endpoints in control or reference samples and site samples	Statistical evaluation to be conducted	Statistical evaluation indicated significant growth reduction in several samples with higher lead concentrations relative to reference sample results
Soil Exposure Protocol - Blood parameters indicate toxicity or lack of toxicity	Statistical difference between blood parameters measured in control or reference samples and site samples	Statistical evaluation to be conducted	No statistical differences were observed in blood parameters in any samples
Secondary Criteria (Qualitative)			
Collection and biological evaluation of native salamanders is applicable for evaluating potential impacts due to metals	Correlation between mesic soil concentrations and histopathological evaluation	Statistical evaluation to be conducted	Native salamanders were not collected so criteria could not be evaluated
Technology transferred to other potential end-users	Presentation at conference or in journal	Results or protocols presented	Peer-reviewed article has been submitted to present soil exposure results. Sediment exposure protocol has been accepted as ASTM guide. Peer-reviewed articles to be prepared.

4.3.1 Soil Exposure Protocol

The 28-day soil exposure protocol was conducted in accordance with the general protocol described in Appendix A. The soils collected from each demonstration site were dried, pulverized, and sifted through 2 screens (Nalgene; 1- mm^2 and 0.5-mm^2 mesh) to homogenize the sample. Each treatment consisted of 10, individually housed salamanders (Figure 4-1). Each animal was placed into an individual Petri dish containing treatment-specific soil. Food consisted of potworms exposed to lead-contaminated soil from the same sample used to expose the salamanders. Animals were observed at least daily for signs of overt toxicity (e.g., lethargy, sensitivity to touch, abnormal behavior) and body weights were measured weekly.

Figure 4-1 Soil Exposure Protocol Test Set-up

On Day 28, surviving salamanders were euthanized using aqueous preparations of MS-222 followed by decapitation. The remaining head and body were preserved in 10% neutral buffered formalin. Cross sections of the head and body were then trimmed, embedded in paraffin, sectioned at 6 microns, stained with hematoxylin and eosin, and examined via routine light microscopy. The histologic sections were of adequate size and quality for the detection of treatment-related changes. Histologic observations and a record of tissues examined were entered into a computer-assisted data retrieval system (StarTox, Graham Laboratories, New Braunfels, TX) at the time of histologic examination. Growth, mortality, and health criteria (blood parameters, histological organ evaluation including quantification of liver melanomacrophages) results were incorporated into the dose response based screening values.

Soil samples were collected at the start (Day 0), mid-point (Day 14), and end of the assay (Day 28) to determine lead concentrations to which the salamanders were exposed during the assays. As described in Section 3.5.2, a protocol deviation resulted in half of the salamanders being exposed

for 29 days instead of 28 days. The necropsies were originally planned over the course of two days with the beginning exposures staggered accordingly; however all test organisms were necropsied on one day. Therefore, half the animals were exposed for an extra day.

4.3.1.1 Results of Field Demonstration

Three samples from each demonstration site were tested, with lead concentrations ranging from 11 mg/kg to nearly 17,000 mg/kg during the test (average of Day 0, Day 14, and Day 28 measurements). Copper levels in these samples measured just after the field effort (Tables 3-6 and 3-8) ranged up to 700 mg/kg; below the survival NOAEC observed during the laboratory validation testing (803 mg/kg; Table 3-3). A laboratory control treatment was not included in the test design so treatment results were compared against the results from the associated reference sample (SlAb-A06 was treated as the reference sample for the salamander tests on the APG samples).

Data from Travis AFB and APG were analyzed separately due to the differences in soil types (see Tables 4-3 and 4-4). The parameters evaluated included total erythrocyte counts, total leukocyte counts, hemoglobin, body weight, and percent change in body weight (calculated as the change from baseline body weight). Normality for all parameters was tested with Kolmogorov-Smirnov and transformed if necessary. Erythrocyte and leukocyte data were transformed with the natural log for analysis. One-way analysis of variance (ANOVA) was performed for all parameters using the lead concentration as the fixed factor. Repeated measures ANOVA was also performed on the weekly body weight measurements and percent change in growth. Tukey's post-hoc was performed if a significant difference ($p<0.05$) was found.

As indicated in Table 4-3, no mortality was observed in any treatments. Statistical evaluations indicated no significant differences among the Travis AFB samples.

The only difference observed among the APG samples was for percent change in body weight on Day 28 (Figure 4-2). Growth was significantly lower for SlAb-A01 (16,967 mg/kg) relative to the APG reference sample (SlAb-A06 with 28 mg/kg lead) ($p=0.009$). The percent change in body weight on Day 28 for the SlAb-A01 sample was also statistically different from the SlAb-A05 sample (260 mg/kg lead) ($p=0.027$).

The final histology report (presented in Appendix C) concluded that there was no toxicity associated with the field-collected (aged) soil exposures. No test article-related histopathologic findings were found.

Table 4–3 Summary of Soil Exposure Results – Survival and Growth

Location ID	Lead Concentration[1] (mg/kg)	TOC (%)	Mean Survival	Mean Body Weight (g)					Mean Change in Weight from Day 0 (%)			
			Day 28	Day 0	Day 7	Day 14	Day 21	Day 28	Day 7	Day 14	Day 21	Day 28
Travis Air Force Base												
SDTBK	10.8	1.5	100%	0.813	0.825	0.818	0.839	0.834	1.244	0.255	2.727	2.308
SST09	1430	1.6	100%	0.801	0.805	0.798	0.804	0.786	0.811	-0.250	0.461	-1.947
SST13	2710	2.3	100%	0.798	0.826	0.814	0.837	0.823	-2.975	1.882	4.360	3.412
Aberdeen Proving Ground												
SlAb-A06	28	1.3	100%	0.812	0.818	0.811	0.818	0.819	0.658	-0.073	0.748	0.755
SlAb-A05	260	0.41	100%	0.808	0.827	0.806	0.820	0.808	2.174	-0.447	1.461	-0.289
SlAb-A01	16967	0.088	100%	0.812	0.862	0.805	0.789	0.760	5.764	-1.025	-2.878	**-6.565**

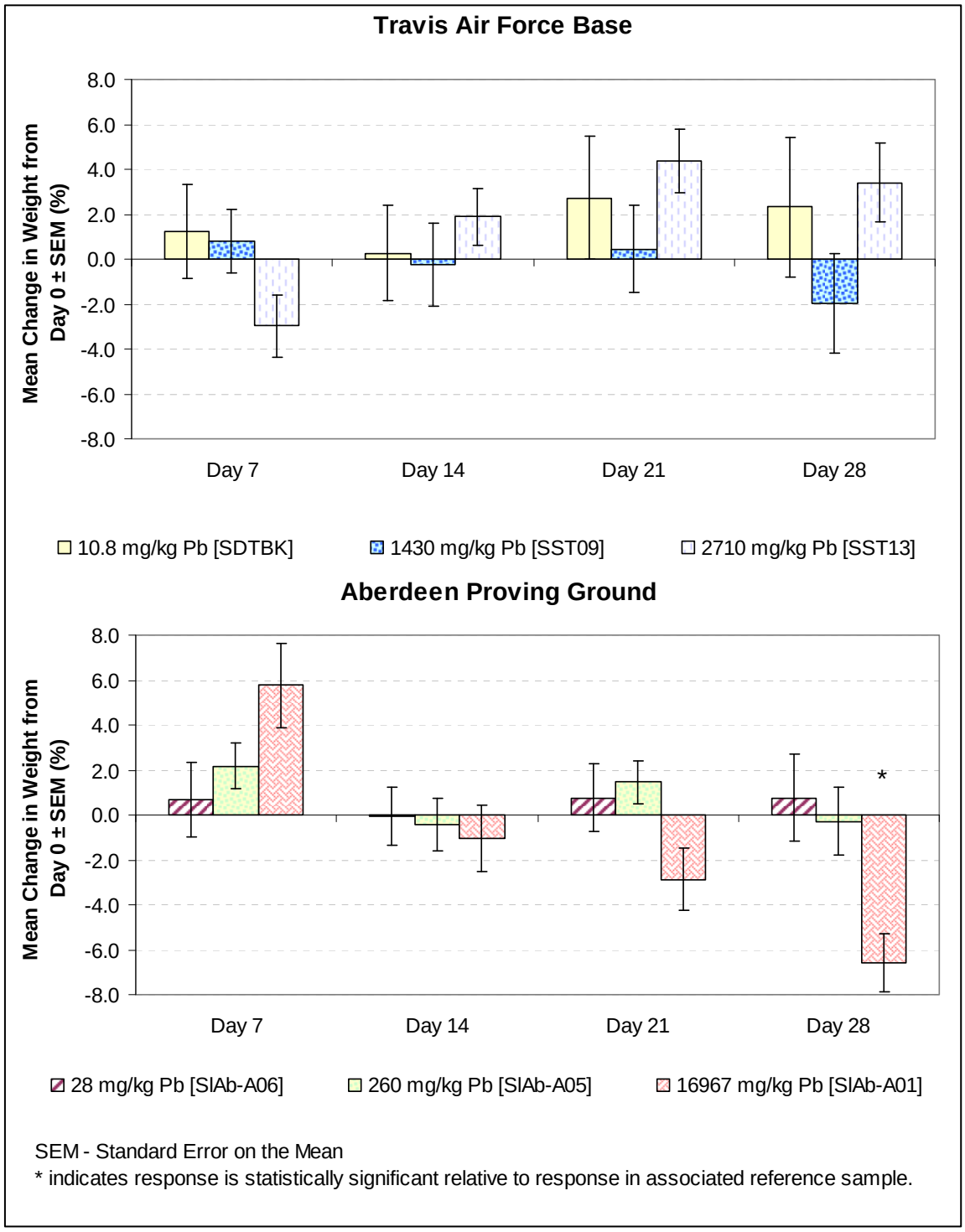
1 - Samples analyzed by CHPPM on Days 0, 14, and 28 of test. Average is presented.
BK in location ID identifies background reference location; all others are site locations.
Bold text indicates result is statistically different from associated reference sample results.

Table 4–4 Summary of Soil Exposure Results – Blood Parameters

Location ID	Lead Concentration[1] (mg/kg)	TOC (%)	Average erythrocyte counts (10x4 cells/ul)	Average leukocyte counts (10x3 cells/ul)	Average Hemoglobin (g/dL)
Travis Air Force Base					
SDTBK	10.8	1.5	9.73	4.06	9.4
SST09	1430	1.6	9.40	4.20	8.8
SST13	2710	2.3	11.44	4.12	8.6
Aberdeen Proving Ground					
SlAb-A06	28	1.3	9.30	4.01	8.3
SlAb-A05	260	0.41	8.95	4.09	9.3
SlAb-A01	16967	0.088	8.98	4.80	8.4

1 - Samples analyzed by CHPPM on Days 0, 14, and 28 of test. Average is presented.
BK in location ID identifies background reference location; all others are site locations.
No results were statistically different from associated reference sample results.

Figure 4-2 Mean % Change in Weight Over 28 Days of Soil Exposure



SEM - Standard Error on the Mean
* indicates response is statistically significant relative to response in associated reference sample.

4.3.1.2 Evaluation of Field Demonstration Results Relative to Previous Studies

In general, the concentrations of lead in the field demonstration soils were similar to the range of concentrations evaluated in the laboratory validation testing. The maximum lead concentration from the APG demonstration site (16,967 mg/kg) was above the maximum concentration (9,167 mg/kg lead) detected in the lead-spiked soil evaluated in the laboratory validation phase of testing.

A comparison of the NOAECs and LOAECs derived from the laboratory validation tests conducted with lead acetate and the field demonstration tests show that less toxicity was observed in the test conducted with field-collected aged soils (Table 4-5). These results indicate that using screening values derived from studies conducted with laboratory-spiked soils may be overly protective of salamanders exposed to weathered metals under field conditions.

Table 4–5 Comparison of Soil NOAECs and LOAECs in Validation Testing and Field Demonstrations

Study	Lead (mg/kg)			
	Survival		Sub-Lethal Endpoint	
	NOAEC	LOAEC	NOAEC	LOAEC
Laboratory Validation [Spiked Soil]	1700	4700	1700	4700 [2]
Travis AFB	2710 [1]	>2710 [1]	2710 [1]	>2710 [1]
Aberdeen Proving Ground	16967 [1]	>16967 [1]	260	16967 [3]
All Field Demonstration Data	16967 [1]	>16967 [1]	2710	16967 [3]

Concentrations based on average of measured values at Days 0, 14, and 28 of test.

1 - No sub-lethal effects were observed at the highest tested concentration at demonstration site.

2 - Reduced leukocyte count observed at 4700 mg/kg lead.

3 - Reduced cumulative average growth observed at 16967 mg/kg lead.

For example, during the validation phase of testing, only 20% survival was recorded for salamanders exposed to the 9,167 mg/kg lead level, resulting in a survival NOAEC of 1,700 mg/kg lead. However, in the field demonstration testing, even at the highest lead concentration (16,967 mg/kg), no mortality was observed. This resulted in a survival NOAEC of 2,710 mg/kg lead for Travis AFB samples and 16,967 mg/kg lead for APG samples (the maximum tested concentration at each site). Since these NOAECs were derived based on a lack of observed toxicity, the actual NOAECs associated with a toxic response would be higher.

A similar trend was observed for the sub-lethal endpoints with toxicity observed at higher concentrations in the field demonstration tests. However, few statistically significant sub-lethal endpoints were observed in either the laboratory validation testing or the field demonstration testing. In the laboratory validation testing, the only sub-lethal response was a reduced leukocyte count observed in the 4,700 mg/kg lead treatment, resulting in a NOAEC of 1,700 mg/kg lead. The only statistically significant sub-lethal effect observed in the field demonstration was a reduction in

body weight (as percent change in body weight relative to Day 0) in the highest lead concentration (Figure 4-2).

Due to the wide distribution in lead concentrations for the APG site, the NOAEC for the percent change in body weight endpoint at APG was 260 mg/kg. It is likely that a higher NOAEC would be derived if additional samples between 260 mg/kg and 16,967 mg/kg lead were tested. If samples from both demonstration sites are evaluated together, the NOAEC is 2710 mg/kg lead (the highest tested sample from Travis AFB).

While these laboratory- and field-based NOAECs and LOAECs may be used as soil screening values, it is difficult to know the significance of the impact that some sub-lethal effects (e.g., reduced leukocyte counts) would have on the health of an individual salamander or a salamander population exposed to lead in the soil. A sub-lethal effect like reduced growth may be better related to population effects than a change in blood parameters.

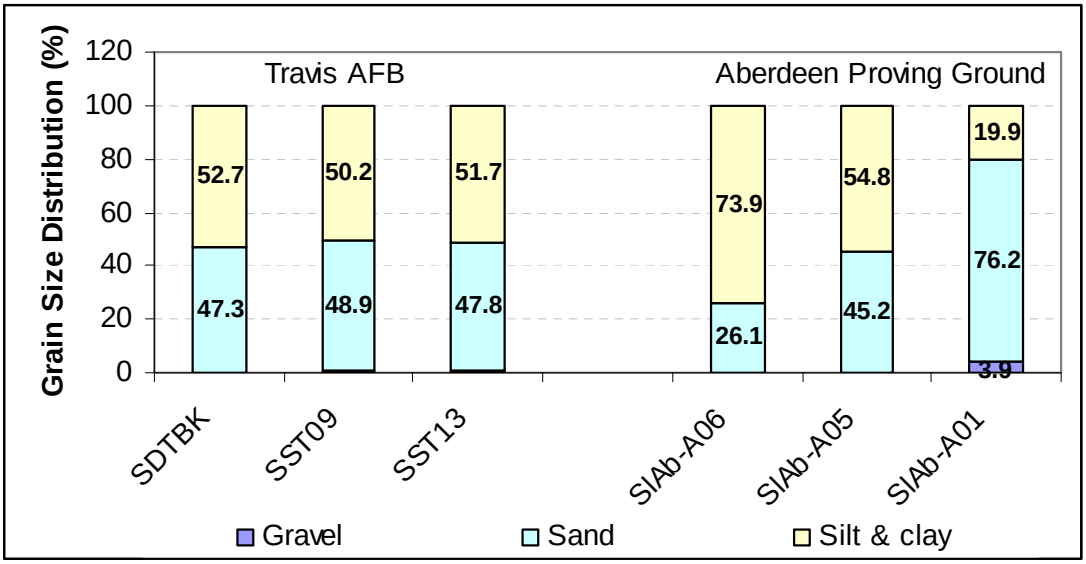
The difference in responses between the laboratory validation testing and the field demonstration testing may be explained by differences in the bioavailability of the lead. In the spiked-soil tests, the nature of the lead used to treat the soil (i.e., lead acetate) is such that the lead is likely to be highly bioavailable.

It appears that the weathering of the lead under field conditions may reduce the bioavailability of lead. Although the Travis AFB samples have weathered for less time than the APG samples (the skeet range at Travis AFB is still active), there did not appear to be a notable difference in bioavailability between the sites.

The level of TOC present in the soil may also have an effect on the observed toxicity with less toxicity expected in samples with higher TOC. The soil used in the laboratory validation testing contained 22.4% organic matter. Although TOC is a sub-set of the organic matter in a sample, there appears to be much more organic carbon in the laboratory validation soils than in the field demonstration soils (maximum TOC in tested soil samples was 2.3%).

The grain size composition may also be impact bioavailability. The laboratory validation soil was comprised of 45.6% sand, 43.6% silt, 10.8% clay. The composition of the laboratory validation soil is similar to the three Travis AFB samples and one of the APG samples (SlAb-A05) (Figure 4-3). The other APG samples had either more silt and clay (SlAb-A06) or more sand (SlAb-A01). It is not unexpected that the sandiest soil (SlAb-A01 at 76% sand) had the lowest TOC (0.088%), the highest lead level (16967 mg/kg) and the only observed toxic response.

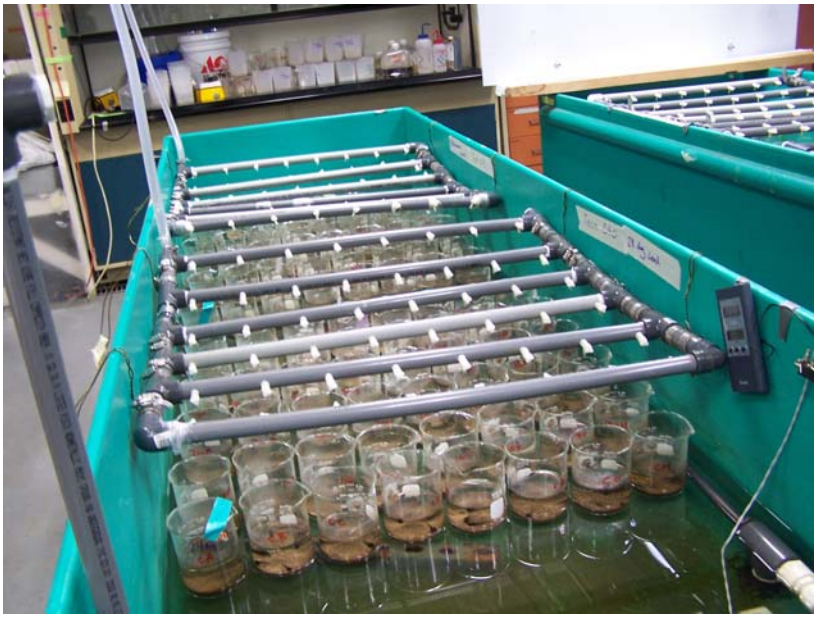
Figure 4-3 Grain Size Distribution of Tested Soils



4.3.2 Sediment Exposure Protocol

Tests were conducted for 10 days (in accordance with the protocol presented in Appendix A) with recently hatched tadpoles (Gosner Stages 17-20). Sediments were homogenized prior to placement in the test vessels. Each tested treatment consisted of eight replicates containing 10 larval tadpoles in each vessel. Figure 4-4 shows the set up of the toxicity test in the water bath (to maintain constant temperature) with the continuous drip flow-through system over the test vessels. Mortality and growth (i.e., body width, body length) were evaluated at test termination (Day 10).

Figure 4-4 Sediment Exposure Protocol Test Set-up



To select samples for the sediment toxicity test, the initial lead levels in each sample were reviewed (Table 3-11). In order to achieve a gradient of lead concentrations for the tests conducted for each demonstration site, dilutions of some sample were generated. For example, in the set of samples collected from the Travis AFB site, three dilutions of a sample with approximately 2000 mg/kg lead were made in order to achieve concentrations between approximately 80 and 850 mg/kg lead. One of the APG samples was also diluted to fill the gap between approximately 1000 and 17000 mg/kg lead.

Due to delays in test organism availability, the field demonstration tests were not started until December 2006 and January 2007. Copper and lead concentrations were analyzed in the fall of 2006 prior to the start of the tests. All sediments were stored in the dark at 4°C until testing began. A laboratory control was run with each set of tests to ensure the quality of the toxicity data.

4.3.2.1 Results of Field Demonstration

As described in Section 3.5.6, both lethal (mortality) and sub-lethal (growth as body width and body length) endpoints were measured in each toxicity study. Normality and homogeneity of variance were evaluated using the chi-square test and Bartlett's Test, respectively, with alpha = 0.01. Survival data were arcsine transformed, and growth data were not transformed. Statistical significance between the tested site samples and the reference site sample was evaluated using Steel's Many-One Rank Test (alpha = 0.05).

Treatments that demonstrated a significant reduction in survival (significantly higher mortality) were excluded from sub-lethal analyses since the sub-lethal measurements of surviving organisms can be skewed by a significant reduction in sample size. For the purposes of determining NOAECs and LOAECs these sub-lethal endpoints were treated as 'toxic' even though statistical analyses were not conducted. Table 4-6 presents the results of the sediment exposure tests for Travis AFB and APG. The results for each demonstration site are discussed below.

Eight samples (including diluted samples) from the Travis AFB site and a laboratory control were tested as part of the Travis AFB field demonstration. Lead concentrations ranged from 15 mg/kg in the reference sample to 3700 mg/kg. Copper concentrations were also analyzed in these samples to rule out another possible chemical stressor. Copper concentrations were low and not expected to be toxic. TOC concentrations were relatively consistent between samples, ranging from 1.5 % to 2.3%.

Survival in one sample from Travis AFB (SST07; 2100 mg/kg lead) was statistically lower than that observed for the reference sample (Figure 4-5). Since survival in this sample was significantly reduced, the sub-lethal endpoints were excluded from further statistical analyses. One sample (the M3 dilution of SST07) had a significant reduction in body length relative to the control.

Although toxicity was observed in the SST07 sample, samples with much higher lead levels did not show toxicity. The SDT14 and SST13 samples contained up to 3700 mg/kg lead with no impacts on survival or growth. Based on these results, the toxicity in the SST07 sample may not have been related to lead, or some characteristic of the sample increased the bioavailability of the lead present in the sample. Survival in the SST07 replicates was also variable with three replicates with total mortality (0% survival) and one replicate with no mortality (100% survival).

A review of the sample characteristics presented in Table 3-6 indicate that TOC, SEM and AVS, bulk density, CEC, and grain size values in the SST07 sample were similar to values for non-toxic samples. Levels of copper were also low in this sample. A review of the water quality parameters measured during the test (Appendix C) indicate that the dissolved oxygen (DO) in this samples was low (down to 3.4 mg/L), but within the acceptable range. Therefore, it is difficult to state with certainty that lead in the sediment was responsible for the toxicity observed in the SST07 sample. However, to be conservative, this sample was considered in the derivation of the LOAECs and NOAECs for the Travis AFB samples.

Eight samples (including diluted samples) from the APG site and a laboratory control were tested as part of the APG field demonstration. Lead concentrations ranged from 26 mg/kg in the reference sample to 17000 mg/kg. Copper concentrations were also analyzed in these samples to rule out another possible chemical stressor. Copper concentrations ranged from 7.7 mg/kg to 1200 mg/kg. During the laboratory validation phase of testing (summarized in Table 3-4), 87 mg/kg copper was sufficient to result in sub-lethal effects under low TOC conditions (TOC was 0.066%). Therefore, it is possible that, in addition to the lead, copper concentrations in the APG samples also contributed to observed toxicity. TOC levels in the APG samples were variable, ranging from 0.36% to 2.0%.

Survival in the SedAb-A3A sample and three dilutions of this sample was significantly reduced relative to the reference sample (Table 4-6; Figure 4-5). Since survival in these samples was significantly reduced, the sub-lethal endpoints were excluded from further statistical analyses. The SedAb-A04 sample had significant reductions in body width and length relative to the reference sample.

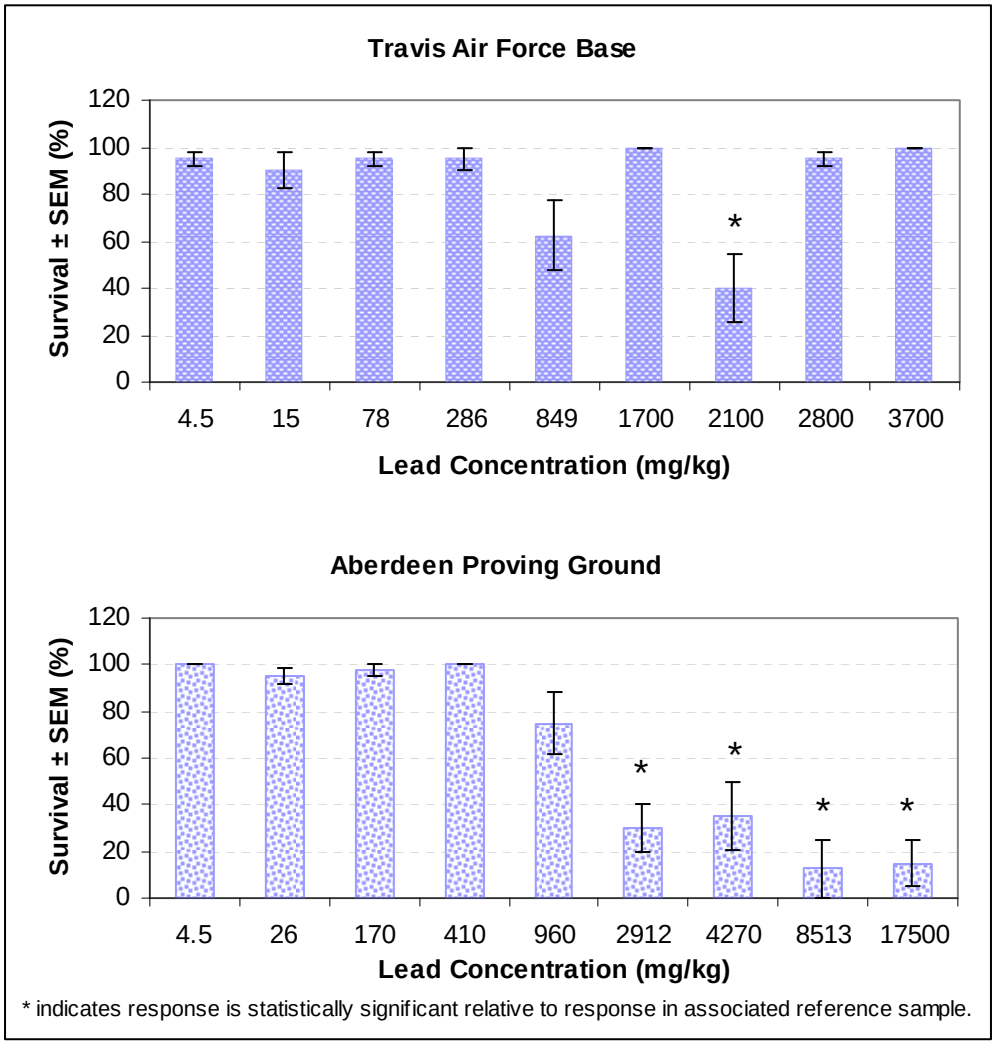
Table 4–6 Summary of Sediment Exposure Results

Location ID	Chemical Concentration [1] (mg/kg)		TOC (%)	Tadpole Results at Test Termination (Day 10)		
	Copper	Lead		Mean Survival (%)	Mean Body Width (mm)	Mean Body Length (mm)
Travis Air Force Base						
Lab Control	7.2	4.5	0.066	95	5.3	7.6
SDTBK	12	15	1.5	90	5.0	7.5
SST07 [M1]	12	78	1.5	95	5.1	7.5
SST07 [M2]	13	286	1.5	95	4.8	7.4
SST07 [M3]	14	849	1.5	62	3.4	**4.9** [2]
SDT04	19	1700	1.8	100	4.9	7.1
SST07	17	2100	1.6	**40** [2]	**2.5** [3]	**3.5** [3]
SDT14	21	2800	1.9	100	5.2	7.3
SST13	13	3700	2.3	95	5.0	7.0
Aberdeen Proving Ground						
Lab Control	7.2	4.5	0.066	100	5.4	9.0
SedAb-ABk11	7.7	26	0.46	95	5.6	9.7
SedAb-A08	16	170	0.36	98	5.7	9.8
SedAb-A07	37	410	1.9	100	5.8	9.9
SedAb-A04	140	960	0.57	75	**3.8** [2]	**6.0** [2]
SedAb-A3A [M1]	210	2912	0.72	**30** [2]	**1.3** [3]	**2.1** [3]
SedAb-A3A [M2]	306	4270	0.84	**35** [2]	**1.5** [3]	**2.4** [3]
SedAb-A3A [M3]	604	8513	1.2	**12** [2]	**0.4** [3]	**0.6** [3]
SedAb-A3A	1200	17000	2.0	**15** [2]	**0.4** [3]	**0.7** [3]

1 - Samples re-analyzed by analytical laboratory prior to toxicity testing .
2 - Indicates result is statistically different from reference sample results.
3 - Excluded from statistical analysis because survival was significantly reduced.
BK or Bk in location ID identifies background reference location; all others are site locations.

M1, M2, M3 concentrations achieved by diluting the following samples-
- for Travis Air Force Base - SST07 diluted with SDTBK
- for Aberdeen Proving Ground - SedAb-A3A diluted with SedAb-ABk11
- Copper, lead and TOC concentrations for these diluted samples are estimated based on the analytical results for the samples included in the dilution

Figure 4-5 Summary of Tadpole Survival Results



* indicates response is statistically significant relative to response in associated reference sample.

4.3.2.2 Evaluation of Field Demonstration Results Relative to Previous Studies

In general, the concentrations of lead detected in the field demonstration sediments were higher than the concentrations evaluated in the laboratory validation testing. The maximum tested lead concentrations from the Travis AFB demonstration site (3700 mg/kg) and the APG demonstration site (17500 mg/kg) were well above the maximum concentration (1200 mg/kg lead) evaluated in the lead-spiked sediments during the laboratory validation phase of testing.

Table 4-7 presents a comparison of the NOAECs and LOAECs derived from the laboratory validation tests and the field demonstration tests. These results show that less toxicity was observed in the field demonstration testing with aged sediment than with the spiked sediments in the validation testing.

Table 4–7 Comparison of Lead Sediment Screening Values in Validation Testing and Field Demonstrations

Study	Lead (mg/kg)			
	Survival		Sub-Lethal Endpoint [1]	
	NOAEC	LOAEC	NOAEC	LOAEC
Laboratory Validation [Spiked Sediment]	1200	>1200 [2]	100	260
Travis AFB	1700	2100 [3]	286	849 [3]
Aberdeen Proving Ground	960	2912	410	960
All Field Demonstration Data	1700	2100 [3]	410	849 [3]

Concentrations based on measured values prior to test initiation.

1 - Based on lower of values for body width and body length endpoints.

2 - No lethal effects were observed at the highest tested concentration.

3 - Acceptable survival and growth results were observed above this concentration; therefore these values may be overly conservative depending on site-specific conditions.

Since no mortality was observed in the maximum tested concentration during the laboratory validation phase of testing, the survival LOAEC was >1200 mg/kg lead. Lethal effects were observed in samples from both demonstration sites (Figure 4-5) resulting in survival LOAECs of 2100 mg/kg and 2912 mg/kg for the Travis AFB and APG sites, respectively. As indicated in Section 4.5.1, it is unclear whether lead is the stressor responsible for the observed toxicity in the Travis AFB sample since samples with higher lead levels did not show a significant reduction in survival (Figure 4-5). Although the survival NOAEC for the APG site is lower than that derived during the laboratory validation testing (1200 mg/kg lead), this is an artifact of the concentration gradient tested during the demonstration testing. No concentrations between 960 mg/kg and 2912 mg/kg were tested during the APG demonstration so the 960 mg/kg value became the NOAEC when survival was impacted in the 2912 mg/kg sample. If all of the sediment demonstration data are considered, the survival NOAEC becomes 1700 mg/kg lead.

The sub-lethal NOAECs and LOAECs were also higher in the field demonstration testing than the laboratory validation testing further indicating that lead is likely less bioavailable under field conditions (Table 4-7). As with the survival results, it is difficult to identify the stressors responsible for the sub-lethal impacts observed in the Travis AFB samples since samples with higher lead levels did not show a reduction in growth endpoints. Therefore, the sub-lethal LOAEC of 849 mg/kg based on the Travis AFB data may be overly conservative. There is more confidence in the sub-lethal LOAEC derived based on the APG results (960 mg/kg) since this data followed a more traditional dose-response curve (i.e., less growth observed in samples with higher lead levels).

These results indicate that using screening values derived from studies conducted with laboratory-spiked sediments is likely to over-estimate potential risks to amphibians. While this level of conservatism is appropriate for screening level risk analysis, the use of these screening values at wetland sites could result in overly conservative cleanup levels requiring remediation of larger wetland areas than may be warranted. The use of the sediment exposure protocol would provide a

site-specific assessment of the bioavailability and toxicity of lead, or other stressors, on larval amphibians that might be present in the wetland.

4.3.3 Assessment of Amphibian ERA Protocol

In 2004 NAVFAC published a guidance manual presenting the framework for a tiered amphibian ERA protocol that could be used to assess potential risks to amphibians as part of site evaluations at DoD (NAVFAC, 2004).

Conducting 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 ERA 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 Navy endorses a tiered approach for ERA (US Navy, 1999) and a tiered approach is consistent with USEPA methods for ERA (USEPA, 1997).

Although formal risk assessments were not completed for either of the field demonstration sites, the tiered approach may be applied using the available data to assess the versatility and applicability of the amphibian ERA protocol.

The Tier I amphibian ERA protocol essentially comprises a screening level ERA. This approach uses readily available information to identify potential amphibian exposure pathways at a site; determine which exposure pathways are complete; and conduct an effects-based screening using available benchmarks to determine whether or not the complete exposure pathways have the potential to pose a significant environmental risk.

The site reconnaissance efforts conducted at each of the sites provided an initial evaluation of amphibian habitat quality. The site selection effort focused on sites that had documented amphibian populations or appeared to contain amphibian habitat in order to assess sites with complete exposure pathways.

The initial habitat assessment at Travis AFB indicated that the site provides suitable habitat for at least one amphibian species. At the time of field sampling in March 2006, the average pool depth was approximately 50 cm with deeper areas over 80 cm. The surface area of the pool was approximately 1,200-square meters. The pool was primarily vegetated with annual hydrophytic grasses; curly dock (*Rumex sp.*), star thistle (*Centaurea solstitialis*), and other herbaceous materials. A Pacific tree frog (*Hyla regilla)* egg mass and several Pacific tree frog tadpoles were observed within the pool during a February 8, 2006 site visit. The calls of at least one adult Pacific tree frog were also heard.

During the March 2006 sampling effort, an ecological inventory was conducted using dip nets and meander surveys. No egg masses were observed and no amphibian species were collected using dip nets.

The initial habitat assessment at the Aberdeen Proving Ground study area indicated that the site provides suitable habitat for numerous amphibian species and other facultative vernal pool species.

At the time of sampling in April 2006, the average pool depth was approximately 60 to 90 cm. The pool was primarily vegetated with soft rush (*Juncus effusus*), broad-leaved cattail (*Typha angustifolia*), sedges (*Carex* spp.), and marsh bedstraw (*Galium palustre*). Ecological inventories at the site included dip netting, meander surveys, hand captures, and egg mass counts. The following species were observed at the site:

- Green frog (*R. clamitans*): vocalizations, hand capture
- Northern Cricket Frog (*Acris crepitans*): hand capture
- Spotted salamander (*Ambystoma maculatum*): 10 egg masses
- American toad (*B. americanus*): vocalizations
- Pickerel frog (*R. palustris*): vocalizations
- Fairy shrimp (*Eubranchipus* sp.): dip net
- Isopoda: dip net
- Unknown water beetle (*Coleoptera*): dip net
- Fishing spider (*Dolomedes* sp.): dip net

An effects-based screening was then conducted using the XRF data that was obtained as part of the reconnaissance effort at each site. Since copper was not detected in the Travis AFB XRF survey and copper XRF data were not collected the APG site, this initial screening focused on the available lead data. Table 4-8 compares the range of lead concentrations within each study area (excluding the reference locations) against literature-based soil and sediment screening values that might typically be used in an ERA conducted for a wetland site. These values were generally derived for the protection of terrestrial (e.g., earthworm, bird) and benthic (e.g., invertebrate) receptors, not for amphibians. The amphibian-based soil and sediment screening values from the laboratory validation phase of testing are also included in the table.

The maximum and average lead concentrations at each site were above the available soil and sediment screening values. The range of lead concentrations at each site was also above the XRF results for the associated reference locations. According to the Tier I amphibian ERA protocol, the presence of complete exposure pathways and concentrations above screening values and background locations would indicate that additional evaluation is warranted in the Tier II assessment.

The Tier II amphibian ERA protocol comprises a refined ERA using site-specific information to evaluate complete exposure pathways and amphibian ecological resources. This protocol provides quantitative measures and/or risk estimates of potential ecological effects associated with amphibian exposure to chemical stressors.

The soil and sediment sampling and analyses efforts conducted at each demonstration site after the XRF surveys would be consistent with the Tier II ERA process. Table 4-9 compares the analytical chemistry results for both copper and lead in field-collected samples (as opposed to the field-measured XRF data presented in Table 4-8) against soil and sediment screening levels indentified in the literature and derived during the validation phase of this project. Table 4-9 indicates that copper levels at the Travis AFB site are low and would not be expected to be toxic to worms, birds, salamanders, or tadpoles. However, levels of copper at APG and lead at both sites exceeded at least some of the screening values, indicating the potential for risk to wetland receptors.

Table 4–8 Screening of Field Demonstration Site XRF Survey Lead Data against Screening Values

				Lead (mg/kg)	
				Travis Air Force Base	**Aberdeen Proving Ground**
Field Demonstration XRF Survey Data			Minimum	464	38
			Average	1837	2265
			Maximum	4025	12387
			Background	20	33
Medium	**Source**	**Value**	**Receptor**	**Travis Air Force Base**	**Aberdeen Proving Ground**
Soil Screening Values	**From Literature**	Eco-SSL	Terrestrial invertebrate	**1700**	**1700**
		Eco-SSL	Vertebrate [bird]	**11**	**11**
	From Validation Testing	Survival NOAEC	Salamander	**1700**	**1700**
		Sub-Lethal NOAEC	Salamander	**1700**	**1700**
Sediment Screening Values	**From Literature**	TEC	Benthic invertebrate	**35.8**	**35.8**
	From Validation Testing	Survival NOAEC	Tadpole	**1200**	**1200**
		Sub-Lethal NOAEC	Tadpole	**100**	**100**

Boldface indicates that maximum site concentration exceeds that screening value.
Validation testing values were presented in Tables 3-3 and 3-4 and are based on measured concentrations.
Copper was either not analyzed (APG) or not detected (Travis APG) in the XRF surveys.
XRF data are not corrected for percent moisture (corrected values would be approximately 25% lower; assuming percent solid is approximately 75%).
Eco-SSL - Ecological-Soil Screening Level (USEPA, 2005b; USEPA, 2007). Vertebrate Eco-SSL is the lower of the avian and mammalian Eco-SSLs.
NOAEC - No Observed Adverse Effect Concentration
TEC - Threshold Effect Concentration (MacDonald, et al, 2000)

Table 4–9 Screening of Field `Demonstration Site Analytical Data against Screening Values

				Travis Air Force Base		Aberdeen Proving Ground	
				Copper (mg/kg)	**Lead (mg/kg)**	**Copper (mg/kg)**	**Lead (mg/kg)**
Field Demonstration Analytical Data			Minimum	16	935	17	35
			Average	18	2117	232	3166
			Maximum	21	4200	1150	17500
Medium	**Source**	**Value**	**Receptor**	**Copper (mg/kg)**	**Lead (mg/kg)**	**Copper (mg/kg)**	**Lead (mg/kg)**
Soil Screening Values	**From Literature**	Eco-SSL	Terrestrial invertebrate	80	**1700**	**80**	**1700**
		Eco-SSL	Vertebrate [bird]	28	**11**	**28**	**11**
	From Validation Testing	Survival NOAEC	Salamander	803	**1700**	**803**	**1700**
		Sub-Lethal NOAEC	Salamander	2700	**1700**	2700	**1700**
Sediment Screening Values	**From Literature**	TEC	Benthic invertebrate	31.6	**35.8**	**31.6**	**35.8**
	From Validation Testing	Survival NOAEC	Tadpole	230	**1200**	**230**	**1200**
		Sub-Lethal NOAEC	Tadpole	21	**100**	**21**	**100**

Boldface indicates that maximum site concentration exceeds that screening value.
Validation testing values were presented in Tables 3-3 and 3-4 and are based on measured concentrations.
Eco-SSL - Ecological-Soil Screening Level (USEPA, 2005b; USEPA, 2007). Vertebrate Eco-SSL is the lower of the avian and mammalian Eco-SSLs.
NOAEC - No Observed Adverse Effect Concentration.
TEC - Threshold Effect Concentration (MacDonald, et al, 2000).

At this point in the Tier II ERA, depending upon stakeholder concerns and regulatory status, additional work might be recommended to further assess potential impacts to amphibians. This could be accomplished using a variety of methods including laboratory toxicity tests or field surveys designed to assess the diversity and abundance of the existing amphibian community. In some cases, bioaccumulation evaluations (e.g., site-specific tissue collection, laboratory exposures) may be warranted if risks to higher trophic level receptors are of concern.

For the field demonstration, laboratory toxicity tests were used to directly assess the bioavailability and toxicity of the soil and sediment samples. As described in Sections 4.3.1.1 and 4.3.2.1, the sub-lethal effects on salamanders and both lethal and sub-lethal impacts on larval amphibians were observed during the testing. In the Tier II ERA, these responses could be used to identify toxic sampling locations that might need further study or remediation or, since a lead concentration gradient was tested, to establish clean up levels based on the NOAECs and LOAECs.

Table 4-10 compares the results of the field demonstration test against the soil and sediment screening values identified in the literature and the screening values derived using spiked soil and sediment in the laboratory validation phase of testing. The field demonstrations observed less toxicity than would have been predicted using screening values typically used in ERAs (e.g. literature based screening values for plants, terrestrial invertebrates, or benthic invertebrates) or using amphibian toxicity data generated using spiked soils and sediments.

This demonstration indicates that the application of the tiered amphibian ERA protocol and laboratory testing with the soil and sediment exposure protocols was appropriate for assessing potential risks to amphibians at both demonstration sites. At both demonstration sites, the use of technologies developed and refined through the ESTPC program identified less potential for risk to amphibians, and therefore less area potentially requiring remediation, than would have been identified by applying the literature based screening levels that have previously been used in wetlands.

Table 4–10 Comparison of Demonstration Testing Results and Screening Values for Lead

Medium	Source	Value	Receptor	Lead (mg/kg)
Soil Screening Values	**From Literature**	Eco-SSL	Terrestrial invertebrate	1700
		Eco-SSL	Vertebrate [bird]	11
	From Validation Testing	Survival NOAEC	Salamander	1700
		Sub-Lethal NOAEC	Salamander	1700
	From Demonstration Testing	Survival NOAEC	Salamander	2710 (TAFB) 16967 (APG)
		Sub-Lethal NOAEC	Salamander	2710 (TAFB) 260 (APG)
Sediment Screening Values	**From Literature**	TEC	Benthic invertebrate	35.8
	From Validation Testing	Survival NOAEC	Tadpole	1200
		Sub-Lethal NOAEC	Tadpole	100
	From Demonstration Testing	Survival NOAEC	Tadpole	1700 (TAFB) 960 (APG)
		Sub-Lethal NOAEC	Tadpole	286 (TAFB) 410 (APG)

Validation testing values were presented in Tables 3-3 and 3-4 and are based on measured concentrations.
Demonstration testing values were presented in Tables 4-5 and 4-7 and are based on measured concentrations.
TAFB - Travis Air Force Base demonstration testing results.
APG - Aberdeen Proving Ground demonstration testing results.
Eco-SSL - Ecological-Soil Screening Level (USEPA, 2005b; USEPA, 2007). Vertebrate Eco-SSL is the lower of the avian and mammalian Eco-SSLs.
NOAEC - No Observed Adverse Effect Concentration.
TEC - Threshold Effect Concentration (MacDonald, et al, 2000).

5.0 Cost Assessment

In addition to assessing the technical performance of the amphibian testing protocols, developing an understanding of cost performance is equally important. Cost considerations to be reported and evaluated include the perceived "real" costs associated with implementing the amphibian testing protocol as part of a larger site characterization effort. These costs are readily quantifiable and are based on site-specific conditions, including but not limited to the regulatory status of the site, size of the impacted site, number of samples, and laboratory testing requirements. Section 5.1 presents additional detail regarding this element of the cost assessment.

In addition, to "real" costs, use of technologies such as the amphibian testing protocol also has "opportunity" cost implications. When the toxicity testing protocols are appropriately applied, the user may avoid potential opportunity cost(s) associated with using a more conservative risk management approach. For instance, the use of inappropriate site characterization technologies in a palustrine wetland may result in costly and unnecessary wetland remediation based on the use of inappropriate endpoints. A more detailed discussion related to the opportunity cost savings presented by this innovative technology are presented in Section 5.2.

5.1 Cost Analysis

A summary of the approximate range of costs associated with implementing the amphibian testing protocols at several progressively sized sites is provided below in Table 5-1. This table further quantifies the use of a tiered amphibian ERA approach presented in a guidance manual published by NAVFAC (NAVFAC, 2004). Tier I of the amphibian ERA protocol represents a screening level ERA, which uses readily available information to identify potential amphibian exposure pathways. The results of the Tier I screening level ERA are typically used to determine whether or not additional amphibian ERA is warranted. Should the results of the Tier I assessment indicate that further amphibian ERA activities are not warranted, the Tier I activities would represent a finite and typically a *de minimus* costs for the end user, in relation to the overall site characterization. In this scenario, the costs associated with the Tier I screening level ERA would represent the extent of costs associated with the application of the amphibian testing technology at a site.

The Tier II portion of the protocol is a refined ERA, and is conducted to evaluate site-specific exposure pathways recommended at the conclusion of the Tier I evaluation. 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 sediment, hydric soil, or surface water samples from within the study area or appropriate background locations. Depending upon site-specific circumstances, collection of sediment or hydric soil for laboratory toxicity testing may also be required. In addition, site-specific amphibian field studies may be warranted. These studies may include determining what amphibian species occur at the site, 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.

Table 5–1 Tier I and Tier II Amphibian ERA Implementation Costs

Cost Category	Sub Category	Details	Costs		
			Site A	Site B	Site C
Tier I ERA Costs					
Screening Costs	Site Characterization/ Screening Level ERA	Review of available information	$ 7,500	$ 17,500	$ 37,500
Tier II ERA Costs					
Start-up Costs	Site Reconnaissance	Labor and travel for 2 people	$ 1,790	$ 3,880	$ 5,970
	Mobilization	Planning, contracting, site preparation, personnel mobilization, supply shipping	$ 4,000	$ 5,000	$ 5,500
Capital Costs	Capital Equipment Purchases	Sampling/ homogenizing	$ 400	$ 900	$ 1,800
Direct Operating Costs	Capital Equipment Rentals	XRF analyzer	$ 600	$ 1,200	$ 3,000
	Toxicity Testing	Amphibian toxicity ($1200/sample)	$ 4,800	$ 10,800	$ 21,600
	Supervision	Labor and travel for 1 person	$ 500	$ 1,145	$ 3,080
	Operator Labor	Labor and travel for 2 people	$ 1,790	$ 3,880	$ 10,150
	Consumables/ Supplies	Sampling/ decontamination	$ 800	$ 1,620	$ 3,240
	Sampling and Analysis	Chemistry analyses ($425/sample)	$ 3,400	$ 7,650	$ 15,300

Table 5–1 Tier I and Tier II Amphibian ERA Implementation Costs (continued)

Cost Category	Sub Category	Details	Costs		
			Site A	Site B	Site C
Indirect Operating Costs	Environmental and Safety Training	OSHA 40 hour training for 2 samplers ($600/person)	$ 1,200	$ 1,200	$ 1,200
Demobilization	Demobilization	Equipment decontamination, shipment of supplies, personnel demobilization	$ 2,000	$ 2,500	$ 2,750
Other	Report Preparation	Evaluate potential for risk and establish remedial goals	$ 20,000	$ 25,000	$ 30,000
Total Implementation Costs					
Cost of Tier I and Tier II ERA			$ 48,780	$ 82,275	$ 141,090

Site A = 2 acres; 4 toxicity testing samples; 8 analytical samples; 1 day of site reconnaissance; 1 day of field sampling
Site B = 15 acres; 9 toxicity testing samples; 18 analytical samples;2 days of site reconnaissance; 2 days of field sampling
Site C = 30 acres; 18 toxicity testing samples; 36 analytical samples; 3 days of site reconnaissance; 5 days of field sampling
All costs are estimates and could vary by up to 50% depending upon site-specific conditions.
Chemical analyses include metals, TOC, grain size, and SEM/AVS.

Assumptions:

8 hour field days with 2 field staff	Travel assumptions:
Field staff rate = $100/hour	No airfare included
Supervisor rate = $150/hour	Hotel = $150/night
Supervisor in the field 50% of the time	Car + mileage = $90/day
XRF rental fee is $600/day	Meals = $50/day

When the early life stage frog (sediment) bioassay protocol is used at a site, as with other toxicity testing procedures, the unit costs are expected to vary somewhat based on market conditions, number of tests being considered, nature of contamination, and other site-specific considerations. The expected costs to implement the 10-day amphibian toxicity testing protocol (ASTM E2591-07 Standard Guide for Conducting Whole Sediment Toxicity Tests with Amphibians) generated through this ESTCP program are expected to be similar to other ASTM and USEPA assays such as the 10-day benthic invertebrate toxicity tests conducted with the midge, *Chironomus tentans,* and the amphipod, *Hyalella azteca*. Actual unit costs for these benthic invertebrate assays (in 2008 dollars) range from approximately $750 to $1500 per 10-day test, depending upon site-specific circumstances, whereas longer term tests are typically proportionately scaled. It is anticipated that the amphibian testing protocol market costs will be within ± 20% of the invertebrate costs.

The costs to implement the amphibian ERA protocol is primarily dependent upon the spatial scale of the area under investigation and the number of samples required to meet the data quality objectives. For the sediment exposure protocol, the duration of the toxicity test can be increased to allow the evaluation of additional sub-lethal endpoints, and this increase in duration will have an impact on the implementation costs. Once the spatial scale of the area has been established, cost drivers are expected to be primarily related to labor, travel, laboratory analytical costs, and laboratory toxicity testing costs, which will vary from site to site.

The size of the site under investigation provide a basis for the number of personnel hours required to conduct the field surveys and collect the soil and/or sediment samples for evaluation. The number of samples submitted for analytical or toxicological evaluation will likely increase with the size of the site and will impact the amount of labor needed to conduct the analyses and the toxicity tests, as well as the level of effort associated with the evaluation of the associated results and generation of the project reports.

The distance of the site from airports, hotels, and the field team's home base will increase costs if the area under investigation is relatively isolated or distant. Costs associated with mobilizing and demobilizing equipment for the field effort are largely dependent upon labor and shipping costs. Labor is likely to be relatively consistent from site to site, but shipping costs, like travel, will vary depending upon distance to the site and method of transportation.

As the size of the site increases, the per sample incremental costs associated with travel, reporting, mobilization and sample collection are driven down by efficiencies associated with economies of scale. For example, Table 5-1 provides a range of costs to conduct the amphibian ERA at three sites with varying acreage and equivalent conditions as they relate to costs (i.e., location from field team base, analytical parameters, labor rates). The savings associated with a larger site can be viewed on a unit basis by dividing the total cost per site by the acreage or samples to be collected and presenting the costs on a per acre or per sample basis, as presented in Table 5-2.

Table 5–2 Incremental Implementation Costs

	Amphibian ERA Incremental Costs	
	Per acre	**Per toxicity testing sample**
Site A[1]	$ 24,390	$ 12,195
Site B[1]	$ 5,485	$ 9,142
Site C[1]	$ 4,703	$ 7,838
1 – Total costs for conducting Tier I and Tier II ERA at each site are detailed in Table 5-1.		

5.2 Opportunity Cost Evaluation

As previously discussed, the cost implications associated with implementing the amphibian ERA protocol as a means to derive ERA-based remedial goals are two dimensional. In many cases, alternative, non-wetland ecological receptors are inappropriately used to derive ERA-based remedial goals at wetland sites. The use of these organisms has the potential to overestimate potential risks and increase project costs, or alternatively to under-estimate potential risks, and thereby result in a less costly, but less protective, risk management decision.

In the absence of the amphibian sediment testing protocol, remedial risk-management decisions in wetlands often rely on site-specific benthic invertebrate toxicity testing using organisms such as the amphipod, *Hyalella azteca,* or the midge, *Chironomus tentans*. While these species may not be present in many of the wetlands in questions, they are commonly accepted surrogates for assessing toxicity. Implementing the amphibian sediment testing protocol could be as much as 20% more costly than these traditional methods (depending upon site-specific circumstances). However, the value in expending this additional amount is achieved when making an informed decision about incurring the financial burdens associated with unnecessary wetland remediation and the preventable loss of valuable wetland resources.

The costs associated with using an inappropriate ERA-based remedial goal to require unnecessary environmental activities has four major cost implications, including: the derivation and negotiation of clean-up goals, the remediation activities, the wetland restoration activities, and the more intangible disturbance associated with disturbing the wetland.

The DoD has historically expended considerable effort and time attempting to assess impacts to amphibians or negotiating more reasonable remedial goals than the ecological screening levels that could serve as an initial overly conservative remedial goal. At Site 22, a 500 acre munitions bunker area in the Inland Area of Naval Weapons Station Seal Beach Detachment Concord in Concord, California, the endangered California tiger salamander (*Ambystoma californiense*) has been identified as an ecological receptor with the potential for exposure to arsenic in shallow soil. However, because there is not an ecological screening value for salamanders exposed to arsenic in soil it has been difficult to quantitatively evaluate the risk to these receptors. The project schedule and budget have been impacted by requests from the regulatory agencies to quantitatively assess risks to the salamanders in the absence of an appropriate soil screening value or an accepted

methodology. This issue has led to an extended comment resolution process on documents, and the project team has expended considerable effort to resolve these comments and stay within the Federal Facilities Agreement (FFA) schedule at this National Priorities List (NPL) site. The risk assessment challenge at this site exemplifies the need for amphibian-based ecological risk assessment methodologies and testing protocols for soil.

The sediment toxicity test protocol using northern leopard frog tadpoles (*R. pipiens*) was included, along with midge sediment toxicity tests, in the 2005 Baseline Ecological Risk Assessment conducted for Tributary 2 of Operable Unit 1 (OU-1) at Cherry Point, North Carolina (CH2M Hill, 2005). Contaminants measured in the sediments included heavy metals, PAHs, pesticides, VOCs, and SVOCs. The sediment toxicity test offered a means to directly evaluate potential risks to amphibians, instead of using other organisms (i.e., aquatic or sediment invertebrates) as surrogates. The results of the toxicity tests indicated that potential impacts to amphibians were expected to be minimal and that potential risks to the midge were greater. At this site the amphibian data were used to show that amphibians were not an at-risk receptor group and that risk management efforts and remediation should focus on the benthic macroinvertebrate community. The use of the amphibian test results was considered "cost effective uncertainty reduction" since it gave the project team site-specific amphibian data on which conclusions could be drawn.

At the Naval Weapons Station (NWS) Yorktown site in York County, Virginia, the sediment toxicity test protocol was included in a toxicity testing program designed to generate preliminary remediation goals for metals (e.g., mercury, arsenic, cadmium, and selenium, silver) found in a palustrine scrub/shrub wetland. The toxicity testing program included testing with green frog tadpoles (*R. clamitans*), the amphipod (*Hyalella azteca*), and the fathead minnow (*Pimephales promelas*). Although remediation has not yet occurred, the arsenic NOAEC from the amphibian test and the mercury NOAEC from the amphipod test will likely be used to help determine the remedial action.

Remediation costs can and will vary significantly from one site to another. Factors such as the type of contaminants, contaminant concentrations, the three dimensional nature of impacts in the subsurface, leachability of the contaminants, accessibility of the site, and local resources available to perform remedial activities can all play a major role in the total remediation costs. Due to the wide variety of factors that can affect remediation costs it is impossible to provide a narrow range since costs can easily range from several thousand to millions of dollars.

Wetland restoration costs vary regionally and by complexity and wetland type. The most costly restoration efforts involve significant soil management activities (i.e., excavation, disposal, backfill, and grading) and hydrologic manipulation (i.e., dewatering, water treatment and disposal, stream diversion, extraction wells, etc.). Wetland restoration costs involving only limited backfill and grading to replace an herbaceous emergent wetland can range from $40,000 to $80,000/acre (reflecting regional variation), while the costs for restoration of a palustrine scrub-shrub or forested wetland complex requiring 2 feet of backfill and hydrologic modifications during construction may approach $85,000 to $135,000/acre. If riparian corridor/stream restoration and the associated armoring or bioengineering structures are also required, costs (excluding soil management and disposal) can range up to $150,000/acre. In comparison, applying the amphibian risk assessment at a 10-acre forested palustrine wetland site would cost approximately $20,000 to $100,000 (depending upon site-specific considerations), and potentially result in a no action finding based on

use of technically appropriate risk assessment endpoints. In comparison, the potential ecological restoration costs (not including soil or sediment management costs, which might even outweigh restoration costs) in the same wetland system may be as high as $1.5 million.

Assigning a monetary value to the disturbance of an ecosystem/wetland when those activities are unwarranted is very difficult to quantify, yet the costs are real. Among the many valuable, but relatively intangible, benefits of a wetland ecosystem system include the improvement of water quality, flood control, recreation, shoreline erosion control, and a habitat for a multitude of species. When reviewing the costs of remediating or restoring a wetland, the ecological costs associated with the disturbance of the wetland habitat need to be considered.

6.0 Implementation Issues

6.1 Environmental Checklist

In general, under CERCLA status, the collection of soil or sediment from most locations would not require any local or state environmental permits. If threatened or endangered species are known to occur within a sampling site, additional care must be taken to avoid injuring protected species or their habitats. Under most state programs, a scientific collection permit would be required if a field program anticipated collection of amphibians for tissue analyses.

All participants in the field effort would be expected to comply with health and safety regulations and all facility-specific requirements while working at the sites.

6.2 Other Regulatory Issues

Neither the skeet shooting range at Travis AFB nor the small arms range at APG is currently part of site characterization or investigation activities. Both sites have been included in previous on-site investigations (i.e., a remedial investigation at Travis AFB, habitat surveys and screening level ERA at APG). The results of the demonstration could be incorporated into the evaluation of corrective actions if such actions are suggested by other site investigations.

6.3 End-User Issues

The primary end-users for the toxicity testing protocols will be site investigators and the regulators that review ecological risk assessments. Other stake-holders involved in the ERA process may include groups like the USFWS and the general public. The sediment exposure protocol has been included and accepted as a component of ERAs conducted at several locations including the Massachusetts Military Reservation and the soil exposure protocol has been used to develop soil screening values for several compounds of interest to the Army.

The sediment exposure protocol and the amphibian ERA framework are both applicable to investigating potential impacts to amphibians due to exposure to a variety of contaminants including metals, pesticides, PAHs, and PCBs associated with sediments or hydric soils in wetlands or other aquatic habitats that may occur on DoD facilities. The soil exposure protocol is expected to be most appropriate for controlled toxicological investigations designed to derive safe soil levels for particular compounds.

The equipment required for this technology (e.g., field survey equipment and laboratory supplies) will generally be commercially-off-the-shelf (COTS) items. However, not all environmental laboratories are set-up to run these types of assays or have experience with these test organisms. It is recommended that end-users thoroughly investigate the qualifications of the toxicity testing laboratory prior to conducting the soil and sediment protocols. The chemical analyses used to characterize the soil and sediment samples are typical for most site investigations. It is recommended that end-users identify a chemistry lab that is accustomed to analyzing samples from hazardous waste sites.

Technology transfer efforts have been on-going over the course of this project. The development of an approved ASTM sediment testing guide is one key component of the technology transfer and will be important in gaining acceptance of this technology by both regulatory agencies and end-users. As discussed in Section 1.2, the draft guide was submitted to the full Biological Effects and Environmental Fate committee in August 2007 and was approved in November 2007. The protocol was published in December 2007 and is presented in Appendix D. When a procedure has been accepted as an ASTM standard for conducting physical, chemical, or biological measurements, it inspires confidence among end users, and facilitates regulatory (e.g., USEPA) acceptance of innovative technologies.

The results of the validation phase of testing have been presented at several conferences and it is anticipated that the results of the field demonstration and species sensitivity testing will be presented at upcoming conferences. These conferences represent opportunities to present the results of this project and discuss the use of the amphibian protocol with site investigators and regulators. Several of these scientific conferences are attended by representatives from universities, federal and state government agencies, and environmental consulting firms from around the world and presenting the ESTCP project in these venues is an important part of publicizing the work and achieving regulatory acceptance.

Through these efforts and others the sediment toxicity testing protocol has been implemented at several DoD facilities (i.e., Cherry Point, North Carolina, Massachusetts Military Reservation, Massachusetts, and NWS Yorktown, Virginia) and private sites (i.e., Massachusetts Highway Department site and site investigation led by USEPA Region 4) under several different regulatory programs. Posters and presentations have been presented at the following venues:

- Tri-Service Ecological Risk Assessment Work Group (TSERAWG) Meetings in May 2005 and May 2006
- ESTCP/SERDP Symposia in December 2006 and 2007
- Society of Environmental Toxicology and Chemistry (SETAC), North America Annual Meeting in November 2006 and November 2008
- University of Massachusetts Annual Conference on Soils, Sediments and Water in October 2006
- In Situ and On-Site Bioremediation Symposium in May 2007
- DoD Operational Range Assessment and Management Meeting in August 2007.

Team members have also presented project information at an EPA Region 3 Biological Technical Assistance Group (BTAG) meeting, at a USGS Patuxent Wildlife Research Center (PWRC) seminar, in the AFCEE Technology Transfer Newsletter that is distributed to over 75,000 regulators, consultants, and members of the DoD, and in an upcoming issue of the Navy's magazine Currents. Presentations are also anticipated at Battelle's February 2009 Fifth International Conference on Remediation of Contaminated Sediments.

An article discussing the toxicological responses of red-backed salamanders (*P.cinereus*) to soil exposures of copper has been accepted by a peer-reviewed journal (Bazar, et al., 2008) and an article discussing the response of the salamanders to lead exposures is in progress.

7.0 References

Bazar, M.A., M.J. Quinn Jr, K. Mozzachio, J.A. Bleiler, C.R. Archer, C.T. Phillips, and M.S. Johnson. 2008. Toxicological responses of red-backed salamanders (*Plethodon cinereus*) to soil exposures of copper. (accepted September 2008). Archives of Environmental Contamination and Toxicology.

Chaney RL, Mielke HW, and Sterrett SB. 1989. Speciation, mobility and bioavailability of soil lead. In: BF. Davies, BG. Wixson editors. Lead in soil: Issues and guidelines. Environmental Geochemistry and Health, Monograph Series 4, Supplement to Volume 9. Northwood, UK: Science Reviews. p 105–129.

CH2M Hill, 2005. Baseline Ecological Risk Assessment Operable Unit 1 (OU-1). Marine Corps Air Station, Cherry Point, North Carolina. Prepared for Department of the Navy NAVFAC Atlantic. August.

Johnson, M.S., H.I. Paulus, C.J. Salice, R.T. Checkai, and M. Simini. 2004. Toxicologic and histopathologic response of the terrestrial salamander: *Plethodon cinereus* to soil exposures of 1,3,5-trinitrohexahydro-1,3,5-triazine (RDX). Archives of Environmental Contamination and Toxicology. 47:496-501.

Loehr, R.C., 1996. The Environmental Impact of Soil Contamination: Bioavailability, Risk Assessment, and Policy Implications. Reason Foundation. Los Angeles, California. Policy Study No. 211.

MacDonald, D.D., Ingersoll, C.G. and T.A. Berger. 2000. Development and Evaluation of Consensus-Based Sediment Quality Guidelines for Freshwater Ecosystems. Archives of Environmental Contamination and Toxicology. 39:20-31.

Naval Facilities Engineering Command (NAVFAC). 2004. Development of a Standardized Approach for Assessing Potential Risks to Amphibians Exposed to Sediments and Hydric Soils. March.

Naval Facilities Engineering Command (NAVFAC). 2005. Laboratory Validation Plan for ESTCP Project CU-0514 Demonstration and Certification of Amphibian Ecological Risk Assessment Protocol. December.

Naval Facilities Engineering Command (NAVFAC). 2006. Site Selection Memorandum for ESTCP Project CU-0514 Demonstration and Certification of Amphibian Ecological Risk Assessment Protocol. February.

Naval Facilities Engineering Command (NAVFAC). 2007a. Field Demonstration Plan for ESTCP Project CU-0514 Demonstration and Certification of Amphibian Ecological Risk Assessment Protocol. May.

Naval Facilities Engineering Command (NAVFAC). 2007b. Test Refinement Interim Report for ESTCP Project CU-0514 Demonstration and Certification of Amphibian Ecological Risk Assessment Protocol. June.

Thorbjornsen, K. and J. Myers. 2007. Identification of Metals Contamination in Firing-Range Soil Using Geochemical Correlation Evaluation. Soil and Sediment Contamination, Volume 16. 4:337-349.

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

US Environmental Protection Agency (USEPA). 2005a. Procedures for the Derivation of Equilibrium Partitioning Sediment Benchmarks (ESBs) for the Protection of Benthic Organisms: Metal Mixtures (Cadmium, Copper, Lead, Nickel, Silver and Zinc). EPA-600-R-02-011. Office of Research and Development. January.

US Environmental Protection Agency (USEPA). 2005b. Ecological Soil Screening Levels for Lead: Interim Final. OSWER Directive 9285.7-70. Office of Solid Waste and Emergency Response, March.

US Environmental Protection Agency (USEPA). 2007. Ecological Soil Screening Levels for Copper: Interim Final. OSWER Directive 9285.7-68. Office of Solid Waste and Emergency Response, Issued July 2006, Revised February 2007.

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

Appendix A

Analytical Methods Supporting the Experimental Design

Standard Operating Procedure – Test Method for Conducting Whole Sediment Toxicity Tests with Amphibians

Animal Use Protocol - Effects of lead exposure to the red-backed salamander (*Plethodon cinereus*)

Animal Use Protocol - Effects of copper exposure to the red-backed salamander (*Plethodon cinereus*)

Standard Operating Procedure – Test Method for Conducting Whole Sediment Toxicity Tests with Amphibians

STANDARD OPERATING PROCEDURE

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.

Where appropriate, this SOP has been designed to be consistent with previously developed methods for assessing sediment toxicity (e.g., *Hyalella azteca* and *Chironomus tentans* assays). Historically, most assessments of contaminated sediment have relied on tests of limited duration (e.g., 10 d; U.S. EPA-USACE, 1991, 1998; U.S. EPA, 2000; ASTM 2001) that evaluate primarily lethality and, perhaps, short-term growth. These short-term tests are useful in identifying "hot spots" of contamination, but may not be sensitive enough to evaluate moderately contaminated areas.

The inclusion of sub-lethal responses like growth (weight or length) or reproduction may be more useful than lethality alone in evaluating moderately contaminated areas. Therefore, this SOP also includes measurements of sub-lethal responses after 10 days of exposure.

The most recent sediment toxicity testing protocols have also encompassed longer duration studies which allow the evaluation of reproduction (USEPA, 2000, 2001). Such tests, because of increased sensitivity of the sublethal endpoints, may also be helpful in evaluating moderately contaminated areas.

Longer duration assays (28 days and through metamorphosis) conducted during the laboratory validation of this SOP did not consistently result in greater statistical effects on the test organisms. That is, endpoints such as the NOEC (calculated with survival, body width or length or weight) were not necessarily lower in the tests of longer duration. Nor was there an indication that metamorphosis was a sensitive indicator of effects. Therefore, the recommended duration for this SOP is 10 days with an assessment of mortality and selected sub-lethal endpoints (i.e., body width, body length or dry weight). However, if site-specific information indicates that the contaminants present are likely to affect other endpoints, then the duration of the assay may be increased through metamorphosis or additional sub-lethal endpoints may be measured (e.g., impaired

behavior, deformities, time-to-metamorphosis).

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 sub-lethal 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 [U.S. EPA 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 Animal Use Protocols

The Department of Defense (DoD) requires that the testing laboratory establish an Animal Use Protocol prior to conducting any DoD-funded testing. This Protocol ensures that all vertebrate animals are treated humanely and do not endure any un-necessary pain. As part of the Protocol, an Institutional Animal Care and Use Committee (IACUC) must be employed to oversee and evaluate the animal care program and ensure that treatment is in compliance with applicable regulations and laws (e.g., animal welfare regulations). Studies may not begin until the IACUC has evaluated and approved the laboratory's Animal Use Protocol. The Animal Use Protocol for this study is currently under review by a project-specific IACUC and will be appended to this SOP when it is received.

4.2 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® or TetraFin®)
- 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).

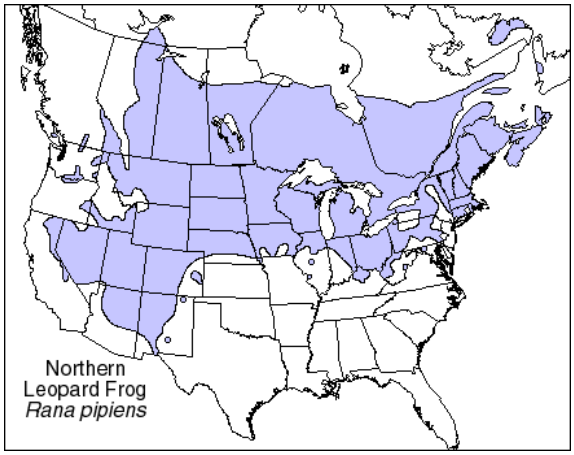


Figure 2 Range of the Northern Leopard Frog in North America

(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.

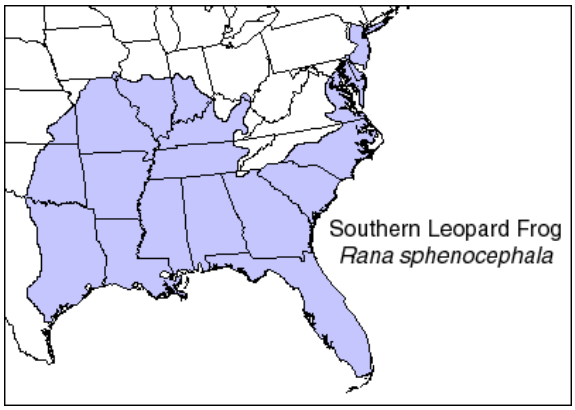


Figure 4 Range of the Southern Leopard Frog in North America.

(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).

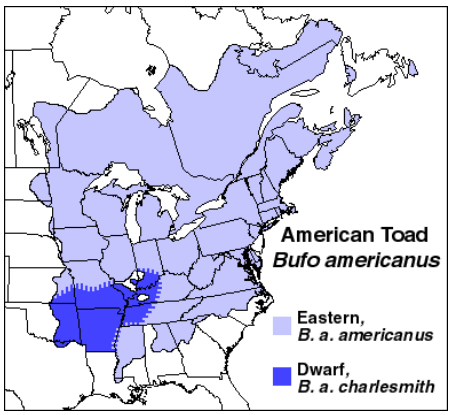


Figure 5 Range of the American Toad in North America

(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.

Longer duration assays (28 days and through metamorphosis) conducted during the laboratory validation of this SOP did not consistently result in greater effects (lower statistical endpoints such as a NOEC), using survival, total and body length, body width, weight, or metamorphic stage. Therefore, the recommended duration for this SOP is 10 days with an assessment of mortality and selected sub-lethal endpoints (i.e., body width, body length or weight). However, if site-specific information indicates that the contaminants present are likely to affect other endpoints, then the duration of the assay may be increased through metamorphosis or additional sub-lethal endpoints may be measured (e.g., impaired behavior,

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® or TetraFin® 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 carefully examined for dead organisms. Tadpoles can sometimes blend in with the color of the sediment, so careful examination is required. If a tadpole is not moving, it can be gently prodded with a blunt object to indce movement. Animals that do not move after repeated gentle prodding should be considered dead. Dead tadpoles must be removed. Live tadpoles are left in the chamber.

At the end of the test (generally at 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 sub-lethal (width and body length, and/or weight if required in the specific test protocol)

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® or TetraFin® per chamber. Adding excess food must 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 (or test termination)

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 sub-lethal 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 2 ml of a 2g/L solution of MS-222 per 30 ml of water. If organisms continue to move after several minutes, add two additional drops of the anesthetic. Tadpoles should not be left in the MS-222 solution for an extended period of time since tissue will begin to disintegrate.

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).

If site-specific information indicates that longer duration assays should be conducted, the daily activities described previously should be followed until test termination. Activities conducted at test termination will be similar to those conducted for the 10 day assay but may also include inspection for deformities, observations of impaired behavior (prior to anesthetizing), or developmental stage. If the assay is to be conducted through to metamorphosis, a platform must be provided which allows the developing tadpoles to crawl out of the water.

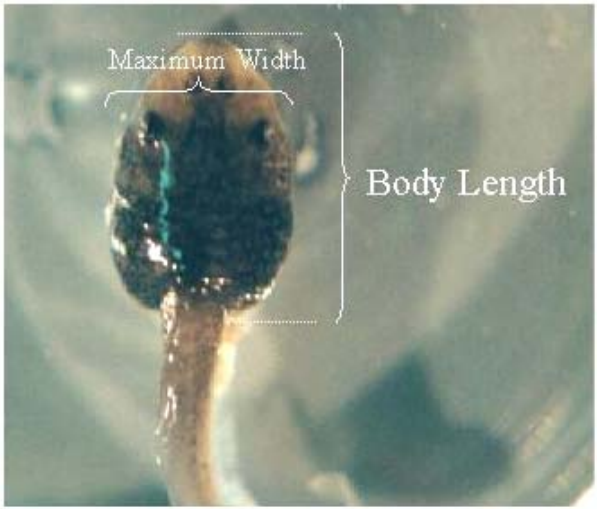


Figure 7 Measurement of Body Width

9.2 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.

9.2.1 Hypothesis Testing

Hypothesis testing should follow the same general structure as described by U.S. EPA (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 sub-lethal 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 ($1-\beta$), 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.

Sub-lethal 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 sub-lethal effects. Sub-lethal measurements are continuous data and therefore do not need to be transformed (arc sine-squareroot) before analysis. With that exception, the analysis of sub-lethal endpoints is the same as for survival.

9.2.2 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 U.S. EPA (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., U.S. EPA 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.

ASTM. 2001. Test Methods for Measuring the Toxicity of Sediment-Associated Contaminants with Freshwater Invertebrates. E 1706-00. pp. 1109-1225 *in* Annual Book of ASTM Standards, Vol. 11.05, Biological Effects and Environmental Fate; Biotechnology; Pesticides. American Society for Testing and Materials, West Conshohocken, PA

Denton, D.L. and T.J. Norberg-King. 1996. Whole Effluent Toxicity Statistics: A Regulatory Perspective. pp. 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 Sub-lethal 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.

U.S. EPA. 1993. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. Fourth Edition. EPA/600/4-90/027F.

U.S. EPA. 1994a. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. Third Edition. EPA/600/4-91/002.

U.S. EPA. 1994b. U.S. EPA Toxicity Data Analysis Software. Version 1.5. United States Environmental Protection Agency, Cincinnati, OH.

U.S. EPA. 2000. Methods for Measuring the Toxicity and Bioaccumulation of Sediment-Associated Contaminants with Freshwater Invertebrates. Second Edition. EPA/600/R-99/064.

U.S. EPA-USACE, 1991. Evaluation of Dredged Material Proposed for Ocean Disposal (Testing Manual). EPA-68-C8-0105. USEPA, Office of Marine and Estuarine Protection, Washington, D.C.

U.S.EPA-USACE, 1998. Evaluation of Dredged Material Proposed for Discharge in Waters of the U.S. Testing Manual. EPA-823-B-98-004. Washington, D.C.

WEST, Inc. and D.D. Gulley. 1996. Toxstat Version 3.5. Western Ecosystems Technology, Inc. Cheyenne, WY.

Table 2
Test Specifications

Test Organism	*Rana pipiens* or small *Bufo* species
Test Organism Age	≤72 hours
Test Duration	10 days [1]
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 (U.S. EPA 1994a))
Test Temperature	23 ± 1°C
Dissolved Oxygen	≥3.0 mg/L
Solution Renewal	Continuous flow-through
Feeding	4 mg TetraMin® or TetraFin® per vessel daily after tadpoles reach stage 25
Test Endpoints	Survival, body width, and body length [2]
Acceptability	Mean control survival of at least 80%

1 - Test duration may be extended to 28 days or until metamorphosis if existing data on possible chemical stressors suggest longer tests are needed to identify effects levels

2 - Additional sub-lethal endpoints (e.g., weight, eye-to-eye width, spinal defects, time until metamorphosis) may also be considered on a project-specific basis.

Animal Use Protocol - Effects of lead exposure to the red-backed salamander (*Plethodon cinereus*)

ANIMAL USE PROTOCOL

TOXICOLOGY DIRECTORATE

U.S. ARMY CENTER FOR HEALTH PROMOTION

AND PREVENTIVE MEDICINE

ABERDEEN PROVING GROUND, MD 21010-5403

PROTOCOL TITLE: Effects of lead exposure to the red-backed salamander (*Plethodon cinereus*)

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I. NON-TECHNICAL SYNOPSIS:

The purpose of this study is to assess the toxicity of soil exposures of lead to the terrestrial red-backed salamander (*Plethodon cinereus*). The use of lead in projectiles, fuzes, and detonators is well known in the Army. Small arms ranges are a necessary requirement for training purposes and as such are common at installations worldwide. Amphibian species are ubiquitously present at many military installations and, because of ecological and physiological characteristics, may experience worst-case exposures from lead in a soil matrix. However, the effects of lead to amphibians in terrestrial environments have not been characterized. In this study, red-backed salamanders will be exposed to four different concentrations of lead in soil. Food will consist of potworms (*Enchytraeids doerjesi*) exposed to contaminated soil of the same stock used to expose

the salamanders. Following 28 days of exposure, salamanders will be euthanized, necropsied, and evaluated for various health effects. These evaluations will consist of a histopathological analysis of main blood conditioning organs (liver and spleen), gut, and gonads. These results will help determine toxic soil concentrations of lead and will be used to develop soil-screening levels for lead appropriate for terrestrial amphibians.

II. BACKGROUND:

II.1. Background: Lead is a soft and highly corrosion resistant metal with widespread military and commercial applications. The use of lead in projectiles, fuzes, and detonators is well known in the Army. The distribution of lead at many U.S. military sites is substantial and sites of contamination include habitats in and around military storage facilities, manufacturing, load and packing plants, open burning/open detonation areas, and firing ranges. Firing ranges in particular are a significant source of lead pollution as lead bullets are the primary projectiles fired on the more than 337 small arms ranges throughout the United States. These bullets are oxidized over time from their original metallic state to carbonate, oxide, sulfate, and chloride forms of the metals. It is estimated that 6% of bullets will oxidize within 20 years while it could take up to 300 years to release all of the contaminant metal. These sites therefore present a mounting risk to humans who inhabit them and the ecosystems and wildlife that include them.

Lead ranks second in the list of prioritized hazardous substances issued by the U.S. Agency for Toxic Substances and Disease Registry (ATSDR) in 2003. Lead uptake induces the synthesis of metal-binding proteins that bind some reactive lead ions, rendering them non-reactive and fostering their excretion. However, lead has the capacity to mimic calcium and is thus substituted into many of the fundamental cellular processes that depend on calcium. This includes activities in the kidneys, liver, brain, central nervous system and bones. Lead deposited in bone can be mobilized and returned to the blood stream during times of physiological stress or increased calcium uptake. Organisms with consistently high exposures may be more likely to experience high lead body burdens through the continued deposition and liberation of lead from mineral tissue. Amphibian species are ubiquitously present at many military installations and, because of ecological and physiological characteristics, may experience worst-case exposures from lead in a soil matrix. Salamanders of the genus plethodon exist primarily in soil (Bishop 1943), have a fairly thin integument compared with that of birds and mammals, prey on soil invertebrates, and are known to constitute a significant portion of the biomass in some ecosystems (Burton and Likens 1975). To date, there have been a variety of studies on the toxicity of lead to mammalian species; however, few data have been generated regarding the toxicity of lead to amphibians.

Our laboratory has conducted studies testing the effects of energetic compounds in soil to species of terrestrial salamanders (Johnson et al. 1999, Johnson et al. 2000a & b, Johnson et al. in prep). These previously used methods include a system to holistically evaluate total soil-borne exposures through the integration of a microcosm design. This study will use the microcosm design in a dose-response scheme to evaluate and ascertain toxic vs. safe soil concentrations of lead.

The study described will be conducted compliant with the principles in the Good Laboratory Practice (GLP) guidelines of the Toxic Substances Control Act (TSCA): 40 CFR (Code of Federal Regulations) 792, plus amendments (2). The investigators and technicians will adhere to The Guide for Care and Use of Laboratory Animals (U.S. Department of Health, Education, and Welfare Publication No. NIH 86-23, 1996) (3).

II.2. Literature Search for Duplication:

II.2.1. Literature Source(s) Searched:

A number of databases were consulted in an effort to obtain information on the acute toxicity of lead in salamanders as well as a look for duplication of effort. The databases were:

a. DTIC: Defense Technical Information Center (1965 – present)

b. MEDLINE®: National Library of Medicine Database (1966 – present)

c. TOMES Plus® (1960 – present)

d. RTECS: Registry of Toxic Effects of Chemical Substances (1960 – present)

e. HSDB: Hazardous Substance Data Bank (1960 – present)

f. CHRIS: Chemical Hazard Response Information (1960 – present)

g. Shepard's Catalog of Teratogenic Effects (1960 – present)

h. TERIS: Teratogen Information System (1960 – present)

i. REPROTOX: Reproductive Toxicology (1960 – present)

j. IRIS: Integrated Risk Information System

k. New Jersey Hazardous Substance Fact Sheets (1960 – present)

l. NIOSH Pocket Guide: National Institute for Safety and Health (current)

m. North American Emergency Response Handbook (1960 – present)

n. ECOTOX®: USEPA Environmental Databases

o. PHYTOTOX: Terrestrial Plant Database (1926 – present)

p. TERRETOX: Terrestrial Animal Database (1969 – present)

q. TOXLINE® (1900 – present)

r. FED.RIP: Federal Reports In Progress (1998 – present)

s. AGRICOLA: USDA National Agricultural Library (1970 – present)

t. BRD: DoD Biomedical Research (1998 – present)

u. CRISP: Computer Retrieval of Information on Scientific Projects (1972 – present)

II.2.2. Date of Search: 11 January 2005

II.2.3. Period of Search:

No date limits were applied to any of the databases. The time periods established for each of the individual databases were the defaults used, as shown above.

II.2.4. Key Words of Search: lead, salamander and lead, salamander and metal, amphibian and lead, amphibian and metal

II.2.5. Results of Search: We found very few studies that evaluated the effects of lead on amphibians in any database. Hits ranged from 0 to 50,000 (TOXLINE) when the search term "lead" was used alone. The AGRICOLA and CRISP searches produced studies that focused both on the effects of habitat on population level effects and on inapplicable aspects of physiology and neurology. Many studies were found that investigated toxicity of exposure to various substances and stressors to salamanders in TOXLINE (inclusive of MEDLINE, BIOSIS, and FEDRIP). However, none did so evaluating dose response relationships of adult amphibians in non-aquatic environments. The paucity of studies evaluating the response of adult amphibians to metals in general was further evidenced by the retrieval of very few studies reporting field exposures from contaminated sites. Multiple reports were found on heavy metal exposures in aquatic amphibians with nearly all of these focusing on larval stages and developmental toxicity. The DTIC search included only the word "salamander" and revealed 14 hits; however most involved installation-specific inventory reports and inapplicable metabolic evaluations. Even though the described literature search was comprehensive, we felt that there might be pertinent government documents and peer reviewed articles not cataloged in the aforementioned databases that would be useful for this study. We additionally performed an independent literature search using a combination of several academic search engines and by manually tracing literature cited within related lead toxicology articles. The following synopses summarize our most relevant findings:

1. In mammals, the majority of circulating lead is effectively bound by metal-binding proteins and excreted. Primary target organs for the remaining portion include blood (carried almost

exclusively by erythrocytes), mineral tissues (bones and teeth), soft tissues (kidney, liver, bone marrow), and the brain and central nervous system. Peripheral neuropathy occurs through a cascade of interactions starting interferences with the dopaminergic, cholinergic and glutamatergic neurotransmission systems.

2. From a comparison between lead levels in frogs (*Xenopus laevis*) and mice (*Peromyscus sp.*), Ireland (1977) reported that frogs had much higher levels and therefore may be important in concentrating lead in the food web.

3. In a study of the response of adult Leopard frogs (*Rana pipiens*) to lead acetate, Kaplan et al. (1967) reported a 96 hour LC50 of 105 mg/L. Lead exposed frogs exhibited a loss of semi-erect posture, sloughing of the integument, muscular twitching, salivation, and sluggishness. The authors concluded that hematopoietic tissues were inhibited by lead acetate resulting in a reduction of read and white blood cells.

4. Frogs exposed to 14 mg/L for 4, 10, and 30 days showed no gross morphological changes but showed lead accumulation in all tissues by the end of the 30 day exposure period (Vogiatzis and Loumbourdis 1999). The accumulation of lead in the liver, kidneys and ventral skin was positively correlated with time of exposure.

5. Frogs (*Xenopus laevis*) exposed to lead contaminated worms showed highest levels of lead in the liver (30.75 mg/kg wet), kidney (81.32 mg/kg) and bone (55.98 mg/kg) with lower levels in the skin and muscle (less than 12.0 mg/kg) (Ireland 1977).

Baudo (1976) collected adults of the edible frog (*Rana esculenta*) from a contaminated site and observed that the highest level of lead within females was in the liver (7.56 mg/kg) whereas males had highest levels in the skin (3.66 mg/kg).

6. The affinity of melanin in the amphibian skin for binding metals was demonstrated in the frog (*Xenopus laevis*) by Ireland et al. (1979). Adult frogs that were dark adapted to increase melanophore number accumulated significantly more lead than those that were light adapted with reduced melanophore number.

III. OBJECTIVE/HYPOTHESIS:

The objective of this research is to determine the Lowest Observable Adverse Effect Level (LOAEL) and the No Observable Adverse Effect Level (NOAEL) from subchronic soil exposures to lead in the red-backed salamander, *Plethodon cinereus* focusing on pathological indicators and biomarkers. *P. cinereus* was chosen since it is terrestrial, amenable to laboratory investigations (Jaeger 1992), and has been reported to constitute a significant portion of the biomass in some ecosystems (Burton and Likens 1975). The usefulness of a biomarker of effects using melanomacrophage density (Johnson et al. 2000a) will be evaluated.

IV. MILITARY RELEVANCE:

Decreases in amphibian populations (Phillips 1990, Wake1991) and increases in developmental abnormalities (Schmidt 1997) have been widely reported and often attributed to environmental contamination. The U.S. Army has 337 small arms ranges, many encompassing large tracts of land. Nearly every installation has at least one range and it has been estimated that small arms ranges total more than 210,000 acres. Lead core bullets are used as the primary projectile. Given the extent and prevalence of the contamination, and the need to balance environmental stewardship while maintaining readiness, it is of great importance that the Army understands the fate of lead exposure and the relative potential for effects if we are to maintain important training activities.

Amphibian species are ubiquitously present at many military installations and, because they are predominantly terrestrial and perform as much as 95% of their gas exchange through cutaneous respiration, their skin differs in its permeability to certain pollutants relative to most other terrestrial vertebrates. Therefore, any comprehensive ecological risk evaluation neglecting the dermal route of exposure in amphibians would likely underestimate the total systemic exposure and result in inaccurate characterization of potential effects. The data collected in this study will be used to determine safe levels of lead in soil to a terrestrial amphibian species and will be used to further develop a toxicity reference value for lead in soil for terrestrial amphibians. Determinations using this information will help provide guidance in regards to training, clean up, and transfer of lands affected from military activity.

V. MATERIALS AND METHODS:

V.1. Experimental Design:

V.1.1. Experiments:

1) 10-day Range Finding Study: A 10-day range finding study will be conducted to determine the appropriate lead concentrations used in the primary study (N=8). This will include one animal per a known concentration; approximately 8 concentrations of lead will be tested. Target soil concentrations will be 0, 50, 350, 800, 1200, 2300, 3750, 4500 mg Pb/kg soil. Only soil exposures to salamanders will be evaluated in this study. Overt signs of stress (e.g., excess mucus production, neuromuscular effects, excitability, etc.) will be used to determine toxicity and dose range for the 28-day exposures.

2) 28-Day Microcosm Toxicity Study: Data from the 10-day Range Finding study will be used to refine treatment groups. The 28-day study will consist of exposures from soil and food whereby soil will be spiked with lead at 5 concentrations, including a control (0 mg Pb/kg soil), chemical concentrations will be determined by the 10–day range finding study. Soil of the Webster type will be collected from a mature oak-beech forest at the Edgewood area of APG where red-backed salamanders are present. Soil will be dried, pulverized, and sifted through 2 screens (Nalgene; 1-mm2 and 0.5-mm2 mesh) to homogenize the sample. This will serve as base soil for the study. A sub-sample of the soil will be characterized (e.g., organic carbon content, pH, cation exchange

capacity, %clay, sand, and silt). Lead acetate will be dissolved into deionized water and the resulting stock solution thoroughly mixed with soil sub-samples in proportion to achieve the desired treatment levels. The treatments levels will be determined through a weight-of-evidence evaluation consisting of time to death, manifestation (etiology) of overt toxicological symptoms, type and characterization of overt effects, and consistency of overt observations with known effects from lead exposure (e.g., neurological in origin). Each treatment will then be allowed to dry overnight in a darkened fume-hood before being mixed individually in a three-dimensional mixer for 18 hours. Approximately 110 g of soil will be added to 5" glass Petri dishes, hydrated, and allowed to sit in a dark room at room temperature for 15 days prior to the start of the study.

At the start of the first day of the 28 exposure period, 100 salamanders (N=100) will be divided evenly by weight into the five treatment groups and placed separately into individual Petri dishes containing treatment specific soil. Each dish/animal will retain its original cage card amended with a treatment designation and affixed to the new Petri dish. Approximately 1 g of soil from each treatment will be grab sampled on day –1 (start), 14 (middle), and 28 (end) and analyzed using an x-ray fluorescence spectrometer (InnovXsys model XT400). Calibration will be conducted using known samples each day and recorded in the laboratory notebook, compliant with U.S. EPA's Office of Solid Waste method 6200 (Field Portable XRF Spectrometry for the Determination of Elemental Concentrations in Soil and Sediment).

Food will consist of potworms exposed to copper contaminated soil of the same stock used to expose the salamanders. Potworms will be exposed to the treated soil prior to feeding and food samples will be collected on day –1 (start), 14 (middle), and 28 (end) to determine levels of lead experienced by salamanders through oral exposure. Should lead concentrations exceed potworm tolerance, wingless fruitflies (*Drosphila melanogaster*) will be used as food and the oral pathway will not be considered. Salamanders will be provided with food once every two days in the manner described in Section V.5.1. To consider changes as a result of at least one day of exposure, salamanders will be weighed on days –1, 7, and weekly thereafter to 0.001 g. Animals will be observed daily for signs of overt toxicity (e.g., lethargy, sensitivity to touch, abnormal behavior). On day 28, salamanders will be euthanized using aqueous preparations of MS-222 and euthanized via decapitation; the remaining body and head will be preserved in 10% formalin.

3) Histopathological Analyses:

a. Saggital body and cross sections of the head will be trimmed, fixed in formalin, and embedded in paraffin. These tissues will then be sectioned at 6 microns, stained with hematoxylin and eosin, and examined via light microscopy.

b. Liver melanomacrophage (MMO) characteristics (density, diameter, area, per unit area) will be quantified using a 3.3 megapixel Q-fire color digital camera (Q-Imaging, Burnaby BC, CA) attached to an Olympus light microscope, model BH-2 (Optical Elements Corp., Dulles VA USA) at 400x. The camera is attached to a Dell Pentium IV PC and images will be evaluated using Image-Pro Plus V4.5 imaging software (Media Cybernetics, Silver Spring MD USA). Three sections will be quantified for each animal and then summed (for area, per unit area and density) or

averaged (diameter). These values will then be used to represent a single data point for each animal and then evaluated by treatment group.

4) Hematological Analyses:

a. Blood Collection: Small amounts (~ 20 ul) may be collected following euthanasia from adult salamanders of sufficient size. Blood will be collected from cranial region following decapitation with a microcapillary tube containing sodium heparin. After collection, the methods below are used. Sample volume will determine which evaluations are conducted.

b. Whole Blood Cellularity: A 10 µl aliquot of blood will be transferred into a tube containing 990 µl of phosphate buffered saline (PBS) and mixed by gentle vortexing for Natt-Herrick staining and whole blood enumeration. Tubes will be gently vortexed and a 10-µl aliquot of the stained blood sample will be placed onto a hemacytometer and the white blood cells will be attempted. The number of red blood cells (RBCs) will also be determined per ml. Based on this relationship, whole blood cellularity will be enumerated and size-analyzed using a hemocytometer or by using a CASY 1 model TTC cell counter + analyzer system (Scharfe System GMbH, Germany).

c. Packed Cell Volume (PCV), Total Protein, and Hemoglobin: A hematocrit capillary tube will be inserted into the tube containing the heparinized blood. Once ¾ filled, the bottom of the tube will be inserted into a clay pad, centrifuged for 5 min., and the PCV determined using a graphic reader card. Following enumeration of the PCV, the tube will be cut above the RBC layer and 2-3 drops (30-50µl) of plasma will be placed onto a refractometer and the total protein determined. Another drop will be placed within a slide cassette and hemoglobin content enumerated using a HemoCue Hemoglobin photometer (HemoCue AB, Angelholm, Sweden).

5) The study described will be conducted compliant with the principles of the Good Laboratory Practice (GLP) guidelines in the Toxic Substances Control Act (TSCA): 40 CFR (Code of Federal Regulations) 792, plus amendments (2). The investigators and technicians will adhere to The Guide for Care and Use of Laboratory Animals (U.S. Department of Health, Education, and Welfare Publication No. NIH 86-23, 1996) (3).

V.2. Data Analysis: All data will be recorded in the laboratory notebook . The weight data will be treated as percent change from baseline and will be tested with a Levene's Equal variance test. The data will then be tested with a two-way repeated measures ANOVA used to compare the percent change from Day 0 weights for treatment groups across the four observation times (mass collected on days 7, 14, 21 and 28). A Tukey's multiple comparison test will be used to assess the differences between treatment groups. Daily observational data may also be tested using the appropriate categorical method (e.g., Chi-square, Fisher's Exact test, etc.). Melanomacrophage data will be tested for normality using a Shapiro-Wilk, W-test at the $p < 0.05$ level. Melanomacrophage measurements are expected to be non-normally distributed, thus we will likely compare across treatments with a Kruskal-Wallis One-way ANOVA on ranks (SigmaStat®

statistical software, SPSS Science, Chicago, Ill.). Multiple pairwise comparisons will be completed using the Dunn's Method at $\alpha = 0.05$ level of significance.

V.3. <u>Laboratory Animals Required and Justification</u>:

V.3.1. Non-animal Alternatives Considered: The objective addressed by this study is focused on the adverse health effects of soil exposures of lead to a terrestrial amphibian species. Non-animal alternatives would not provide the necessary information.

V.3.2. Animal Model and Species Justification: In order to address questions involving the heath effects from exposure to amphibian species that inhabit military installations where lead is present in the soil, it is essential to establish the toxicity of lead to a representative amphibian species. The red-backed salamander was chosen given its terrestrial life history. They are amenable to laboratory investigations (Jaeger 1992), and are widely distributed in the eastern North America (Jaeger 1980, Bishop 1943).

V.3.3. Laboratory Animals:

V.3.3.1. Genus and Species: *Plethodon cinereus*

V.3.3.2. Strain/Stock: N/A

V.3.3.3. Source/Vendor: Locally collected by hand in the spring between March and mid May in the Gunpowder watershed (MD. DNR Permit No. SCO-3558) or obtained from Connecticut Valley Biological Supply Company, Southhampton, MA. Care will be taken to choose larger animals (> 0.5g) and to not extirpate local populations (e.g., not to collect more than approximately 60% found).

V.3.3.4. Age: Unknown

V.3.3.5. Weight: Varying (0.5 – 2.0 g; 60 – 110 mm)

V.3.3.6. Sex: Both males and females will be tested (sex is not determined reliably)

V.3.3.7. Special Considerations: None

V.3.4. Number of Animals Required (By Species):

1. Red-backed Salamander (*Plethodon cinereus*) – 120

Twenty animals per treatment will be required. There are five treatments (including a control) and a 10-day range-finding study that will require 8 animals. Moreover, this selection of 108 from the original pool of 120 will allow for fewer animals to be needed to achieve meaningful results.

The number of animals required has been determined through an evaluation of variability in sensitive responses from data collected from previous work with salamanders (Johnson et al. 2004, Johnson et al. 2000a & b) and also based upon judgment of the investigators considering the effort involved in maintaining and gathering data for the study. When the sample size in each of the 5 groups is 20, a one-way analysis of variance will have 80% power to detect at the 0.05 level a difference in mean percent change in weights ranging from 100% to 85% (similar to those observed in a previous RDX study), assuming that the common standard deviation is 10.5 or less.

Treatment Group	**Test Animals**
Off study/training[1]	12
10-day range-finding	8
Control (0mg/kg)	20
TBD	20
TBD	20
TBD	20
TBD	20
Total	**120**

Since these animals are wild-caught (i.e., outbred of various ages) an additional twelve animals will be needed to choose animals of a similar size and roughly similar age to achieve a degree of homogeneity in the treatment groups. This is not needed in locally-collected situations since only those animals exceeding about 0.5 g in mass will be collected. These animals will be used for training purposed and assigned accordingly. These animals will be used for training purposed and assigned accordingly.

If the proper number and quality of animals is not obtained, a statistician will be consulted to determine if a revision is necessary. If a change in the design is necessary, a modification and approval through the IACUC will be completed.

[1] Animals from vendor of sub-optimal weight for 28-d exposures.

V.3.5. Refinement, Reduction, Replacement:

V.3.5.1. Refinement: This protocol is based on what has been learned from earlier work in our lab (Johnson 1999, Johnson et al. 2000a & b, Johnson et al. 2004) and the methods developed by Jaeger (1992). This background information will be used to optimize husbandry conditions, thus minimize distress to our animals.

V.3.5.2. Reduction: Based on the results of earlier investigations, an optimal number of animals will be used (Johnson et al. 2004). Additionally, by conducting a 10-day range-finding study, it will help to determine the concentrations at which to reduce mortality and determine the Lowest Observed Adverse Effect Level.

V.3.5.3. Replacement: No replacement alternatives are appropriate for this study. In order to address questions involving the health effects from exposure to amphibian species that inhabit military installations where lead is present in the soil, it is essential to establish the toxicity of lead to a representative amphibian species. The red-backed salamander was chosen given its terrestrial life history. They are amenable to laboratory investigations (Jaeger 1992), and are widely distributed in the eastern North America. (Jaeger 1980, Bishop 1943).

V.4. <u>Technical Methods</u>:

V.4.1. Pain/Distress Assessment: Considering that the intention of this research is to understand the concentration of lead in soil that causes toxicity, pain may be experienced by animals in the two highest exposure groups. This assessment is based on the reports of previous studies where indications of distress (for e.g., excessive mucus production, loss of righting reflex, excessive buccopharyngeal pumping) and/or mortality/morbidity has occurred at the highest dose. However, this is a conservative assessment as previous experiments with TNT and the nitroaromatic analog DNT produced no adverse effects.

V.4.1.1. APHIS Form 7023 Information

V.4.1.1.1. Number of animals:

V.4.1.1.1.1. Column C: 76 (67%)

V.4.1.1.1.2. Column D: 0

V.4.1.1.1.3. Column E: 44 (33%) (Conservative possibility - see V.4.1. for rationale)

V.4.1.2. Pain Relief/Prevention: Since the goal of this investigation is to determine the effects from exposure, no other substances that could potentially interfere with the interpretation of these results will be administered. However, moribund or animals in overt pain will be humanely euthanized as described in section V.4.6. (Euthanasia). Based on previous experience with these

models we will consider the following as indications of distress where recover is unlikely at continued exposure regimes:

1) Appear extremely listless/lethargic and exhibit excessive mucus production

2) Experience a continued loss of appetite.

3) Are unresponsive and do not demonstrate a righting response

A weight of evidence determination will be made on a case by case basis after consultation with the principal investigator and the attending veterinarian.

V.4.1.2.1. Anesthesia/Analgesia Tranquilization: n/a

V.4.1.2.2. Pre- and Post-procedural Provisions: None

V.4.1.2.3. Paralytics: None

V.4.1.3. Literature Search for Alternatives to Painful or Distressful Procedures:

V.4.1.3.1. Sources Searched:

a. NORINA: Norwegian Reference Centre Alternatives Database

b. Altweb

c. Scirus

d. STINET: Scientific and Technical Information Network

e. DTIC: Defense Technical Information Center

f. MEDLINE®: National Library of Medicine Database

g. ECOTOX®: USEPA Environmental Databases

h. TERRETOX: Terrestrial Animal Database

i. TOXLINE®

j. AGRICOLA: USDA National Agricultural Library

k. CRISP: Computer Retrieval of Information on Scientific Projects

V.4.1.3.2. Date of Search: 11 January 2005

V.4.1.3.3. Period of Search: The period of search of the databases is the same as that stated in section II.2.3.

V.4.1.3.4. Key Words of Search: Amphibian and anesthe?, amphibian and euthanas?, salamander and alternative and model, salamander and metal and simulate, amphibian and pain, amphibian and distress

V.4.1.3.5. Results of Search: We found very few papers discussing amphibian anesthesia or euthanasia however, of the databases listed above, Altweb was most productive and produced several articles discussing anesthesia/euthanasia and pain/distress in amphibians. We additionally performed an independent literature search using a combination of several academic search engines and by manually tracking down literature cited in captive care literature. This search was highly productive and yielded several order specific anesthesia/euthanasia review papers. The following synopses summarize our most relevant findings:

1. There are no alternatives to live salamanders for addressing the purpose of this study.

2. The housing and husbandry design is both appropriate to the study and ideal in meeting the life maintenance and enrichment needs of the red-backed salamander.

3. The few studies that have evaluated the effects of amphibians and nitroaromatics have shown no induced adverse affects or indications of pain. Therefore, no substances can be reliably used to alleviate pain since the toxicodynamic properties (i.e. factors that may confound the evaluation of the toxicity of lead exposure) of such have not yet been determined and may compromise the results.

4. Tricaine methane sulphanate (MS-222) is an anesthetic and alleviates pain in amphibians prior to euthanasia. The use of MS-222 has been recommended for anesthesia and euthanasia in amphibians by many researchers (Cooper et al. 1989, Johnson 1992, Schaeffer 1994) as well as the American Veterinary Medical Association (AVMA 2001), the Canadian Council on Animal Care (CCAC 1980), and a collection of major herpetological societies (ASIH et al. 2001). A review of its pharmacology and use in amphibians has been published (Downes 1995).

V.4.1.4. Unalleviated Painful/Distressful Procedure Justification: Since the goal of this investigation is to determine the effects from exposure, no other substances that could potentially interfere with the interpretation of these results will be administered. However, moribund or animals in overt pain will be humanely euthanized as described in section V.4.5. (Study Endpoint).

V.4.2. Prolonged Restraint: Not applicable

V.4.3. Surgery: Not applicable

V.4.3.1. Pre-surgical Provisions: Not applicable

V.4.3.2. Procedure: Not applicable

V.4.3.3. Post-surgical Provisions: Not applicable

V.4.3.4. Location: Not applicable

V.4.3.5. Surgeon: Not applicable

V.4.3.6. Multiple Major Survival Operative Procedures: Not applicable

V.4.3.6.1. Procedures: Not applicable

V.4.3.6.2. Scientific Justification: Not applicable

V.4.4. Animal Manipulations: Not applicable

V.4.4.1. Injections: Not applicable

V.4.4.2. Biosamples: Not applicable

V.4.4.3. Adjuvants: Not applicable

V.4.4.4. Monoclonal Antibody (MAbs) Production: Not applicable

V.4.4.5. Animal Identification: Individual animals will be identified by cage card according to SOP No.003.04 (TOX 2004).

V.4.4.6. Behavioral Studies: Not applicable

V.4.4.7. Other Procedures: Animals will be observed every 2 hours for the first 8 hours, and then daily thereafter as part of the initial range finding study. During the 28 day study, animals will be observed at least daily for signs of overt toxicity (e.g., excess mucus production, neuromuscular effects, excitability, etc.) by the principal investigator and/or co-investigators.

V.4.4.8. Tissue Sharing: Not applicable

V.4.5. Study Endpoint: The study will be terminated on Day 28 after initial exposure, after anesthesia, euthanasia, and necropsy of animals. Euthanasia is required to histologically evaluate

the potential for toxic effects from combined exposures to lead. Early euthanasia will occur when a weight of evidence determination (as described in V.4.1.2) indicates that animals are experiencing significant pain or distress.

V.4.6. Euthanasia: On Day 28, salamanders will be euthanized by immersing them in a beaker containing a buffered aqueous preparation of MS-222 at 2g/l. Deep anesthesia (determined through the lack of a response to tail pinch) will be followed with decapitation. The remaining body and head will be preserved in 10% formalin. The humane use of a buffered MS-222 solution of 2 g/L has been recommended for anesthesia and euthanasia in amphibians by the American Veterinary Medical Association (AVMA 2001), the Canadian Council on Animal Care (CCAC 1980), a collection of major herpetological societies (ASIH et al. 2001), and by many researchers (Cooper et al. 1989, Johnson 1992, Schaeffer 1994).

V.5. <u>Veterinary Care</u>:

V.5.1. Husbandry Considerations: Salamanders will be singly housed in 5" borosilicate glass petri dishes as recommended by Jaeger (1992). During the acclimation period, each dish will contain only a sheet of filter paper hydrated with deionized water. Filter paper (Q8, 12.5 cm dia., coarse porosity, Cat. No. 09-790E, Fisher Scientific, Atlanta, GA) will be changed as necessary. The acclimatization period will last at least 14 days. During the study, the filter paper will be replaced with soil, depending upon the treatment. Hydration with deionized water will occur as needed. All animals will be kept in conditions of constant photoperiod (12 hrs light/12 hrs dark), humidity and temperature. All uneaten feed will be collected daily. Salamanders will be housed in AAALAC accredited facility at USACHPPM where humidity and temperature will be monitored. Humidity will be maintained between 30 and 70% and the temperature will be maintained between 62 and 75 ˚F. A small piece of sphagnum moss (approx. 1-2g) will be added for environmental enrichment and to help stabilize within container humidity.

Salamanders will be field collected or obtained from a commercial vendor and placed on moistened filter paper in individual Petri dishes. Each dish/animal will be identified via a cage card affixed to the petri dish according to SOP No.003.04 (TOX 2004a). They will be maintained and acclimated to laboratory conditions for a minimum of two weeks prior to the onset of experiments (Jaeger 1992). Salamanders will be kept in conditions of constant photoperiod (12 hrs light/12 hrs dark), humidity and temperature. Following acclimation, animals will be weighed and distributed to experimental groups. Sex cannot be determined reliably in this group of animals and thus will remain unknown at the initiation of treatment.

Salamanders will be provided with food once every two days. Food will consist of potworms (*Enchytraeids doerjesi*) manually offered to animals via forceps. Previous investigations in our laboratory have shown salamanders to accept this food type readily, however, the feasibility of this feeding method has yet to be determined. All uneaten food will be collected and discarded. Additionally, we will maintain a culture of fruit flies (*Drossophila melanogaster*) that, should incorrigible complications arise with the primary food source (e.g., culture loss, etc.), will become the sole food item used for the remainder of the study. Salamanders will be housed in an

AAALAC accredited facility at USACHPPM where humidity and temperature within the animal rooms will be monitored.

V.5.1.1. Study Room: Housing- as assigned, E2100/ E-2101.

Dosing- rm. E2100 / E2101

Necropsy- rm. 3201, E2100

V.5.1.2. Special Husbandry Provisions: Not applicable

V.5.1.3. Exceptions: None

V.5.2. Veterinary Medical Care:

V.5.2.1. Routine Veterinary Medical Care: All animals will be observed at least twice daily by the animal care staff and once on weekend days and holidays. Appropriate methods of animal care shall be maintained to prevent, control, diagnose and treat diseases and injuries. Animal users are trained in the handling and euthanasia of the red-backed salamander. If it is determined that an animal is under distress, comatose, or moribund then the observer will report to the principal investigator or the attending veterinarian for an examination of its disposition and medical condition. If the animal is judged (by the PI through consultation with the attending veterinarian) unlikely to recover, as indicated by having met criteria spelled out in V.4.1.2., then the animal will be humanely euthanized as described.

V.5.2.2. Emergency Veterinary Medical Care: Animals will be observed at least once daily by the animal care staff on weekend days and holidays. If an animal is noted to be ill, the Attending Veterinarian will be contacted. Should the animal require euthanasia as described previously, the Attending Veterinarian will contact the PI/study director. If the PI/study director or other co-investigators are not available, the Attending Veterinarian will euthanize the animal and take as many required samples as possible from the animal. The animal carcass will be held at the facility under refrigeration for a minimum of three days in order for investigators to take any additional required samples.

V.5.3. Environmental Enrichment:

V.5.3.1. Enrichment Strategy: Each dish will contain a small piece of hydrated sphagnum moss as environmental enrichment and to moderate humidity within the enclosure.

V.5.3.2. Enrichment Restriction:

VI. STUDY PERSONNEL QUALIFICATIONS AND TRAINING:

All investigators named in this study have demonstrated an understanding of the humane care and use of research animals. They have taken part in discussions of pertinent laws and regulations concerning the use of animals in biomedical research in the Department of Defense as required by Public Laws 89-544, 91-579, and 99-198 (The Animal Welfare Act and Amendments, DoD Directives, and Army Regulations). They are familiar with the concepts for the reduction or elimination of the use of animals and have concluded that there is a need for the use of animals in this study. They have been familiarized in the proper methods for minimizing and/or alleviating pain in the animal species selected for study.

If their training in required procedures has not been of a formal, documented nature, they will demonstrate proficiency in these procedures prior to performing them on study animals. The Attending Veterinarian will observe them and determine proficiency or additional training needs.

Personnel working on this study have been advised on the animal care and use policy at this institution and are aware of the established reporting mechanisms for observed deficiencies in animal care and treatment.

The following is a table of participating investigators listing training and qualifications:

Staff Member	**Procedure(s)**	**Years of Experience**	**Qualifications**
Johnson	Collection, handling, feeding, weighing, euthanasia, necropsy	10+, Animal Research. Refined the salamander microcosm model and pioneered its use in mainstream eco-toxicology assessment	Ph.D, Biology; D.A.B.T.
Bazar	All	6+, Animal Research. On the job training (via MSJ) specific to salamanders.	MS, Biology
Ilg Paulus	All	6+, Animal Research. On the job training (via MSJ) specific to salamanders.	BS, Biology
Walker	All	3+, Animal Research. On the job training (via MSJ) specific to salamanders.	Ph.D, Biology

Dr. Mark Johnson has a background in population ecology and veterinary medical science. He has worked in the area of environmental risk assessment and has over 30 publications in the field of wildlife toxicology and ecology. He has been working in this field at CHPPM for the last 10 years. Mr. Matthew Bazar is trained in both Biology and Environmental Science and has first hand experience with field collection and captive care of herpetofauna. He has specific experience with amphibian handling and husbandry gained on the job during earlier in-house salamander studies and through field work studies at Towson State University. Ms. Heidi Ilg Paulus has been the co-investigator on other toxicity studies of this type and has been trained and experienced in the use of red-backed salamanders in research. She is the co-author of previous peer-reviewed manuscripts on this subject. She has had animal care and use training and training in the requirements of GLPs while at this institution. Mr. Matthew Bazar has extensive experience in conducting environmental risk assessments and has been working in the field for the last 5 years. Johnson, Ilg-Paulus, and Bazar have all had animal care or specific salamander care training.

Other supporting personnel include Jamie Suski, Pat Beall (Necropsies), MAJ Ann Schiavetta (Veterinary Care), and Michael P. Kefauver (Quality Assurance). The study will be archived according to institution procedures (reference DTOX SOP archival of samples).

VII. BIOHAZARD/SAFETY:

Normal adherence to standard chemical and animal handling procedures will be required during the performance of these studies. Lead is a potentially hazardous material will be handled in accordance with SOP No.083.04 (TOX 2004b).

Several strains of pathogenic bacteria have been cultured from wild caught amphibians (e.g., *Salmonella, Pseduomonas, Vibrio*, others). Care will be taken to ensure animal handlers will wash their hands and wear latex gloves while working with these animals. Masks will be worn while in the laboratory to reduce the probability of hand-to-mouth exposures.

VIII. ENCLOSURES:

IX. STUDY TIME FRAME:

IX.1. <u>Estimated Experimental Initiation Date</u>: March 2005

IX.2. <u>Estimated Experimental Completion Date</u>: July 2005

X. ASSURANCES: The law specifically requires several written assurances from the Study Director/Principal Investigator. Please read and sign the assurances as indicated.

As the Study Director/Principal Investigator on this protocol, I acknowledge my responsibilities and provide assurances for the following:

A. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a modification is specifically approved by the IACUC prior to its implementation.

B. Duplication of Effort: I have made every effort to ensure that this protocol is not an unnecessary duplication of previous experiments.

C. Statistical Assurance: : I assure that I have consulted with a qualified individual who evaluated the experimental design with respect to the statistical analysis, and that the minimum number of animals needed for scientific validity will be used.

D. Biohazard/Safety: I have taken into consideration and made the proper coordinations regarding all applicable rules and regulations concerning radiation protection, biosafety, recombinant issues, and so forth, in the preparation of this protocol.

E. Training: I verify that the personnel performing the animal procedures/manipulations/observations described in this protocol are technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused to the animals as a result of the procedures/manipulations.

F. Responsibility: I acknowledge the inherent moral, ethical and administrative obligations associated with the performance of this animal use protocol, and I assure that all individuals associated with this project will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to conduct this study in the spirit of the fourth "R," namely "Responsibility," which the DoD has embraced for implementing animal use alternatives where feasible and conducting humane and lawful research.

G. Scientific Review: This proposed animal use protocol has received appropriate peer scientific review and is consistent with good scientific research practice.

H. Painful Procedures: (A signature for this assurance is required by the Study Director/Principal Investigator if the research being conducted has the potential to cause more than momentary or slight pain or distress even if an anesthetic or analgesic is used to relieve the pain and/or distress.)

I am conducting biomedical experiments, which may potentially cause more than momentary or slight pain or distress to animals. This potential pain and/or distress WILL NOT be

relieved with the use of anesthetics, analgesics, and/or tranquilizers. I have considered alternatives to such procedures; however, I have determined that alternative procedures are not available to accomplish the objectives of this proposed experiment.

Mark S. Johnson

2005/01/08

Signature

Date (YYYYMMDD)

APPENDIX

References:

AVMA 2001. Report of the AVMA Panel on Euthanasia. JAVMA 218:669-696.

Baudo, R. 1976. Heavy metals concentrations (Cr, Cu, Mn, and Pb) in tadpoles and adults of *Rana esculenta*. L Mem Ist Ital Idrobiol 33:325-344 as cited in Harfenist et al. 1989.

Bishop, S.C. 1943. Handbook of Salamanders. Cornell University Press, Ithaca, NY.

Burton, T.M., and G.E. Likens. 1975. Salamander populations and biomass in the Hubbard Brook experimental forest. Copiea 3:541-546.

Jaeger, R.G. 1980. Density-Dependent and Density-Independent Causes of Extinction of a Salamander Population. Evolution 34(4): 617-621.

Jaeger, R.G. 1992. Housing, handling, and nutrition of salamanders. In: The Care and Use of Amphibians, Reptiles and Fish in Research, (Shaeffer, D.O, K.M. Kleinow, and L. Krulisch, eds). Scientist Center For Animal Welfare, Bethesda, MD, pp. 25-29.

Johnson, J.H. 1992. Anesthesia, Analgesia and Euthansia in Reptiles and Amphibians. In: The Care and Use of Amphibians, Reptiles and Fish in Research, (Shaeffer, D.O, K.M. Kleinow, and L. Krulisch, eds). Scientist Center For Animal Welfare, Bethesda, MD, pp. 49-51.

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. 18:873-878.

Johnson, M.S., J.W. Ferguson, and S.D. Holladay. 2000a. Immune effects of oral 2,4,6-trinitrotoluene (TNT) exposure to the white-footed mouse, Peromyscus leucopus. Int. J. Toxicol. 19: 5-11.

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

Johnson, M.S., H.I. Paulus, C.J. Salice, R.T. Checkai, and M. Simini. 2004. Toxicologic and histopathologic response of the terrestrial salamander *Plethodon cinereus* to soil exposures of 1,3,5-trinitrohexahydro-1,3,5-triazine. Arch. Environ. Contam. Toxicol. 47:496-501.

Harfenist, A., T. Power, K.L. Clark, and D.B. Peakall. 1989. A review and evaluation of the amphibian toxicological literature. Technical Report Series No. 61, Canadian Wildlife Service, Headquarters, Ottawa, Canada.

Ireland, M.P. 1977. Lead retention in toads *Xenopus laevis* fed increasing levels of lead-contaminated earthworms. Environ Pollut 12:85-92.

Ireland, M.P., K.S. Richards, and A.P. Gwynn, I. 1979. The localization of lead in the skin of light and dark adapted *Xenopus laevis*. Histochemistry 65:31-40.

Kaplan, H.M., T.J. Arnholt, and J.E. Payne. 1967. Toxicity of lead nitrate solution for frogs (*Rana pipiens*). Lab Animal Care 17:240-246.

Phillips, K. 1990. Where have all the frogs and toads gone? BioScience 40: 22-424.

Schmidt, C.W. 1997. Amphibian deformities continue to puzzle researchers. Environ. Sci. Technol. 31: 324-326.

TOX 2004a, SOP No.003.04, Individual Animal Identification.

TOX 2004b, SOP No.083.04, Health and Safety of Laboratory Personnel

Vogiatzis, A.K., and N.S. Loumbourdis. 1999. Exposure of *Rana ridibunda* to lead: I. Study of lead accumulation in various tissues and hepatic delta-aminolevulinic acid dehydratase activity. Journal of Appl. Toxicol. 19:25-29.

Wake, D.B. 1991. Declining amphibian populations. Science 253: 860.

US ARMY CENTER FOR HEALTH PROMOTION AND PREVENTIVE MEDICINE
PROTOCOL REVIEW, SUPPORT, APPROVAL SHEET

PROTOCOL NUMBER:

TITLE: Effects of lead exposure to the red-backed salamander (*Plethodon cinereus*)

PROTOCOL REVIEW:

1. **Scientific Merit (peer review)**

Dr. Desmond I. Bannon — Toxicologist
Printed Name (first, mi, last) — Title

MCHB-TS-THE, HERP
Division

2005/01/28
Date (YYYYMMDD)

Signature: Desmond Bannon

2. **Statistician**

Robyn B. Lee — Statistician
Printed Name (first, mi, last) — Title

MCHB-TS, DTS
Division

2005/02/09
Date (YYYYMMDD)

Signature:

3. **Quality Assurance**

Michael P. Kefauver,
Printed Name (first, mi, last)

Quality Assurance
Title

MCHB-TS-QSO, QSO
Division

2005/01/31
Date (YYYYMMDD)

Signature: Michael P. Kef

PROTOCOL SUPPORT:

4. **Program Manager, TEP/HERP**

Dr. Michael A. Major
Printed Name (first, mi, last)

Toxicologist
Title

MCHB-TS-THE, HERP
Division

20050131
Date (YYYYMMDD)

Signature:

5. **Attending Veterinarian**

MAJ Ann M. Schiavetta,
Printed Name (first, mi, last)

Veterinarian
Title

MCHB-TS-TTE, TEP
Division

20050131
Date (YYYYMMDD)

Signature:

6. **Chemistry**

Geraldine Miles
Printed Name (first, mi, last)

Chemist
Title

MCHB-TS-LAD, ASD
Division

20050128
Date (YYYYMMDD)

Signature:

PROTOCOL APPROVAL:

7. **Safety Manager**

Roy A. Valiant,
Printed Name (first, mi, last)

Safety Manager
Title

MCHB-CG-SEM, SEMO
Division

20050131
Date (YYYYMMDD)

Signature:

8. **Chairman, IACUC**

Dr. Joseph J Knapik
Printed Name (first, mi, last)

IACUC Chair
Title

MCHB-TS-DI, IPP
Division

23MAR05
Date (YYYYMMDD)

Animal Use Protocol – Effects of ~~copper~~ lead exposure to red-backed salamanders

E-Mail

Signature:

9. **Study Director**

Dr. Mark S. Johnson
Printed Name (first, mi, last)

Toxicologist
Title

MCHB-TS-THE, HERP
Division

20050322
Date (YYYYMMDD)

Signature:

10. **Sponsor**

Amy L. Hawkins
Printed Name (first, mi, last)

Program manager
Title

Naval Facilities Engineering Service Center
Division

20050323
Date (YYYYMMDD)

Signature

Animal Use Protocol - Effects of copper exposure to the red-backed salamander (*Plethodon cinereus*)

ANIMAL USE PROTOCOL

TOXICOLOGY DIRECTORATE

U.S. ARMY CENTER FOR HEALTH PROMOTION

AND PREVENTIVE MEDICINE

ABERDEEN PROVING GROUND, MD 21010-5403

PROTOCOL TITLE: Effects of copper exposure to the red-backed salamander (*Plethodon cinereus*)

PROTOCOL NUMBER: 03U9-64-05-03-02

PRINCIPAL INVESTIGATOR/STUDY DIRECTOR:

Mark S. Johnson, Ph.D.

Health Effects Research Program

Toxicology Directorate

U.S. Army Center for Health Promotion and Preventive Medicine

Aberdeen Proving Ground, MD 21010-5403

CO-INVESTIGATOR(S):

Matthew Bazar, M.S.

Toxicity Evaluation Program

Toxicology Directorate

U.S. Army Center for Health Promotion and Preventive Medicine

Aberdeen Proving Ground, MD 21010-5403

Heidi Ilg Paulus, B.S.

Toxicity Evaluation Program

Toxicology Directorate

U.S. Army Center for Health Promotion and Preventive Medicine

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Karen Walker, Ph.D.

Health Effects Research Program

Toxicology Directorate

U.S. Army Center for Health Promotion and Preventive Medicine

Aberdeen Proving Ground, MD 21010-5403

SPONSOR:

Mrs. Amy L. Hawkins (through ESTCP funded work unit CU-0514)

Naval Facilities Engineering Service Center, ESC413

1100 23rd Ave.

Port Hueneme

CA, 93043

SERDP/ESTCP POC:

Dr. Andrea Leeson

SERDP/ESTCP Cleanup Program Manager

901 N. Stuart Street

Suite 303

Arlington, VA 22203

I. NON-TECHNICAL SYNOPSIS:

The purpose of this study is to assess the toxicity of soil exposures of copper to the terrestrial red-backed salamander (*Plethodon cinereus*). The use of copper as a protective jacket for lead projectiles is well known in the Army. Small arms ranges are a necessary requirement for training purposes and as such are common at installations worldwide. Amphibian species are ubiquitously present at many military installations and, because of ecological and physiological characteristics, may experience worst-case exposures from copper in a soil matrix. However, the effects of copper to amphibians in terrestrial environments has not been characterized. In this study, red-backed salamanders will be exposed to four different concentrations of copper in soil. Food will consist of potworms (*Enchytraeids doerjesi*) exposed to contaminated soil of the same stock used to expose the

salamanders. Following 28 days of exposure, salamanders will be euthanized, necropsied, and evaluated for various health effects. These evaluations will consist of a pathological analysis of main blood conditioning organs (liver and spleen), gut, and gonads. These results will help determine toxic soil concentrations of copper and will be used to develop soil-screening levels for copper appropriate for terrestrial amphibians.

II. BACKGROUND:

II.1. <u>Background</u>: Copper is a malleable and highly corrosion resistant metal with widespread military and commercial application. The use of copper as a protective casing for lead projectiles is well known in the Army. The distribution of copper at many U.S. military sites is substantial and sites of contamination include habitats in and around military storage facilities, manufacturing, load and packing plants, open burning/open detonation areas, and firing ranges. Firing ranges in particular are a significant source of copper pollution as copper jacketed lead bullets are the primary projectiles fired on the more than 337 small arms ranges throughout the United States. These copper casings are oxidized over time from their original metallic state to carbonate, oxide, sulfate, and chloride forms of the metals. It is estimated that 6% of bullets will oxidize within 20 years while it could take up to 300 years to release all of the contaminant metal. These sites therefore present a mounting risk to humans who inhabit them and the ecosystems and wildlife that include them.

Copper is a biologically important metal and is a nutritionally essential element for most animals. Copper uptake induces the synthesis of metal-binding proteins that bind reactive copper ions, rendering them non-reactive and fostering their excretion. This action prevents copper ions from undergoing noxious chemical interactions within the organism, thus increasing tissue concentrations do not necessarily result in increased adverse effects. However, toxic effects do arise when the capacity of this metal-binding regulatory system is overcharged. Organisms with consistently high exposures may be more likely to experience copper body burdens that surpass the threshold capacity of metal-binding proteins. Amphibian species are ubiquitously present at many military installations and, because of ecological and physiological characteristics, may experience worst-case exposures from copper in a soil matrix. Salamanders of the genus *plethodon* exist primarily in soil (Bishop 1943), have a fairly thin integument compared with that of birds and mammals, prey on soil invertebrates, and are known to constitute a significant portion of the biomass in some ecosystems (Burton and Likens 1975). To date, there have been a variety of studies on the toxicity of copper to mammalian species, however, few data have been generated regarding the toxicity of copper to amphibians.

Our laboratory has conducted studies testing the effects of energetic compounds in soil to species of terrestrial salamanders (Johnson et al. 1999, Johnson et al. 2000a & b, Johnson et al. in prep). These previously used methods include a system to holistically evaluate total soil-borne exposures through the integration of a microcosm design. This study will use the microcosm design in a dose-response scheme to evaluate and ascertain toxic vs. safe soil concentrations of copper.

The study described will be conducted compliant with the principles in the Good Laboratory Practice (GLP) guidelines of the Toxic Substances Control Act (TSCA): 40 CFR (Code of Federal Regulations) 792, plus amendments (2). The investigators and technicians will adhere to The Guide for Care and Use of Laboratory Animals (U.S. Department of Health, Education, and Welfare Publication No. NIH 86-23, 1996) (3).

II.2. Literature Search for Duplication:

II.2.1. Literature Source(s) Searched:

A number of databases were consulted in an effort to obtain information on the acute toxicity of copper in salamanders as well as a look for duplication of effort. The databases were:

a. DTIC: Defense Technical Information Center (1965 – present)

b. MEDLINE®: National Library of Medicine Database (1966 – present)

c. TOMES Plus® (1960 – present)

d. RTECS: Registry of Toxic Effects of Chemical Substances (1960 – present)

e. HSDB: Hazardous Substance Data Bank (1960 – present)

f. CHRIS: Chemical Hazard Response Information (1960 – present)

g. Shepard's Catalog of Teratogenic Effects (1960 – present)

h. TERIS: Teratogen Information System (1960 – present)

i. REPROTOX: Reproductive Toxicology (1960 – present)

j. IRIS: Integrated Risk Information System

k. New Jersey Hazardous Substance Fact Sheets (1960 – present)

l. NIOSH Pocket Guide: National Institute for Safety and Health (current)

m. North American Emergency Response Handbook (1960 – present)

n. ECOTOX®: USEPA Environmental Databases

o. PHYTOTOX: Terrestrial Plant Database (1926 – present)

p. TERRETOX: Terrestrial Animal Database (1969 – present)

q. TOXLINE® (1900 – present)

r. FED.RIP: Federal Reports In Progress (1998 – present)

s. AGRICOLA: USDA National Agricultural Library (1970 – present)

t. BRD: DoD Biomedical Research (1998 – present)

u. CRISP: Computer Retrieval of Information on Scientific Projects (1972 – present)

II.2.2. Date of Search: 11 January 2005

II.2.3. Period of Search:

No date limits were applied to any of the databases. The time periods established for each of the individual databases were the defaults used, as shown above.

II.2.4. Key Words of Search: copper, salamander and copper, salamander and metal, amphibian and copper, amphibian and metal

II.2.5. Results of Search: We found very few studies that evaluated the effects of copper on amphibians in any database. Hits ranged from 0 to 29,323 (TOXLINE) when the search term "copper" was used alone. The AGRICOLA and CRISP searches produced studies that focused both on the effects of habitat on population level effects and on inapplicable aspects of physiology and neurology. Many studies were found that investigated toxicity of exposure to various substances and stressors to salamanders in TOXLINE (inclusive of MEDLINE, BIOSIS, and FEDRIP). However, none did so evaluating dose response relationships of adult amphibians in non-aquatic environments. The paucity of studies evaluating the response of adult amphibians to metals in general was further evidenced by the retrieval of very few studies reporting field exposures from contaminated sites. Multiple reports were found on heavy metal exposures in aquatic amphibians with nearly all of these focusing on larval stages and developmental toxicity. The DTIC search included only the word "salamander" and revealed 14 hits; however most involved installation-specific inventory reports and inapplicable metabolic evaluations. Even though the described literature search was comprehensive, we felt that there might be pertinent government documents and peer reviewed articles not cataloged in the aforementioned databases that would be useful for this study. We additionally performed an independent literature search using a combination of several academic search engines and by manually tracing literature cited within related copper toxicology articles. The following synopses summarize our most relevant findings:

1. In mammals, about half of the copper consumed is not absorbed through the intestine (Harris 1997). Primary target organ for the absorbed portion is the liver where,

when the metal-binding regulatory system remains in balance, another two-thirds is released in bile. Hepatic necrosis results when the capacity of metal-binding proteins is overwhelmed.

2. Kaplan and Yoh (1961) reported an LC50 of 6368 μg/L for copper sulfate in the adult leopard frog (*Rana pipiens*).

3. Arhem (1980) reported that copper slows down the kinetics of the potassium system in myelinated nerve fibers of the frog (*Xenopus* laevis).

III. OBJECTIVE/HYPOTHESIS:

The objective of this research is to determine the Lowest Observable Adverse Effect Level (LOAEL) and the No Observable Adverse Effect Level (NOAEL) from subchronic soil exposures to copper in the red-backed salamander, *Plethodon cinereus* focusing on pathological indicators and biomarkers. *P. cinereus* was chosen since it is terrestrial, amenable to laboratory investigations (Jaeger 1992), and has been reported to constitute a significant portion of the biomass in some ecosystems (Burton and Likens 1975). The usefulness of a biomarker of effects using melanomacrophage density (Johnson et al. 2000a) will be evaluated.

IV. MILITARY RELEVANCE:

Decreases in amphibian populations (Phillips 1990, Wake1991) and increases in developmental abnormalities (Schmidt 1997) have been widely reported and often attributed to environmental contamination. The U.S. Army has 337 small arms ranges, many encompassing large tracts of land. Nearly every installation has at least one range and it has been estimated that small arms ranges total more than 210,000 acres. Copper jacketed bullets are used as the primary projectile. Given the extent and prevalence of the contamination, and the need to balance environmental stewardship while maintaining readiness, it is of great importance that the Army understands the fate of copper exposure and the relative potential for effects if we are to maintain important training activities.

Amphibian species are ubiquitously present at many military installations and, because they are predominantly terrestrial and perform as much as 95% of their gas exchange through cutaneous respiration, their skin differs in its permeability to certain pollutants relative to most other terrestrial vertebrates. Therefore, any comprehensive ecological risk evaluation neglecting the dermal route of exposure in amphibians would likely underestimate the total systemic exposure and result in inaccurate characterization of potential effects. The data collected in this study will be used to determine safe levels of copper in soil to a terrestrial amphibian species and will be used to further develop a toxicity reference value for copper in soil for terrestrial amphibians. Determinations using this information will help provide guidance in regards to training, clean up, and transfer of lands affected from military activity.

V. MATERIALS AND METHODS:

V.1. Experimental Design:

V.1.1. Experiment:

1) 10-day Range Finding Study: A 10-day range finding study will be conducted to determine the appropriate copper sulfate concentrations used in the primary study (N=8). This will include one animal per a known concentration; approximately 8 concentrations of copper will be tested. Target soil concentrations will be 0, 0.2, 1.0, 100, 400, 1000, and 3000, 10,000 mg copper/kg soil. Only soil exposures to salamanders will be evaluated in this study. Overt signs of stress (e.g., excess mucus production, neuromuscular effects, excitability, etc.) will be used to determine toxicity and dose range for the 28-day exposures.

2) 28-Day Microcosm Toxicity Study: Data from the 10-day Range Finding study will be used to refine treatment groups, whereby a weigh-of-evidence procedure will be used. Time to death, types and prevalence of overt toxicological symptoms, proximate etiology, and consistency with known targets will be the evidence that will be used to determine the 28-day copper sulfate concentrations for each treatment. The 28-day study will consist of exposures from soil and food whereby soil will be spiked with copper sulfate at 5 concentrations, including a control (0 mg/kg), chemical concentrations will be determined by the 10–day range finding study. Soil of the Windsor type will be collected from a mature oak-beech forest at the Edgewood area of APG where red-backed salamanders are present. Soil will be dried, pulverized, and sifted through 2 screens (Nalgene; 1-mm2 and 0.5-mm2 mesh) to homogenize the sample. This will serve as base soil for the study. A sub-sample of the soil will be characterized (e.g., organic carbon content, pH, cation exchange capacity, %clay, sand, and silt). A copper salt will be dissolved into deionized water and the resulting stock solution thoroughly mixed with soil sub-samples in proportion to achieve the desired treatment levels. Each treatment will then be allowed to dry overnight in a darkened fume-hood before being mixed individually in a three-dimensional mixer for 18 hours. Approximately 110 g of soil will be added to 5” glass Petri dishes, hydrated, and allowed to sit in a dark room at room temperature for 15 days prior to the start of the study.

At the start of the first day of the 28 exposure period, 100 salamanders (N=100) will be divided evenly by weight into the five treatment groups and placed separately into individual Petri dishes containing treatment specific soil. Each dish/animal will retain its original cage card amended with a treatment designation and affixed to the new Petri dish. Approximately 1 g of soil from each treatment will be grab sampled on day –1 (start), 14 (middle), and 28 (end) and analyzed using an x-ray fluorescence spectrometer (InnovXsys model XT400). Calibration will be conducted using known samples each day and recorded in the laboratory notebook, compliant with U.S. EPA's Office of Solid Waste method 6200 (Field Portable XRF Spectrometry for the Determination of Elemental Concentrations in Soil and Sediment).

Food will consist of potworms exposed to copper contaminated soil of the same stock used to expose the salamanders. Potworms will be exposed to the treated soil prior to feeding and food samples will be collected on day –1 (start), 14 (middle), and 28 (end) to determine levels of copper experienced by salamanders through oral exposure. Should copper concentrations exceed potworm tolerance, wingless fruitflies (*Drosphila melanogaster*) will be used as food and the oral pathway will not be considered. Salamanders will be provided with food once every two days in the manner described in Section (V.5.1.). To consider changes as a result of at least one day of exposure, salamanders will be weighed on days –1, 7, and weekly thereafter to 0.001 g. Animals will be observed daily for signs of overt toxicity (e.g., lethargy, sensitivity to touch, abnormal behavior). On day 28, salamanders will be euthanized using aqueous preparations of MS-222 and euthanized via decapitation; the remaining body and head will be preserved in 10% formalin.

3) Histopathological Analyses

a. Saggital body and cross sections of the head will be trimmed, fixed in formalin, and embedded in paraffin. These tissues will then be sectioned at 6 microns, stained with hematoxylin and eosin, and examined via light microscopy.

b. Liver melanomacrophage (MMO) characteristics (density, diameter, area, per unit area) will be quantified using a 3.3 megapixel Q-fire color digital camera (Q-Imaging, Burnaby BC, CA) attached to an Olympus light microscope, model BH-2 (Optical Elements Corp., Dulles VA USA) at 400x. The camera is attached to a Dell Pentium IV PC and images will be evaluated using Image-Pro Plus V4.5 imaging software (Media Cybernetics, Silver Spring MD USA). Three sections will be quantified for each animal and then summed (for area, per unit area and density) or averaged (diameter). These values will then be used to represent a single data point for each animal and then evaluated by treatment group.

4) Hematological Analyses

a. <u>Blood Collection</u>: Small amounts (~ 20 ul) may be collected following euthanasia from adult salamanders of sufficient size. Blood will be collected from cranial region following decapitation with a microcapillary tube containing sodium heparin. After collection, the methods below are used. Sample volume will determine which evaluations are conducted.

b. <u>Whole Blood Cellularity</u>: A 10 µl aliquot of blood will be transferred into a tube containing 990 µl of phosphate buffered saline (PBS) and mixed by gentle vortexing for Natt-Herrick staining and whole blood enumeration. Tubes will be gently vortexed and a 10-µl aliquot of the stained blood sample will be placed onto a hemacytometer and the white blood cells will be attempted. The number of red blood cells (RBCs) will also be determined per ml. Based on this relationship, whole blood cellularity will be enumerated and size-analyzed using a hemocytometer or by using a CASY 1 model TTC cell counter + analyzer system (Scharfe System GMbH, Germany).

c. Packed Cell Volume (PCV), Total Protein, and Hemoglobin: A hematocrit capillary tube will be inserted into the tube containing the heparinized blood. Once ¾ filled, the bottom of the tube will be inserted into a clay pad, centrifuged for 5 min., and the PCV determined using a graphic reader card. Following enumeration of the PCV, the tube will be cut above the RBC layer and 2-3 drops (30-50µl) of plasma will be placed onto a refractometer and the total protein determined. Another drop will be placed within a slide cassette and hemoglobin content enumerated using a HemoCue Hemoglobin photometer (HemoCue AB, Angelholm, Sweden).

5) The study described will be conducted compliant with the principles of the Good Laboratory Practice (GLP) guidelines in the Toxic Substances Control Act (TSCA): 40 CFR (Code of Federal Regulations) 792, plus amendments (2). The investigators and technicians will adhere to The Guide for Care and Use of Laboratory Animals (U.S. Department of Health, Education, and Welfare Publication No. NIH 86-23, 1996) (3).

V.2. Data Analysis: All data will be recorded in the laboratory notebook. The weight data will be treated as percent change from baseline and will be tested with a Levene's Equal variance test. The data will then be tested with a two-way repeated measures ANOVA used to compare the percent change from Day 0 weights for treatment groups across the four observation times (mass collected on days 7, 14, 21 and 28). A Tukey's multiple comparison test will be used to assess the differences between treatment groups. Daily observational data may also be tested using the appropriate categorical method (e.g., Chi-square, Fisher's Exact test, etc.). Melanomacrophage data will be tested for normality using a Shapiro-Wilk, W-test at the $p < 0.05$ level. Melanomacrophage measurements are expected to be non-normally distributed, thus we will likely compare across treatments with a Kruskal-Wallis One-way ANOVA on ranks (SigmaStat® statistical software, SPSS Science, Chicago, Ill.). Multiple pairwise comparisons will be completed using the Dunn's Method at $\alpha = 0.05$ level of significance.

V.3. Laboratory Animals Required and Justification:

V.3.1. Non-animal Alternatives Considered: The objective addressed by this study is focused on the adverse health effects of soil exposures of copper to a terrestrial amphibian species. Non-animal alternatives would not provide the necessary information.

V.3.2. Animal Model and Species Justification: In order to address questions involving the heath effects from exposure to amphibian species that inhabit military installations where copper is present in the soil, it is essential to establish the toxicity of copper to a representative amphibian species. The red-backed salamander was chosen given its terrestrial life history. They are amenable to laboratory investigations (Jaeger 1992), and are widely distributed in the eastern North America (Jaeger 1980, Bishop 1943).

V.3.3. Laboratory Animals:

V.3.3.1. Genus and Species: *Plethodon cinereus*

V.3.3.2. Strain/Stock: N/A

V.3.3.3. Source/Vendor: Salamanders will be locally collected by hand in the spring between March and mid May in the Gunpowder watershed (MD. DNR Permit No. SCO-3558) or obtained from Connecticut Valley Biological Supply Company, Southhampton, MA. Care will be taken to choose larger animals (> 0.5g) and to not extirpate local populations (e.g., not to collect more than approximately 60% found).

V.3.3.4. Age: Unknown

V.3.3.5. Weight: Varying (0.5 – 2.0 g; 60 – 110 mm)

V.3.3.6. Sex: Both males and females will be tested (sex is not determined reliably)

V.3.3.7. Special Considerations: None

V.3.4. Number of Animals Required (By Species):

1. Red-backed Salamander (*Plethodon cinereus*) – 120

Twenty animals per treatment will be required. There are five treatments (including a control) and a 10-day range-finding study that will require 8 animals. Since these animals are wild-caught (i.e., outbred of various ages) an additional twelve animals will be needed to choose animals of a similar size and roughly similar age to achieve a degree of homogeneity in the treatment groups. Moreover, this selection of 108 from the original pool of 120 will allow for fewer animals to be needed to achieve meaningful results.

The number of animals required has been determined through an evaluation of variability in sensitive responses from data collected from previous work with salamanders (Johnson et al. 2004, Johnson et al. 2000a & b) and also based upon judgment of the investigators considering the effort involved in maintaining and gathering data for the study. When the sample size in each of the 5 groups is 20, a one-way analysis of variance will have 80% power to detect at the 0.05 level a difference in mean percent change in weights similar to those observed in a previous RDX study, assuming that the common standard deviation is 10.5 or less.

Treatment Group	Test Animals
Off study/training[2]	12
10-day range-find	8
Control (0 mg/kg)	20
TBD	20
TBD	20
TBD	20
TBD	20
Total	**120**

Since these animals are wild-caught (i.e., outbred of various ages) an additional twelve animals will be needed to choose animals of a similar size and roughly similar age to achieve a degree of homogeneity in the treatment groups. This is not needed in locally-collected situations since only those animals exceeding about 0.5 g in mass will be collected. These animals will be used for training purposed and assigned accordingly.

If the proper number and quality of animals is not obtained, a statistician will be consulted to determine if a revision is necessary. If a change in the design is necessary, a modification and approval through the IACUC is will be completed.

V.3.5. Refinement, Reduction, Replacement:

V.3.5.1. Refinement: This protocol is based on what has been learned from earlier work in our lab (Johnson 1999, Johnson et al. 2000a & b, Johnson et al. 2004) and the methods developed by Jaeger (1992). This background information will be used to optimize husbandry conditions, thus minimize distress to our animals.

V.3.5.2. Reduction: Based on the results of earlier investigations, an optimal number of animals will be used (Johnson et al. 2004). Additionally, by conducting a 10-day range-finding study, it will help to determine the concentrations at which to reduce mortality and determine the Lowest Observed Adverse Effect Level.

V.3.5.3. Replacement: No replacement alternatives are appropriate for this study. In order to address questions involving the health effects from exposure to amphibian species that inhabit military installations where copper is present in the soil, it is essential to

[2] Animals from vendor of sub-optimal weight for 28-d exposures.

establish the toxicity of copper to a representative amphibian species. The red-backed salamander was chosen given its terrestrial life history. They are amenable to laboratory investigations (Jaeger 1992), and are widely distributed in the eastern North America. (Jaeger 1980, Bishop 1943).

V.4. Technical Methods:

V.4.1. Pain/Distress Assessment: Considering that the intention of this research is to understand the concentration of copper in soil that causes toxicity, pain may be experienced by animals in the two highest exposure groups. This assessment is based on the reports of previous studies where indications of distress (for e.g., excessive mucus production, loss of righting reflex, excessive buccopharyngeal pumping) and/or mortality/morbidity has occurred at the highest dose. However, this is a conservative assessment as previous experiments with TNT and the nitroaromatic analog DNT produced no adverse effects.

V.4.1.1. APHIS Form 7023 Information

V.4.1.1.1. Number of animals:

V.4.1.1.1.1. Column C: 76 (67%)

V.4.1.1.1.2. Column D: 0

V.4.1.1.1.3. Column E: 44 (33%) (Conservative possibility - see V.4.1. for rationale)

V.4.1.2. Pain Relief/Prevention: Since the goal of this investigation is to determine the effects from exposure, no other substances that could potentially interfere with the interpretation of these results will be administered. However, moribund or animals in overt pain will be humanely euthanized as described in section V.4.6. (Euthanasia). Based on previous experience with these models we will consider the following as indications of distress where recover is unlikely at continued exposure regimes:

1) Appear extremely listless/lethargic and exhibit excessive mucus production

2) Experience a continued loss of appetite.

3) Are unresponsive and do not demonstrate a righting response

A weight of evidence determination will be made on a case by case basis after consultation with the principal investigator and the attending veterinarian.

V.4.1.2.1. Anesthesia/Analgesia Tranquilization: n/a

V.4.1.2.2. Pre- and Post-procedural Provisions: None

V.4.1.2.3. Paralytics: None

V.4.1.3. Literature Search for Alternatives to Painful or Distressful Procedures:

V.4.1.3.1. Sources Searched:

a. NORINA: Norwegian Reference Centre Alternatives Database

b. Altweb

c. Scirus

d. STINET: Scientific and Technical Information Network

e. DTIC: Defense Technical Information Center

f. MEDLINE®: National Library of Medicine Database

g. ECOTOX®: USEPA Environmental Databases

h. TERRETOX: Terrestrial Animal Database

i. TOXLINE®

j. AGRICOLA: USDA National Agricultural Library

k. CRISP: Computer Retrieval of Information on Scientific Projects

V.4.1.3.2. Date of Search: 11 January 2005

V.4.1.3.3. Period of Search: The period of search of the databases is the same as that stated in section II.2.3.

V.4.1.3.4. Key Words of Search: Amphibian and anesthe?, amphibian and euthanas?, salamander and alternative and model, salamander and metal and simulate, amphibian and pain, amphibian and distress

V.4.1.3.5. Results of Search: We found very few papers discussing amphibian anesthesia or euthanasia however, of the databases listed above, Altweb was most productive and produced several articles discussing anesthesia/euthanasia and pain/distress in amphibians. We additionally performed an independent literature search using a combination of several academic search engines and by manually tracking down literature

cited in captive care literature. This search was highly productive and yielded several order specific anesthesia/euthanasia review papers. The following synopses summarize our most relevant findings:

1. There are no alternatives to live salamanders for addressing the purpose of this study.

2. The housing and husbandry design is both appropriate to the study and ideal in meeting the life maintenance and enrichment needs of the red-backed salamander.

3. The few studies that have evaluated the effects of amphibians and nitroaromatics have shown no induced adverse affects or indications of pain. Therefore, no substances can be reliably used to alleviate pain since the toxicodynamic properties (i.e. factors that may confound the evaluation of the toxicity of copper exposure) of such have not yet been determined and may compromise the results.

4. Tricaine methane sulphanate (MS-222) is an anesthetic and alleviates pain in amphibians prior to euthanasia. The use of MS-222 has been recommended for anesthesia and euthanasia in amphibians by many researchers (Cooper et al. 1989, Johnson 1992, Schaeffer 1994) as well as the American Veterinary Medical Association (AVMA 2001), the Canadian Council on Animal Care (CCAC 1980), and a collection of major herpetological societies (ASIH et al. 2001). A review of its pharmacology and use in amphibians has been published (Downes 1995).

V.4.1.4. Unalleviated Painful/Distressful Procedure Justification: Since the goal of this investigation is to determine the effects from exposure, no other substances that could potentially interfere with the interpretation of these results will be administered. However, moribund or animals in overt pain will be humanely euthanized as described in section V.4.5. (Study Endpoint).

V.4.2. Prolonged Restraint: Not applicable

V.4.3. Surgery: Not applicable

V.4.3.1. Pre-surgical Provisions: Not applicable

V.4.3.2. Procedure: Not applicable

V.4.3.3. Post-surgical Provisions: Not applicable

V.4.3.4. Location: Not applicable

V.4.3.5. Surgeon: Not applicable

V.4.3.6. Multiple Major Survival Operative Procedures: Not applicable

V.4.3.6.1. Procedures: Not applicable

V.4.3.6.2. Scientific Justification: Not applicable

V.4.4. Animal Manipulations: Not applicable

V.4.4.1. Injections: Not applicable

V.4.4.2. Biosamples: Not applicable

V.4.4.3. Adjuvants: Not applicable

V.4.4.4, Monoclonal Antibody (MAbs) Production: Not applicable

V.4.4.5. Animal Identification: Individual animals will be identified by cage card according to SOP No.003.04 (TOX 2004).

V.4.4.6. Behavioral Studies: Not applicable

V.4.4.7. Other Procedures: Animals will be observed every 2 hours for the first 8 hours, and then daily thereafter as part of the initial range finding study. During the 28 day study, animals will be observed at least daily for signs of overt toxicity (e.g., excess mucus production, neuromuscular effects, excitability, etc.) by the principal investigator and/or co-investigators.

V.4.4.8. Tissue Sharing: Not applicable

V.4.5. Study Endpoint: The study will be terminated on Day 28 after initial exposure, after anesthesia, euthanasia, and necropsy of animals. Euthanasia is required to histologically evaluate the potential for toxic effects from combined exposures to copper. Early euthanasia will occur when a weight of evidence determination (as described in V.4.1.2) indicates that animals are experiencing significant pain or distress.

V.4.6. Euthanasia: On day 28, salamanders will be euthanized by immersing them in a beaker containing a buffered aqueous preparation of MS-222 at 2g/l. Deep anesthesia (determined through the lack of a response to tail pinch) will be followed with decapitation. The remaining body and head will be preserved in 10% formalin. The humane use of a buffered MS-222 solution of 2 g/L has been recommended for anesthesia and euthanasia in amphibians by the American Veterinary Medical Association (AVMA 2001), the Canadian Council on Animal Care (CCAC 1980), a collection of major herpetological societies (ASIH et al. 2001), and by many researchers (Cooper et al. 1989, Johnson 1992, Schaeffer 1994).

V.5. <u>Veterinary Care</u>:

V.5.1. Husbandry Considerations: Salamanders will be singly housed in 5" borosilicate glass petri dishes as recommended by Jaeger (1992). During the acclimation period, each dish will contain only a sheet of filter paper hydrated with deionized water. Filter paper (Q8, 12.5 cm dia., coarse porosity, Cat. No. 09-790E, Fisher Scientific, Atlanta, GA) will be changed as necessary. The acclimatization period will last at least 14 days. During the study, the filter paper will be replaced with soil, depending upon the treatment. Hydration with deionized water will occur as needed. All animals will be kept in conditions of constant photoperiod (12 hrs light/12 hrs dark), humidity and temperature. All uneaten feed will be collected daily. Salamanders will be housed in AAALAC accredited facility at USACHPPM where humidity and temperature will be monitored. Humidity will be maintained between 30 and 70% and the temperature will be maintained between 62 and 75 ˚F. A small piece of sphagnum moss (approx. 1-2g) will be added for environmental enrichment and to help stabilize within container humidity.

Salamanders will be field collected or obtained from a commercial vendor and placed on moistened filter paper in individual Petri dishes. Each dish/animal will be identified via a cage card affixed to the petri dish according to SOP No.003.04 (TOX 2004a). They will be maintained and acclimated to laboratory conditions for a minimum of two weeks prior to the onset of experiments (Jaeger 1992). Salamanders will be kept in conditions of constant photoperiod (12 hrs light/12 hrs dark), humidity and temperature. Following acclimation, animals will be weighed and distributed to experimental groups. Sex cannot be determined reliably in this group of animals and thus will remain unknown at the initiation of treatment.

Salamanders will be provided with food once every two days. Food will consist of potworms (*Enchytraeids doerjesi*) manually offered to animals via forceps. Previous investigations in our laboratory have shown salamanders to accept this food type readily, however, the feasibility of this feeding method has yet to be determined. All uneaten food will be collected and discarded. Additionally, we will maintain a culture of fruit flies (*Drossophila melanogaster*) that, should incorrigible complications arise with the primary food source (e.g., culture loss, etc.), will become the sole food item used for the remainder of the study. Salamanders will be housed in an AAALAC accredited facility at USACHPPM where humidity and temperature within the animal rooms will be monitored.

V.5.1.1. Study Room: Housing- as assigned, E2100/ E-2101.

Dosing- rm. E2100 / E2101

Necropsy- rm. 3201, E2100

V.5.1.2. Special Husbandry Provisions: Not applicable

V.5.1.3. Exceptions: None

V.5.2. Veterinary Medical Care:

V.5.2.1. Routine Veterinary Medical Care: All animals will be observed at least twice daily by the animal care staff and once on weekend days and holidays. Appropriate methods of animal care shall be maintained to prevent, control, diagnose and treat diseases and injuries. Animal users are trained in the handling and euthanasia of the red-backed salamander. If it is determined that an animal is under distress, comatose, or moribund then the observer will report to the principal investigator or the attending veterinarian for an examination of its disposition and medical condition. If the animal is judged (by the PI through consultation with the attending veterinarian) unlikely to recover, as indicated by having met criteria spelled out in V.4.1.2., then the animal will be humanely euthanized as described.

V.5.2.2. Emergency Veterinary Medical Care: Animals will be observed at least once daily by the animal care staff on weekend days and holidays. If an animal is noted to be ill, the Attending Veterinarian will be contacted. Should the animal require euthanasia as described previously, the Attending Veterinarian will contact the PI/study director. If the PI/study director or other co-investigators are not available, the Attending Veterinarian will euthanize the animal and take as many required samples as possible from the animal. The animal carcass will be held at the facility under refrigeration for a minimum of three days in order for investigators to take any additional required samples.

V.5.3. Environmental Enrichment:

V.5.3.1. Enrichment Strategy: Each dish will contain a small piece of hydrated sphagnum moss as environmental enrichment and to moderate humidity within the enclosure.

V.5.3.2. Enrichment Restriction:

VI. STUDY PERSONNEL QUALIFICATIONS AND TRAINING:

All investigators named in this study have demonstrated an understanding of the humane care and use of research animals. They have taken part in discussions of pertinent laws and regulations concerning the use of animals in biomedical research in the Department of Defense as required by Public Laws 89-544, 91-579, and 99-198 (The Animal Welfare Act and Amendments, DoD Directives, and Army Regulations). They are familiar with the concepts for the reduction or elimination of the use of animals and have concluded that there is a need for the use of animals in this study. They have been familiarized in the proper methods for minimizing and/or alleviating pain in the animal species selected for study.

If their training in required procedures has not been of a formal, documented nature, they will demonstrate proficiency in these procedures prior to performing them on study

animals. The Attending Veterinarian will observe them and determine proficiency or additional training needs.

Personnel working on this study have been advised on the animal care and use policy at this institution and are aware of the established reporting mechanisms for observed deficiencies in animal care and treatment.

The following is a table of participating investigators listing training and qualifications:

Staff Member	**Procedure(s)**	**Years of Experience**	**Qualifications**
Johnson	Collection, handling, feeding, weighing, euthanasia, necropsy	10+, Animal Research. Refined the salamander microcosm model and pioneered its use in mainstream eco-toxicology assessment	Ph.D, Biology; D.A.B.T.
Bazar	All	6+, Animal Research. On the job training (via MSJ) specific to salamanders.	MS, Biology
Ilg Paulus	All	6+, Animal Research. On the job training (via MSJ) specific to salamanders.	BS, Biology
Walker	All	3+, Animal Research. On the job training (via MSJ) specific to salamanders.	Ph.D, Biology

Dr. Mark Johnson has a background in population ecology and veterinary medical science. He has worked in the area of environmental risk assessment and has over 30 publications in the field of wildlife toxicology and ecology. He has been working in this field at CHPPM for the last 10 years. Mr. Matthew Bazar is trained in both Biology and Environmental Science and has first hand experience with field collection and captive care of herpetofauna. He has specific experience with amphibian handling and husbandry gained on the job during earlier in-house salamander studies and through field work studies at Towson State University. Ms. Heidi Ilg Paulus has been the co-investigator on other toxicity studies of this type and has been trained and experienced in the use of red-backed salamanders in research. She is the co-author of previous peer-reviewed manuscripts on this subject. She has had animal care and use training and training in the requirements of GLPs while at this institution. Mr. Matthew Bazar has extensive experience in conducting environmental risk assessments and has been working in the field for the last 5 years. Johnson, Ilg Paulus, and Bazar have all had animal care or specific salamander care training.

Other supporting personnel include Jamie Suski, Pat Beall (Necropsies), MAJ Ann Schiavetta (Veterinary Care), and Micheal P. Kefauver (Quality Assurance). The study will be archived according to institution procedures (reference DTOX SOP archival of samples).

VII. BIOHAZARD/SAFETY:

Normal adherence to standard chemical and animal handling procedures will be required during the performance of these studies. Copper is a potentially hazardous material will be handled in accordance with SOP No.083.04 (TOX 2004b).

Several strains of pathogenic bacteria have been cultured from wild caught amphibians (e.g., *Salmonella, Pseduomonas, Vibrio*, others). Care will be taken to ensure animal handlers will wash their hands and wear latex gloves while working with these animals. Masks will be worn while in the laboratory to reduce the probability of hand-to-mouth exposures.

VIII. ENCLOSURES:

IX. STUDY TIME FRAME:

IX.1. Estimated Experimental Initiation Date: March 2005

IX.2. Estimated Experimental Completion Date: June 2005

X. ASSURANCES: The law specifically requires several written assurances from the Study Director/Principal Investigator. Please read and sign the assurances as indicated.

As the Study Director/Principal Investigator on this protocol, I acknowledge my responsibilities and provide assurances for the following:

A. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a modification is specifically approved by the IACUC prior to its implementation.

B. Duplication of Effort: I have made every effort to ensure that this protocol is not an unnecessary duplication of previous experiments.

C. Statistical Assurance: : I assure that I have consulted with a qualified individual who evaluated the experimental design with respect to the statistical analysis, and that the minimum number of animals needed for scientific validity will be used.

D. Biohazard/Safety: I have taken into consideration and made the proper coordinations regarding all applicable rules and regulations concerning radiation protection, biosafety, recombinant issues, and so forth, in the preparation of this protocol.

E. Training: I verify that the personnel performing the animal procedures/manipulations/observations described in this protocol are technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused to the animals as a result of the procedures/manipulations.

F. Responsibility: I acknowledge the inherent moral, ethical and administrative obligations associated with the performance of this animal use protocol, and I assure that all individuals associated with this project will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to conduct this study in the spirit of the fourth "R," namely "Responsibility," which the DoD has embraced for implementing animal use alternatives where feasible and conducting humane and lawful research.

G. Scientific Review: This proposed animal use protocol has received appropriate peer scientific review and is consistent with good scientific research practice.

H. Painful Procedures: (A signature for this assurance is required by the Study Director/Principal Investigator if the research being conducted has the potential to cause more than momentary or slight pain or distress even if an anesthetic or analgesic is used to relieve the pain and/or distress.)

I am conducting biomedical experiments, which may potentially cause more than momentary or slight pain or distress to animals. This potential pain and/or distress WILL NOT be relieved with the use of anesthetics, analgesics, and/or tranquilizers. I have

considered alternatives to such procedures; however, I have determined that alternative procedures are not available to accomplish the objectives of this proposed experiment.

Mark S. Johnson

Signature

2005/01/08

Date (YYYYMMDD)

US ARMY CENTER FOR HEALTH PROMOTION AND PREVENTIVE MEDICINE
PROTOCOL REVIEW, SUPPORT, APPROVAL SHEET

PROTOCOL NUMBER: 03U9-64-05-03-02

TITLE: Effects of copper exposure to the red-backed salamander (*Plethodon cinereus*)

PROTOCOL REVIEW:

1. **Scientific Merit (peer review)**

Dr. Desmond I. Bannon
Printed Name (first, mi, last)

Toxicologist
Title

MCHB-TS-THE, HERP
Division

2005/1/28
Date (YYYYMMDD)

Signature: Desmond Bannon

2. **Statistician**

Robyn B. Lee
Printed Name (first, mi, last)

Statistician
Title

MCHB-TS, DTS
Division

2/9/2005
Date (YYYYMMDD)

Signature:

3. **Quality Assurance**

Michael P. Kefauver,
Printed Name (first, mi, last)

Quality Assurance
Title

MCHB-TS-QSO, QSO
Division

2005/01/31
Date (YYYYMMDD)

Signature: Michael P. Kefauver

PROTOCOL SUPPORT:

4. **Program Manager, TEP/HERP**

Dr. Michael A. Major
Printed Name (first, mi, last)

Toxicologist
Title

MCHB-TS-THE, HERP
Division

20050131
Date (YYYYMMDD)

Signature:

5. **Attending Veterinarian**

MAJ Ann M. Schiavetta,
Printed Name (first, mi, last)

Veterinarian
Title

MCHB-TS-TTE, TEP
Division

20050131
Date (YYYYMMDD)

Signature:

6. **Chemistry**

Geraldine Miles
Printed Name (first, mi, last)

Chemist
Title

MCHB-TS-LAD, ASD
Division

20050128
Date (YYYYMMDD)

Signature: Geraldine Miles

PROTOCOL APPROVAL:

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APPENDIX

References:

Arhem, P. 1980. Effects of some heavy metal ions on the ionic currents of myelinated fibers from *Xenopus laevis*. J Physiol 306:219-231.

AVMA 2001. Report of the AVMA Panel on Euthanasia. JAVMA 218:669-696.

Bishop, S.C. 1943. Handbook of Salamanders. Cornell University Press, Ithaca, NY.

Burton, T.M., and G.E. Likens. 1975. Salamander populations and biomass in the Hubbard Brook experimental forest. Copiea 3:541-546.

Harris, E.D. 1997. Copper. in B.L. O'Dell and R.A. Sunde (eds): *Handbook of nutritionally essential mineral elements*. New York: Marcel Dekker, pp 231-273.

Jaeger, R.G. 1980. Density-Dependent and Density-Independent Causes of Extinction of a Salamander Population. Evolution 34(4): 617-621.

Jaeger, R.G. 1992. Housing, handling, and nutrition of salamanders. In: The Care and Use of Amphibians, Reptiles and Fish in Research, (Shaeffer, D.O, K.M. Kleinow, and L. Krulisch, eds). Scientist Center For Animal Welfare, Bethesda, MD, pp. 25-29.

Johnson, J.H. 1992. Anesthesia, Analgesia and Euthansia in Reptiles and Amphibians. In: The Care and Use of Amphibians, Reptiles and Fish in Research, (Shaeffer, D.O, K.M. Kleinow, and L. Krulisch, eds). Scientist Center For Animal Welfare, Bethesda, MD, pp. 49-51.

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. 18:873-878.

Johnson, M.S., J.W. Ferguson, and S.D. Holladay. 2000a. Immune effects of oral 2,4,6-trinitrotoluene (TNT) exposure to the white-footed mouse, *Peromyscus leucopus*. Int. J. Toxicol. 19: 5-11.

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

Johnson, M.S., H.I. Paulus, C.J. Salice, R.T. Checkai, and M. Simini. 2004. Toxicologic and histopathologic response of the terrestrial salamander *Plethodon cinereus* to soil exposures of 1,3,5-trinitrohexahydro-1,3,5-triazine. Arch. Environ. Contam. Toxicol. 47:496-501.

Kaplan, H.M. and L. Yoh. 1961. Toxicity of copper for frogs. Herpetologica 17:131-135.

Phillips, K. 1990. Where have all the frogs and toads gone? BioScience 40: 22-424.

Schmidt, C.W. 1997. Amphibian deformities continue to puzzle researchers. Environ. Sci. Technol. 31: 324-326.

TOX 2004a, SOP No.003.04, Individual Animal Identification.

TOX 2004b, SOP No.083.04, Health and Safety of Laboratory Personnel

Wake, D.B. 1991. Declining amphibian populations. Science 253: 860.

Appendix B

Quality Assurance Project Plan

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1.0 Purpose and Scope of the Plan

This Quality Assurance Project Plan (QAPP) presents the organization, objectives, planned activities, and specific quality assurance/quality control (QA/QC) procedures associated with the field demonstration of this ESTCP project (*Demonstration and Certification of Amphibian Ecological Risk Assessment Protocol, CU-0514*). Specific protocols for sampling, sample handling and storage, chain-of-custody, and laboratory and field analyses will be described.

This QAPP has been prepared in accordance with the following documents:

- United States Environmental Protection Agency. 1995. QA/QC Guidance for Sampling and Analysis of Sediments, Water, and Tissues for Dredged Material Evaluations – Chemical Evaluations. EPA 823-B-95-001. Office of Water, Office of Science and Technology, Standards and Applied Science Division, Washington, DC.
- United States Environmental Protection Agency. 1997. Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, SW-846. Third Edition. May 1986, revised June 1997.
- United States Environmental Protection Agency. 2002. EPA Guidance for Quality Assurance Project Plans. EPA QA/G-5. December 2002

2.0 Quality Assurance Responsibilities

The laboratories providing physical testing, chemical analyses, and toxicity testing support to this project are listed below.

Organization	Contact	Tasks
USACHPPM Aberdeen Proving Ground, MD	Dr. Mark Johnson	Conduct toxicity testing according to soil exposure protocol
ENSR Fort Collins Toxicology Lab 4303 W. LaPorte Avenue Fort Collins, CO 80521	Dr. David Pillard	Conduct toxicity testing according to sediment exposure protocol
Paragon Analytics 225 Commerce Drive Fort Collins, CO 80524	Debbie Fazio	Bulk sediment/soil chemistry analyses
Mitkem Corporation 175 Metro Center Blvd. Warwick, RI 02886	Ben Dodge	Bulk sediment/soil SEM/AVS analyses
Huffman Laboratories, Inc. 4630 Indiana Street Golden, CO 80403	Ed Huffman	Bulk sediment/soil total organic carbon analyses
GeoTesting Express 1145 Massachusetts Ave. Boxborough, MA 01719	Gary Torosian	Bulk sediment/soil grain size, bulk density, and cation exchange capacity tests

The Laboratory Manager at each facility is ultimately responsible for the data produced by the laboratory. The Laboratory QA Coordinator is responsible for assessing and maintaining the laboratory QA manual implementation within the facility operations and performing QA assessments. The Laboratory Project Manager is the primary point of contact between the laboratory and ENSR. The Laboratory Project Manager is responsible for monitoring analytical and QA project requirements for the specified project, reviewing project data packages for completeness and compliance to client needs, and monitoring, reviewing, and evaluating the progress and performance of projects.

The ENSR Field Team Leaders (FTL) have overall responsibility for completion of all field activities in accordance with the QAPP and are the communication links between ENSR project management and the respective field teams. Specific responsibilities of the ENSR FTLs include: coordinating activities at the demonstration sites, assigning specific duties to field team members, mobilizing and demobilizing of the field team, resolving any logistical problems that could potentially hinder field activities (e.g., equipment malfunctions or availability, personnel conflicts, weather dependent working conditions), and implementing field QC including issuance and tracking of measurement and test equipment; the proper labeling, handling, storage, shipping, and chain-of-custody procedures used at the time of sampling; and control and collection of all field documentation.

The field staff reports directly to the ENSR FTL and the responsibilities of the field team include: collecting samples, conducting field measurements, and decontaminating equipment according to documented procedures stated in the QAPP, ensuring that field instruments are properly operated, calibrated, and maintained, and that adequate documentation is kept for all instruments, collecting the required QC samples and thoroughly documenting QC sample collection, ensuring that field documentation and data are complete and accurate, and communicating any nonconformance or potential data quality issues to the ENSR FTL.

3.0 Data Quality Parameters

The program will consist of field sampling activities and physical, chemical, and biological testing. The Field Demonstration Plan outlines a sampling design to be performed and specifies the use of collection and handling procedures that will ensure the representativeness and integrity of the samples. Furthermore, the analytical program is designed to generate definitive data of sufficient quality and sensitivity to meet the project objectives.

Measures to ensure representativeness, completeness, comparability, accuracy and precision of the data are discussed below.

Representativeness – Representativeness expresses the degree to which data accurately and precisely represent a characteristic of a population, parameter variations at a sampling point, a process condition, or an environmental condition within a defined spatial and/or temporal boundary. Representativeness is dependent upon the proper design of the sampling program and will be satisfied by ensuring that the Field Demonstration Plan and QAPP are followed and that proper sampling techniques are used.

Representativeness in the laboratory is ensured by using the proper analytical procedures, appropriate methods, meeting sample holding times, and analyzing and assessing blank samples.

Completeness - Completeness is a measure of the amount of valid data obtained from a measurement system compared to the amount that was expected to be obtained under normal conditions. "Normal conditions" are defined as the conditions expected if the sampling plan was implemented as planned.

Field completeness is a measure of the amount of valid samples obtained during all sampling for the project. The field completeness objective is greater than 95 percent.

Laboratory completeness is a measure of the amount of valid measurements obtained from all the measurements taken in the project. The laboratory completeness objective is greater than 95 percent.

Comparability – Comparability expresses the confidence with which one data set can be compared to another. Planned analytical data will be comparable when similar sampling and analytical methods are used as documented in the Field Demonstration Plan and the QAPP.

Accuracy - Accuracy is the degree of agreement between the observed value and an accepted reference or true value. Accuracy in the field is assessed through the use of equipment rinsate blanks and through the adherence to all sample handling, preservation, and holding time requirements. The objective for equipment blanks is that no target analytes are present above the reporting limit.

Laboratory accuracy is assessed through the analysis of MS/MSDs, laboratory control samples (LCSs), and the subsequent determination of percent recoveries (%Rs). For spiked samples the accuracy objectives will be the laboratory control limits that are current at the time of analyses.

Precision - Precision is a measure of the degree to which two or more measurements are in agreement. Precision will be measured through the calculation of relative percent difference (RPD).

The objectives for field precision RPDs are 30% RPD for aqueous samples and 50% RPD for solid samples.

Precision in the laboratory is assessed through the calculation of RPD for duplicate samples, either as matrix spike/matrix spike duplicates (MS/MSDs) or as laboratory duplicates, depending on the method. The control limits generated by the laboratories that are current at the time of analyses will be utilized.

4.0 Calibration Procedures, Quality Control Checks, and Corrective Action

4.1 Instrument/Equipment Testing, Inspection, and Maintenance

The field equipment for this project may include a grab sampler for sediment collection, small scoops for soil collection, and spoons and bowls for sample homogenizing . Field instruments will include a DGPS, an XRF unit, and YSI multi-parameter meter. The ENSR FTL will be responsible for ensuring that equipment and instruments are free from obvious defects, damage, and contamination and are properly functioning. At a minimum, this will entail checking the equipment or instrument prior to shipment to the field and performing daily operational checks and calibration as described below. Routine maintenance and trouble-shooting procedures will be performed as described in the manufacturer's instructions.

Routine testing and preventive maintenance is performed by the laboratory as part of their QA program. Details on the type of checks, frequencies, and corrective actions are included in the laboratory QA manuals.

4.2 Instrument/Equipment Calibration and Frequency

In general, field instruments will be calibrated prior to daily or initial use, and will be checked every 15 samples and at the end of the day. Calibration procedures will be consistent with the manufacturer's recommendations. All calibration procedures will be documented in the field records. Calibration records will include the date/time of calibration, name of the person performing the calibration, reference standard used, and the results of the calibration.

Calibration procedures for laboratory instruments will consist of initial calibrations, initial calibration verifications, and continuing calibration verification. The SOP for each analysis performed in the laboratory describes the calibration procedures, their frequency, acceptance criteria, and the conditions that will require recalibration. This information is summarized in the laboratory QA Manuals.

The laboratory maintains documentation for each instrument which includes the following information: instrument identification, serial number, date of calibration, analyst, calibration solutions, and the samples associated with these calibrations.

4.3 Inspection/Acceptance of Supplies and Consumables

For this project, critical supplies for field activities will be tracked through ENSR's system in the following manner.

Critical Supplies and Consumables	Inspection Requirements and Acceptance Criteria	Responsible Individual
Sample containers	Visually inspected upon receipt for cracks, breakage, cleanliness. Must be accompanied by certificate of analysis.	ENSR FTL
Chemicals and reagents	Visually inspected for proper labeling, expiration dates, appropriate grade	ENSR FTL
Field measurement equipment	Functional checks to ensure proper calibration and operating capacity	ENSR FTL
Sampling equipment	Visually inspected for obvious defects, damage, and contamination	ENSR FTL

Supplies and consumables not meeting acceptance criteria will initiate the appropriate corrective action. Corrective measures may include repair or replacement of measurement equipment, and/or notification of vendor and subsequent replacement of defective or inappropriate materials. All actions will be documented in the project files.

The laboratory system of inspection and acceptance of supplies and consumable is documented in the laboratory QA Manuals.

4.4 Corrective Actions

Corrective action is the process of identifying, recommending, approving, and implementing measures to counter unacceptable procedures or out-of-limit QC performance that can affect data quality. Corrective action can occur during field activities, laboratory analyses, data validation, and data assessment. All corrective action proposed and implemented should be documented in the QA reports to management. Corrective action should only be implemented after approval by the ENSR Project Manager

4.4.1 Field Corrective Action

Corrective action in the field may be needed when the sample network is changed (i.e., more/less samples, sampling locations other than those specified in the Field Demonstration Plan, etc.), or when sampling procedures and/or field analytical procedures require modification, etc. due to unexpected conditions. The field team may identify the need for corrective action. The ENSR FTL will approve the corrective action and notify the ENSR Project Manager. The ENSR Project Manager will approve the corrective measure. The ENSR FTL will ensure that the corrective measure is implemented by the field team.

Corrective actions will be implemented and documented in the field record book. Documentation will include:

- A description of the circumstances that initiated the corrective action,
- The action taken in response,
- The final resolution, and
- Any necessary approvals.

No staff member will initiate corrective action without prior communication of findings through the proper channels.

4.4.2 Laboratory Corrective Action

Corrective action in the laboratory may occur prior to, during, and after initial analyses. A number of conditions such as broken sample containers, multiple phases, low/high pH readings, and potentially high concentration samples may be identified during sample log-in or analysis. Following consultation with laboratory analysts and supervisory personnel, it may be necessary for the Laboratory QA Coordinator to approve the implementation of corrective action. If the nonconformance causes project objectives not to be achieved, the ENSR QA Officer will be notified.

These corrective actions are performed prior to release of the data from the laboratory. The corrective action will be documented in both the laboratory's corrective action files, and in the narrative data report sent from the laboratory to the ENSR QA Officer. If the corrective action does not rectify the situation, the laboratory will contact the ENSR QA Officer, who will determine the action to be taken and inform the appropriate personnel.

4.4.3 Corrective Action During Data Validation and Data Assessment

The need for corrective action may be identified during either data validation or data assessment. Potential types of corrective action may include resampling by the field team or reinjection/reanalysis of samples by the laboratory. These actions are dependent upon the ability to mobilize the field team and whether the data to be collected is necessary to meet the required QA objectives. If the QA Officer identifies a corrective action situation, the ENSR Project Manager will be responsible for informing the appropriate personnel.

5.0 Demonstration Procedures

This section presents the planned activities and specific QA/QC procedures associated with the field demonstration phase of this project. The field demonstrations will be conducted at two locations: Travis Air Force Base in California and Aberdeen Proving Ground in Maryland. At both locations, the previously developed laboratory toxicity test protocols (i.e., the soil and sediment exposure protocols presented in Appendix A) will be applied to evaluate the potential impact of on-site contamination on existing or future amphibian populations. In addition to toxicity testing, these field evaluations may also include conducting tissue and histological evaluations of laboratory exposed and field-collected amphibians, performing field surveys to evaluate habitat and amphibian populations, and comparing media concentrations against screening values developed during the laboratory validation testing.

The method and number of sediment and soil samples collected will be dependent upon site conditions, although it is anticipated that up to 3 soil samples and 6 to 8 sediment samples will be collected from each site. An attempt will be made to identify and sample from copper and/or lead concentration gradients. Sediment and soil samples will be shipped on ice directly to the chemical and toxicity testing laboratories.

The sediment and soil toxicity testing will be conducted at the ENSR FCETL and the USACHPPM toxicity laboratories, respectively. The sediment and soil exposure protocols were developed and validated at these laboratories and both facilities have been involved in conducting similar types of tests for many years. Measurements at the end of the toxicity tests will include lethal (i.e., mortality) and sub-lethal (e.g., body length, body width, weight, histological measurements). Collection of additional chemical samples at test termination (water, sediment, tissue) may also be conducted. Chemical and physical analyses will be conducted by Paragon Laboratory, Huffman Laboratories, Inc., Mitkem Corporation, and GeoTesting Express.

5.1 QC Sample Collection

QC samples collected during the field sampling effort will include equipment rinsate blanks, field duplicates, DI water blank, and MS/MSDs as appropriate for the parameter and media sampled.

Equipment rinsate blanks – Equipment rinsate blanks will be collected at a frequency of one per 20 samples on non-dedicated equipment in association with the soil and sediment sampling. Soil and sediment equipment rinsate blanks will include the grab samplers and the sample homogenization equipment. Equipment rinsate blanks associated with sediment and soil samples will be analyzed for metals only.

Field duplicates – Field duplicates will be collected at a frequency of one per 20 samples by alternately filling two sets of identical sample containers from the same homogenate or surface water sample.

DI water blank – One DI water blank will be collected for the field sampling program. This sample will consist of the DI water used for decontamination and equipment rinsate blank collection. The DI water blank will be analyzed for metals only.

MS/MSDs – MS/MSD samples will be collected at a frequency of one for every 20 or less investigative samples. For those samples designated as MS/MSDs, sufficient additional volume (based on the individual laboratory's requirements) will be collected.

5.2 Equipment Decontamination

During handling of soil and sediment samples, it is critical that cross contamination between samples, or contamination from extraneous sources, does not occur. All sample handling tools will be constructed of inert materials wherever possible (stainless steel, polypropylene, or Teflon, as appropriate) and will be decontaminated between sampling locations.

Decontamination will generally consist of a tap water rinse to remove gross contamination (if needed), followed by a non-phosphate detergent (e.g., Alconox) water rinse, a rinse with deionized water, and followed by another deionized water rinse. If equipment is to be stored or transported, it should be wrapped in aluminum foil after air-drying. Water generated during decontamination of sampling equipment will be containerized and disposed of according to DoD facility instructions.

5.3 Sample Handling and Custody

The sampling handling and custody procedures will apply to both the sediments and soils collected during the field effort, and any analytical samples collected during, or at termination of, the toxicity tests.

5.3.1 Sample Containers, Preservation, and Holding Times

Sample bottles and chemical preservatives will be provided by the analytical laboratories. The containers will be cleaned by the manufacturer to meet or exceed all analyte specifications established in the latest U.S. EPA's Specifications and Guidance for Contaminant-Free Sample Containers. Certificates of analysis will be maintained on file to document conformance to EPA specifications. Sample container, preservation, and holding time requirements are presented in Table 1.

5.3.2 Sample Numbering System

Each sample will be assigned a unique six-digit sample identifier. This identifier will be used throughout collection, analysis, and reporting activities. The sample identifier will be clearly shown on the chain-of-custody form and sample container labels and tags. The sample identifier will be cross-referenced to the field identification of the sample point in both field notebooks and the project database management system.

The first two characters of the six-character alphanumeric code will define the sample type:

SD Sediment

SS Surface Soil

AQ Aqueous

TS Tissue

The third character will define the location of the field demonstration

T Travis Air Force Base

A Aberdeen Proving Ground

The fourth and fifth characters will be a number that corresponds to a specific location on the site map.

The sixth and seventh digits will identify the analysis planned for the sample:

ST Soil toxicity testing (salamander assay)

DT Sediment toxicity testing (tadpole assay)

SC Sediment/Soil chemistry

AC Aqueous chemistry

TC Tissue chemistry

The eighth digit will be a number identifying the type of sample:

A Investigative sample

B Field duplicate

C Equipment rinsate blank

The DI water blank will be identified as “DI” followed by the date. Matrix spike/matrix spike duplicate samples will be indicated by appending an "MS/MSD" to the eight-digit sample identification number.

5.3.3 Sample Labels

Immediately upon collection, each sample will be labeled with a pre-printed adhesive label, which includes test to be performed, preservation conditions and a unique identifier. Field personnel will mark the date and time of collection and sampler’s initials once the label is affixed to the sampling container with a permanent marker.

5.3.4 Chain-of-Custody Records

Samples will be accompanied by a properly completed chain-of-custody form. When transferring the possession of samples, the individuals relinquishing and receiving will sign, date, and note the time on the record. This record documents the transfer of custody of samples from the sampler, to another person, to a mobile laboratory, to the permanent laboratory, or to/from a secure storage location.

Minimum information recorded on the chain-of-custody record in addition to the signatures and dates of all custodians will include:

- Client/project name,
- Project location,
- Project number,

- Field logbook number,
- Chain-of-custody tape numbers,
- The person to whom results should be reported,
- Field sampling number/identification,
- Sampling date and time,
- Type of sample (grab or composite),
- Identification of sample collector and his/her affiliation,
- Sample container number, size, and material,
- Sample description (matrix),
- Sample preservative,
- The performance of field filtration, and
- Analyses to be performed.

The field sampler will be personally responsible for the care and custody of the samples until the samples are transferred or dispatched properly. As few people as possible should handle the samples.

Each sample container will have a pre-affixed label. This label will be completed in the field with a unique sample identifier, the site name, sample collection date and time, analysis requested, and preservative and will be signed by the sampler. The FTL will review field activities to determine whether proper custody procedures were followed during the field work and will decide if additional samples are required.

5.3.5 Sample Packaging and Shipping Requirements

Samples will be packaged properly for shipment and dispatched to the laboratories for analysis with a separate signed custody record enclosed in each sample cooler. Shipping containers will be locked or secured with strapping tape and sealed with custody seals. The preferred procedure is to attach a custody seal to the front right and back left of the cooler. The cooler will be taped closed with fiberglass tape covering the chain-of-custody seals.

Samples will be shipped daily from the field to the laboratory using an overnight courier or onsite pickup by the laboratory. All shipments will be accompanied by the chain-of-custody record identifying the contents. The back copy will be detached and kept as part of the field records. The original record and remaining copies will accompany the shipment.

5.4 Analytical Methods

Table 2 presents the analytical methods to be used. The methods specified must be adhered to for these evaluations. Sediment and soil chemistry results are to be reported on a dry weight basis. Table 3 presents the quantitation limits for each parameter.

5.5 Laboratory QA/QC Measures

Each laboratory has a QC program in place to ensure the reliability and validity of the analysis performed at the laboratory. All analytical procedures are documented in writing as SOPs and each SOP includes the minimum requirements for the procedure. The QC programs for the individual laboratories are described in their respective QA Manuals.

Laboratory QA/QC measures are performed by the analytical laboratories to ensure that all environmental efforts to produce the data are technically sound and legally defensible. The quality assurance measures include standard operating procedures, state certification, training records, internal audits, and preservation and container checks. Laboratory quality control is measured by analysis of the following types of samples:

- Method Blanks – used to define the level of laboratory background and reagent contamination;
- Lab Control Spikes (LCS) – used to determine method accuracy;
- Matrix Spikes – used to indicate appropriateness of the method for the matrix;
- Duplicate Samples – used to verify laboratory consistency and precision; and
- Calibrations – necessary for accurate quantification.

For the toxicity testing laboratories, laboratory controls will be conducted for both sediment and soil tests and reference toxicant tests will be completed and fall within the acceptable results range.

Table 1 Sample Container, Preservation, and Holding Time Requirements

	Sample Container [1]	Preservation	Holding Time[2]
Sediment/Soil Parameters			
Metals [3]	2 oz. glass jar	Cool 4°C	6 months
TOC	2 oz. glass jar	Cool 4°C	14 days
pH	2 oz. glass jar	None	As soon as possible, same day as sample collection
SEM/AVS	8 oz. amber glass jar	Cool 4°C	14 days
Grain size	Double zipper-lock bag	None	None
Cation exchange capacity	Double zipper-lock bag	None	None
Bulk density	Double zipper-lock bag	None	None
Surface Water Parameters			
Metals (dissolved)	125 mL plastic bottle	Filter immediately and preserve with HNO_3 to pH <2; cool 4°C	6 months
Metals (total recoverable) and hardness	125 mL plastic bottle	HNO_3 to pH <2; cool 4°C	6 months
DOC	250 mL amber glass jar	Filter immediately and preserve with H_2SO_4 or HCl to pH <2; cool 4°C	28 days
TOC	250 mL amber glass jar	H_2SO_4 or HCl to pH <2; cool 4°C	28 days
Tissue Parameters			
Metals	2 oz. glass jar (at least 1 gram of tissue needed for analysis)	Cool 4°C then freeze (-20°C) upon arrival at laboratory	6 months

1 - Additional volume will be collected for MS/MSD samples.
2 - Holding time begins from date of sample collection.
3 - Calcium included in metals analyses.

Table 2 Analytical Methodology

Parameter	Methodology
Sediment/Soil	
Metals [1]	SW-846 6010B
TOC	Lloyd Kahn
SEM/AVS	EPA 821/R-91-100 Draft
pH	SW-846 9045C
Grain size	ASTM D-422
Cation exchange capacity	SW-846 9080
Bulk density	ASTM D-2937
Surface Water	
Total/Dissolved Metals	SW-846 6010B
Hardness	Standard Methods 2340B by calculation
pH [2]	SW-846 9040B
TOC/DOC	EPA 415.1
Tissue	
Metals	SW-846 6010B

1 - Calcium included in metals analyses.

2 - pH to be measured by toxicity lab prior to test termination.

Table 3 Quantitation Limits

Parameter	Quantitation Limit
Sediment/Soil	
Copper	1 mg/kg
Lead	0.3 mg/kg
Calcium	100 mg/kg
TOC	0.05 %
AVS	0.499 umol/gram
Cadmium (SEM)	0.00111 umol/gram
Copper (SEM)	0.00983 umol/gram
Lead (SEM)	0.00072 umol/gram
Mercury (SEM)	0.00006 umol/gram
Nickel (SEM)	0.01704 umol/gram
Zinc (SEM)	0.03823 umol/gram
Surface Water	
Copper	0.01 mg/L
Lead	0.003 mg/L
Calcium	1 mg/L
TOC/DOC	1 mg/L
Tissue	
Copper	0.5 mg/kg
Lead	0.15 mg/kg

6.0 Calculation of Data Quality Indicators

The field and laboratory data collected during this investigation will be used to achieve the objectives identified in the Field Demonstration Plan. The QC results associated with each analytical parameter for each matrix will be compared to the measurement objectives presented in Section 3.0. Only data generated in association with QC results meeting the stated acceptance criteria (i.e., data determined to be valid) will be considered usable for decision making purposes.

6.1 Accuracy Assessment

One measure of accuracy will be percent recovery (%Rs), which is calculated for matrix spikes, and LCSs. Percent recoveries for matrix spike results will be determined according to the following equation:

$$\%R = \frac{(Amount\ in\ Spiked\ Sample - Amount\ in\ Sample)}{Known\ \text{Amount}\ \text{Added}} x\,100$$

Percent recoveries for LCS results will be determined according to the following equation:

$$\%R = \frac{Experimental\ Concentration}{Known\ Amount\ \text{Added}} x\,100$$

An additional measure of accuracy is blank contamination. The blanks associated with this project include laboratory method blanks, DI water blanks, and equipment rinsate blanks. The results of the laboratory and field blanks will be compared to the objectives in stated Section 3.0 of the QAPP. Failure to meet these objectives may indicate a systematic laboratory or field problem that should be investigated and resolved immediately. Associated data may have limitations placed on its use, depending on the magnitude of the problem.

6.2 Precision Assessment

The RPD between the matrix spike and matrix spike duplicate, or sample and sample duplicate in the case of some of the inorganic parameters, and field duplicate pair is calculated to compare to precision objectives (Section 3.0 of this QAPP). The RPD will be calculated according to the following formula.

$$RPD = \frac{(Amount\ in\ Sample\,1 - Amount\ in\ Sample\,2)}{0.5\,(Amount\ in\ Sample\,1 + Amount\ in\ Sample\,2)} x\,100$$

Failure to achieve precision objectives may result in the associated data having limitations placed upon its use.

6.3 Completeness Assessment

Completeness is the ratio of the number of valid sample results to the total number of samples analyzed with a specific matrix and/or analysis. Following completion of the analytical testing, the percent completeness will be calculated by the following equation:

$$Completeness = \frac{(number\ of\ valid\ measurements)}{(number\ of\ measurements\ planned)} x100$$

Failure to meet the completeness objective will require an assessment to determine if the missing or invalid data are critical to achieving the project objectives. Corrective actions may include resampling or re-analysis, depending on the type of problem, logistical constraints, etc.

6.4 Comparison to Project Objectives

In addition, the data obtained will be both qualitatively and quantitatively assessed on a project-wide, matrix-specific, and parameter-specific basis. Factors to be considered in this assessment of field and laboratory data will include, but not necessarily be limited to, the following.

- Conformance to the field methodologies and SOPs proposed in the QAPP;
- Conformance to the analytical methodologies provided in the QAPP;
- Adherence to proposed sampling strategy;
- Presence of elevated detection limits due to matrix interferences or contaminants present at high concentrations;
- Unusable data sets based on the data review results;
- Data sets identified as usable for limited purposes based on the data review results;
- Status of all issues requiring corrective action, as presented in the QA reports to management;
- Effect of nonconformance (procedures or requirements) on project objectives;
- Adequacy of the data as a whole in meeting the project objectives; and
- Identification of any remaining data gaps and need to reevaluate project decision rules.

This assessment will be performed by the ENSR technical team, in conjunction with the ENSR Project QA Officer, and the results presented and discussed in detail in the final report.

7.0 Performance and System Audit

The types of planned assessments pertinent to this program include technical surveillance audits (TSAs) of field and laboratory activities, and data package audits.

7.1 Field Activity TSA

A TSA of field activities will be conducted by the ENSR Project QA Officer or his/her designate at the request of the ENSR Project Manager. Ideally, this audit will be conducted at the beginning of the sampling program to ensure that the approved procedures documented in the Field Demonstration Plan and QAPP are being followed. The TSA will include examination of field sampling records, field measurement results, field instrument operating and calibration records, sample collection, handling, and packaging procedures, QA procedures, chain-of-custody, sample documentation, etc. If significant deficiencies are noted, follow-up audits will be conducted.

During the audit, the auditor will keep detailed notes of audit findings. Preliminary results of the audit will be reviewed with the ENSR FTL while on site to ensure that deficiencies adversely affecting data quality are immediately identified and corrective measures initiated. Upon completion of the audit, the ENSR Project QA Officer will prepare a written audit report, which summarizes the audit findings, identifies deficiencies and recommends corrective actions. This report will be submitted to the ENSR Project Manager, who will be responsible for ensuring that corrective measures are implemented and documented. The results of the audit process will be included in the QA reports to management, as described in Section 8.0.

7.2 Laboratory TSA

Laboratory TSAs are conducted periodically by ENSR as part of their analytical subcontractor monitoring program. A typical laboratory TSA includes a review of the following areas:

- QA organization and procedures,
- Personnel training and qualifications,
- Sample log-in procedures,
- Sample storage facilities,
- Analyst technique
- Adherence to laboratory SOPs and project QAPP,
- Compliance with QA/QC objectives,
- Instrument calibration and maintenance,
- Data recording, reduction, review, and reporting, and
- Cleanliness and housekeeping.

Preliminary results of the TSA are discussed with the Laboratory Manager, Laboratory Project Manager, and Laboratory QA Coordinator. A written report that summarizes audit findings and recommends corrective actions is prepared and submitted to the Laboratory Manager for response, and to the ENSR Project Manager.

7.3 Data Package Audits

Audits of analytical data packages will be conducted for 100% of the packages received as part of the data validation process. The review will include an evaluation of the package to ensure that (1) all required deliverables are provided, and (2) the package contains the information necessary to reproduce the reported results. Any deficiencies will be communicated to the laboratory and documented in the project files.

8.0 Quality Assurance Reports

QA reports will be submitted to the ENSR Project Manager to ensure that any problems identified during the sampling and analysis programs are investigated and the proper corrective measures taken in response. The QA reports will include:

- All results of field and laboratory audits; and
- Significant QA/QC problems, recommended corrective actions, and the outcome of corrective actions.

QA reports will be prepared by the ENSR Project QA Officer and submitted on an as-needed basis.

9.0 Data Format

All data generated during the conduct of the field demonstration shall be recorded directly, promptly, and legibly in ink. The exception will be for data generated by an automated data collection system, if applicable. In these cases, the individual responsible for direct data input should be identified at the time of data input. The data format for field records and laboratory deliverables are described in the following sub-sections.

9.1 Field Records

Documentation of field activities will be performed as described in ENSR SOP 7515, Recording of Field Data. Field logbooks will provide the means of recording the data collecting activities performed during the investigation. As such, entries will be described in as much detail as possible so that persons going to the facility could reconstruct a particular situation without reliance on memory.

Field logbooks will be bound field survey books or notebooks. Logbooks will be assigned to field personnel, but will be stored in the project files when not in use. Each logbook will be identified by the project-specific document number.

The title page of each logbook will contain the following:

- Person to whom the logbook is assigned,
- The logbook number,
- Project name and number,
- Project start date, and
- End date.

Entries into the logbook will contain a variety of information. At the beginning of each entry, the date, start time, weather, names of all sampling team members present, and the signature of the person making the entry will be entered. The names of any visitors, and the purpose of their visit, will also be recorded in the field logbook.

Field logbooks will be supplemented by standardized forms. All measurements made and samples collected will be recorded. All entries will be made in permanent ink, signed, and dated and no erasures or obliterations will be made. If an incorrect entry is made, the information will be crossed out with a single strike mark which is signed and dated by the sampler. Whenever a sample is collected, or a measurement is made, a detailed description of the sampling location, which includes compass and distance measurements, or, latitude and longitude information (e.g., obtained by using a global positioning system) will be recorded. The number of photographs taken of the sampling location, if any, will be noted. All equipment used to make measurements will be identified, along with the date of calibration.

9.2 Laboratory Records and Deliverables

Laboratory data reduction procedures will be performed according to the following protocol. All information related to analysis will be documented in controlled laboratory logbooks, instrument printouts, or other approved forms. All entries that are not generated by an automated data system will be made neatly and legibly in permanent, waterproof ink. Information will not be erased or obliterated. Corrections will be made by drawing a single line through the error and entering the correct information adjacent to the cross-out. All changes will be initialed, dated, and, if appropriate, accompanied by a brief explanation. Unused pages or portions of pages will be crossed out to prevent future data entry. Analytical laboratory records will be reviewed by the supervisory personnel on a regular basis, and by the Laboratory QA Coordinator periodically, to verify adherence to documentation requirements.

Chemical sediment and soil data will be reported on a dry weight basis. Tissue data, if applicable, will be reported on both a dry weight and wet weight basis. The detection limits associated with all nondetected results will be corrected for sample-specific factors such as analytical dilutions, percent moisture, sample volume, etc.

Metals data will be reported down to the method detection limits (MDLs). Data between the MDL and the reporting limit will be flagged as estimated by the laboratory. Nondetected results (i.e., values below the MDL) will be reported as nondetect at the reporting limit.

Data deliverables will be provided within standard turnaround time. The laboratory will provide at least one copy of hard copy report and one copy of an electronic diskette deliverable (EDD). The hard copy data package will include the information summarized below:

- Case narrative (see description below)
- Cross reference of field sample IDs and laboratory IDs
- Method summary
- Chain-of-custody documentation
- Dates of sample extraction and analysis
- Description of any data qualifiers used
- Sample results, including units
- Sample preparation information
- Results for MS/MSDs, method or preparation/calibration blanks, LCSs, and laboratory duplicates

The case narrative will include the client name, project name and number, date of issuance, and a discussion of any deviations from analytical strategy, technical problems, and QC failures or nonconformances. The report will be signed by the Laboratory Project Manager.

Physical testing results will be provided as a hard copy report. The report will include a summary of results, method citations, and QC summary.

Reports of the results of the bioassays will contain a signature and approval page, executive summary, summary of data collected, references, statistical analyses, and copies of laboratory bench sheets. A hard copy and PDF version of the report will be provided.

10.0 Data Storage and Archiving Procedures

The project files will be the central repository for all documents which constitute evidence relevant to sampling and analysis activities as described in this QAPP. ENSR is the custodian of the project files and will maintain the contents of the project files for the investigation, including all relevant records, reports, logs, field notebooks, pictures, subcontractor reports, and data reviews in a secured, limited access area and under custody of the ENSR Project Manager.

The project files will include at a minimum:

- Field logbooks,
- Field data and data deliverables,
- Photographs,
- Drawings,
- Sample collection logs,
- Data validation reports,
- Data assessment reports,
- Progress reports, QA reports, interim project reports, final reports, etc.,

All custody documentation (tags, forms, airbills, bottle certifications, etc.)

Appendix C

Laboratory Data

Analytical Results for Field Demonstration Tests

USACHPPM Histopathology Report

FCETL Sediment Toxicity Testing Reports

Analytical Results for Field Demonstration Tests

			Task Code	TravisAFB	TravisAFB	TravisAFB	Aberdeen
			Sample Date	3/28/2006	3/28/2006	3/27/2006	4/12/2006
			Sample ID	PCB Pest EQB	TALEQB	AQT01ACEB	AQAb-EQB
			Sample Matrix	WQ	WQ	WS	WQ
			Sample Type	EB	EB	EB	EB
Analytical Method	CHEMICAL NAME	Total/Dissolved	Unit				
EPA415.1	DISSOLVED ORGANIC CARBON	D	MG/L			1 U	1 U
EPA415.1	TOTAL ORGANIC CARBON	T	MG/L			1 U	1 U
SW6010	ALUMINUM (FUME OR DUST)	D	MG/L				0.2 U
SW6010	ANTIMONY	D	MG/L				0.02 U
SW6010	ARSENIC	D	MG/L				0.01 U
SW6010	BARIUM	D	MG/L				0.1 U
SW6010	BERYLLIUM	D	MG/L				0.005 U
SW6010	CADMIUM	D	MG/L				0.005 U
SW6010	CALCIUM METAL	D	MG/L			1 U	1 U
SW6010	CHROMIUM (TOTAL)	D	MG/L				0.01 U
SW6010	COBALT	D	MG/L				0.01 U
SW6010	COPPER	D	MG/L			0.01 U	0.01 U
SW6010	HARDNESS (AS CACO3)	D	MG/L			1 U	2.5 U
SW6010	IRON	D	MG/L				0.1 U
SW6010	LEAD	D	MG/L			0.003 U	0.003 U
SW6010	MAGNESIUM	D	MG/L			1 U	1 U
SW6010	MANGANESE	D	MG/L				0.01 U
SW7470	MERCURY	D	MG/L				0.0002 U
SW6010	NICKEL	D	MG/L				0.02 U
SW6010	POTASSIUM	D	MG/L				1 U
SW6010	SELENIUM	D	MG/L				0.005 U
SW6010	SILVER	D	MG/L				0.01 U
SW6010	SODIUM	D	MG/L				1 U
SW6010	THALLIUM	D	MG/L				0.01 U
SW6010	VANADIUM	D	MG/L				0.01 U
SW6010	ZINC	D	MG/L				0.02 U
SW6010	ALUMINUM	T	MG/L		0.2 U	0.2 U	0.2 U
SW6010	ANTIMONY	T	MG/L		0.02 U	0.02 U	0.02 U
SW6010	ARSENIC	T	MG/L		0.01 U	0.01 U	0.01 U
SW6010	BARIUM	T	MG/L		0.1 U	0.1 U	0.1 U
SW6010	BERYLLIUM	T	MG/L		0.005 U	0.005 U	0.005 U
SW6010	CADMIUM	T	MG/L		0.005 U	0.005 U	0.005 U
SW6010	CALCIUM METAL	T	MG/L		1 U	1 U	1 U
SW6010	CHROMIUM (TOTAL)	T	MG/L		0.01 U	0.01 U	0.01 U
SW6010	COBALT	T	MG/L		0.01 U	0.01 U	0.01 U
SW6010	COPPER	T	MG/L		0.01 U	0.01 U	0.01 U
SW6010	HARDNESS (AS CACO3)	T	MG/L			1 U	
SW6010	IRON	T	MG/L		0.1 U	0.1 U	0.1 U
SW6010	LEAD	T	MG/L		0.003 U	0.003 U	0.003 U
SW6010	MAGNESIUM	T	MG/L		1 U	1 U	1 U
SW6010	MANGANESE	T	MG/L		0.01 U	0.01 U	0.01 U
SW7470	MERCURY	T	MG/L		0.0002 U	0.0002 U	0.0002 U
SW6010	NICKEL	T	MG/L		0.02 U	0.02 U	0.02 U
SW6010	POTASSIUM	T	MG/L		1 U	1 U	1 U
SW6010	SELENIUM	T	MG/L		0.005 U	0.005 U	0.005 U
SW6010	SILVER	T	MG/L		0.01 U	0.01 U	0.01 U
SW6010	SODIUM	T	MG/L		1 U	1 U	1 U
SW6010	THALLIUM	T	MG/L		0.01 U	0.01 U	0.01 U
SW6010	VANADIUM	T	MG/L		0.01 U	0.01 U	0.01 U
SW6010	ZINC	T	MG/L		0.02 U	0.02 U	0.04

			Task Code	TravisAFB	TravisAFB	TravisAFB	Aberdeen
			Sample Date	3/28/2006	3/28/2006	3/27/2006	4/12/2006
			Sample ID	PCB Pest EQB	TALEQB	AQT01ACEB	AQAb-EQB
			Sample Matrix	WQ	WQ	WS	WQ
			Sample Type	EB	EB	EB	EB
Analytical Method	CHEMICAL NAME	Total/Dissolved	Unit				
SW8081	4,4-DDD	T	UG/L	0.047 U			0.048 U
SW8081	4,4-DDE	T	UG/L	0.047 U			0.048 U
SW8081	4,4-DDT	T	UG/L	0.047 U			0.048 U
SW8081	ALDRIN	T	UG/L	0.047 U			0.048 U
SW8081	ALPHA-BHC	T	UG/L	0.047 U			0.048 U
SW8081	ALPHA-CHLORDANE	T	UG/L	0.047 U			0.048 U
SW8081	BETA-BHC	T	UG/L	0.047 U			0.048 U
SW8081	DELTA-BHC	T	UG/L	0.047 U			0.048 U
SW8081	DIELDRIN	T	UG/L	0.047 U			0.048 U
SW8081	ENDOSULFAN I	T	UG/L	0.047 U			0.048 U
SW8081	ENDOSULFAN II	T	UG/L	0.047 U			0.048 U
SW8081	ENDOSULFAN SULFATE	T	UG/L	0.047 U			0.048 U
SW8081	ENDRIN	T	UG/L	0.047 U			0.048 U
SW8081	ENDRIN ALDEHYDE	T	UG/L	0.047 U			0.048 U
SW8081	ENDRIN KETONE	T	UG/L	0.047 U			0.048 U
SW8081	GAMMA-BHC (LINDANE)	T	UG/L	0.047 U			0.048 U
SW8081	GAMMA-CHLORDANE	T	UG/L	0.047 U			0.048 U
SW8081	HEPTACHLOR	T	UG/L	0.047 U			0.048 U
SW8081	HEPTACHLOR EPOXIDE	T	UG/L	0.047 U			0.048 U
SW8081	METHOXYCHLOR	T	UG/L	0.23 U			0.24 U
SW8081	TOXAPHENE	T	UG/L	2.3 U			2.4 U
SW8082	AROCLOR 1016	T	UG/L	0.94 U			0.96 U
SW8082	AROCLOR 1221	T	UG/L	1.9 U			1.9 U
SW8082	AROCLOR 1232	T	UG/L	0.94 U			0.96 U
SW8082	AROCLOR 1242	T	UG/L	0.94 U			0.96 U
SW8082	AROCLOR 1248	T	UG/L	0.94 U			0.96 U
SW8082	AROCLOR 1254	T	UG/L	0.94 U			0.96 U
SW8082	AROCLOR 1260	T	UG/L	0.94 U			0.96 U

		Task Code	Travis	Travis	Travis - dup	Travis	Aberdeen	Aberdeen	Aberdeen	Aberdeen - dup
		Sample Date	3/27/2006	3/27/2006	3/27/2006	3/27/2006	4/12/2006	4/12/2006	4/12/2006	4/12/2006
		Sample ID	AQTBKACA	AQT01ACA	AQT01ACB [1]	AQT02ACA	AQAb-Abk	AQAb-A01	AQAb-A02	AQ-Ab-B02 [2]
		Sample Matrix	WS	WS	WS	WS	WS	WS	WS	WS
Analytical Method	CHEMICAL NAME	Total/Dissolved								
EPA415.1	DISSOLVED ORGANIC CARBON	D	15	11		12	12	13	12	12
EPA415.1	TOTAL ORGANIC CARBON	T	22	12	13	13	14	16	16	15
SW6010	ALUMINUM	D					0.2 U	0.2 U	0.2 U	0.2 U
SW6010	ANTIMONY	D					0.02 U	0.02 U	0.02 U	0.02 U
SW6010	ARSENIC	D					0.01 U	0.01 U	0.01 U	0.01 U
SW6010	BARIUM	D					0.1 U	0.1 U	0.1 U	0.1 U
SW6010	BERYLLIUM	D					0.005 U	0.005 U	0.005 U	0.005 U
SW6010	CADMIUM	D					0.005 U	0.005 U	0.005 U	0.005 U
SW6010	CALCIUM METAL	D	4.5	14		15	2.6	3.7	4.1	4.1
SW6010	CHROMIUM (TOTAL)	D					0.01 U	0.01 U	0.01 U	0.01 U
SW6010	COBALT	D					0.01 U	0.01 U	0.01 U	0.01 U
SW6010	COPPER	D	0.01 U	0.01 U		0.01 U	0.01 U	0.04	0.038	0.038
SW6010	HARDNESS (AS CACO3)	D	17	47		50	13	15	14	14
SW6010	IRON	D					0.92	0.41	0.23	0.23
SW6010	LEAD	D	0.003 U	0.012		0.0078	0.003 U	0.027	0.039	0.04
SW6010	MAGNESIUM	D	1.3	3.1		3.3	1.6	1.4	1	1
SW6010	MANGANESE	D					0.043	0.05	0.011	0.012
SW7470	MERCURY	D					0.0002 U	0.0002 U	0.0002 U	0.0002 U
SW6010	NICKEL	D					0.02 U	0.02 U	0.02 U	0.02 U
SW6010	POTASSIUM	D					1 U	1 U	1.1	1.1
SW6010	SELENIUM	D					0.005 U	0.005 U	0.005 U	0.005 U
SW6010	SILVER	D					0.01 U	0.01 U	0.01 U	0.01 U
SW6010	SODIUM	D					2.5	1 U	1 U	1 U
SW6010	THALLIUM	D					0.01 U	0.01 U	0.01 U	0.01 U
SW6010	VANADIUM	D					0.01 U	0.01 U	0.01 U	0.01 U
SW6010	ZINC	D					0.025	0.06	0.071	0.062
SW6010	ALUMINUM (FUME OR DUST)	T	0.5	0.2 U	0.2 U	0.2 U	0.2 U	0.2 U	0.2 U	0.2 U
SW6010	ANTIMONY	T	0.02 U	0.02 U	0.02 U	0.02 U	0.02 U	0.02 U	0.02 U	0.02 U
SW6010	ARSENIC	T	0.01 U	0.01 U	0.01 U	0.01 U	0.01 U	0.01 U	0.01 U	0.01 U
SW6010	BARIUM	T	0.38	0.15	0.14	0.15	0.1 U	0.1 U	0.1 U	0.1 U
SW6010	BERYLLIUM	T	0.005 U	0.005 U	0.005 U	0.005 U	0.005 U	0.005 U	0.005 U	0.005 U
SW6010	CADMIUM	T	0.005 U	0.005 U	0.005 U	0.005 U	0.005 U	0.005 U	0.005 U	0.005 U
SW6010	CALCIUM METAL	T	6.1	14	13	14	2.7	3.7	4.1	4.4
SW6010	CHROMIUM (TOTAL)	T	0.01 U	0.01 U	0.01 U	0.01 U	0.01 U	0.01 U	0.01 U	0.01 U
SW6010	COBALT	T	0.01 U	0.01 U	0.01 U	0.01 U	0.01 U	0.01 U	0.01 U	0.01 U
SW6010	COPPER	T	0.01 U	0.01 U	0.01 U	0.01 U	0.01 U	0.045	0.049	0.062
SW6010	HARDNESS (AS CACO3)	T	22	47	46	50	13	15	15	16
SW6010	IRON	T	5.1	1.7	1.5	1.8	1.6	0.87	0.74	0.86
SW6010	LEAD	T	0.003 U	0.019	0.015	0.016	0.004	0.063	0.11	0.17
SW6010	MAGNESIUM	T	1.5	3.1	3.1	3.3	1.6	1.4	1.1	1.1
SW6010	MANGANESE	T	1.1	0.27	0.16	0.26	0.08	0.11	0.037	0.064
SW7470	MERCURY	T	0.0002 U	0.0002 U	0.0002 U	0.0002 U	0.0002 U	0.0002 U	0.0002 U	0.0002 U
SW6010	NICKEL	T	0.02 U	0.02 U	0.02 U	0.02 U	0.02 U	0.02 U	0.02 U	0.02 U
SW6010	POTASSIUM	T	1 U	1.3	1.3	1.3	1 U	1 U	1.1	1.3
SW6010	SELENIUM	T	0.005 U	0.005 U	0.005 U	0.005 U	0.005 U	0.005 U	0.005 U	0.005 U
SW6010	SILVER	T	0.01 U	0.01 U	0.01 U	0.01 U	0.01 U	0.01 U	0.01 U	0.01 U
SW6010	SODIUM	T	3.3	3.7	3.6	3.7	2.4	1 U	1 U	1 U
SW6010	THALLIUM	T	0.01 U	0.01 U	0.01 U	0.01 U	0.01 U	0.01 U	0.01 U	0.01 U
SW6010	VANADIUM	T	0.01 U	0.01 U	0.01 U	0.01 U	0.01 U	0.01 U	0.01 U	0.01 U
SW6010	ZINC	T	0.02 U	0.02 U	0.02 U	0.02 U	0.025	0.07	0.07	0.075

Notes:
1 - Duplicate of AQT01ACA
2 - Duplicate of AQAb-A02
D - Dissolved
T - Total
All units are mg/L

		Task Code	Travis	Travis	Travis-dup	Travis	Travis	Travis	Travis	Travis	Travis	Travis
		Sample Date	3/28/2006	3/28/2006	3/28/2006	3/28/2006	3/28/2006	3/28/2006	3/28/2006	3/28/2006	3/28/2006	3/28/2006
		Sample ID	SDTBKSCA	SST04SCA	SST04SCB [1]	SDT04SCA	SST09SCA	SST07SCA	SDT01SCA	SDT02SCA	SDT14SCA	SST13SCA
		Sample Matrix	SE	SS	SS	SE	SS	SS	SE	SE	SE	SS
Analytical Method	Chemical Name	Unit										
D2216	SOLIDS, PERCENT	%	71.7	77.5	76.4	71.3	78.4	68.9	63.8	73.5	65.2	67.6
E415.1	TOTAL ORGANIC CARBON	mg/kg	15000 EH	17000 EH	18000 EH	18000 EH	16000 EH	16000 EH	20000 EH	16000 EH	19000 EH	23000 EH
SW6010	ALUMINUM	MG/KG	5900							7500		6600
SW6010	ANTIMONY	MG/KG	3.2 U							19		12
SW6010	ARSENIC	MG/KG	7.8							21		25
SW6010	BARIUM	MG/KG	340							310		350
SW6010	BERYLLIUM	MG/KG	0.81 U							0.74 U		0.81 U
SW6010	CADMIUM	MG/KG	0.81 U							0.74 U		1.6 U
SW6010	CALCIUM METAL	MG/KG	1700							2100		1900
SW6010	CHROMIUM (TOTAL)	MG/KG	15							16		20
SW6010	COBALT	MG/KG	7.3							7.9		8.4
SW6010	COPPER	MG/KG	15	15	16	21	16	17	20	20	17	19
SW6010	IRON	MG/KG	15000							18000		19000
SW6010	LEAD	MG/KG	14	770	1100	1500	1600	2000	2100	2100	2500	4200
SW6010	MAGNESIUM	MG/KG	950							1400		1100
SW6010	MANGANESE	MG/KG	550							440		600
SW6010	NICKEL	MG/KG	14							17		17
SW6010	POTASSIUM	MG/KG	450							670		710
SW6010	SELENIUM	MG/KG	0.81 U							0.74 U		1.6 U
SW6010	SILVER	MG/KG	1.6 U							1.5 U		1.6 U
SW6010	SODIUM	MG/KG	160 U							150 U		160 U
SW6010	THALLIUM	MG/KG	1.6 U							1.5 U		3.2 U
SW6010	VANADIUM	MG/KG	37							37		42
SW6010	ZINC	MG/KG	26							42		38
SW7471	MERCURY	MG/KG	0.054 U							0.048 U		0.055 U
SW8081	4,4-DDD	UG/KG	14 U							12 U		14 U
SW8081	4,4-DDE	UG/KG	14 U							12 U		14 U
SW8081	4,4-DDT	UG/KG	14 U							12 U		14 U
SW8081	ALDRIN	UG/KG	14 U							12 U		14 U
SW8081	ALPHA-BHC	UG/KG	14 U							12 U		14 U
SW8081	ALPHA-CHLORDANE	UG/KG	14 U							12 U		14 U
SW8081	BETA-BHC	UG/KG	14 U							12 U		14 U
SW8081	DELTA-BHC	UG/KG	14 U							12 U		14 U
SW8081	DIELDRIN	UG/KG	14 U							12 U		14 U
SW8081	ENDOSULFAN I	UG/KG	14 U							12 U		14 U
SW8081	ENDOSULFAN II	UG/KG	14 U							12 U		14 U
SW8081	ENDOSULFAN SULFATE	UG/KG	14 U							12 U		14 U
SW8081	ENDRIN	UG/KG	14 U							12 U		14 U
SW8081	ENDRIN ALDEHYDE	UG/KG	14 U							12 U		14 U
SW8081	ENDRIN KETONE	UG/KG	14 U							12 U		14 U
SW8081	GAMMA-BHC (LINDANE)	UG/KG	14 U							12 U		14 U
SW8081	GAMMA-CHLORDANE	UG/KG	14 U							12 U		14 U
SW8081	HEPTACHLOR	UG/KG	14 U							12 U		14 U
SW8081	HEPTACHLOR EPOXIDE	UG/KG	14 U							12 U		14 U
SW8081	METHOXYCHLOR	UG/KG	69 U							62 U		70 U
SW8081	TOXAPHENE	UG/KG	690 U							620 U		700 U

		Task Code	Travis	Travis	Travis-dup	Travis	Travis	Travis	Travis	Travis	Travis	Travis
		Sample Date	3/28/2006	3/28/2006	3/28/2006	3/28/2006	3/28/2006	3/28/2006	3/28/2006	3/28/2006	3/28/2006	3/28/2006
		Sample ID	SDTBKSCA	SST04SCA	SST04SCB [1]	SDT04SCA	SST09SCA	SST07SCA	SDT01SCA	SDT02SCA	SDT14SCA	SST13SCA
		Sample Matrix	SE	SS	SS	SE	SS	SS	SE	SE	SE	SS
Analytical Method	Chemical Name	Unit										
SW8082	AROCLOR 1016	UG/KG	55 U							50 U		56 U
SW8082	AROCLOR 1221	UG/KG	110 U							100 U		110 U
SW8082	AROCLOR 1232	UG/KG	55 U							50 U		56 U
SW8082	AROCLOR 1242	UG/KG	55 U							50 U		56 U
SW8082	AROCLOR 1248	UG/KG	55 U							50 U		56 U
SW8082	AROCLOR 1254	UG/KG	55 U							50 U		56 U
SW8082	AROCLOR 1260	UG/KG	55 U							50 U		56 U
SW8270	2-METHYLNAPHTHALENE	UG/KG	1100 U									
SW8270	ACENAPHTHENE	UG/KG	1100 U									
SW8270	ACENAPHTHYLENE	UG/KG	1100 U									
SW8270	ANTHRACENE	UG/KG	1100 U									
SW8270	BENZO(A)ANTHRACENE	UG/KG	1100 U									
SW8270	BENZO(A)PYRENE	UG/KG	1100 U									
SW8270	BENZO(B)FLUORANTHENE	UG/KG	1100 U									
SW8270	BENZO(G,H,I)PERYLENE	UG/KG	1100 U									
SW8270	BENZO(K)FLUORANTHENE	UG/KG	1100 U									
SW8270	CHRYSENE	UG/KG	1100 U									
SW8270	DIBENZ(A,H)ANTHRACENE	UG/KG	1100 U									
SW8270	FLUORANTHENE	UG/KG	1100 U									
SW8270	FLUORENE	UG/KG	1100 U									
SW8270	INDENO(1,2,3-CD)PYRENE	UG/KG	1100 U									
SW8270	NAPHTHALENE	UG/KG	1100 U									
SW8270	PHENANTHRENE	UG/KG	1100 U									
SW8270	PYRENE	UG/KG	1100 U									
SW6010 (AVS-SEM)	CADMIUM	mg/kg	0.28 U	0.25 U	0.3 B	0.27 U	0.24 U	0.28 U	0.29 U	0.25 U	0.29 U	0.28 U
SW6010 (AVS-SEM)	COPPER	mg/kg	6.1 B	9.2	6.7 B	8.4 B	7.1 B	6.9 B	11	12.7	8.3 B	7.4 B
SW6010 (AVS-SEM)	LEAD	mg/kg	11.2	1080	1700	3300	1650	1560	2110	3470	4090	3770
SW6010 (AVS-SEM)	NICKEL	mg/kg	5.2 B	6 B	5.3 B	5.9 B	6.9 B	5.3 B	6.1 B	7.8 B	6.2 B	5.7 B
SW6010 (AVS-SEM)	ZINC	mg/kg	10.1	12.8	11.2	30.7	11	28.2	25.8	19.8	14.8	13.1
SW7470 (AVS-SEM)	MERCURY	mg/kg	0.015 B	0.011 B	0.0097 B	0.0054 B	0.017 B	0.017 B	0.0037 U	0.009 B	0.0078 B	0.0054 B
AVS	SULFIDE	mg/kg	44.1	35.2	54.4	21.4 U	58.2	22.7	58.4	20.3 U	46.7	61.2

Notes:
1 - Duplicate of SST04SCA
2 - Duplicate of SlAb-A05
3 - Duplicate of SedAb-02B
4 - Duplicate of SedAb-A3A

		Task Code	Aberdeen	Aberdeen	Aberdeen	Aberdeen	Aberdeen-dup	Aberdeen	Aberdeen	Aberdeen-dup	Aberdeen	Aberdeen	Aberdeen
		Sample Date	4/12/2006	4/12/2006	4/12/2006	4/12/2006	4/12/2006	4/12/2006	4/12/2006	4/12/2006	4/12/2006	4/12/2006	4/12/2006
		Sample ID	SedAb-ABk11	SlAb-A06	SedAb-A08	SlAb-A05	SLAB-B05 [2]	SedAb-A10	SedAb-A07	SedAb-02B	SedAb02B [3]	SedAb-A04	SedAb-A09
		Sample Matrix	SE	SS	SE	SS	SO	SE	SE	SE	SE	SE	SE
Analytical Method	Chemical Name	Unit											
D2216	SOLIDS, PERCENT	%	78.7	78.3	93	88.2	81.3	69.1	71	79.7		84.3	90.4
E415.1	TOTAL ORGANIC CARBON	mg/kg	4600 EH	13000 EH	3600 H	4100 EH	4000 EH	6200 EH	19000 EH	5500 EH		5700 EH	1100 H
SW6010	ALUMINUM	MG/KG		9900						6200	5600		
SW6010	ANTIMONY	MG/KG		2.4 U						5	24		
SW6010	ARSENIC	MG/KG		5.2						3.6	3.4		
SW6010	BARIUM	MG/KG		44						36	32		
SW6010	BERYLLIUM	MG/KG		0.61 U						0.6 U	0.67 U		
SW6010	CADMIUM	MG/KG		0.61 U						0.6 U	0.67 U		
SW6010	CALCIUM METAL	MG/KG		620						350	390		
SW6010	CHROMIUM (TOTAL)	MG/KG		14						9.2	8.7		
SW6010	COBALT	MG/KG		6.5						3.2	2.9		
SW6010	COPPER	MG/KG	8.4	17	21	55		27	34	110	130	120	73
SW6010	IRON	MG/KG		20000						12000	11000		
SW6010	LEAD	MG/KG	34	35	200	260		310	460	650	1900	850	870
SW6010	MAGNESIUM	MG/KG		1700						810	670		
SW6010	MANGANESE	MG/KG		250						51	49		
SW6010	NICKEL	MG/KG		11						7.1	5.7		
SW6010	POTASSIUM	MG/KG		440						270	250		
SW6010	SELENIUM	MG/KG		0.61 U						0.6 U	0.67 U		
SW6010	SILVER	MG/KG		1.2 U						1.2 U	1.3 U		
SW6010	SODIUM	MG/KG		120 U						120 U	130 U		
SW6010	THALLIUM	MG/KG		1.2 U						1.2 U	1.3 U		
SW6010	VANADIUM	MG/KG		21						15	14		
SW6010	ZINC	MG/KG		37						45	46		
SW7471	MERCURY	MG/KG		0.043 U						0.041 U	0.044 U		
SW8081	4,4-DDD	UG/KG		11 U						10 U	11 U		
SW8081	4,4-DDE	UG/KG		11 U						3.3 J	9 J		
SW8081	4,4-DDT	UG/KG		11 U						10 U	11 U		
SW8081	ALDRIN	UG/KG		11 U						10 U	11 U		
SW8081	ALPHA-BHC	UG/KG		11 U						10 U	11 U		
SW8081	ALPHA-CHLORDANE	UG/KG		11 U						10 U	11 U		
SW8081	BETA-BHC	UG/KG		11 U						10 U	11 U		
SW8081	DELTA-BHC	UG/KG		11 U						10 U	11 U		
SW8081	DIELDRIN	UG/KG		11 U						10 U	11 U		
SW8081	ENDOSULFAN I	UG/KG		11 U						10 U	11 U		
SW8081	ENDOSULFAN II	UG/KG		11 U						10 U	11 U		
SW8081	ENDOSULFAN SULFATE	UG/KG		11 U						10 U	11 U		
SW8081	ENDRIN	UG/KG		11 U						10 U	11 U		
SW8081	ENDRIN ALDEHYDE	UG/KG		11 U						10 U	11 U		
SW8081	ENDRIN KETONE	UG/KG		11 U						10 U	11 U		
SW8081	GAMMA-BHC (LINDANE)	UG/KG		11 U						10 U	11 U		
SW8081	GAMMA-CHLORDANE	UG/KG		11 U						10 U	11 U		
SW8081	HEPTACHLOR	UG/KG		11 U						10 U	11 U		
SW8081	HEPTACHLOR EPOXIDE	UG/KG		11 U						10 U	11 U		
SW8081	METHOXYCHLOR	UG/KG		53 U						50 U	56 U		
SW8081	TOXAPHENE	UG/KG		530 U						500 U	560 U		

		Task Code	Aberdeen	Aberdeen	Aberdeen	Aberdeen	Aberdeen-dup	Aberdeen	Aberdeen	Aberdeen-dup	Aberdeen	Aberdeen	Aberdeen
		Sample Date	4/12/2006	4/12/2006	4/12/2006	4/12/2006	4/12/2006	4/12/2006	4/12/2006	4/12/2006	4/12/2006	4/12/2006	4/12/2006
		Sample ID	SedAb-ABk11	SlAb-A06	SedAb-A08	SlAb-A05	SLAB-B05 [2]	SedAb-A10	SedAb-A07	SedAb-02B	SedAb02B [3]	SedAb-A04	SedAb-A09
		Sample Matrix	SE	SS	SE	SS	SO	SE	SE	SE	SE	SE	SE
Analytical Method	Chemical Name	Unit											
SW8082	AROCLOR 1016	UG/KG		42 U						40 U	45 U		
SW8082	AROCLOR 1221	UG/KG		85 U						81 U	90 U		
SW8082	AROCLOR 1232	UG/KG		42 U						40 U	45 U		
SW8082	AROCLOR 1242	UG/KG		42 U						40 U	45 U		
SW8082	AROCLOR 1248	UG/KG		42 U						40 U	45 U		
SW8082	AROCLOR 1254	UG/KG		42 U						40 U	45 U		
SW8082	AROCLOR 1260	UG/KG		42 U						40 U	45 U		
SW8270	2-METHYLNAPHTHALENE	UG/KG											
SW8270	ACENAPHTHENE	UG/KG											
SW8270	ACENAPHTHYLENE	UG/KG											
SW8270	ANTHRACENE	UG/KG											
SW8270	BENZO(A)ANTHRACENE	UG/KG											
SW8270	BENZO(A)PYRENE	UG/KG											
SW8270	BENZO(B)FLUORANTHENE	UG/KG											
SW8270	BENZO(G,H,I)PERYLENE	UG/KG											
SW8270	BENZO(K)FLUORANTHENE	UG/KG											
SW8270	CHRYSENE	UG/KG											
SW8270	DIBENZ(A,H)ANTHRACENE	UG/KG											
SW8270	FLUORANTHENE	UG/KG											
SW8270	FLUORENE	UG/KG											
SW8270	INDENO(1,2,3-CD)PYRENE	UG/KG											
SW8270	NAPHTHALENE	UG/KG											
SW8270	PHENANTHRENE	UG/KG											
SW8270	PYRENE	UG/KG											
SW6010 (AVS-SEM)	CADMIUM	mg/kg	0.13 U	0.12 U	0.18 B	0.1 U	0.12 U	0.14 U	0.13 U	0.12 U		0.11 U	0.11 U
SW6010 (AVS-SEM)	COPPER	mg/kg	6.7 B	7.9	8.4	63.5	46.5	12.2	21.7	77.5		77.9	34.8
SW6010 (AVS-SEM)	LEAD	mg/kg	22	26.7	147	277	1350	228	386	474		696	789
SW6010 (AVS-SEM)	NICKEL	mg/kg	4.8 B	0.75 B	0.53 U	0.74 B	0.6 U	3.1 B	2.5 B	0.58 U		0.56 U	1.7 B
SW6010 (AVS-SEM)	ZINC	mg/kg	10.3	9.9	3.6 B	14.1	11.1	21.9	21.2	17.1		16	7.3
SW7470 (AVS-SEM)	MERCURY	mg/kg	0.0031 U	0.014 B	0.003 B	0.015 B	0.014 B	0.0035 U	0.0033 U	0.0029 U		0.02 B	0.0028 U
AVS	SULFIDE	mg/kg	20.1 U	18.9 U	33.6	33.4	19.2 U	22.1 U	52.6	18.6		17.9 U	26.5

Notes:
1 - Duplicate of SST04SCA
2 - Duplicate of SlAb-A05
3 - Duplicate of SedAb-02B
4 - Duplicate of SedAb-A3A

		Task Code	Aberdeen	Aberdeen	Aberdeen-dup
		Sample Date	4/12/2006	4/12/2006	4/12/2006
		Sample ID	SlAb-A01	SedAb-A3A	SedAb-B3A [4]
		Sample Matrix	SO	SE	SO
Analytical Method	Chemical Name	Unit			
D2216	SOLIDS, PERCENT	%	87.3	63.5	75.4
E415.1	TOTAL ORGANIC CARBON	mg/kg	880 H	16000 EH	24000 EH
SW6010	ALUMINUM	MG/KG			
SW6010	ANTIMONY	MG/KG			
SW6010	ARSENIC	MG/KG			
SW6010	BARIUM	MG/KG			
SW6010	BERYLLIUM	MG/KG			
SW6010	CADMIUM	MG/KG			
SW6010	CALCIUM METAL	MG/KG			
SW6010	CHROMIUM (TOTAL)	MG/KG			
SW6010	COBALT	MG/KG			
SW6010	COPPER	MG/KG	700	1200	1100
SW6010	IRON	MG/KG			
SW6010	LEAD	MG/KG	9900	18000	17000
SW6010	MAGNESIUM	MG/KG			
SW6010	MANGANESE	MG/KG			
SW6010	NICKEL	MG/KG			
SW6010	POTASSIUM	MG/KG			
SW6010	SELENIUM	MG/KG			
SW6010	SILVER	MG/KG			
SW6010	SODIUM	MG/KG			
SW6010	THALLIUM	MG/KG			
SW6010	VANADIUM	MG/KG			
SW6010	ZINC	MG/KG			
SW7471	MERCURY	MG/KG			
SW8081	4,4-DDD	UG/KG			
SW8081	4,4-DDE	UG/KG			
SW8081	4,4-DDT	UG/KG			
SW8081	ALDRIN	UG/KG			
SW8081	ALPHA-BHC	UG/KG			
SW8081	ALPHA-CHLORDANE	UG/KG			
SW8081	BETA-BHC	UG/KG			
SW8081	DELTA-BHC	UG/KG			
SW8081	DIELDRIN	UG/KG			
SW8081	ENDOSULFAN I	UG/KG			
SW8081	ENDOSULFAN II	UG/KG			
SW8081	ENDOSULFAN SULFATE	UG/KG			
SW8081	ENDRIN	UG/KG			
SW8081	ENDRIN ALDEHYDE	UG/KG			
SW8081	ENDRIN KETONE	UG/KG			
SW8081	GAMMA-BHC (LINDANE)	UG/KG			
SW8081	GAMMA-CHLORDANE	UG/KG			
SW8081	HEPTACHLOR	UG/KG			
SW8081	HEPTACHLOR EPOXIDE	UG/KG			
SW8081	METHOXYCHLOR	UG/KG			
SW8081	TOXAPHENE	UG/KG			

		Task Code	Aberdeen	Aberdeen	Aberdeen-dup
		Sample Date	4/12/2006	4/12/2006	4/12/2006
		Sample ID	SlAb-A01	SedAb-A3A	SedAb-B3A [4]
		Sample Matrix	SO	SE	SO
Analytical Method	Chemical Name	Unit			
SW8082	AROCLOR 1016	UG/KG			
SW8082	AROCLOR 1221	UG/KG			
SW8082	AROCLOR 1232	UG/KG			
SW8082	AROCLOR 1242	UG/KG			
SW8082	AROCLOR 1248	UG/KG			
SW8082	AROCLOR 1254	UG/KG			
SW8082	AROCLOR 1260	UG/KG			
SW8270	2-METHYLNAPHTHALENE	UG/KG			
SW8270	ACENAPHTHENE	UG/KG			
SW8270	ACENAPHTHYLENE	UG/KG			
SW8270	ANTHRACENE	UG/KG			
SW8270	BENZO(A)ANTHRACENE	UG/KG			
SW8270	BENZO(A)PYRENE	UG/KG			
SW8270	BENZO(B)FLUORANTHENE	UG/KG			
SW8270	BENZO(G,H,I)PERYLENE	UG/KG			
SW8270	BENZO(K)FLUORANTHENE	UG/KG			
SW8270	CHRYSENE	UG/KG			
SW8270	DIBENZ(A,H)ANTHRACENE	UG/KG			
SW8270	FLUORANTHENE	UG/KG			
SW8270	FLUORENE	UG/KG			
SW8270	INDENO(1,2,3-CD)PYRENE	UG/KG			
SW8270	NAPHTHALENE	UG/KG			
SW8270	PHENANTHRENE	UG/KG			
SW8270	PYRENE	UG/KG			
SW6010 (AVS-SEM)	CADMIUM	mg/kg	0.11 U	0.25 B	0.31 B
SW6010 (AVS-SEM)	COPPER	mg/kg	1410	947	689
SW6010 (AVS-SEM)	LEAD	mg/kg	8350	10100	7710
SW6010 (AVS-SEM)	NICKEL	mg/kg	0.55 U	0.99 B	0.62 U
SW6010 (AVS-SEM)	ZINC	mg/kg	134	107	73.8
SW7470 (AVS-SEM)	MERCURY	mg/kg	0.0085 B	0.014 B	0.0085 B
AVS	SULFIDE	mg/kg	17.7 U	25.1 U	19.7 U

Notes:
1 - Duplicate of SST04SCA
2 - Duplicate of SlAb-A05
3 - Duplicate of SedAb-02B
4 - Duplicate of SedAb-A3A

	Task Code	Travis	Travis	Travis-dup	Travis	Travis	Travis	Travis	Travis	Travis	Travis	Aberdeen	Aberdeen	Aberdeen
	Sample Date	3/28/2006	3/28/2006	3/28/2006	3/28/2006	3/28/2006	3/28/2006	3/28/2006	3/28/2006	3/28/2006	3/28/2006	4/12/2006	4/12/2006	4/12/2006
	Sample ID	SDTBKSCA	SST04SCA	SST04SCB [1]	SDT04SCA	SST09SCA	SST07SCA	SDT01SCA	SDT02SCA	SDT14SCA	SST13SCA	SedAb-ABk11	SlAb-A06	SedAb-A08
	Sample Matrix	SE	SS	SS	SE	SS	SS	SE	SE	SE	SS	SE	SS	SE
Chemical Name	Unit													
TOTAL ORGANIC CARBON	foc	0.015	0.017	0.018	0.018	0.016	0.016	0.020	0.016	0.019	0.023	0.0046	0.013	0.0036
SULFIDE	umol/g	1.4	1.1	1.7	ND	1.8	0.71	1.8	ND	1.5	1.9	ND	ND	1.0
CADMIUM	umol/g	0.0012	0.0011	0.0027	0.0012	0.0011	0.0012	0.0013	0.0011	0.0013	0.0012	0.00058	0.00053	0.0016
COPPER	umol/g	0.10	0.14	0.11	0.13	0.11	0.11	0.17	0.20	0.13	0.12	0.11	0.12	0.13
LEAD	umol/g	0.054	5.2	8.2	15.9	8.0	7.5	10.2	16.7	19.7	18.2	0.11	0.13	0.71
NICKEL	umol/g	0.089	0.10	0.090	0.10	0.12	0.090	0.10	0.13	0.11	0.097	0.082	0.013	0.005
ZINC	umol/g	0.15	0.20	0.17	0.47	0.17	0.43	0.39	0.30	0.23	0.20	0.16	0.15	0.06
Sum SEM	umol/g	0.39	5.7	8.6	16.6	8.4	8.2	10.9	17.4	20.2	18.6	0.45	0.42	0.90
Sum SEM - AVS	umol/g	-1.0	4.6	6.9	NC	6.5	7.5	9.0	NC	18.7	16.7	NC	NC	-0.15
SUM SEM/AVS	unitless	0.29	5.2	5.1	NC	4.6	11.5	6.0	NC	13.9	9.7	NC	NC	0.86
[Sum SEM - AVS]/foc	umol/goc	-65.4	268	382	NC	409	466	452	NC	987	726	NC	NC	-40.3

Notes:
1 - Duplicate of SST04SCA
2 - Duplicate of SlAb-A05
3 - Duplicate of SedAb-A3A

One-half detection limit used in calculation for non-detect SEM analytes. Indicated with shading.
AVS - Acid Volatile Sulfides
foc - fraction organic carbon
goc - gram organic carbon
NC - Not calculated; Calculations not completed if AVS was not detected.
SEM - Simultaneously Extracted Metals
TOC - Total Organic Carbon

	Task Code	Travis	Travis	Travis	Travis	Travis	Travis	Travis	Travis	Travis
	Sample Date	3/28/2006	3/28/2006	3/28/2006	3/28/2006	3/28/2006	3/28/2006	3/28/2006	3/28/2006	3/28/2006
	Sample ID	SDTBKSCA	SST04SCA	SDT04SCA	SST09SCA	SST07SCA	SDT01SCA	SDT02SCA	SDT14SCA	SST13SCA
	Sample Matrix	SE	SS	SE	SS	SS	SE	SE	SE	SS
Chemical Name	Unit									
Bulk Density	lb/ft3	110	112	106	116	110	100	114	110	106
Gravel	%	0	0.8	0.4	0.9	0.3	0	0	0	0.5
Sand	%	47.3	46.7	45	48.9	48.1	47.5	47.4	45.3	47.8
Silt & Clay	%	52.7	52.5	54.6	50.2	51.6	52.5	52.6	54.7	51.7
Cation Exchange Capacity	meq/100g	9.2	1.5	13.4	1.3	8.0	2.1	1.5	11.9	1.8

	Aberdeen	Aberdeen	Aberdeen	Aberdeen	Aberdeen	Aberdeen	Aberdeen	Aberdeen	Aberdeen	Aberdeen	Aberdeen
	4/12/2006	4/12/2006	4/12/2006	4/12/2006	4/12/2006	4/12/2006	4/12/2006	4/12/2006	4/12/2006	4/12/2006	4/12/2006
	SedAb-ABk11	SlAb-A06	SedAb-A08	SlAb-A05	SedAb-A10	SedAb-A07	SedAb-02B	SedAb-A04	SedAb-A09	SlAb-A01	SedAb-A3A
	SE	SS	SE	SS	SE	SE	SE	SE	SE	SO	SE
Chemical Name											
Bulk Density	123	119	101	102	112	106	128	117	121	125	100
Gravel	0.2	0	3.3	0	0.3	3.9	2.3	2.9	1.6	3.9	0
Sand	46.2	26.1	87.7	45.2	14.2	36.4	59.5	47.7	82.9	76.2	23.3
Silt & Clay	53.6	73.9	9	54.8	85.5	59.7	38.2	49.4	15.5	19.9	76.7
Cation Exchange Capacity	7.3	9	3.1	5.1	13.8	10.9	1.6	4.3	0.6	0.9	5.8

	Task Code	Aberdeen	Aberdeen-dup	Aberdeen	Aberdeen	Aberdeen	Aberdeen	Aberdeen	Aberdeen	Aberdeen	Aberdeen-dup
	Sample Date	4/12/2006	4/12/2006	4/12/2006	4/12/2006	4/12/2006	4/12/2006	4/12/2006	4/12/2006	4/12/2006	4/12/2006
	Sample ID	SlAb-A05	SLAB-B05 [2]	SedAb-A10	SedAb-A07	SedAb-02B	SedAb-A04	SedAb-A09	SlAb-A01	SedAb-A3A	SedAb-B3A [3]
	Sample Matrix	SS	SO	SE	SE	SE	SE	SE	SO	SE	SO
Chemical Name	Unit										
TOTAL ORGANIC CARBON	foc	0.0041	0.0040	0.0062	0.019	0.0055	0.0057	0.0011	0.0009	0.0160	0.0240
SULFIDE	umol/g	1.0	ND	ND	1.6	0.6	ND	0.8	ND	ND	ND
CADMIUM	umol/g	0.00044	0.00053	0.00062	0.00058	0.00053	0.00049	0.00049	0.00049	0.0022	0.0028
COPPER	umol/g	1.0	0.73	0.19	0.34	1.2	1.2	0.55	22.2	14.9	10.8
LEAD	umol/g	1.3	6.5	1.1	1.9	2.3	3.4	3.8	40.3	48.7	37.2
NICKEL	umol/g	0.013	0.0051	0.053	0.043	0.0049	0.0048	0.029	0.0047	0.017	0.0053
ZINC	umol/g	0.22	0.17	0.33	0.32	0.26	0.24	0.11	2.0	1.6	1.1
Sum SEM	umol/g	2.6	7.4	1.7	2.6	3.8	4.8	4.5	64.5	65.3	49.2
Sum SEM - AVS	umol/g	2	NC	NC	0.93	3.2	NC	3.7	NC	NC	NC
SUM SEM/AVS	unitless	2	NC	NC	1.6	6.5	NC	5.4	NC	NC	NC
[Sum SEM - AVS]/foc	umol/goc	371	NC	NC	49.0	581	NC	3336	NC	NC	NC

Notes:
1 - Duplicate of SST04SCA
2 - Duplicate of SlAb-A05
3 - Duplicate of SedAb-A3A

One-half detection limit used in calculation for non-detect SEM analytes. Indicated with shading.
AVS - Acid Volatile Sulfides
foc - fraction organic carbon
goc - gram organic carbon
NC - Not calculated; Calculations not completed if AVS was not detected.
SEM - Simultaneously Extracted Metals
TOC - Total Organic Carbon

USACHPPM Histopathology Report

U.S. ARMY CENTER FOR HEALTH PROMOTION AND PREVENTIVE MEDICINE

STUDY NO. 03U9-64-05-03-03:

EFFECTS OF LEAD EXPOSURE TO THE RED-BACKED SALAMANDER (*Plethodon cinereus*) – 28-DAY

HISTOPATHOLOGY REPORT

REPORT DATE: 03 NOVEMBER 2006

Submitted by:

Kristie Mozzachio, DVM, DACVP

STUDY NO. 03U9-64-05-03-03:

EFFECTS OF LEAD EXPOSURE TO THE RED-BACKED SALAMANDER (*Plethodon cinereus*) – 28-DAY

HISTOPATHOLOGY REPORT

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INTRODUCTION:

The purpose of this study was to assess the toxicity of and to determine the Lowest Observable Adverse Effect Level (LOAEL) and the No Observable Adverse Effect Level (NOAEL) from subchronic soil exposures to lead in the terrestrial red-backed salamander (*Plethodon cinereus*). Field-collected (aged) soils were used in this study to determine whether aged soils are as toxic as spiked soils.

MATERIALS AND METHODS:

Wild-caught salamanders in this 28-day study were divided evenly by weight into the following treatment groups:

Treatment Group	Number of Animals
APG: 50.3 mg/kg lead	10
APG: 348.7 mg/kg lead	10
APG: 11723.9 mg/kg lead	10
TAB: 24.1 mg/kg lead	10
TAB: 2010.1 mg/kg lead	10
TAB: 4093.0 mg/kg lead	10

Note: TAB and APG designate location of field-collected soils from an Air Force and an Army small arms range.

Each animal was placed into an individual Petri dish containing treatment specific soil. Food consisted of potworms exposed to lead-contaminated soil of the same stock used to expose the salamanders. Soil and potworm preparation and sampling were performed according to protocol specifications. Animals were observed at least daily for signs of overt toxicity (e.g., lethargy, sensitivity to touch, abnormal behavior).

On Day 28 or 29, salamanders were euthanized using aqueous preparations of MS-222 followed by decapitation. The remaining head and body were preserved in 10% neutral buffered formalin. Cross sections of the head and body were then trimmed, embedded in paraffin, sectioned at 6 microns, stained with hematoxylin and eosin, and examined via routine light microscopy. The histologic sections were of adequate size and quality for the detection of treatment-related changes. Histologic observations and a record of tissues examined were entered into a computer-

assisted data retrieval system (StarTox, Graham Laboratories, New Braunfels, TX) at the time of histologic examination. The attached tabulation of tissues examined and histologic findings in those tissues serve as the basis for this narrative report.

SURVIVAL:

All salamanders survived to the scheduled termination (study day 28 or 29).

GROSS NECROPSY OBSERVATIONS:

There were no reported gross necropsy observations for this study.

HISTOPATHOLOGIC FINDINGS:

There were no test article-related histopathologic findings. Histologic findings were considered to be incidental, manifestations of spontaneous disease or related to some aspect of experimental manipulation other than exposure to the test article. There was no test article-related alteration in the incidence, severity or histologic character of these incidental and spontaneous tissue alterations.

CONCLUSIONS:

Based on the results of this study, there was no toxicity associated with field-collected (aged) soil exposure to APG 50.3, 348.7 and 11723.9 mg/kg lead or TAB 24.1, 2010.1, and 4093.0 mg/kg lead for 28 or 29 days in terrestrial red-backed salamanders.

Submitted by: _____ Date: _____

Kristie Mozzachio, DVM, DACVP

Study Number: 03U9-64-05-03-03 (28 Day)
Microscopic Incidence Table
Final Histopathology Report
Date Printed: 03 Nov 2006 11:42 AM

Organs Group:	*50.3 mg/kg*				*348.7 mg/kg*			
Diagnoses Sacrifice:	*Dos*		*Sac*		*Dos*		*Sac*	
Modifiers Sex:	*M*	*F*	*M*	*F*	*M*	*F*	*M*	*F*
Total Animals Selected:	[0]	[0]	[2]	[8]	[0]	[0]	[6]	[4]
Cavity, Abdominal	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
Parasite(s)	0	0	0	0	0	0	0	0
Cavity, Oral	(0)	(0)	(1)	(3)	(0)	(0)	(1)	(1)
Inflammation	0	0	1	3	0	0	1	1
focal, minimal	0	0	0	1	0	0	0	0
multifocal, minimal	0	0	1	2	0	0	1	1
Eye, Lens	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)
Degeneration	0	0	0	1	0	0	0	0
mild, unilateral	0	0	0	1	0	0	0	0
Gastrointestinal Tract	(0)	(0)	(2)	(8)	(0)	(0)	(6)	(4)
Inflammation	0	0	0	0	0	0	0	1
focal, minimal	0	0	0	0	0	0	0	1
Within Normal Limits	0	0	2	8	0	0	6	3
Kidney	(0)	(0)	(2)	(8)	(0)	(0)	(6)	(4)
Dilatation, tubular	0	0	0	1	0	0	0	0
minimal	0	0	0	1	0	0	0	0
Infiltration, mononuclear	0	0	0	0	0	0	1	0
focal, minimal	0	0	0	0	0	0	1	0
Mineralization	0	0	0	0	0	0	0	0
multifocal, minimal	0	0	0	0	0	0	0	0
Not examined, not in plane of section	0	0	0	0	0	0	1	0
Pigment, brown	0	0	0	0	0	0	0	0
minimal	0	0	0	0	0	0	0	0
Section inadequate	0	0	0	0	0	0	0	0
Within Normal Limits	0	0	2	7	0	0	4	4
Liver	(0)	(0)	(2)	(8)	(0)	(0)	(6)	(4)
Glycogen depletion	0	0	0	1	0	0	0	1
minimal	0	0	0	0	0	0	0	0
mild	0	0	0	1	0	0	0	1
Infiltration, mononuclear	0	0	0	0	0	0	0	0
multifocal, minimal	0	0	0	0	0	0	0	0
Within Normal Limits	0	0	2	7	0	0	6	3
Muscle, Skeletal	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
Infiltration, mononuclear	0	0	0	0	0	0	0	0
focal, minimal	0	0	0	0	0	0	0	0
Ovary	(0)	(0)	(0)	(8)	(0)	(0)	(0)	(4)
Section inadequate	0	0	0	0	0	0	0	0
Within Normal Limits	0	0	0	8	0	0	0	4
Skin	(0)	(0)	(0)	(1)	(0)	(0)	(1)	(0)
Hyperplasia, epithelial	0	0	0	1	0	0	0	0
focal, minimal	0	0	0	1	0	0	0	0
Inflammation	0	0	0	1	0	0	1	0
focal, minimal	0	0	0	1	0	0	1	0
Spleen	(0)	(0)	(2)	(8)	(0)	(0)	(6)	(4)
Congestion	0	0	0	0	0	0	0	0
minimal	0	0	0	0	0	0	0	0
Not examined, not in plane of section	0	0	1	1	0	0	1	1
Within Normal Limits	0	0	1	7	0	0	5	3
Testis	(0)	(0)	(2)	(0)	(0)	(0)	(6)	(0)
Hypoplasia	0	0	0	0	0	0	1	0
Not examined, not in plane of section	0	0	0	0	0	0	0	0
Section inadequate	0	0	0	0	0	0	0	0
Within Normal Limits	0	0	2	0	0	0	5	0

Dos=Died on Study
Sac=Scheduled Sacrifice

Study Number: 03U9-64-05-03-03 (28 Day)
Microscopic Incidence Table
Final Histopathology Report
Date Printed: 03 Nov 2006 11:42 AM

Organs Group:	*11723.9 mg/kg*				*24.1 mg/kg*			
Diagnoses Sacrifice:	*Dos*		*Sac*		*Dos*		*Sac*	
Modifiers Sex:	*M*	*F*	*M*	*F*	*M*	*F*	*M*	*F*
Total Animals Selected:	[0]	[0]	[6]	[4]	[0]	[0]	[6]	[4]
Cavity, Abdominal	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
Parasite(s)	0	0	0	0	0	0	0	0
Cavity, Oral	(0)	(0)	(2)	(3)	(0)	(0)	(4)	(2)
Inflammation	0	0	2	3	0	0	4	2
focal, minimal	0	0	1	1	0	0	2	0
multifocal, minimal	0	0	1	2	0	0	2	2
Eye, Lens	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
Degeneration	0	0	0	0	0	0	0	0
mild, unilateral	0	0	0	0	0	0	0	0
Gastrointestinal Tract	(0)	(0)	(6)	(4)	(0)	(0)	(6)	(4)
Inflammation	0	0	0	0	0	0	1	0
focal, minimal	0	0	0	0	0	0	1	0
Within Normal Limits	0	0	6	4	0	0	5	4
Kidney	(0)	(0)	(6)	(4)	(0)	(0)	(6)	(4)
Dilatation, tubular	0	0	0	1	0	0	0	0
minimal	0	0	0	1	0	0	0	0
Infiltration, mononuclear	0	0	0	0	0	0	0	0
focal, minimal	0	0	0	0	0	0	0	0
Mineralization	0	0	1	0	0	0	0	0
multifocal, minimal	0	0	1	0	0	0	0	0
Not examined, not in plane of section	0	0	1	0	0	0	0	0
Pigment, brown	0	0	0	0	0	0	0	1
minimal	0	0	0	0	0	0	0	1
Section inadequate	0	0	0	0	0	0	0	0
Within Normal Limits	0	0	4	3	0	0	6	3
Liver	(0)	(0)	(6)	(4)	(0)	(0)	(6)	(4)
Glycogen depletion	0	0	0	1	0	0	0	0
minimal	0	0	0	1	0	0	0	0
mild	0	0	0	0	0	0	0	0
Infiltration, mononuclear	0	0	0	0	0	0	1	0
multifocal, minimal	0	0	0	0	0	0	1	0
Within Normal Limits	0	0	6	3	0	0	5	4
Muscle, Skeletal	(0)	(0)	(1)	(0)	(0)	(0)	(0)	(0)
Infiltration, mononuclear	0	0	1	0	0	0	0	0
focal, minimal	0	0	1	0	0	0	0	0
Ovary	(0)	(0)	(0)	(4)	(0)	(0)	(0)	(4)
Section inadequate	0	0	0	1	0	0	0	0
Within Normal Limits	0	0	0	3	0	0	0	4
Skin	(0)	(0)	(1)	(1)	(0)	(0)	(1)	(0)
Hyperplasia, epithelial	0	0	0	0	0	0	0	0
focal, minimal	0	0	0	0	0	0	0	0
Inflammation	0	0	1	1	0	0	1	0
focal, minimal	0	0	1	1	0	0	1	0
Spleen	(0)	(0)	(6)	(4)	(0)	(0)	(6)	(4)
Congestion	0	0	2	0	0	0	0	0
minimal	0	0	2	0	0	0	0	0
Not examined, not in plane of section	0	0	2	1	0	0	1	0
Within Normal Limits	0	0	2	3	0	0	5	4
Testis	(0)	(0)	(6)	(0)	(0)	(0)	(6)	(0)
Hypoplasia	0	0	0	0	0	0	0	0
Not examined, not in plane of section	0	0	0	0	0	0	0	0
Section inadequate	0	0	1	0	0	0	0	0
Within Normal Limits	0	0	5	0	0	0	6	0

Dos=Died on Study
Sac=Scheduled Sacrifice

Study Number: 03U9-64-05-03-03 (28 Day)
Microscopic Incidence Table
Final Histopathology Report
Date Printed: 03 Nov 2006 11:42 AM

Organs Group:	*2010.1 mg/kg*				*4093.0 mg/kg*			
Diagnoses Sacrifice:	*Dos*		*Sac*		*Dos*		*Sac*	
Modifiers Sex:	*M*	*F*	*M*	*F*	*M*	*F*	*M*	*F*
Total Animals Selected:	[0]	[0]	[6]	[4]	[0]	[0]	[6]	[4]
Cavity, Abdominal	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)
Parasite(s)	0	0	0	1	0	0	0	0
Cavity, Oral	(0)	(0)	(3)	(1)	(0)	(0)	(3)	(2)
Inflammation	0	0	3	1	0	0	3	2
focal, minimal	0	0	3	1	0	0	1	2
multifocal, minimal	0	0	0	0	0	0	2	0
Eye, Lens	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
Degeneration	0	0	0	0	0	0	0	0
mild, unilateral	0	0	0	0	0	0	0	0
Gastrointestinal Tract	(0)	(0)	(6)	(4)	(0)	(0)	(6)	(4)
Inflammation	0	0	0	0	0	0	0	0
focal, minimal	0	0	0	0	0	0	0	0
Within Normal Limits	0	0	6	4	0	0	6	4
Kidney	(0)	(0)	(6)	(4)	(0)	(0)	(6)	(4)
Dilatation, tubular	0	0	1	0	0	0	0	0
minimal	0	0	1	0	0	0	0	0
Infiltration, mononuclear	0	0	0	0	0	0	0	0
focal, minimal	0	0	0	0	0	0	0	0
Mineralization	0	0	0	0	0	0	0	0
multifocal, minimal	0	0	0	0	0	0	0	0
Not examined, not in plane of section	0	0	0	0	0	0	2	0
Pigment, brown	0	0	0	0	0	0	0	0
minimal	0	0	0	0	0	0	0	0
Section inadequate	0	0	1	0	0	0	0	0
Within Normal Limits	0	0	4	4	0	0	4	4
Liver	(0)	(0)	(6)	(4)	(0)	(0)	(6)	(4)
Glycogen depletion	0	0	0	0	0	0	1	0
minimal	0	0	0	0	0	0	0	0
mild	0	0	0	0	0	0	1	0
Infiltration, mononuclear	0	0	0	0	0	0	0	0
multifocal, minimal	0	0	0	0	0	0	0	0
Within Normal Limits	0	0	6	4	0	0	5	4
Muscle, Skeletal	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
Infiltration, mononuclear	0	0	0	0	0	0	0	0
focal, minimal	0	0	0	0	0	0	0	0
Ovary	(0)	(0)	(0)	(4)	(0)	(0)	(0)	(4)
Section inadequate	0	0	0	0	0	0	0	0
Within Normal Limits	0	0	0	4	0	0	0	4
Skin	(0)	(0)	(1)	(0)	(0)	(0)	(0)	(0)
Hyperplasia, epithelial	0	0	0	0	0	0	0	0
focal, minimal	0	0	0	0	0	0	0	0
Inflammation	0	0	1	0	0	0	0	0
focal, minimal	0	0	1	0	0	0	0	0
Spleen	(0)	(0)	(6)	(4)	(0)	(0)	(6)	(4)
Congestion	0	0	0	0	0	0	0	0
minimal	0	0	0	0	0	0	0	0
Not examined, not in plane of section	0	0	1	0	0	0	0	1
Within Normal Limits	0	0	5	4	0	0	6	3
Testis	(0)	(0)	(6)	(0)	(0)	(0)	(6)	(0)
Hypoplasia	0	0	0	0	0	0	0	0
Not examined, not in plane of section	0	0	0	0	0	0	1	0
Section inadequate	0	0	0	0	0	0	0	0
Within Normal Limits	0	0	6	0	0	0	5	0

Dos=Died on Study
Sac=Scheduled Sacrifice

Gp: (01) 50.3 mg/kg Sex: Female Animal Number: 06-25
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 29

Microscopic Results

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Ovary, Spleen

Gp: (01) 50.3 mg/kg Sex: Female Animal Number: 06-50
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 28

Microscopic Results

Cavity, Oral	Inflammation, multifocal, minimal
Eye, Lens	Degeneration, mild, unilateral
Kidney	Dilatation, tubular, minimal

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Liver, Ovary, Spleen

Gp: (01) 50.3 mg/kg Sex: Female Animal Number: 06-51
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 29

Microscopic Results

Liver	Glycogen depletion, mild

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Ovary, Spleen

Gp: (01) 50.3 mg/kg Sex: Female Animal Number: 06-55
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 29

Microscopic Results

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Ovary, Spleen

Gp: (01) 50.3 mg/kg Sex: Female Animal Number: 06-62
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 29

Microscopic Results

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Ovary, Spleen

Gp: (01) 50.3 mg/kg Sex: Female Animal Number: 06-63
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 28

Microscopic Results

Cavity, Oral	Inflammation, multifocal, minimal
Spleen	Not examined, not in plane of section

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Ovary

Gp: (01) 50.3 mg/kg Sex: Female Animal Number: 06-65
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 28

Microscopic Results

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Ovary, Spleen

Gp: (01) 50.3 mg/kg Sex: Female Animal Number: 06-88
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 28

Microscopic Results

Cavity, Oral	Inflammation, focal, minimal
Skin	Hyperplasia, epithelial, focal, minimal
	Inflammation, focal, minimal

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Ovary, Spleen

Gp: (01) 50.3 mg/kg Sex: Male Animal Number: 06-43
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 29

Microscopic Results

Spleen	Not examined, not in plane of section

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Testis

Gp: (01) 50.3 mg/kg Sex: Male Animal Number: 06-52
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 28

Microscopic Results

Cavity, Oral	Inflammation, multifocal, minimal

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Spleen, Testis

Gp: (02) 348.7 mg/kg Sex: Female Animal Number: 06-28
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 28

Microscopic Results

Cavity, Oral	Inflammation, multifocal, minimal
Gastrointestinal Tract	Inflammation, focal, minimal
	serosa

The following organs were found to be Within Normal Limits:
Kidney, Liver, Ovary, Spleen

Gp: (02) 348.7 mg/kg Sex: Female Animal Number: 06-48
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 28

Microscopic Results

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Ovary, Spleen

Gp: (02) 348.7 mg/kg Sex: Female Animal Number: 06-70
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 29

Microscopic Results

Liver	Glycogen depletion, mild

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Ovary, Spleen

Gp: (02) 348.7 mg/kg Sex: Female Animal Number: 06-78
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 29

Microscopic Results

Spleen	Not examined, not in plane of section

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Ovary

Gp: (02) 348.7 mg/kg Sex: Male Animal Number: 06-42
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 28

Microscopic Results

Testis	Hypoplasia *with one ovarian follicle*

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Spleen

Gp: (02) 348.7 mg/kg Sex: Male Animal Number: 06-47
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 29

Microscopic Results

Spleen	Not examined, not in plane of section

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Testis

Gp: (02) 348.7 mg/kg Sex: Male Animal Number: 06-57
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 29

Microscopic Results

Kidney	Infiltration, mononuclear, focal, minimal

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Liver, Spleen, Testis

Gp: (02) 348.7 mg/kg Sex: Male Animal Number: 06-67
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 28

Microscopic Results

Cavity, Oral	Inflammation, multifocal, minimal

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Spleen, Testis

Gp: (02) 348.7 mg/kg Sex: Male Animal Number: 06-74
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 29

Microscopic Results

Kidney	Not examined, not in plane of section

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Liver, Spleen, Testis

Gp: (02) 348.7 mg/kg Sex: Male Animal Number: 06-81
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 28

Microscopic Results

Skin	Inflammation, focal, minimal

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Spleen, Testis

Gp: (03) 11723.9 mg/kg Sex: Female Animal Number: 06-38
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 29

Microscopic Results

Ovary	Section inadequate *insufficient tissue present*

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Spleen

Gp: (03) 11723.9 mg/kg Sex: Female Animal Number: 06-49
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 28

Microscopic Results

Cavity, Oral	Inflammation, focal, minimal
Kidney	Dilatation, tubular, minimal
Skin	Inflammation, focal, minimal

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Liver, Ovary, Spleen

Gp: (03) 11723.9 mg/kg Sex: Female Animal Number: 06-83
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 29

Microscopic Results

Cavity, Oral	Inflammation, multifocal, minimal
Spleen	Not examined, not in plane of section

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Ovary

Gp: (03) 11723.9 mg/kg Sex: Female Animal Number: 06-86
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 29

Microscopic Results

Cavity, Oral	Inflammation, multifocal, minimal
Liver	Glycogen depletion, minimal

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Ovary, Spleen

Gp: (03) 11723.9 mg/kg Sex: Male Animal Number: 06-32
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 28

Microscopic Results

Spleen	Congestion, minimal

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Testis

Gp: (03) 11723.9 mg/kg Sex: Male Animal Number: 06-34
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 28

Microscopic Results

Cavity, Oral	Inflammation, focal, minimal

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Spleen, Testis

Gp: (03) 11723.9 mg/kg Sex: Male Animal Number: 06-36
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 29

Microscopic Results

Kidney	Mineralization, multifocal, minimal
Muscle, Skeletal	Infiltration, mononuclear, focal, minimal
Skin	Inflammation, focal, minimal
Spleen	Not examined, not in plane of section
Testis	Section inadequate *insufficient tissue present*

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Liver

Gp: (03) 11723.9 mg/kg Sex: Male Animal Number: 06-37
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 29

Microscopic Results

Spleen	Congestion, minimal

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Testis

Gp: (03) 11723.9 mg/kg Sex: Male Animal Number: 06-66
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 28

Microscopic Results

Cavity, Oral	Inflammation, multifocal, minimal
Spleen	Not examined, not in plane of section

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Testis

Gp: (03) 11723.9 mg/kg Sex: Male Animal Number: 06-79
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 28

Microscopic Results

Kidney	Not examined, not in plane of section

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Liver, Spleen, Testis

Gp: (04) 24.1 mg/kg Sex: Female Animal Number: 06-31
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 28

Microscopic Results

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Ovary, Spleen

Gp: (04) 24.1 mg/kg Sex: Female Animal Number: 06-46
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 29

Microscopic Results

Kidney	Pigment, brown, minimal

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Liver, Ovary, Spleen

Gp: (04) 24.1 mg/kg Sex: Female Animal Number: 06-56
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 28

Microscopic Results

Cavity, Oral	Inflammation, multifocal, minimal

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Ovary, Spleen

Gp: (04) 24.1 mg/kg Sex: Female Animal Number: 06-85
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 29

Microscopic Results

Cavity, Oral	Inflammation, multifocal, minimal

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Ovary, Spleen

Gp: (04) 24.1 mg/kg Sex: Male Animal Number: 06-39
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 28

Microscopic Results

Liver	Infiltration, mononuclear, multifocal, minimal

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Spleen, Testis

Gp: (04) 24.1 mg/kg Sex: Male Animal Number: 06-44
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 29

Microscopic Results

Cavity, Oral	Inflammation, multifocal, minimal

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Spleen, Testis

Gp: (04) 24.1 mg/kg Sex: Male Animal Number: 06-53
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 29

Microscopic Results

Skin	Inflammation, focal, minimal

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Spleen, Testis

Gp: (04) 24.1 mg/kg Sex: Male Animal Number: 06-60
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 28

Microscopic Results

Cavity, Oral	Inflammation, focal, minimal
Spleen	Not examined, not in plane of section

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Testis

Gp: (04) 24.1 mg/kg Sex: Male Animal Number: 06-77
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 29

Microscopic Results

Cavity, Oral	Inflammation, focal, minimal

Gp: (04) 24.1 mg/kg Sex: Male Animal Number: 06-77
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 29

Microscopic Results (continued)

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Spleen, Testis

Gp: (04) 24.1 mg/kg Sex: Male Animal Number: 06-87
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 28

Microscopic Results

Cavity, Oral	Inflammation, multifocal, minimal
Gastrointestinal Tract	Inflammation, focal, minimal *serosa*

The following organs were found to be Within Normal Limits:
Kidney, Liver, Spleen, Testis

Gp: (05) 2010.1 mg/kg Sex: Female Animal Number: 06-27
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 29

Microscopic Results

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Ovary, Spleen

Gp: (05) 2010.1 mg/kg Sex: Female Animal Number: 06-68
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 28

Microscopic Results

Cavity, Oral	Inflammation, focal, minimal

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Ovary, Spleen

Gp: (05) 2010.1 mg/kg Sex: Female Animal Number: 06-82
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 28

Microscopic Results

Cavity, Abdominal	Parasite(s) *free in coelom*

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Ovary, Spleen

Gp: (05) 2010.1 mg/kg Sex: Female Animal Number: 06-84
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 29

Microscopic Results

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Ovary, Spleen

Gp: (05) 2010.1 mg/kg Sex: Male Animal Number: 06-33
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 28

Microscopic Results

Cavity, Oral	Inflammation, focal, minimal

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Spleen, Testis

Gp: (05) 2010.1 mg/kg Sex: Male Animal Number: 06-54
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 29

Microscopic Results

Kidney	Section inadequate *insufficient tissue present*
Spleen	Not examined, not in plane of section

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Liver, Testis

Gp: (05) 2010.1 mg/kg Sex: Male Animal Number: 06-59
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 29

Microscopic Results

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Spleen, Testis

Gp: (05) 2010.1 mg/kg Sex: Male Animal Number: 06-61
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 28

Microscopic Results

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Spleen, Testis

Gp: (05) 2010.1 mg/kg Sex: Male Animal Number: 06-75
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 28

Microscopic Results

Cavity, Oral	Inflammation, focal, minimal
Kidney	Dilatation, tubular, minimal

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Liver, Spleen, Testis

Gp: (05) 2010.1 mg/kg Sex: Male Animal Number: 06-76
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 29

Microscopic Results

Cavity, Oral	Inflammation, focal, minimal
Skin	Inflammation, focal, minimal

Gp: (05) 2010.1 mg/kg Sex: Male Animal Number: 06-76
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 29

Microscopic Results (continued)

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Spleen, Testis

Gp: (06) 4093.0 mg/kg Sex: Female Animal Number: 06-40
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 29

Microscopic Results

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Ovary, Spleen

Gp: (06) 4093.0 mg/kg Sex: Female Animal Number: 06-64
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 29

Microscopic Results

Spleen	Not examined, not in plane of section

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Ovary

Gp: (06) 4093.0 mg/kg Sex: Female Animal Number: 06-72
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 29

Microscopic Results

Cavity, Oral	Inflammation, focal, minimal

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Ovary, Spleen

Gp: (06) 4093.0 mg/kg Sex: Female Animal Number: 06-80
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 29

Microscopic Results

Cavity, Oral	Inflammation, focal, minimal

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Ovary, Spleen

Gp: (06) 4093.0 mg/kg Sex: Male Animal Number: 06-26
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 28

Microscopic Results

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Spleen, Testis

Gp: (06) 4093.0 mg/kg Sex: Male Animal Number: 06-41
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 29

Microscopic Results

Cavity, Oral	Inflammation, multifocal, minimal

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Spleen, Testis

Gp: (06) 4093.0 mg/kg Sex: Male Animal Number: 06-45
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 28

Microscopic Results

Kidney	Not examined, not in plane of section

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Liver, Spleen, Testis

Gp: (06) 4093.0 mg/kg Sex: Male Animal Number: 06-58
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 28

Microscopic Results

Kidney	Not examined, not in plane of section
Testis	Not examined, not in plane of section **SEX UNKNOWN**

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Liver, Spleen

Gp: (06) 4093.0 mg/kg Sex: Male Animal Number: 06-71
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 28

Microscopic Results

Cavity, Oral	Inflammation, multifocal, minimal
Liver	Glycogen depletion, mild

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Spleen, Testis

Gp: (06) 4093.0 mg/kg Sex: Male Animal Number: 06-89
Fate: (TS) Terminal Sacrifice Species: Salamander Days on Study: 28

Microscopic Results

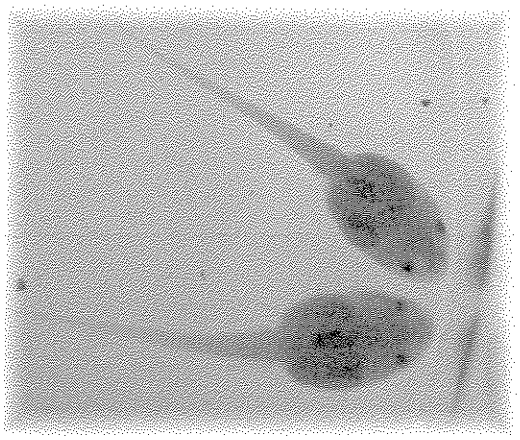
Cavity, Oral	Inflammation, focal, minimal

The following organs were found to be Within Normal Limits:
Gastrointestinal Tract, Kidney, Liver, Spleen, Testis

End of Report

FCETL Sediment Toxicity Testing Reports

ENSR
Westford, MA
(09070-056)



Report of Short-Term Toxicity of Whole Sediment to *Rana pipiens*
(Sediments collected from Travis Air Force Base)

Prepared by

ENSR | AECOM

ENSR
Environmental Toxicology
Fort Collins, CO

Laboratory Project 08503-128-019-(086-093)
ENSR Project 09070-056
January 2007

ENSR | AECOM

Report of Short-Term Toxicity of Whole Sediment to Rana pipiens

Project ID: 08503-128-019-(086-093)
January 2007

Sponsor and Laboratory Information

Sponsor	ENSR 2 Technology Park Drive Westford, MA 01886-3140 (978) 589-3000
ENSR Project Officer	John Bleiler
Testing Facility	ENSR Fort Collins Environmental Toxicology Laboratory 4303 West LaPorte Ave. Fort Collins, CO 80521 Fax: (970) 490-2963 State of Florida NELAP Laboratory ID E87972
Study Director	David A. Pillard, Ph.D.

Test Information

Test	Short-term chronic screening toxicity test of sediment	
Basis	NAVFAC (2004)	
Test Protocol	RP3ESTCP.TIE019.001	
Test Period	January 13, 2007 @ 1040 to January 23, 2007 @ 1130	
Test Length	10 days	
Species	*Rana pipiens*	
Test Material	Whole sediment	
Sediment ID	Sample (Field) ID	ENSR Laboratory ID
	M1	(19381+19383)
	M2	(19381+19383)
	M3	(19381+19383)
	SDT04DTA	19386
	SDT07DTA	19381
	SST13DTA	19994
	SDT14DTA	19385
Background (Reference) Sediment	SDTBKSTA	19383
Control Sediment	Cache la Poudre River Sediment	
Overlying water	Laboratory process water from Horsetooth Reservoir	
Test Concentrations	0 (control), SDTBKSTA, SDT04DTA, SDT07DTA, SST13DTA and SDT14DTA 100% of each test sediment. M1 = (3% SDT07DTA and 97% SDTBKSTA) M2 = (13% SDT07DTA and 87% SDTBKSTA) M2 = (40% SDT07DTA and 60% SDTBKSTA)	

Sediment Collection and Receipt

Sample ID	Collection Date and Time	ENSR No.	Date of Receipt	Cooler Air Temp. at Arrival (°C)
SDTBKSTA[a]	3/28/06 @ 0950	19383	3/30/06	9
SDT04DTA	3/28/06 @ 1415	19386	3/31/06	11
SDT07DTA	3/28/06 @ 1205	19381	3/30/06	9
SST13DTA	3/28/06 @ 1130	19994	10/31/06	12
SDT14DTA	3/28/06 @ 1415	19385	3/31/06	11

[a] Background sample
Note: See Appendix A for copies of chain of custody records

Control Sediment

The control sediment was from the Cache la Poudre River northwest of Fort Collins, CO. The river sediment was washed through a No. 60 sieve with laboratory process water from Horsetooth Reservoir until the water ran clear. It was then air-dried before use.

Test Sediment Preparation

Sample ID	Date Homogenized	Time Homogenized
M1	1/12/07	1319-1322
M2		1339-1342
M3		1358-1401
SDT04DTA		1417-1420
SDT07DTA		1451-1454
SST13DTA		1515-1518
SDT14DTA		1439-1442
SDTBKSTA		1506-1509

Test Conditions

Test Type	Static sediment with continuous replacement of overlying water for 10 days
Overlying Water Delivery System	Continuous renewal (flow-through) [a]
Test Endpoints	Survival, Body Width and Length
Test Chambers	500-ml glass beakers
Test Sediment Volume	100 ml
Overlying Water Volume	175 ml
Replicates per Treatment	8
Organisms per Replicate	5
Test Temperature	23 ± 1°C
Lighting	Fluorescent, 16 hours light:8 hours dark
Chamber Placement	Randomized
Test Sediment Renewal	None
Test Overlying Water Renewal	Approximately two volume additions per test chamber per day

[a] Continuous replacement via a drip system
Note: See Appendix B for the Test Protocol

Test Organism

One the day of test initiation, five *Rana pipiens* were taken from the lot used in the test and examined to determine developmental stage. Based on gill development, tail length, and ventral appearance, it was determined that tadpoles were at stage 21, which is immediately post-hatch.

Species and Lot Number	*Rana pipiens*, Lot 07-003
Age	Stage 21 (Gosner 1960)
Source	Carolina Biological, Burlington, NC
Feeding	Approx. 3 mg TetraMin daily in each chamber, after reaching stage 25
Reference Toxicant Testing	Initiated January 13, 2007 using sodium chloride (NaCl)

TEST RESULTS

Biological Data – Mean Survival and Body Width and Length per Original Organism

Sample ID	Mean Percent Survival	Mean Body Width (mm)	Mean Body Length (mm)
Poudre River Sediment Control	95	5.3	7.6
M1	95	5.1	7.5
M2	95	4.8	7.4
M3	62	3.4	4.9[a,b]
SDT04DTA	100	4.9	7.1
SDT07DTA	40[a,b]	2.5[c]	3.5[c]
SST13DTA	95	5.0	7.0
SDT14DTA	100	5.2	7.3
SDTBKSTA	90	5.0	7.5
Control Performance	Acceptable	N/A	N/A

[a] Significantly (α=0.05) less than the Poudre River Control
[b] Significantly (α=0.05) less than the Background Sediment (SDTBKSTA)
[c] Excluded form statistical analysis because survival was significantly reduced
Note: See Appendix C for test data sheets

Data Analysis

Biological Endpoint	Comparison[a]	Procedure
Survival	Normality	Chi-Square Test (α=0.01)
	Homogeneity of Variance	Bartlett's Test (α=0.01)
	Significant Reduction Relative to the Poudre River Sediment Control or SDTBKSTA	Steel's Many-One Rank Test (α=0.05)
Body Width	Normality	Chi-Square Test (α=0.01)
	Homogeneity of Variance	Bartlett's Test (α=0.01)
	Significant Reduction Relative to the Poudre River Sediment Control or SDTBKSTA	Steel's Many-One Rank Test (α=0.05)
Body Length	Normality	Chi-Square Test (α=0.01)
	Homogeneity of Variance	Bartlett's Test (α=0.01)
	Significant Reduction Relative to the Poudre River Sediment Control or SDTBKSTA	Steel's Many-One Rank Test (α=0.05)

[a] Using Toxstat Version 3.5 (WEST, Inc. and Gulley 1996)

Physical and Chemical Data

Sample ID	pH (units)	DO (mg/L)	Conductivity (µS/cm)	Temperature (°C)[a]	Ammonia as N (mg/L)	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)
Poudre R. Control	7.5-7.9	6.0-6.8	100-150	22-23	<1.0	42-90	36-36
M1	7.2-7.8	4.2-5.7	177-307	22-23	2.9-7.2	72-130	78-112
M2	7.2-7.7	4.2-6.0	159-298	22-23	2.2-7.1	64-120	72-112
M3	7.2-7.8	3.6-6.2	171-361	22-23	2.2-8.9	72-170	74-132
SDT04DTA	7.1-7.6	5.2-6.3	108-166	22-23	<1.0-1.6	38-100	38-64
SDT07DTA	7.3-7.8	3.4-5.5	191-512	22-23	1.9-12.6	78-220	82-184
SST13DTA	6.7-7.8	4.6-6.0	98-118	22-23	<1.0-2.2	40-70	38-40
SDT14DTA	7.4-7.8	4.3-6.1	220-230	22-23	3.9-7.5	86-90	88-98
SDTBKSTA	7.2-7.8	4.6-5.8	191-252	22-23	2.4-5.5	78-100	82-92

[a] Temperature in test chambers

Concentration of Copper and Lead in Test Sediments

Sample ID	Copper (mg/kg dry weight)	Lead (mg/kg dry weight)
Poudre R.[a] Control	7.2	4.5
M1	12[b]	78[a]
M2	13[b]	286[a]
M3	14[b]	849[a]
SDT04DTA	19	1700
SDT07DTA[c]	17	2100
SST13DTA	15	3700
SDT14DTA	21	2800
SDTBKSTA	12	15

[a] Poudre River Sediment has the field sample number of "001s" in the analytical report from Paragon Analytics (report cover letter dated January 17, 2007).
[b] Expected concentrations calculated using known concentrations of SDTBKSTA and SDT07DTA
[c] Label on sample jar said "SST07DTA" but "SDT07DTA" was written on cap. Sample identified as "SST07DTA" in the analytical report from Paragon Analytics (report cover letter dated November 14, 2006).
Note: See Appendix D for raw analytical data from Paragon Analytics

Reference Toxicant Test Results for *R. pipiens*

Organism Lot Number	Test Date	96-Hour LC_{50} (mg/L Cl^-)	ENSR/FCETL Historical 95% Control Limits	
			Low	High
07-003	January 13, 2007	3625	NA	NA

Note: There are less than five data points in the database, therefore historical limits are not reported

Protocol Deviations

In addition to 100% sediment as specified in the protocol, three mixtures of SDTBKSTA and SDT07DTA were tested.

Ammonia was not measured in each treatment on days 3 and 7 as specified in the protocol. Instead, it was measured only on days 0 and 10. This deviation had no adverse effect on the biological data.

References

Gosner, K. L. 1960. A Simplified Table for Staging Anuran Embryos and Larvae with Notes on Identification. Herpetologica, 16:183-190.

NAVFAC. 2004. Development of a Standardized Approach for Assessing Potential Risks to Amphibians Exposed to Sediment and Hydric Soils. Prepared for Naval Facilities Engineering Service Center (NFESC), Port Hueneme, CA.

WEST, Inc. and D.D. Gulley. 1996. Toxstat Version 3.5. Western EcoSystems Technology, Inc., Cheyenne, WY.

Statement of Procedural Compliance

I certify that this document and all attachments were prepared under my direction or supervision in accordance with a system designed to assure that qualified personnel properly gather and evaluate the information submitted. Based on my inquiry of the person or persons who manage the system, or those persons directly responsible for gathering the information, the information submitted is, to the best of my knowledge, accurate and complete.

David A. Pillard — 12 Feb 2007

David A. Pillard, Ph.D. — Date
Study Director

Statement of Quality Assurance

The test data were reviewed by the Quality Assurance Unit to assure that the study was performed in accordance with the protocol and standard operating procedures, and that the resulting data and report meet the requirements of the NELAC standards. This report is an accurate reflection of the raw data.

Anita Rehmer — February 9, 2007

Quality Assurance Unit — Date

APPENDIX A

Chain of Custody

ENSR INTERNATIONAL

CHAIN OF CUSTODY RECORD

Client/Project Name: ESTCP

Project Location: Travis AFB

Project Number: 09070-056

Field Logbook No.: 0306A A91

Sampler (Print Name)/(Affiliation): A. Desilets ENSR - Westford, MA

Chain of Custody Tape No.: 27541

Signature: [signature]

Send Results/Report to: L. Herbenich, ENSR

Analysis Requested

Field Sample No./ Identification	Date	Time	Grab	Comp	Sample Container (Size/Mat'l)	Sample Type (Liquid, Sludge, Etc.)	Preservative	Field Filtered	Tot.							Lab I.D.	Remarks
SST04 DTA	3/28/06	12:10	✓	✓	1g. poly	sed.	—	—	✓							19380	
SD107 DTA	3/28/06	12:05	✓	✓	1g. poly	sed.	—	—	✓							19381	
SD101 DTA	3/28/06	12:35	✓	✓	1g. poly	sed.	—	—	✓							19382	
SDTBK STA	3/28/06	09:00	✓	✓	1g. poly	sed.	—	—	✓							19383	

Relinquished by: (Print Name)/(Affiliation)	Date:	~~Relinquished~~ Received by: (Print Name)/(Affiliation)	Date:
Signature: [signature]	Time: 0700	David Pillard Signature: [signature]	3-30-06 Time: 1200
Relinquished by: (Print Name)/(Affiliation) Signature:	Date: Time:	Relinquished by: (Print Name)/(Affiliation) Signature:	Date: Time:
Relinquished by: (Print Name)/(Affiliation) Signature:	Date: Time:	Relinquished by: (Print Name)/(Affiliation) Signature:	Date: Time:

Analytical Laboratory (Destination):

Cooler Air Temp = 9°C #22

Fed Ex

Serial No. 38468

FM01008a_902

ENSR INTERNATIONAL

CHAIN OF CUSTODY RECORD

Client/Project Name: ESTCP

Project Location: Travis AFB

Project Number: 09070 USCG

Field Logbook No.: 0306A291

Sampler (Print Name)/(Affiliation): A Desiletts ENSR Westford, MA

Chain of Custody Tape No.:

Signature: AMDesiletts

Send Results/Report to: ~~[illegible]~~ i Herberich. ENSR

Analysis Requested: Rt.

Field Sample No./ Identification	Date	Time	Grab	Comp	Sample Container (Size/Mat'l)	Sample Type (Liquid, Sludge, Etc.)	Preservative	Field Filtered	Rt.							Lab I.D.	Remarks
SDT02DTA	3/30/06	13:30	✓	✓	1g poly	sed.	—	—	✓							19384	
SDT14DTA	3/30/06	14:15	✓	✓	1g poly	sed.	—	—	✓							19385	
SDT04DTA	3/30/06	14:15	✓	✓	1g poly	sed.	—	—	✓							19386	

Relinquished by: (Print Name)/(Affiliation)	Date	Relinquished by: (Print Name)/(Affiliation)	Date
Signature: AMDesiletts	3/31/06 Time: 0700	David A. Pillard Signature: David A. Pillard	3-31-06 Time: 1300
Relinquished by: (Print Name)/(Affiliation) Signature:	Date: Time:	Relinquished by: (Print Name)/(Affiliation) Signature:	Date: Time:
Relinquished by: (Print Name)/(Affiliation) Signature:	Date: Time:	Relinquished by: (Print Name)/(Affiliation) Signature:	Date: Time:

Analytical Laboratory (Destination):

Note: The COC ~~for~~ was wadded up and stuck in cupholder on outside of cooler. DAP

Air Temp inside cooler = 11°C

22

Serial No. 38467

FM01008a_902

CHAIN-OF-CUSTODY RECORD

The Proponent of This Form Is the Directorate of Toxicology

RELEASING LABORATORY: USACHPPM - DTOX
PROTOCOL #: 0349-64-05-03-03
PROTOCOL TITLE: Salamander Pb

STUDY DIRECTOR: Matthew Bazar
PURPOSE OF MATERIAL RELEASE: left over soil for additional testing
RECEIVING LABORATORY: ENSR - Fort Collins Toxicology Lab

NOTE: For use of this form, see DTOX SOP # 52.

TEST SYSTEM	IDENTIFICATION NUMBER	TOTAL #	BOTTLES OUT	BOTTLES IN	~~CASSETTES~~ OUT ENSR Lab #	CASSETTES IN	BLOCKS OUT	BLOCKS IN	SLIDES OUT	SLIDES IN	HISTO RECORD	REPORT
	SSTBKSTA		1		19995							
	SST13DTA		1		19994							
	Total	2										

DATE SHIPPED: 30 Oct 06 CARRIER USED: Fed Ex CARRIER COMMENTS:

RELINQUISHED BY	DATE/TIME	RECEIVED BY	DATE/TIME	COMMENTS
Matthew Bazar	30 Oct 06	Kelly Tapp	10/31/06 @ 1100	rec'd via Fed ex @ 12°C (cooler temp) chain of custody seal (intact)

REMARKS ① 3/7/07 CFLE container had sample times as 3/28/06 @ 1130 this data was not on COC.

APPENDIX B

Test Protocol

Title: Toxicity of Sediment to Amphibian Larvae, *Rana pipiens*

Study Sponsor:	ENSR 2 Technology Park Drive Westford, MA 01886-3140
Testing Facility	Fort Collins Environmental Toxicology Laboratory 4303 West LaPorte Avenue Fort Collins, Colorado 80521

1.0 INTRODUCTION

1.1 Objective

To determine the toxicity of sediment samples to the Northern Leopard Frog, *Rana pipiens*.

1.2 Experimental Approach

Sediment samples will be visually examined for the presence of endemic organisms, which will be removed. Tadpoles will be added to the overlying water and test chambers maintained under test conditions until test termination. Test chambers will be examined for surviving organisms, which will then be measured for body width and length.

1.3 Test Material

The test material will be a field-collected sediment. Samples will be stored under refrigeration (4°C) until used in testing. Each sample will be homogenized mechanically or by hand, prior to testing.

2.0 BASIS AND TEST ORGANISM

2.1 Basis

This protocol is based on USEPA (2002) and NAVFAC (2004), where appropriate.

2.2 Test Organism

1. Species – *Rana pipiens*
2. Age – Organisms used to initiate the tests will be recently hatched tadpoles at approximately Gosner stage 18 (Gosner 1960).
3. Source - Test organisms will be obtained from a commercial supplier.
4. Feeding – Tadpoles will be fed approximately 3 mg TetraMin per chamber daily after stage 25.

3.0 TEST SYSTEM

3.1 Artificial Sediment

If the laboratory control sediment is an artificial sediment, it will consist of washed natural sediment or washed silica sand. Control sediment may be amended with the addition of organic matter (e.g., peat).

3.2 Overlying Water

The overlying water used in the toxicity test will be uncontaminated surface water from Horsetooth Reservoir.

3.3 Test Temperature

Test temperature will be 23 ± 1°C. Testing will be conducted in an environmental chamber or a temperature controlled water bath.

3.4 Test Containers

Test containers will be 500-ml beakers containing 100 ml of sediment and 175 ml of overlying water.

3.5 Photoperiod

The photoperiod will be 16-hours light and 8-hours dark.

3.6 Dissolved Oxygen Concentrations

Dissolved oxygen concentrations in the overlying water will be maintained ≥ 3.0 mg/L. If the dissolved oxygen concentration in the overlying water approaches this level, all test chambers will be gently aerated throughout the remainder of the test. If aeration is initiated, the aeration pipette will be appropriately positioned so as to avoid disturbance of the sediment.

3.7 Reference Toxicant Testing

In addition to the test material exposures, reference toxicant tests will be conducted using sodium chloride (NaCl) or copper chloride ($CuCl_2$) to determine the sensitivity range of the test organisms. Reference toxicant exposures will be conducted at the time of test initiation for commercially-supplied organisms.

4.0 TEST DESIGN

4.1 Test Treatments

The test concentration will be 100 percent of each test sediment. A 100 percent laboratory control sediment (see section 4.3) exposure will be conducted concurrently. A reference sediment collected at the study site may also be included.

4.2 Sediment/Water Mixture

Sediment will be placed in each test chamber at least one day (day –1) before test initiation. Because of uncertainty in the availability of the amphibian embryos and hatching time, sediment may remain in the chambers for up to three days prior to test initiation. After addition of sediment, the overlying water will be carefully poured into each beaker. The beakers will be left static and unaerated overnight to allow sediment to settle and to reduce turbidity prior to addition of test organisms.

4.3 Reference/Control Sediment

In addition to the field-collected samples, at least one control sediment will be tested concurrently. Control sediment will be either an artificial laboratory sediment (silica sand, see section 3.1) or natural sediment collected from the Cache la Poudre River (northwest of Fort Collins, CO).

4.4 Number of Test Organisms

Forty tadpoles will be exposed to each treatment. Five organisms will be assigned to each test chamber and eight replicates will be tested per treatment.

4.5 Analytical Sampling

Aliquots of sediment for chemical analysis will be removed by the study sponsor prior to shipping samples to ENSR's laboratory. No analytical sampling of the test chambers themselves is planned.

4.6 Test Initiation/Renewal Frequency

Testing will be initiated by addition of the test organisms after the overnight sediment settling period. Renewal of overlying water will be accomplished either by a flow-through system or with a renewal box. Each chamber will be renewed with a minimum of 2 volume additions per day.

4.7 Chemical and Physical Monitoring

At a minimum, the following measurements will be made:

1. Dissolved oxygen, temperature, and pH will be measured in the overlying water of each treatment and the control each day of testing.

2. Hardness, alkalinity, conductivity, and ammonia will be measured in the overlying water of each treatment on day 0.

3. Hardness, alkalinity, conductivity, and ammonia will be measured in overlying water from each treatment at test initiation (just prior to renewal on day 0 or 1) and at test termination.

4. Ammonia will also be measured in each treatment on days 3 and 7.

4.8 Biological Monitoring

After ten days of exposure, sediment from each test chamber will be removed and sieved or sorted (if needed) to recover living test organisms. Organisms not recovered at test termination will be presumed dead. At test termination, the surviving organisms in each test chamber will be counted and the width of the body (at the widest point) and length of the body (snout to base of tail) will be determined.

4.9 Test Duration

The test duration will be 10 days.

4.10 Calculations

Survival data will be transformed by arcsine squareroot. Normality and homogeneity assumptions for survival data will be evaluated by the Shapiro-Wilk's (or Chi-Square) test and Bartlett's test, respectively (α = 0.01). Data will then be evaluated (α = 0.05) using either parametric or nonparametric methods depending upon the outcome of the normality and homogeneity assessments.

Organism width and length will be statistically compared in treatments not having significantly reduced survival. Analysis will occur in the same manner as for survival, although the width and length measurements will not be transformed using arcsine squareroot.

4.11 Quality Criterion

The test will not be considered acceptable if mortality in the control sediment exceeds 20 percent.

5.0 TEST REPORT

The report will be a typed document describing the results of the test and will be signed by the Study Director and Quality Assurance Unit. The report will include, but not be limited to, the following:

- A copy of all raw data.
- Name of test, Study Director, and laboratory, and date test was begun.
- Time and date of sediment collection.
- The source of the overlying water and a description of any pretreatment.
- Test organism scientific name and age.
- A description of the experimental design, manner in which the test was initiated, the number of organisms per treatment, replicates per treatment and the lighting.
- A description of any aeration performed on test solutions before or during the test.
- Definition of the criterion used to determine the effect and a summary of general observations on other effects or symptoms.
- Percentage of organisms that died or showed an effect.
- The minimum dissolved oxygen concentration, range in tests temperature and pH, all visual observations.
- Any deviations from the protocol.

6.0 LITERATURE CITED

Gosner, K.L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*. 16:183-190.

NAVFAC. 2004. Development of a Standardized Approach for Assessing Potential Risks to Amphibians Exposed to Sediment and Hydric Soils.

USEPA. 2002. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. Fourth Edition. EPA-821-R-02-013.

7.0 PROCEDURAL COMPLIANCE

All test procedures, documentation, records, and reports will comply with USEPA (2002) general guidance on quality assurance related to effluent toxicity testing. To this end, random audits of the test may be scheduled while the test is in progress. The raw data will be checked and compared to protocol requirements and Standard Operating Procedures, and the final report will be audited for accuracy and signed, if satisfactory, by both the Study Director and an individual from the Quality Assurance Unit.

8.0 PROTOCOL AMENDMENTS AND DEVIATIONS

All changes (i.e., amendments, deviations, and final report revisions) of the approved protocol plus the reasons for the changes must be documented in writing. The changes will be signed and dated by the Study Director and maintained with the protocol. All amendments must be authorized in advance by the Sponsor.

9.0 SPONSOR AND STUDY DIRECTOR APPROVAL

Study Director: David A. Pillard	Date: 10 Jan 2007

APPENDIX C

Data Sheets

DAP 2/12/07
CA: AR 02-01-07

Rana pipiens 10-day Survival and Growth, Testing Cover Page

Project Number: 8503-128-019-0 Task #: 086-093 Protocol #: RP3ESTCP.TIE19.001
Test Substance: Sediment
Test Species: *Rana pipiens* Lot #: 07-003 Age: Stage 21 (24 hrs) Supplier: ① ~~ABS~~ carolina Biological Supply
Test Type: 10 Day Chronic
Dilution Water: Filtered Horsetooth water Investigators: DAP/TD/JG/JMH
Sampling Date(s): 3-28-06 Sampling Time(s):
FCETL Sample #(s): 19383 (BESTA), 19386 (SDT04DTA), 19381 (SDT07DTA), 19384 (SST13DTA), 19385 (SDT14DTA)
Test Initiation Date/Time: 1-13-07 / 1040
Test Termination Date/Time: 1/23/07 / 1130

Renewal Frequency: Cont. drip, 2+ vol/da Feeding Freq: daily after stage 25 Food Type/Amount: 3 mg Tetrafin Test Temp: 23 +/- 1 deg C
Test Chamber Capacity: 500-ML Test Soltn. Vol: 100 mL sed/175 mL # Repl's/Trtmnt: 8
Test Duration: 10 days # Org.'s/Repl: 5 Env. Chmbr/Bath: 3

Water Characterization: Minimum of Hardness , Alkalinity, & Ammonia on days 0 and 10; No TRC; Conductivity weekly; pH & DO3 times weekly on composites, temp daily on composites
record daily observations of mortality; aerate if dissolved oxygen <2.5 mg/L

Test Sediment (s):

1)	Control	2)	SDTBKSTA	3)	M1
4)	M2	5)	M3	6)	SDT04DTA
7)	SDT07DTA	8)	~~SDT13DTA~~ SST13DTA	9)	SDT14DTA
10)		11)			

Reference Tox. Dates: 1/13-~~20~~ 17 ①/07 LC50: (96-h) 3625 Hist. Limits: Method:
Study Director Initials: DAP Date: 1/13/06

Overlying water added at a minimum of 2 volume additions/day; equivalent to >350 ml/day or >0.24 ml/min

① AR for DAP 02-01-07 cf
② P 1/31/07 E

FCETL QA Form No. 116
Revision: 0
Effective: 8/99

QA: AR020107
DAP 3/12/07

BIOLOGICAL DATA (Day 0)

Date: 1/13/07

Project Number: 8503-128-019-086 to -093

Test Species (Circle): *Rana pipiens*

Other(Specify):

Sediment	Observation at Test Initiation	Replicate A	B	C	D	E	F	G	H	Time (Range)	Remarks
control	# Organisms Added	5	5	5	5	5	5	5	5	1020 – 1040	
	Initials	DAP	→	→	→	→	→	→	→		
SDT BKSTA	# Organisms Added	5	5	5	5	5	5	5	5	1020 – 1040	
	Initials	DAP	→	→	→	→	→	→	→		
M1	# Organisms Added	5	5	5	5	5	5	5	5	1020 – 1040	
	Initials	DAP	→	→	→	→	→	→	→		
M2	# Organisms Added	5	5	5	5	5	5	5	5	1020 – 1040	
	Initials	DAP	→	→	→	→	→	→	→		
M3	# Organisms Added	5	5	5	5	5	5	5	5	1020 – 1040	
	Initials	DAP	→	→	→	→	→	→	→		
SDTOYDTA	# Organisms Added	5	5	5	5	5	5	5	5	1020 – 1040	
	Initials	DAP	→	→	→	→	→	→	→		
SDTO7DTA	# Organisms Added	5	5	5	5	5	5	5	5	1020 – 1040	
	Initials	DAP	→	→	→	→	→	→	→		

FCETL QA Form No. 116
Revision: 0
Effective: 8/99

BIOLOGICAL DATA (Day 0)

Date: 1-13-07

Project Number: 8503-128-019-086 to-093 Test Species (Circle): *Rana pipiens* Other(Specify):

QA: AR 02-07-07 DAP 2/12/07

Sediment	Observation at Test Initiation	Replicate A	B	C	D	E	F	G	H	Time (Range)	Remarks
① ~~SDT13DTA~~ SST13DTA	# Organisms Added	5	5	5	5	5	5	5	5	1020-1040	
	Initials	DAP	→	→	→	→	→	→	→		
SDT14DTA	# Organisms Added	5	5	5	5	5	5	5	5	1020-1040	
	Initials	DAP	→	→	→	→	→	→	→		
	# Organisms Added										
	Initials										
	# Organisms Added										
	Initials										
	# Organisms Added										
	Initials										
	# Organisms Added										
	Initials										
	# Organisms Added										
	Initials										

① 2/3/07 [illegible]

FCETL QA Form No. 116
Revision: 0
Effective: 8/99

BIOLOGICAL DATA (Day 10)

Date: 1-23-07

Project Number: 8503-128-019- 086 to-093 Test Species (Circle): *Rana pipiens* Other(Specify):

QAP 2/12/07
QA: AR020107

Sediment	Observation at Test Termination	Replicate A	B	C	D	E	F	G	H	Time (Range)	Remarks
Control	# Surviving	5	5	5	5	5	5	4	4	1200-1210	
	# Observed Dead	0	0	0	0	0	0	0	0		
	# Not Found	0	0	0	0	0	0	1	1		
	Initials	[illegible]									
SDT BKSTA	# Surviving	5	5	5	4	5	5	2	5	1210-1220	
	# Observed Dead	0	0	0	0	0	0	0	0		
	# Not Found	0	0	0	1	0	0	3	0		
	Initials	[illegible]									
M1	# Surviving	5	5	5	4	5	5	5	4	1220-1230	
	# Observed Dead	0	0	0	0	0	0	0	0		
	# Not Found	0	0	0	1	0	0	0	1		
	Initials	[illegible]									
M2	# Surviving	5	5	5	5	5	5	5	3	1230-1240	
	# Observed Dead	0	0	0	0	0	0	0	0		
	# Not Found	0	0	0	0	0	0	0	2		
	Initials	[illegible]									
M3	# Surviving	0	1	5	1	4	5	5	4	1250-1300	WATER MURKY
	# Observed Dead	0	0	0	0	0	0	0	0		
	# Not Found	5	4	0	4	1	0	0	1		
	Initials	[illegible]									
SDTOY DTA	# Surviving	5	5	5	5	5	5	5	5*	1300-1310	* Organism w/ crooked tail
	# Observed Dead	0	0	0	0	0	0	0	0		small plants in sediment in all chambers
	# Not Found	0	0	0	0	0	0	0	0		
	Initials	[illegible]									
SDTOY DTA	# Surviving	3	0	0	3	0	5	4	1	1310-1320	WATER MURKY
	# Observed Dead	0	2	0	0	0	0	0	0		
	# Not Found	2	3	5	2	5	0	1	4		
	Initials	[illegible]									

FCETL QA Form No. 116
Revision: 0
Effective: 8/99

BIOLOGICAL DATA (Day 10)

Date: 1-23-07

Project Number: 8503-128-019-086 to-043

Test Species (Circle): *Rana pipiens*

Other(Specify): DSP 2/12/07 QA: AR02-07-07

Sediment	Observation at Test Termination	Replicate A	B	C	D	E	F	G	H	Time (Range)	Remarks
~~SDT13DTA~~ SST13DTA	# Surviving	5	5	4	5 *	5	4	5 *	5	1320-1320	* 1 organism w/ crooked tail
	# Observed Dead	0	0	0	0	0	0	0	0		
	# Not Found	0	0	1	0	0	1	0	0		
	Initials										
SDT14DTA	# Surviving	5	5	5	5	5	5	5	5	1330-1340	
	# Observed Dead	0	0	0	0	0	0	0	0		
	# Not Found	0	0	0	0	0	0	0	0		
	Initials										
	# Surviving										
	# Observed Dead										
	# Not Found										
	Initials										
	# Surviving										
	# Observed Dead										
	# Not Found										
	Initials										
	# Surviving										
	# Observed Dead										
	# Not Found										
	Initials										
	# Surviving										
	# Observed Dead										
	# Not Found										
	Initials										
	# Surviving										
	# Observed Dead										
	# Not Found										
	Initials										

@ 2/3/07 LGE

DA: AR 02/01/07
DAP 2/12/07

CHEMICAL DATA (Composite of Overlying Water) *Rana pipiens* 10 Day Chronic Project 8503-128-019-086 to -093

Parameter	Sediment	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day	Meter	Date	Time	Initials
Dissolved Oxygen (mg/l)	Control	6.0	6.5	6.4	6.4	6.8	6.6	6.4	6.2	6.5	6.7	6.4	0	~~4.6~~	1/13/07	1030	TD
	SDTBKSTA	4.6	4.9	5.3	5.3	5.5	5.3	5.8	5.3	5.8	5.8	5.4	1	5	1/14/07	1445	JG
	M1	5.3	4.2	5.6	4.8	5.2	4.6	5.5	5.3	5.1	5.7	5.2	2	5	1/15/07	1030	P
	M2	4.9	4.2	5.7	5.4	5.9	6.0	5.8	4.9	4.8	5.4	5.3	3	5	1/16/07	1300	P
	M3	4.0	2.3 3.6	3.8	4.2	5.3	6.1	6.2	5.1	5.3	5.2	5.0	4	5	1/17/07	1015	P
	SDT04DTA	5.8	5.8	5.8	5.8	5.6	5.7	6.2	5.8	6.3	5.8	5.2	5	5	1/18/07	0930	TD
	SDT07DTA	4.4	5.1	5.4	3.9	4.1	3.4	5.0	4.8	5.5	4.5	4.0	6	5	1/19/07	1345	P
	~~SDT13DTA~~ SDT13DTA (3)	4.9	5.3	5.2	4.6	5.9	4.7	6.0	5.8	6.0	4.9	5.0	7	5	1/20/07	1340	MH
	SDT14DTA	4.3	5.8	5.6	5.9	5.0	4.6	5.6	5.4	5.9	6.1	4.5	8	5	1/21/07	1315	TD
													9	5	1/22/07	1035	P
													10	5 (2)	1/23/07	1130	P
Temp (deg C)	Control	23	22	22	22	22	23	22	22	22	22	22	0	D32	1/13/07	0930	DAP
	SDTBKSTA	23	22	22	22	22	22	22	22/23	22	22	22	1	D35	1/14/07	1445	JG
	M1	23	22	22	22	22	22	22	22/23	22	22	22	2	D32	1/15/07	1030	P
	M2	23	22	22	22	23	22	22	22	22	22	22	3	D32	1/16/07	1300	P
	M3	23	22	22	22	23	23	22	22	22	22	22	4	D33	1/17/07	1015	P
	SDT04DTA	23	22	22	23	22	22	22	22	22	22	22	5	D-31	1/18/07	0930	TD
	SDT07DTA	23	22	22	22	22	22	22	22	22	22	22	6	D-31	1/19/07	1345	P
	~~SDT13DTA~~ SDT13DTA (3)	23	22	22	22	22	22	22	22	22	22	22	7	D35	1/20/07	1340	MH
	SDT14DTA	23	22	22	22	22	22	22	22/23	22	22	22	8	D31	1/21/07	1315	TD
													9	D-31	1/22/07	1035	P
													10	D-35	1/23/07	1130	P
pH	Control	7.5	7.7	7.8	7.8	7.9	7.9	7.8	7.8	7.7	7.8	7.8	0	16	1/13/07	1030	TD
	SDTBKSTA	7.2	7.4	7.5	7.5	7.5	7.6	7.6	7.7	7.8	7.5	7.8	1	16	1/14/07	1445	JG
	M1	7.2	7.4	7.6	7.6	7.6	7.5	7.6	7.6	7.7	7.5	7.8	2	16	1/15/07	1030	P
	M2	7.2	7.5	7.6	7.7	7.7	7.7	7.5	7.5	7.7	7.6	7.6	3	16	1/16/07	1300	P
	M3	7.2	7.6	7.6	7.8	7.6	7.6	7.6	7.7	7.7	7.6	7.8	4	16	1/17/07	1015	P
	SDT04DTA	7.1	7.2	7.4	7.5	7.4	7.4	7.6	7.5	7.6	7.6	7.5	5	16	1/18/07	0930	TD
	SDT07DTA	7.3	7.6	7.7	7.6	7.6	7.7	7.5	7.5	7.6	7.7	7.8	6	16	1/19/07	1345	P
	~~SDT13DTA~~ SDT13DTA (3)	6.7	7.1	7.3	7.3	7.8	7.5	7.4	7.5	7.5	7.5	7.5	7	16	1/20/07	1340	MH
	SDT14DTA	7.4	7.5	7.7	7.6	7.7	7.8	7.8	7.8	7.8	7.6	7.8	8	16	1/21/07	1315	TD
													9	16	1/22/07	1035	P
													10	12	1/23/07	1130	P

(2) JG 1/14/07 E
(3) MH 1/20/07 E CF
(4) AR for PD 02/01/07 E
(5) P 2/13/07 E

DTD 1/13/07 E

OVERLYING WATER CHARACTERIZATION *Rana pipiens* Chronic, Static Renewal Project No. 8503-128-019- 086 to 093

QA: AR 02-01-07
DAP 2/12/07

	Conductivity (uS/cm)			Hardness (mg/l as CaCO3)			Alkalinity (mg/l as CaCO3)			Ammonia (mg/l)		
Sediment	Day 0	Day	Day 10	Day 0	Day	Day 10	Day 0	Day	Day 10	Day 0	Day	Day 10
Control	150		100	90		42	36		36	<1.0		<1.0
SDTBKSTA	252		191	100		78	92		82	5.5		2.4
M1	307		177	130		72	112		78	7.2		2.9
M2	298		159	120		64	112		72	7.1		2.2
M3	361		171	170		72	132		74	8.4		2.2
SDT04DTA	166		108	100		38	64		38	1.6		<1.0
SDT07DTA	512		191	220		78	184		82	12.6		1.9
① ~~SDT13DTA~~ SDT 13DTA	118		98	70		40	40		38	2.2		<1.0
SDT14DTA	230		220	90		86	88		98	7.5		3.9
Meter #	15		15	NA		NA	NA		NA	3		3
Date:	1/13/07		1/23/07	1/13/07		1/23/07	1/13/07		1/23/07	1/13/07		1/23/07
Time:	1200		1130	1250		1130	1200		1130	1200		1130
Initials:	DAP		P	DAP		P	DAP		P	DAP		P

② P 2/8/07

① AR 02-01-07 E

DAILY TESTING LOG *Rana pipiens* 10 Day Chronic Project No. 8503-128-019- 086 to -093

QA#: AR02-01-07 DAP 3/12/07

Day -1	- Sediments homogenized 1319-1518 & placed in test chambers DAP 1/12/07 - Tempered Horsetooth water added to each chamber. - Chambers randomly placed in water bath						
Day 0				Feeding:	None	Initials/Date:	DAP 1/13/07
Day 1	Bath CT = 23.4 °C	Range =	23.0 - 24.0 °C	Feeding:	NONE	Initials/Date:	JG 1/14/07
Day 2	Bath CT = 23.4 °C	Range =	23.0 - 24.0 °C	Feeding:	None	Initials/Date:	[illegible] 1/15/07
Day 3	Bath CT = 23.4 °C	Range =	23.2 - 24.0 °C	Feeding:	~3mg Tetrafin @ 1320	Initials/Date:	[illegible] 1/16/07
Day 4	Bath CT = 23.4	Range =	23.0 - 23.6 °C	Feeding:	~3mg Tetrafin @ 1030	Initials/Date:	[illegible] 1/17/07
Day 5	Bath CT = 23.4 °C	Range =	23.2 - 24.0 °C	Feeding:	~3mg tetrafin @ 0940	Initials/Date:	TD 1/18/07
Day 6	Bath CT = 23.4 °C	Range =	23.2 - 23.6 °C	Feeding:	~3mg Tetrafin @ 1420	Initials/Date:	[illegible] 1/19/07
Day 7	Bath CT = 23.4 °C	Range =	23.2 - 24.0 °C	Feeding:	~3mg Tetrafin @ 1350	Initials/Date:	MH 1/20/07
Day 8	Bath CT = 23.4 °C	Range =	23.2 - 24.0 °C	Feeding:	~3mg Tetrafin @ 1320	Initials/Date:	TD 1/21/07
Day 9	Bath CT = 23.4 °C	Range =	23.2 - 24.0 °C	Feeding:	~3mg Tetrafin @ 1045	Initials/Date:	[illegible] 1/22/07
Day 10	Bath CT = 23.2 °C	Range =	23.0 - 23.6 °C	Feeding:	None	Initials/Date:	[illegible] 1/23/07

NINE TREATMENTS EIGHT REPLICATES
RANDOM CHAMBER LOCATION "Aeon"

QA: AR020707
DAP 2/12/07

Project No. 8503-128-019-086 to-093

	A	B	C	D	E	F	G	H
1	6E	3F	6H	4H	8F	5F	7D	7C
2	1D	3A	2D	5C	2E	2F	1B	2G
3	9E	5E	4C	8C	3E	5H	2H	1G
4	2A	5D	1A	7G	5G	8D	7E	1E
5	7A	4A	2C	1C	3D	4F	8A	5B
6	8E	1H	6G	4D	6D	1F	9A	7B
7	2B	6C	9H	6F	3B	3H	9G	7H
8	9C	9D	7F	6B	5A	6A	4G	4E
9	4B	8H	3G	3C	8B	9B	8G	9F

1 = Control
2 = SDTBKSTA
3 = M1
4 = M2
5 = M3
6 = SDT04DTA
7 = SDT07DTA
8 = 0SS ~~SDT~~13DTA
9 = SDT14DTA

① 02/13/07 LE

SEDIMENT/SOIL PREPARATION

QA: MR020107
DAP 3/12/07

Artificial soil						
Constituent/source						Amount added (units)
	SDT07-DTA		SDT BK-STA		Total	
M1 =	45g	+	1455g	=	1500g	
M2 =	195g	+	1305g	=	1500g	
M3 =	600g	+	900g	=	1500g	

Notes:

Soil/sediment	FCETL#	Homogenization			
		Date	From	To	Analyst
M1 (Mix 1)*	19381/19383	1/12/07	1319	1322	
M2 (Mix 2)	↓ / ↓	1/12/07	1334	1342	
M3 (Mix 3)	↓ / ↓	1/12/07	1355	1400	
SDT 04 DTA	19386	1/12/07	1417	1420	
SDT 14 DTA	19385	1/12/07	1439	1442	
SDT 07 DTA	19381	1/12/07	1451	1454	
SDT BKSTA*	19383	1/12/07	1506	1509	
~~SDT~~ SST 13 DTA	19994	1/12/07	1515	1518	

* Used all of sample, container disposed off
DAP for JN 2/3/07 E

Tadpole Sublethal Measurements

QA: AR02-0107
DAP 2/12/07

Test Number: 8503-128-019-086 to -093	Test Description: 10 day Chronic
Test Dates: 1/13/07 → 1/23/07	Test Species: Rana pipiens

Treat/ Concn	Rep	Body Width (mm)	Body Length (S-V lngth) (mm)	Eye Width (mm)	Comments
cont	A	6	8		
	A	6	9		
	A	5	7		
	A	6	8		
	A	6	9		
	B	6	8		
	B	6	8		
	B	6	9		
	B	5	8		
	B	5	8		
	C	5	8		
	C	5	8		
	C	5	7		
	C	5	7		
	C	5	7		
	D	6	8		
	D	6	8		
	D	6	8		
	D	6	8		
	D	6	8		
Date		1/23/07	1/23/07		
Time		1400	1400		
Inits		DP	DP		

QA: AR02-01-07
DAP 2/12/07

Tadpole Sublethal Measurements

Test Number:	8503-128-019-086 to 093	Test Description:	10 day Chronic
Test Dates:	1/13/07 - 1/23/07	Test Species:	Rana pipiens

Treat/ Concn	Rep	Body Width (mm)	Body Length (S-V lngth) (mm)	Eye Width (mm)	Comments
cont	E	5	8		
	E	5	8		
	E	6	8		
	E	5	8		
	E	5	7		
	F	6	8		
	F	6	8		
	F	6	9		
	F	5	8		
	F	6	9		
	G	6	9		
	G	6	8		
	G	5	7		
	G	6	9		
	G	—	—		
	H	6	8		
	H	5	8		
	H	6	8		
	H	6	9		
	H	—	—		
Date		1/23/07	1/23/07		
Time		1400	1400		
Inits		DP	DP		

Tadpole Sublethal Measurements

QA: AR02-01-07
DAP 2/12/07

Test Number:	6503-128-019-086 to 093	Test Description:	10 day Chronic
Test Dates:	1/13/07 → 1/23/07	Test Species:	Rana pipiens

Treat/ Concn	Rep	Body Width (mm)	Body Length (S-V lngth) (mm)	Eye Width (mm)	Comments
SDTBKSTA	A	5	8		
	A	6	9		
	A	6	9		
	A	5	8		
	A	6	8		
	B	6	9		
	B	6	9		
	B	6	9		
	B	6	8		
	B	5	8		
	C	5	8		
	C	5	8		
	C	6	8		
	C	6	8		
	C	6	8		
	D	6	8		
	D	6	8		
	D	6	8		
	D	4	7		
	D	—	—		
Date		1/23/07	1/23/07		
Time		1410	1410		
Inits		DP	DP		

Tadpole Sublethal Measurements

QA: AR020107
SAP 2/12/07

Test Number:	8503-128-019-086 to -093	Test Description:	10 day Chronic
Test Dates:	1/13/07 → 1/23/07	Test Species:	Rana pipiens

Treat/ Concn	Rep	Body Width (mm)	Body Length (S-V lngth) (mm)	Eye Width (mm)	Comments
SDT BKSTA	E	6	9		
	E	6	9		
	E	6	9		
	E	6	9		
	E	6	9		
	F	5	8		
	F	5	8		
	F	5	8		
	F	5	8		
	F	5	8		
	G	7	10		
	G	6	8		
	G	—	—		
	G	—	—		
	G	—	—		
	H	4	8		
	H	5	8		
	H	6	8		
	H	5	8		
	H	5	8		
Date		1/23/07	1/23/07		
Time		1415	1415		
Inits		[illegible]	[illegible]		

Tadpole Sublethal Measurements

QA: AR02-0107
DAP 2/12/07

Test Number:	8503-128-019-086 to -093	Test Description:	10 day Chronic
Test Dates:	1/13/07 → 1/23/07	Test Species:	Rana pipiens

Treat/ Concn	Rep	Body Width (mm)	Body Length (S-V lngth) (mm)	Eye Width (mm)	Comments
M1	A	5	7		
	A	5	7		
	A	5	7		
	A	5	7		
	A	4	7		
	B	6	8		
	B	5	8		
	B	6	9		
	B	5	8		
	B	6	8		
	C	6	7		
	C	5	8		
	C	6	8		
	C	6	8		
	C	5	7		
	D	6	8		
	D	6	8		
	D	6	9		
	D	6	9		
	D	—	—		
Date		1/23/07	1/23/07		
Time		1500	1500		
Inits		P	P		

Tadpole Sublethal Measurements

QA: NR02-0107
DAP 2/12/07

Test Number:	8583-128-019-086 to-093	Test Description:	10 day Chronic
Test Dates:	1/13/07 – 1/23/07	Test Species:	Rana pipiens

Treat/ Concn	Rep	Body Width (mm)	Body Length (S-V lngth) (mm)	Eye Width (mm)	Comments
M1	E	5	8		
	E	6	9		
	E	5	8		
	E	5	8		
	E	6	9		
	F	6	8		
	F	6	8		
	F	5	8		
	F	5	8		
	F	6	8		
	G	5	8		
	G	5	8		
	G	6	8		
	G	5	7		
	G	5	7		
	H	5	8		
	H	5	8		
	H	5	8		
	H	5	7		
	H	—	—		
Date		1/23/07	1/23/07		
Time		1510	1510		
Inits		DP	DP		

Tadpole Sublethal Measurements

QA: AR02-0107
JAP 2/12/07

Test Number:	8513-128-019-086 to-093	Test Description:	10 day Chronic
Test Dates:	1/13/07 → 1/23/07	Test Species:	Rana pipiens

Treat/ Concn	Rep	Body Width (mm)	Body Length (S-V lngth) (mm)	Eye Width (mm)	Comments
MZ	A	5	7		
	A	5	8		
	A	6	8		
	A	6	9		
	A	6	8		
	B	4	7		
	B	4	7		
	B	4	7		
	B	4	7		
	B	4	7		
	C	4	6		
	C	4	7		
	C	4	7		
	C	5	7		
	~~B~~ C	4	7		
	D	5	8		
	D	5	8		
	D	5	8		
	D	5	8		
	D	5	8		
Date		1/23/07	1/23/07		
Time		1530	1530		
Inits		JP	JP		

JP 1/23/07 WP

QA: AR02-01-07
DAP 2/12/07

Tadpole Sublethal Measurements

Test Number:	8503-128-019-086 to 093	Test Description:	10 day Chronic
Test Dates:	1/13/07 → 1/23/07	Test Species:	Rana pipiens

Treat/ Concn	Rep	Body Width (mm)	Body Length (S-V lngth) (mm)	Eye Width (mm)	Comments
MZ	E	5	8		
	E	5	7		
	E	5	7		
	E	4	6		
	E	4	6		
	F	6	8		
	F	5	8		
	F	5	8		
	F	5	8		
	F	5	8		
	G	5	8		
	G	5	8		
	G	6	9		
	G	6	8		
	G	5	8		
	H	7	11		
	H	7	10		
	H	6	9		
	H				
	H				
Date		1/23/07	1/23/07		
Time		1540	1540		
Inits		DP	DP		

Tadpole Sublethal Measurements

QA: AR02-0107
DAP 2/12/07

Test Number:	8503-128-019-08610-093	Test Description:	10 day Chronic
Test Dates:	1/13/07 → 1/23/07	Test Species:	Rana pipiens

Treat/ Concn	Rep	Body Width (mm)	Body Length (S-V lngth) (mm)	Eye Width (mm)	Comments
M3	A	—	—		
	A	—	—		
	A	—	—		
	A	—	—		
	A	—	—		
	B	6	10		
	B	—	—		
	B	—	—		
	B	—	—		
	B	—	—		
	C	5	8		
	C	5	8		
	C	5	7		
	C	4	7		
	C	5	7		
	D	7	10		
	D	—	—		
	D	—	—		
	D	—	—		
	D	—	—		
Date		1/23/07	1/23/07		
Time		1600	1600		
Inits		DP	DP		

Tadpole Sublethal Measurements

QA: AR02-01-07
DAP 2/12/07

Test Number:	8503-128-019-086 to 093	Test Description:	10 day Chronic
Test Dates:	1/13/07 → 1/23/07	Test Species:	Rana pipiens

Treat/ Concn	Rep	Body Width (mm)	Body Length (S-V lngth) (mm)	Eye Width (mm)	Comments
M3	E	6	9		
	E	6	8		
	E	6	8		
	E	6	8		
	E	—	—		
	F	6	8		
	F	6	8		
	F	6	8		
	F	4	7		
	F	5	8		
	G	5	7		
	G	6	7		
	G	4	7		
	G	5	8		
	G	5	8		
	H	5	7		
	H	5	7		
	H	6	8		
	H	5	8		
	H	—	—		
Date		1/23/07	1/23/07		
Time		1610	1610		
Inits		DP	DP		

Tadpole Sublethal Measurements

QA: AR02-0107
DAP 2/12/07

Test Number:	8503-129-019-086to-093	Test Description:	10 day Chronic
Test Dates:	1/13/07 → 1/23/07	Test Species:	Rana pipiens

Treat/ Concn	Rep	Body Width (mm)	Body Length (S-V lngth) (mm)	Eye Width (mm)	Comments
50to4 DTA	A	6	8		
	A	5	7		
	A	5	7		
	A	5	7		
	A	5	7		
	B	5	7		
	B	5	7		
	B	4	6		
	B	5	7		
	B	5	7		
	C	4	6		
	C	4	6		
	C	5	7		
	C	5	7		
	C	5	7		
	D	4	6		
	D	5	7		
	D	5	7		
	D	5	7		
	D	5	7		
Date		1/23/07	1/27/07		
Time		1620	1620		
Inits		[illegible]	[illegible]		

Tadpole Sublethal Measurements

QA: AR02-0107
DAP 2/12/07

Test Number:	8503-128-019-086 to 093	Test Description:	10 day Chronic
Test Dates:	1/13/07 → 1/23/07	Test Species:	Rana pipiens

Treat/ Concn	Rep	Body Width (mm)	Body Length (S-V lngth) (mm)	Eye Width (mm)	Comments
SDTCMDTA	E	5	8		
	E	5	7		
	E	5	7		
	E	5	6		
	E	5	8		
	F	5	8		
	F	5	7		
	F	5	7		
	F	5	7		
	F	5	7		
	G	5	7		
	G	5	8		
	G	5	7		
	G	5	8		
	G	5	7		
	H	6	8		
	H*	3	5		* orig had cracked tail
	H	5	8		
	H	6	9		
	H	5	9		
Date		1/23/07	1/23/07		
Time		1635	1635		
Inits		P	P		

QA: AR02-0107
DAP 2/12/07

Tadpole Sublethal Measurements

Test Number: 8503-128-019-086 to-093	Test Description: 10 day chronic
Test Dates: 1/13/07 - 1/23/07	Test Species: Rana pipiens

Treat/ Concn	Rep	Body Width (mm)	Body Length (S-V lngth) (mm)	Eye Width (mm)	Comments
07	A	6	9		
	A	6	8		
	A	6	8		
	A	—	—		
	A	—	—		
	B	~~6~~	~~9~~ ①		
	B	~~6~~	~~9~~ ①		
	B	~~6~~	~~9~~ ①		
	B	—	—		
	B	—	—		
	C	—	—		
	C	—	—		
	C	—	—		
	C	—	—		
	C	—	—		
	D	6	9		
	D	6	9		
	D	6	9		
	D	—	—		
	D	—	—		
Date		1/23/07	1/23/07		
Time		1615	1615		
Inits		RT	RT		

① RT, 1/23/07, E

Tadpole Sublethal Measurements

QA: ARDZ-01-07
DAP 2/12/07

Test Number: 8503-128-019-086 to-093	Test Description: 16 day chronic
Test Dates: 1/13/07 → 1/23/07	Test Species: Rana pipiens

Treat/ Concn	Rep	Body Width (mm)	Body Length (S-V lngth) (mm)	Eye Width (mm)	Comments
07	E	—	—		
	E	—	—		
	E	—	—		
	E	—	—		
	E	—	—		
	F	6	9		
	F	6	9		
	F	6	9		
	F	6	8		
	F	6	8		
	G	—	—		
	G	6	9		
	G	6	8		
	G	6	8		
	G	6	9		
	H	—	—		
	H	—	—		
	H	—	—		
	H	—	—		
	A	9	12		
Date		1/23/07	1/23/07		
Time		1615	1615		
Inits		RT	RT		

Tadpole Sublethal Measurements

QA: NR02-0107
DAC 2/12/07

Test Number: ES03-128-019 -088 to -093	Test Description: 10 day chronic
Test Dates: 1/13/07 → 1/23/07	Test Species: Rana pipiens

Treat/ Concn	Rep	Body Width (mm)	Body Length (S-V lngth) (mm)	Eye Width (mm)	Comments
13	A	7	9		
	A	6	8		
	A	5	8		
	A	5	7		
	A	6	9		
	B	5	6		
	B	5	8		
	B	5	8		
	B	5	7		
	B	5	7		
	C	6	8		
	C	6	7		
	C	5	7		
	C	6	9		
	C	—	—		
	D	6	7		
	D*	3	5		* crooked tail on organism
	D	5	8		
	D	6	8		
	D	6	7		
Date		1/23/07	1/23/07		
Time		1515	1515		
Inits		RT	RT		

Tadpole Sublethal Measurements

QA: AK 02-01-07
DAP 2/12/07

Test Number: 8503-128-019 → 086 to -093	Test Description: 10 day chronic
Test Dates: 1/13/07 → 1/23/07	Test Species: Rana Pipiens

Treat/ Concn	Rep	Body Width (mm)	Body Length (S-V lngth) (mm)	Eye Width (mm)	Comments
13	E	5	7		
	E	5	7		
	E	4	6		
	E	5	7		
	E	5	7		
	F	7	9		
	F	5	7		
	F	6	8		
	F	5	8		
	F	—	—		
	G	5	7		
	G*	4	5		* organism had curved tail
	G	5	8		
	G	6	8		
	G	5	8		
	H	5	8		
	H	5	7		
	H	5	5		
	H	4	7		
	H	5	7		
Date		1/23/07	1/23/07		
Time		1515	1515		
Inits		RT	RT		

DAP 2/12/07
QA: RR02-01-07

Tadpole Sublethal Measurements

Test Number: BS03-128-019-086 to 093	Test Description: 10 day chronic
Test Dates: 1/13/07 → 1/23/07	Test Species: Rana pipiens

Treat/ Concn	Rep	Body Width (mm)	Body Length (S-V lngth) (mm)	Eye Width (mm)	Comments
14	A	5	7		
	A	5	8		
	A	5	8		
	A	5	8		
	A	5	7		
	B	5	7		
	B	5	7		
	B	5	7		
	B	5	6		
	B	5	7		
	C	5	8		
	C	6	8		
	C	5	7		
	C	5	8		
	C	5	7		
	D	6	9		
	D	3	4		
	D	6	8		
	D	5	7		
	D	6	8		
Date		1/23/07	1/23/07		
Time		1415	1415		
Inits		RT	RT		

Tadpole Sublethal Measurements

QA: N202-0107
DAP 2/12/07

Test Number: 8503-128-019	Test Description: 10 day chronic
Test Dates: 1/13/07 → 1/23/07	Test Species: Rana pipiens

Treat/ Concn	Rep	Body Width (mm)	Body Length (S-V lngth) (mm)	Eye Width (mm)	Comments
14	E	5	7		
	E	6	8		
	E	6	8		
	E	4	6		
	E	5	7		
	F	6	8		
	F	6	8		
	F	5	7		
	F	① ~~7~~ 5	7		
	F	6	8		
	G	5	6		
	G	6	7		
	G	6	9		
	G	5	8		
	G	5	8		
	H	5	6		
	H	5	7		
	H	6	8		
	H	5	7		
	H	5	7		
Date		1/23/07	1/23/07		
Time		1445	1445		
Inits		RT	RT		

① RT, 1/23/07, E

QA: MC02-01-07
DAP 2/12/07

08503-128-019-086-093 Rana pipiens Survival and Sublethal Results

Sediment	Replicate	Survival	Body Width	Body Length	Mean Survival	Replicate Mean Width per Original	Replicate Mean Length per Original
Control	A	1	6	8	100	5.8	8.2
Control	A	1	6	9			
Control	A	1	5	7			
Control	A	1	6	8			
Control	A	1	6	9			
Control	B	1	6	8	100	5.6	8.2
Control	B	1	6	8			
Control	B	1	6	9			
Control	B	1	5	8			
Control	B	1	5	8			
Control	C	1	5	8	100	5	7.4
Control	C	1	5	8			
Control	C	1	5	7			
Control	C	1	5	7			
Control	C	1	5	7			
Control	D	1	6	8	100	6	8
Control	D	1	6	8			
Control	D	1	6	8			
Control	D	1	6	8			
Control	D	1	6	8			
Control	E	1	5	8	100	5.2	7.8
Control	E	1	5	8			
Control	E	1	6	8			
Control	E	1	5	8			
Control	E	1	5	7			
Control	F	1	6	8	100	5.8	8.4
Control	F	1	6	8			
Control	F	1	6	9			
Control	F	1	5	8			
Control	F	1	6	9			
Control	G	1	6	9	80	4.6	6.6
Control	G	1	6	8			
Control	G	1	5	7			
Control	G	1	6	9			
Control	G	0	0	0			
Control	H	1	6	8	80	4.6	6.4
Control	H	1	5	8			
Control	H	1	6	8			
Control	H	1	6	8			
Control	H	0	0	0			
SDTBKSTA	A	1	5	8	100	5.6	8.4

QA: AR020107
DAP 2/12/07

08503-128-019-086-093 Rana pipiens Survival and Sublethal Results

Sediment	Replicate	Survival	Body Width	Body Length	Mean Survival	Replicate Mean Width per Original	Replicate Mean Length per Original
SDTBKSTA	A	1	6	9			
SDTBKSTA	A	1	6	9			
SDTBKSTA	A	1	5	8			
SDTBKSTA	A	1	6	8			
SDTBKSTA	B	1	6	9	100	5.8	8.6
SDTBKSTA	B	1	6	9			
SDTBKSTA	B	1	6	9			
SDTBKSTA	B	1	6	8			
SDTBKSTA	B	1	5	8			
SDTBKSTA	C	1	5	8	100	5.6	8
SDTBKSTA	C	1	5	8			
SDTBKSTA	C	1	6	8			
SDTBKSTA	C	1	6	8			
SDTBKSTA	C	1	6	8			
SDTBKSTA	D	1	6	8	80	4.4	6.2
SDTBKSTA	D	1	6	8			
SDTBKSTA	D	1	6	8			
SDTBKSTA	D	1	4	7			
SDTBKSTA	D	0	0	0			
SDTBKSTA	E	1	6	9	100	6	9
SDTBKSTA	E	1	6	9			
SDTBKSTA	E	1	6	9			
SDTBKSTA	E	1	6	9			
SDTBKSTA	E	1	6	9			
SDTBKSTA	F	1	5	8	100	5	8
SDTBKSTA	F	1	5	8			
SDTBKSTA	F	1	5	8			
SDTBKSTA	F	1	5	8			
SDTBKSTA	F	1	5	8			
SDTBKSTA	G	1	7	10	40	2.6	3.6
SDTBKSTA	G	1	6	8			
SDTBKSTA	G	0	0	0			
SDTBKSTA	G	0	0	0			
SDTBKSTA	G	0	0	0			
SDTBKSTA	H	1	4	8	100	5	8
SDTBKSTA	H	1	5	8			
SDTBKSTA	H	1	6	8			
SDTBKSTA	H	1	5	8			
SDTBKSTA	H	1	5	8			
M1	A	1	5	7	100	4.8	7
M1	A	1	5	7			

QA: N202-05-07
DAP 3/12/07

08503-128-019-086-093 Rana pipiens Survival and Sublethal Results

Sediment	Replicate	Survival	Body Width	Body Length	Mean Survival	Replicate Mean Width per Original	Replicate Mean Length per Original
M1	A	1	5	7			
M1	A	1	5	7			
M1	A	1	4	7			
M1	B	1	6	8	100	5.6	8.2
M1	B	1	5	8			
M1	B	1	6	9			
M1	B	1	5	8			
M1	B	1	6	8			
M1	C	1	6	7	100	5.6	7.6
M1	C	1	5	8			
M1	C	1	6	8			
M1	C	1	6	8			
M1	C	1	5	7			
M1	D	1	6	8	80	4.8	6.8
M1	D	1	6	8			
M1	D	1	6	9			
M1	D	1	6	9			
M1	D	0	0	0			
M1	E	1	5	8	100	5.4	8.4
M1	E	1	6	9			
M1	E	1	5	8			
M1	E	1	5	8			
M1	E	1	6	9			
M1	F	1	6	8	100	5.6	8
M1	F	1	6	8			
M1	F	1	5	8			
M1	F	1	5	8			
M1	F	1	6	8			
M1	G	1	5	8	100	5.2	7.6
M1	G	1	5	8			
M1	G	1	6	8			
M1	G	1	5	7			
M1	G	1	5	7			
M1	H	1	5	8	80	4	6.2
M1	H	1	5	8			
M1	H	1	5	8			
M1	H	1	5	7			
M1	H	0	0	0			
M2	A	1	5	7	100	5.6	8
M2	A	1	5	8			
M2	A	1	6	8			

QA: AR02-01-07
DAP 2/12/07

08503-128-019-086-093 Rana pipiens Survival and Sublethal Results

Sediment	Replicate	Survival	Body Width	Body Length	Mean Survival	Replicate Mean Width per Original	Replicate Mean Length per Original
M2	A	1	6	9			
M2	A	1	6	8			
M2	B	1	4	7	100	4	7
M2	B	1	4	7			
M2	B	1	4	7			
M2	B	1	4	7			
M2	B	1	4	7			
M2	C	1	4	6	100	4.2	6.8
M2	C	1	4	7			
M2	C	1	4	7			
M2	C	1	5	7			
M2	C	1	4	7			
M2	D	1	5	8	100	5	8
M2	D	1	5	8			
M2	D	1	5	8			
M2	D	1	5	8			
M2	D	1	5	8			
M2	E	1	5	8	100	4.6	6.8
M2	E	1	5	7			
M2	E	1	5	7			
M2	E	1	4	6			
M2	E	1	4	6			
M2	F	1	6	8	100	5.2	8
M2	F	1	5	8			
M2	F	1	5	8			
M2	F	1	5	8			
M2	F	1	5	8			
M2	G	1	5	8	100	5.4	8.2
M2	G	1	5	8			
M2	G	1	6	9			
M2	G	1	6	8			
M2	G	1	5	8			
M2	H	1	7	11	60	4	6
M2	H	1	7	10			
M2	H	1	6	9			
M2	H	0	0	0			
M2	H	0	0	0			
M3	A	0	0	0	0	0	0
M3	A	0	0	0			
M3	A	0	0	0			
M3	A	0	0	0			

QA: AR02-01-07

DAP 2/12/07

08503-128-019-086-093 Rana pipiens Survival and Sublethal Results

Sediment	Replicate	Survival	Body Width	Body Length	Mean Survival	Replicate Mean Width per Original	Replicate Mean Length per Original
M3	A	0	0	0			
M3	B	1	6	10	20	1.2	2
M3	B	0	0	0			
M3	B	0	0	0			
M3	B	0	0	0			
M3	B	0	0	0			
M3	C	1	5	8	100	4.8	7.4
M3	C	1	5	8			
M3	C	1	5	7			
M3	C	1	4	7			
M3	C	1	5	7			
M3	D	1	7	10	20	1.4	2
M3	D	0	0	0			
M3	D	0	0	0			
M3	D	0	0	0			
M3	D	0	0	0			
M3	E	1	6	9	80	4.8	6.6
M3	E	1	6	8			
M3	E	1	6	8			
M3	E	1	6	8			
M3	E	0	0	0			
M3	F	1	6	8	100	5.4	7.8
M3	F	1	6	8			
M3	F	1	6	8			
M3	F	1	4	7			
M3	F	1	5	8			
M3	G	1	5	7	100	5	7.4
M3	G	1	6	7			
M3	G	1	4	7			
M3	G	1	5	8			
M3	G	1	5	8			
M3	H	1	5	7	80	4.2	6
M3	H	1	5	7			
M3	H	1	6	8			
M3	H	1	5	8			
M3	H	0	0	0			
SDT04DTA	A	1	6	8	100	5.2	7.2
SDT04DTA	A	1	5	7			
SDT04DTA	A	1	5	7			
SDT04DTA	A	1	5	7			
SDT04DTA	A	1	5	7			

OA: ARD2-01-07
DAP 2/12/07

08503-128-019-086-093 Rana pipiens Survival and Sublethal Results

Sediment	Replicate	Survival	Body Width	Body Length	Mean Survival	Replicate Mean Width per Original	Replicate Mean Length per Original
SDT04DTA	B	1	5	7	100	4.8	6.8
SDT04DTA	B	1	5	7			
SDT04DTA	B	1	4	6			
SDT04DTA	B	1	5	7			
SDT04DTA	B	1	5	7			
SDT04DTA	C	1	4	6	100	4.6	6.6
SDT04DTA	C	1	4	6			
SDT04DTA	C	1	5	7			
SDT04DTA	C	1	5	7			
SDT04DTA	C	1	5	7			
SDT04DTA	D	1	4	6	100	4.8	6.8
SDT04DTA	D	1	5	7			
SDT04DTA	D	1	5	7			
SDT04DTA	D	1	5	7			
SDT04DTA	D	1	5	7			
SDT04DTA	E	1	5	8	100	5	7.2
SDT04DTA	E	1	5	7			
SDT04DTA	E	1	5	7			
SDT04DTA	E	1	5	6			
SDT04DTA	E	1	5	8			
SDT04DTA	F	1	5	8	100	5	7.2
SDT04DTA	F	1	5	7			
SDT04DTA	F	1	5	7			
SDT04DTA	F	1	5	7			
SDT04DTA	F	1	5	7			
SDT04DTA	G	1	5	7	100	5	7.4
SDT04DTA	G	1	5	8			
SDT04DTA	G	1	5	7			
SDT04DTA	G	1	5	8			
SDT04DTA	G	1	5	7			
SDT04DTA	H	1	6	8	100	5	7.8
SDT04DTA	H	1	3	5			
SDT04DTA	H	1	5	8			
SDT04DTA	H	1	6	9			
SDT04DTA	H	1	5	9			
SDT07DTA	A	1	6	9	60	3.6	5
SDT07DTA	A	1	6	8			
SDT07DTA	A	1	6	8			
SDT07DTA	A	0	0	0			
SDT07DTA	A	0	0	0			
SDT07DTA	B	0	0	0	0	0	0

QA: AR02-05-07
DAP 2/12/07

08503-128-019-086-093 Rana pipiens Survival and Sublethal Results

Sediment	Replicate	Survival	Body Width	Body Length	Mean Survival	Replicate Mean Width per Original	Replicate Mean Length per Original
SDT07DTA	B	0	0	0			
SDT07DTA	B	0	0	0			
SDT07DTA	B	0	0	0			
SDT07DTA	B	0	0	0			
SDT07DTA	C	0	0	0	0	0	0
SDT07DTA	C	0	0	0			
SDT07DTA	C	0	0	0			
SDT07DTA	C	0	0	0			
SDT07DTA	C	0	0	0			
SDT07DTA	D	1	6	9	60	3.6	5.4
SDT07DTA	D	1	6	9			
SDT07DTA	D	1	6	9			
SDT07DTA	D	0	0	0			
SDT07DTA	D	0	0	0			
SDT07DTA	E	0	0	0	0	0	0
SDT07DTA	E	0	0	0			
SDT07DTA	E	0	0	0			
SDT07DTA	E	0	0	0			
SDT07DTA	E	0	0	0			
SDT07DTA	F	1	6	9	100	6	8.6
SDT07DTA	F	1	6	9			
SDT07DTA	F	1	6	9			
SDT07DTA	F	1	6	8			
SDT07DTA	F	1	6	8			
SDT07DTA	G	1	6	9	80	4.8	6.8
SDT07DTA	G	1	6	8			
SDT07DTA	G	1	6	8			
SDT07DTA	G	1	6	9			
SDT07DTA	G	0	0	0			
SDT07DTA	H	1	9	12	20	1.8	2.4
SDT07DTA	H	0	0	0			
SDT07DTA	H	0	0	0			
SDT07DTA	H	0	0	0			
SDT07DTA	H	0	0	0			
SST13DTA	A	1	7	9	100	5.8	8.2
SST13DTA	A	1	6	8			
SST13DTA	A	1	5	8			
SST13DTA	A	1	5	7			
SST13DTA	A	1	6	9			
SST13DTA	B	1	5	6	100	5	7.2
SST13DTA	B	1	5	8			

QA: AR02-0507
DAP 2/12/07

08503-128-019-086-093 Rana pipiens Survival and Sublethal Results

Sediment	Replicate	Survival	Body Width	Body Length	Mean Survival	Replicate Mean Width per Original	Replicate Mean Length per Original
SST13DTA	B	1	5	8			
SST13DTA	B	1	5	7			
SST13DTA	B	1	5	7			
SST13DTA	C	1	6	8	80	4.6	6.2
SST13DTA	C	1	6	7			
SST13DTA	C	1	5	7			
SST13DTA	C	1	6	9			
SST13DTA	C	0	0	0			
SST13DTA	D	1	6	7	100	5.2	7
SST13DTA	D	1	3	5			
SST13DTA	D	1	5	8			
SST13DTA	D	1	6	8			
SST13DTA	D	1	6	7			
SST13DTA	E	1	5	7	100	4.8	6.8
SST13DTA	E	1	5	7			
SST13DTA	E	1	4	6			
SST13DTA	E	1	5	7			
SST13DTA	E	1	5	7			
SST13DTA	F	1	7	9	80	4.6	6.4
SST13DTA	F	1	5	7			
SST13DTA	F	1	6	8			
SST13DTA	F	1	5	8			
SST13DTA	F	0	0	0			
SST13DTA	G	1	5	7	100	5	7.2
SST13DTA	G	1	4	5			
SST13DTA	G	1	5	8			
SST13DTA	G	1	6	8			
SST13DTA	G	1	5	8			
SST13DTA	H	1	5	8	100	4.8	6.8
SST13DTA	H	1	5	7			
SST13DTA	H	1	5	5			
SST13DTA	H	1	4	7			
SST13DTA	H	1	5	7			
SDT14DTA	A	1	5	7	100	5	7.6
SDT14DTA	A	1	5	8			
SDT14DTA	A	1	5	8			
SDT14DTA	A	1	5	8			
SDT14DTA	A	1	5	7			
SDT14DTA	B	1	5	7	100	5	6.8
SDT14DTA	B	1	5	7			
SDT14DTA	B	1	5	7			

QA: ARO2-01-07
DAP 2/12/07

08503-128-019-086-093 Rana pipiens Survival and Sublethal Results

Sediment	Replicate	Survival	Body Width	Body Length	Mean Survival	Replicate Mean Width per Original	Replicate Mean Length per Original
SDT14DTA	B	1	5	6			
SDT14DTA	B	1	5	7			
SDT14DTA	C	1	5	8	100	5.2	7.6
SDT14DTA	C	1	6	8			
SDT14DTA	C	1	5	7			
SDT14DTA	C	1	5	8			
SDT14DTA	C	1	5	7			
SDT14DTA	D	1	6	9	100	5.2	7.2
SDT14DTA	D	1	3	4			
SDT14DTA	D	1	6	8			
SDT14DTA	D	1	5	7			
SDT14DTA	D	1	6	8			
SDT14DTA	E	1	5	7	100	5.2	7.2
SDT14DTA	E	1	6	8			
SDT14DTA	E	1	6	8			
SDT14DTA	E	1	4	6			
SDT14DTA	E	1	5	7			
SDT14DTA	F	1	6	8	100	5.6	7.6
SDT14DTA	F	1	6	8			
SDT14DTA	F	1	5	7			
SDT14DTA	F	1	5	7			
SDT14DTA	F	1	6	8			
SDT14DTA	G	1	5	6	100	5.4	7.6
SDT14DTA	G	1	6	7			
SDT14DTA	G	1	6	9			
SDT14DTA	G	1	5	8			
SDT14DTA	G	1	5	8			
SDT14DTA	H	1	5	6	100	5.2	7
SDT14DTA	H	1	5	7			
SDT14DTA	H	1	6	8			
SDT14DTA	H	1	5	7			
SDT14DTA	H	1	5	7			

8503-128-019-086-093
Rana pipiens
Background vs Control Length

DA: A1202-01-07
DAP 2/12/07

Title: 8503-128-019-086-093 Background vs Control Length
File: 086lcb Transform: NO TRANSFORMATION
Number of Groups: 2

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	8.2000	8.2000
1	Control	2	8.2000	8.2000
1	Control	3	7.4000	7.4000
1	Control	4	8.0000	8.0000
1	Control	5	7.8000	7.8000
1	Control	6	8.4000	8.4000
1	Control	7	6.6000	6.6000
1	Control	8	6.4000	6.4000
2	Background	1	8.4000	8.4000
2	Background	2	8.6000	8.6000
2	Background	3	8.0000	8.0000
2	Background	4	6.2000	6.2000
2	Background	5	9.0000	9.0000
2	Background	6	8.0000	8.0000
2	Background	7	3.6000	3.6000
2	Background	8	8.0000	8.0000

Title: 8503-128-019-086-093 Background vs Control Length
File: 086lcb Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Control	8	6.4000	8.4000	7.6250
2	Background	8	3.6000	9.0000	7.4750

8503-128-019-086-093
Rana pipiens
Background vs Control Length

QA: NR02-0107
DAP 2/12/07

Title: 8503-128-019-086-093 Background vs Control Length
File: 086lcb Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	Control	0.5764	0.7592	0.2684	9.9571
2	Background	3.1307	1.7694	0.6256	23.6707

8503-128-019-086-093
Rana pipiens
Background vs Control Length

QA: NR02-0107
DAP 2/12/07

Title: 8503-128-019-086-093 Background vs Control Length
File: 086lcb Transform: NO TRANSFORMATION

Shapiro - Wilk's Test for Normality

D = 25.9500
W = 0.8068

Critical W = 0.8440 (alpha = 0.01 , N = 16)
W = 0.8870 (alpha = 0.05 , N = 16)

Data FAIL normality test (alpha = 0.01). Try another transformation.

Warning - The F-test of homogeneity is sensitive to non-normality and should not be performed with this data as is.

Title: 8503-128-019-086-093 Background vs Control Length
File: 086lcb Transform: NO TRANSFORMATION

Wilcoxon's Rank Sum Test w/ Bonferroni Adjustment Ho: Control<Treatment

GROUP	IDENTIFICATION	MEAN IN ORIGINAL UNITS	RANK SUM	CRIT. VALUE	REPS	SIG 0.05
1	Control	7.6250				
2	Background	7.4750	73.00	51	8	

Critical values are 1 tailed (k = 1)

8503-128-019-086-093
Rana pipiens
Poudre River Sediment as Control, Survival

QA: ACO20107
DAP 2/12/07

Title: 8503-128-019-086-093 Survival vs Control
File: 086surc Transform: NO TRANSFORMATION
Number of Groups: 8

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	1.0000	1.0000
1	Control	2	1.0000	1.0000
1	Control	3	1.0000	1.0000
1	Control	4	1.0000	1.0000
1	Control	5	1.0000	1.0000
1	Control	6	1.0000	1.0000
1	Control	7	0.8000	0.8000
1	Control	8	0.8000	0.8000
2	M1	1	1.0000	1.0000
2	M1	2	1.0000	1.0000
2	M1	3	1.0000	1.0000
2	M1	4	0.8000	0.8000
2	M1	5	1.0000	1.0000
2	M1	6	1.0000	1.0000
2	M1	7	1.0000	1.0000
2	M1	8	0.8000	0.8000
3	M2	1	1.0000	1.0000
3	M2	2	1.0000	1.0000
3	M2	3	1.0000	1.0000
3	M2	4	1.0000	1.0000
3	M2	5	1.0000	1.0000
3	M2	6	1.0000	1.0000
3	M2	7	1.0000	1.0000
3	M2	8	0.6000	0.6000
4	M3	1	0.0000	0.0000
4	M3	2	0.2000	0.2000
4	M3	3	1.0000	1.0000
4	M3	4	0.2000	0.2000
4	M3	5	0.8000	0.8000
4	M3	6	1.0000	1.0000
4	M3	7	1.0000	1.0000
4	M3	8	0.8000	0.8000
5	SDT04DTA	1	1.0000	1.0000
5	SDT04DTA	2	1.0000	1.0000
5	SDT04DTA	3	1.0000	1.0000

an: AR 02-01-07
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Rana pipiens
Poudre River Sediment as Control, Survival

5	SDT04DTA	4	1.0000	1.0000
5	SDT04DTA	5	1.0000	1.0000
5	SDT04DTA	6	1.0000	1.0000
5	SDT04DTA	7	1.0000	1.0000
5	SDT04DTA	8	1.0000	1.0000
6	SDT07DTA	1	0.6000	0.6000
6	SDT07DTA	2	0.0000	0.0000
6	SDT07DTA	3	0.0000	0.0000
6	SDT07DTA	4	0.6000	0.6000
6	SDT07DTA	5	0.0000	0.0000
6	SDT07DTA	6	1.0000	1.0000
6	SDT07DTA	7	0.8000	0.8000
6	SDT07DTA	8	0.2000	0.2000
7	SDT13DTA	1	1.0000	1.0000
7	SDT13DTA	2	1.0000	1.0000
7	SDT13DTA	3	0.8000	0.8000
7	SDT13DTA	4	1.0000	1.0000
7	SDT13DTA	5	1.0000	1.0000
7	SDT13DTA	6	0.8000	0.8000
7	SDT13DTA	7	1.0000	1.0000
7	SDT13DTA	8	1.0000	1.0000
8	SDT14DTA	1	1.0000	1.0000
8	SDT14DTA	2	1.0000	1.0000
8	SDT14DTA	3	1.0000	1.0000
8	SDT14DTA	4	1.0000	1.0000
8	SDT14DTA	5	1.0000	1.0000
8	SDT14DTA	6	1.0000	1.0000
8	SDT14DTA	7	1.0000	1.0000
8	SDT14DTA	8	1.0000	1.0000

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Rana pipiens
Poudre River Sediment as Control, Survival

QA: AR02-01-07
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Title: 8503-128-019-086-093 Survival vs Control
File: 086surc Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Control	8	0.8000	1.0000	0.9500
2	M1	8	0.8000	1.0000	0.9500
3	M2	8	0.6000	1.0000	0.9500
4	M3	8	0.0000	1.0000	0.6250
5	SDT04DTA	8	1.0000	1.0000	1.0000
6	SDT07DTA	8	0.0000	1.0000	0.4000
7	SDT13DTA	8	0.8000	1.0000	0.9500
8	SDT14DTA	8	1.0000	1.0000	1.0000

Title: 8503-128-019-086-093 Survival vs Control
File: 086surc Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	Control	0.0086	0.0926	0.0327	9.7455
2	M1	0.0086	0.0926	0.0327	9.7455
3	M2	0.0200	0.1414	0.0500	14.8865
4	M3	0.1764	0.4200	0.1485	67.2054
5	SDT04DTA	0.0000	0.0000	0.0000	0.0000
6	SDT07DTA	0.1600	0.4000	0.1414	100.0000
7	SDT13DTA	0.0086	0.0926	0.0327	9.7455
8	SDT14DTA	0.0000	0.0000	0.0000	0.0000

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Rana pipiens
Poudre River Sediment as Control, Survival

QA: AR02-01-07
DAP 2/12/07

Title: 8503-128-019-086-093 Survival vs Control
File: 086surc Transform: ARC SINE(SQUARE ROOT(Y))

Chi-Square Test for Normality

Actual and Expected Frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	4.2880	15.4880	24.4480	15.4880	4.2880
OBSERVED	8	5	28	22	1

Chi-Square = 16.0908 (p-value = 0.0029)

Critical Chi-Square = 13.277 (alpha = 0.01 , df = 4)
= 9.488 (alpha = 0.05 , df = 4)

Data FAIL normality test (alpha = 0.01). Try another transformation.

Warning - The first three homogeneity tests are sensitive to non-normality and should not be performed with this data as is.

Title: 8503-128-019-086-093 Survival vs Control
File: 086surc Transform: ARC SINE(SQUARE ROOT(Y))

Hartley's Test for Homogeneity of Variance
Bartlett's Test for Homogeneity of Variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

8503-128-019-086-093
Rana pipiens
Poudre River Sediment as Control,Survival

QA: AR02-01-07
DAP 2/12/07

Title: 8503-128-019-086-093 Survival vs Control
File: 086surc Transform: ARC SINE(SQUARE ROOT(Y))

Steel's Many-One Rank Test - Ho: Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	DF	SIG 0.05
1	Control	1.2858				
2	M1	1.2858	68.00	45.00	8.00	
3	M2	1.2879	71.00	45.00	8.00	
4	M3	0.9254	53.00	45.00	8.00	
5	SDT04DTA	1.3453	76.00	45.00	8.00	
6	SDT07DTA	0.6706	42.00	45.00	8.00	*
7	SDT13DTA	1.2858	68.00	45.00	8.00	
8	SDT14DTA	1.3453	76.00	45.00	8.00	

Critical values are 1 tailed (k = 7)

8503-128-019-086-093
Rana pipiens
Background as Control, Survival

QA: NR02-0107
DAP 2/12/07

Title: 8503-128-019-086-093 Survival vs Background
File: 086surb Transform: NO TRANSFORMATION
Number of Groups: 8

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Background	1	1.0000	1.0000
1	Background	2	1.0000	1.0000
1	Background	3	1.0000	1.0000
1	Background	4	0.8000	0.8000
1	Background	5	1.0000	1.0000
1	Background	6	1.0000	1.0000
1	Background	7	0.4000	0.4000
1	Background	8	1.0000	1.0000
2	M1	1	1.0000	1.0000
2	M1	2	1.0000	1.0000
2	M1	3	1.0000	1.0000
2	M1	4	0.8000	0.8000
2	M1	5	1.0000	1.0000
2	M1	6	1.0000	1.0000
2	M1	7	1.0000	1.0000
2	M1	8	0.8000	0.8000
3	M2	1	1.0000	1.0000
3	M2	2	1.0000	1.0000
3	M2	3	1.0000	1.0000
3	M2	4	1.0000	1.0000
3	M2	5	1.0000	1.0000
3	M2	6	1.0000	1.0000
3	M2	7	1.0000	1.0000
3	M2	8	0.6000	0.6000
4	M3	1	0.0000	0.0000
4	M3	2	0.2000	0.2000
4	M3	3	1.0000	1.0000
4	M3	4	0.2000	0.2000
4	M3	5	0.8000	0.8000
4	M3	6	1.0000	1.0000
4	M3	7	1.0000	1.0000
4	M3	8	0.8000	0.8000
5	SDT04DTA	1	1.0000	1.0000
5	SDT04DTA	2	1.0000	1.0000
5	SDT04DTA	3	1.0000	1.0000
5	SDT04DTA	4	1.0000	1.0000

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Rana pipiens
Background as Control, Survival

QA: MR 02-01-07
DAP 2/12/07

5	SDT04DTA	5	1.0000	1.0000
5	SDT04DTA	6	1.0000	1.0000
5	SDT04DTA	7	1.0000	1.0000
5	SDT04DTA	8	1.0000	1.0000
6	SDT07DTA	1	0.6000	0.6000
6	SDT07DTA	2	0.0000	0.0000
6	SDT07DTA	3	0.0000	0.0000
6	SDT07DTA	4	0.6000	0.6000
6	SDT07DTA	5	0.0000	0.0000
6	SDT07DTA	6	1.0000	1.0000
6	SDT07DTA	7	0.8000	0.8000
6	SDT07DTA	8	0.2000	0.2000
7	SDT13DTA	1	1.0000	1.0000
7	SDT13DTA	2	1.0000	1.0000
7	SDT13DTA	3	0.8000	0.8000
7	SDT13DTA	4	1.0000	1.0000
7	SDT13DTA	5	1.0000	1.0000
7	SDT13DTA	6	0.8000	0.8000
7	SDT13DTA	7	1.0000	1.0000
7	SDT13DTA	8	1.0000	1.0000
8	SDT14DTA	1	1.0000	1.0000
8	SDT14DTA	2	1.0000	1.0000
8	SDT14DTA	3	1.0000	1.0000
8	SDT14DTA	4	1.0000	1.0000
8	SDT14DTA	5	1.0000	1.0000
8	SDT14DTA	6	1.0000	1.0000
8	SDT14DTA	7	1.0000	1.0000
8	SDT14DTA	8	1.0000	1.0000

8503-128-019-086-093
Rana pipiens
Background as Control, Survival

QA: AR02-01-07
DAP 2/12/07

Title: 8503-128-019-086-093 Survival vs Background
File: 086surb Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Background	8	0.4000	1.0000	0.9000
2	M1	8	0.8000	1.0000	0.9500
3	M2	8	0.6000	1.0000	0.9500
4	M3	8	0.0000	1.0000	0.6250
5	SDT04DTA	8	1.0000	1.0000	1.0000
6	SDT07DTA	8	0.0000	1.0000	0.4000
7	SDT13DTA	8	0.8000	1.0000	0.9500
8	SDT14DTA	8	1.0000	1.0000	1.0000

Title: 8503-128-019-086-093 Survival vs Background
File: 086surb Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	Background	0.0457	0.2138	0.0756	23.7566
2	M1	0.0086	0.0926	0.0327	9.7455
3	M2	0.0200	0.1414	0.0500	14.8865
4	M3	0.1764	0.4200	0.1485	67.2054
5	SDT04DTA	0.0000	0.0000	0.0000	0.0000
6	SDT07DTA	0.1600	0.4000	0.1414	100.0000
7	SDT13DTA	0.0086	0.0926	0.0327	9.7455
8	SDT14DTA	0.0000	0.0000	0.0000	0.0000

8503-128-019-086-093
Rana pipiens
Background as Control, Survival

QA: MR 02-01-07
DAP 2/12/07

Title: 8503-128-019-086-093 Survival vs Background
File: 086surb Transform: ARC SINE(SQUARE ROOT(Y))

Chi-Square Test for Normality

Actual and Expected Frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	4.2880	15.4880	24.4480	15.4880	4.2880
OBSERVED	7	6	34	16	1

Chi-Square = 13.7978 (p-value = 0.0080)

Critical Chi-Square = 13.277 (alpha = 0.01 , df = 4)
= 9.488 (alpha = 0.05 , df = 4)

Data FAIL normality test (alpha = 0.01). Try another transformation.

Warning - The first three homogeneity tests are sensitive to non-normality and should not be performed with this data as is.

Title: 8503-128-019-086-093 Survival vs Background
File: 086surb Transform: ARC SINE(SQUARE ROOT(Y))

Hartley's Test for Homogeneity of Variance
Bartlett's Test for Homogeneity of Variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

8503-128-019-086-093
Rana pipiens
Background as Control, Survival

AR: AR02-01-07
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Title: 8503-128-019-086-093 Survival vs Background
File: 086surb Transform: ARC SINE(SQUARE ROOT(Y))

Steel's Many-One Rank Test - Ho: Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	DF	SIG 0.05
1	Background	1.2329				
2	M1	1.2858	69.00	45.00	8.00	
3	M2	1.2879	72.00	45.00	8.00	
4	M3	0.9254	54.00	45.00	8.00	
5	SDT04DTA	1.3453	76.00	45.00	8.00	
6	SDT07DTA	0.6706	44.50	45.00	8.00	*
7	SDT13DTA	1.2858	69.00	45.00	8.00	
8	SDT14DTA	1.3453	76.00	45.00	8.00	

Critical values are 1 tailed (k = 7)

8503-128-019-086-093
Rana pipiens
Background as Control Width

Ch: AR02-01-07
DAP 2/12/07

Title: 8503-128-019-086-093 Background as Control Width
File: 086wb Transform: NO TRANSFORMATION
Number of Groups: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Background	1	5.6000	5.6000
1	Background	2	5.8000	5.8000
1	Background	3	5.6000	5.6000
1	Background	4	4.4000	4.4000
1	Background	5	6.0000	6.0000
1	Background	6	5.0000	5.0000
1	Background	7	2.6000	2.6000
1	Background	8	5.0000	5.0000
2	M1	1	4.8000	4.8000
2	M1	2	5.6000	5.6000
2	M1	3	5.6000	5.6000
2	M1	4	4.8000	4.8000
2	M1	5	5.4000	5.4000
2	M1	6	5.6000	5.6000
2	M1	7	5.2000	5.2000
2	M1	8	4.0000	4.0000
3	M2	1	5.6000	5.6000
3	M2	2	4.0000	4.0000
3	M2	3	4.2000	4.2000
3	M2	4	5.0000	5.0000
3	M2	5	4.6000	4.6000
3	M2	6	5.2000	5.2000
3	M2	7	5.4000	5.4000
3	M2	8	4.0000	4.0000
4	M3	1	0.0000	0.0000
4	M3	2	1.2000	1.2000
4	M3	3	4.8000	4.8000
4	M3	4	1.4000	1.4000
4	M3	5	4.8000	4.8000
4	M3	6	5.4000	5.4000
4	M3	7	5.0000	5.0000
4	M3	8	4.2000	4.2000
5	SDT04DTA	1	5.2000	5.2000
5	SDT04DTA	2	4.8000	4.8000
5	SDT04DTA	3	4.6000	4.6000
5	SDT04DTA	4	4.8000	4.8000

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Rana pipiens
Background as Control Width

QA: AR02-01-07
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5	SDT04DTA	5	5.0000	5.0000
5	SDT04DTA	6	5.0000	5.0000
5	SDT04DTA	7	5.0000	5.0000
5	SDT04DTA	8	5.0000	5.0000
6	SDT13DTA	1	5.8000	5.8000
6	SDT13DTA	2	5.0000	5.0000
6	SDT13DTA	3	4.6000	4.6000
6	SDT13DTA	4	5.2000	5.2000
6	SDT13DTA	5	4.8000	4.8000
6	SDT13DTA	6	4.6000	4.6000
6	SDT13DTA	7	5.0000	5.0000
6	SDT13DTA	8	4.8000	4.8000
7	SDT14DTA	1	5.0000	5.0000
7	SDT14DTA	2	5.0000	5.0000
7	SDT14DTA	3	5.2000	5.2000
7	SDT14DTA	4	5.2000	5.2000
7	SDT14DTA	5	5.2000	5.2000
7	SDT14DTA	6	5.6000	5.6000
7	SDT14DTA	7	5.4000	5.4000
7	SDT14DTA	8	5.2000	5.2000

8503-128-019-086-093
Rana pipiens
Background as Control Width

QA: AR02-01-07
DAP 2/12/07

Title: 8503-128-019-086-093 Background as Control Width
File: 086wb Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Background	8	2.6000	6.0000	5.0000
2	M1	8	4.0000	5.6000	5.1250
3	M2	8	4.0000	5.6000	4.7500
4	M3	8	0.0000	5.4000	3.3500
5	SDT04DTA	8	4.6000	5.2000	4.9250
6	SDT13DTA	8	4.6000	5.8000	4.9750
7	SDT14DTA	8	5.0000	5.6000	5.2250

Title: 8503-128-019-086-093 Background as Control Width
File: 086wb Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	Background	1.2114	1.1006	0.3891	22.0130
2	M1	0.3193	0.5651	0.1998	11.0254
3	M2	0.4086	0.6392	0.2260	13.4568
4	M3	4.5000	2.1213	0.7500	63.3230
5	SDT04DTA	0.0336	0.1832	0.0648	3.7203
6	SDT13DTA	0.1536	0.3919	0.1386	7.8770
7	SDT14DTA	0.0393	0.1982	0.0701	3.7934

8503-128-019-086-093
Rana pipiens
Background as Control Width

QA: MRC02-01-07
DAP 2/12/07

Title: 8503-128-019-086-093 Background as Control Width
File: 086wb Transform: NO TRANSFORMATION

Chi-Square Test for Normality

Actual and Expected Frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	3.7520	13.5520	21.3920	13.5520	3.7520
OBSERVED	4	14	19	16	3

Chi-Square = 0.8916 (p-value = 0.9258)

Critical Chi-Square = 13.277 (alpha = 0.01 , df = 4)
= 9.488 (alpha = 0.05 , df = 4)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: 8503-128-019-086-093 Background as Control Width
File: 086wb Transform: NO TRANSFORMATION

Bartlett's Test for Homogeneity of Variance

Calculated B1 statistic = 56.4484 (p-value = 0.0000)

Data FAIL B1 homogeneity test at 0.01 level. Try another transformation.

Critical B = 16.8119 (alpha = 0.01, df = 6)
= 12.5916 (alpha = 0.05, df = 6)

8503-128-019-086-093
Rana pipiens
Background as Control Width

AR: AR02-01-07
DAY 2/12/07

Title: 8503-128-019-086-093 Background as Control Width
File: 086wb Transform: NO TRANSFORMATION

Steel's Many-One Rank Test - Ho: Control<Treatment

GROUP	IDENTIFICATION	MEAN IN ORIGINAL UNITS	RANK SUM	CRIT. VALUE	DF	SIG 0.05
1	Background	5.0000				
2	M1	5.1250	64.00	46.00	8.00	
3	M2	4.7500	57.00	46.00	8.00	
4	M3	3.3500	48.00	46.00	8.00	
5	SDT04DTA	4.9250	58.00	46.00	8.00	
6	SDT13DTA	4.9750	60.50	46.00	8.00	
7	SDT14DTA	5.2250	67.00	46.00	8.00	

Critical values are 1 tailed (k = 6)

8503-128-019-086-093
Rana pipiens
Poudre River Sediment as Control Width

QA: AR02-01-07
DSP 2/12/07

Title: 8503-128-019-086-093 Poudre as Contol Width
File: 086wc Transform: NO TRANSFORMATION
Number of Groups: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	5.8000	5.8000
1	Control	2	5.6000	5.6000
1	Control	3	5.0000	5.0000
1	Control	4	6.0000	6.0000
1	Control	5	5.2000	5.2000
1	Control	6	5.8000	5.8000
1	Control	7	4.6000	4.6000
1	Control	8	4.6000	4.6000
2	M1	1	4.8000	4.8000
2	M1	2	5.6000	5.6000
2	M1	3	5.6000	5.6000
2	M1	4	4.8000	4.8000
2	M1	5	5.4000	5.4000
2	M1	6	5.6000	5.6000
2	M1	7	5.2000	5.2000
2	M1	8	4.0000	4.0000
3	M2	1	5.6000	5.6000
3	M2	2	4.0000	4.0000
3	M2	3	4.2000	4.2000
3	M2	4	5.0000	5.0000
3	M2	5	4.6000	4.6000
3	M2	6	5.2000	5.2000
3	M2	7	5.4000	5.4000
3	M2	8	4.0000	4.0000
4	M3	1	0.0000	0.0000
4	M3	2	1.2000	1.2000
4	M3	3	4.8000	4.8000
4	M3	4	1.4000	1.4000
4	M3	5	4.8000	4.8000
4	M3	6	5.4000	5.4000
4	M3	7	5.0000	5.0000
4	M3	8	4.2000	4.2000
5	SDT04DTA	1	5.2000	5.2000
5	SDT04DTA	2	4.8000	4.8000
5	SDT04DTA	3	4.6000	4.6000
5	SDT04DTA	4	4.8000	4.8000

QA: AR02-01-07
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Rana pipiens
Poudre River Sediment as Control Width

5	SDT04DTA	5	5.0000	5.0000
5	SDT04DTA	6	5.0000	5.0000
5	SDT04DTA	7	5.0000	5.0000
5	SDT04DTA	8	5.0000	5.0000
6	SDT13DTA	1	5.8000	5.8000
6	SDT13DTA	2	5.0000	5.0000
6	SDT13DTA	3	4.6000	4.6000
6	SDT13DTA	4	5.2000	5.2000
6	SDT13DTA	5	4.8000	4.8000
6	SDT13DTA	6	4.6000	4.6000
6	SDT13DTA	7	5.0000	5.0000
6	SDT13DTA	8	4.8000	4.8000
7	SDT14DTA	1	5.0000	5.0000
7	SDT14DTA	2	5.0000	5.0000
7	SDT14DTA	3	5.2000	5.2000
7	SDT14DTA	4	5.2000	5.2000
7	SDT14DTA	5	5.2000	5.2000
7	SDT14DTA	6	5.6000	5.6000
7	SDT14DTA	7	5.4000	5.4000
7	SDT14DTA	8	5.2000	5.2000

8503-128-019-086-093
Rana pipiens
Poudre River Sediment as Control Width

QA: AR 02-01-07
DJP 2/12/07

Title: 8503-128-019-086-093 Poudre as Contol Width
File: 086wc Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Control	8	4.6000	6.0000	5.3250
2	M1	8	4.0000	5.6000	5.1250
3	M2	8	4.0000	5.6000	4.7500
4	M3	8	0.0000	5.4000	3.3500
5	SDT04DTA	8	4.6000	5.2000	4.9250
6	SDT13DTA	8	4.6000	5.8000	4.9750
7	SDT14DTA	8	5.0000	5.6000	5.2250

Title: 8503-128-019-086-093 Poudre as Contol Width
File: 086wc Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	Control	0.3079	0.5548	0.1962	10.4197
2	M1	0.3193	0.5651	0.1998	11.0254
3	M2	0.4086	0.6392	0.2260	13.4568
4	M3	4.5000	2.1213	0.7500	63.3230
5	SDT04DTA	0.0336	0.1832	0.0648	3.7203
6	SDT13DTA	0.1536	0.3919	0.1386	7.8770
7	SDT14DTA	0.0393	0.1982	0.0701	3.7934

8503-128-019-086-093
Rana pipiens
Poudre River Sediment as Control Width

QA: AR02-01-07
DAP 2/12/07

Title: 8503-128-019-086-093 Poudre as Contol Width
File: 086wc Transform: NO TRANSFORMATION

Chi-Square Test for Normality

Actual and Expected Frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	3.7520	13.5520	21.3920	13.5520	3.7520
OBSERVED	3	16	19	15	3

Chi-Square = 1.1658 (p-value = 0.8837)

Critical Chi-Square = 13.277 (alpha = 0.01 , df = 4)
= 9.488 (alpha = 0.05 , df = 4)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: 8503-128-019-086-093 Poudre as Contol Width
File: 086wc Transform: NO TRANSFORMATION

Bartlett's Test for Homogeneity of Variance

Calculated B1 statistic = 58.7735 (p-value = 0.0000)

Data FAIL B1 homogeneity test at 0.01 level. Try another transformation.

Critical B = 16.8119 (alpha = 0.01, df = 6)
= 12.5916 (alpha = 0.05, df = 6)

8503-128-019-086-093
Rana pipiens
Poudre River Sediment as Control Width

QA: AR202-01-07
DAP 2/12/07

Title: 8503-128-019-086-093 Poudre as Contol Width
File: 086wc Transform: NO TRANSFORMATION

Steel's Many-One Rank Test - Ho: Control<Treatment

GROUP	IDENTIFICATION	MEAN IN ORIGINAL UNITS	RANK SUM	CRIT. VALUE	DF	SIG 0.05
1	Control	5.3250				
2	M1	5.1250	61.00	46.00	8.00	
3	M2	4.7500	51.50	46.00	8.00	
4	M3	3.3500	46.50	46.00	8.00	
5	SDT04DTA	4.9250	54.50	46.00	8.00	
6	SDT13DTA	4.9750	56.50	46.00	8.00	
7	SDT14DTA	5.2250	63.50	46.00	8.00	

Critical values are 1 tailed (k = 6)

AR
QA: 02-0107
DAP 2/12/07

8503-128-019-086-093
Rana pipiens
Background vs Control Width

Title: 8503-128-019-086-093 Background vs Control Width
File: 086wcb Transform: NO TRANSFORMATION
Number of Groups: 2

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	5.8000	5.8000
1	Control	2	5.6000	5.6000
1	Control	3	5.0000	5.0000
1	Control	4	6.0000	6.0000
1	Control	5	5.2000	5.2000
1	Control	6	5.8000	5.8000
1	Control	7	4.6000	4.6000
1	Control	8	4.6000	4.6000
2	Background	1	5.6000	5.6000
2	Background	2	5.8000	5.8000
2	Background	3	5.6000	5.6000
2	Background	4	4.4000	4.4000
2	Background	5	6.0000	6.0000
2	Background	6	5.0000	5.0000
2	Background	7	2.6000	2.6000
2	Background	8	5.0000	5.0000

File: 086wcb Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Control	8	4.6000	6.0000	5.3250
2	Background	8	2.6000	6.0000	5.0000

Summary Statistics on Data TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	Control	0.3079	0.5548	0.1962	10.4197
2	Background	1.2114	1.1006	0.3891	22.0130

8503-128-019-086-093
Rana pipiens
Background vs Control Width

QA: NR02-05-07
DAP 2/12/07

```
  Title:  8503-128-019-086-093 Background vs Control Width
  File:            086wcb            Transform:            NO TRANSFORMATION

                    Shapiro - Wilk's Test for Normality
 ---------------------------------------------------------------------------
      D =  10.6350
      W =   0.8656

      Critical W  = 0.8440 (alpha = 0.01 , N = 16)
                W  = 0.8870 (alpha = 0.05 , N = 16)
 ---------------------------------------------------------------------------

  Data PASS normality test (alpha = 0.01). Continue analysis.


                    F-Test for Equality of Two Variances
 ---------------------------------------------------------------------------
          GROUP    IDENTIFICATION          VARIANCE           F
          -----    --------------        ------------     ---------
            1             Control           0.3079
            2          Background           1.2114          3.9350
 ---------------------------------------------------------------------------
                                                (p-value = 0.0912)
    Critical F = 8.8854   (P=0.01, 7, 7)
                 4.9949   (P=0.05, 7, 7)

    Since F <= Critical F, FAIL TO REJECT Ho: Equal Variances (alpha = 0.01).
```

8503-128-019-086-093
Rana pipiens
Background vs Control Width

OA: AR02-0107
DAP 2/12/07

Title: 8503-128-019-086-093 Background vs Control Width
File: 086wcb Transform: NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	1	0.4225	0.4225	0.5562
Within (Error)	14	10.6350	0.7596	
Total	15	11.0575		

(p-value = 0.4681)

Critical F = 8.8616 (alpha = 0.01, df = 1,14)
= 4.6001 (alpha = 0.05, df = 1,14)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)

File: 086wcb Transform: NO TRANSFORMATION

2 Sample t-Test - TABLE 1 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	t STAT	SIG 0.05
1	Control	5.3250	5.3250		
2	Background	5.0000	5.0000	0.7458	

Equal Var: t critical value = 1.7613 (1 Tailed, alpha = 0.05, df = 14)
(p-value = 0.2341)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG 0.05
1	Control	5.3250	5.3250		
2	Background	5.0000	5.0000	0.7458	

Unequal Var: t critical value = 1.8125 (1 Tailed, alpha = 0.05, df = 10)
(p-value = 0.2365)

8503-128-019-086-093
Rana pipiens
Background vs Control Width

QA: AR02-01-07
DAP 2/12/07

Title: 8503-128-019-086-093 Background vs Control Width
File: 086wcb Transform: NO TRANSFORMATION

2 Sample t-Test - TABLE 2 OF 2 Ho: Control<Treatment

Equal Variances:

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	Control	8			
2	Background	8	0.7676	14.4	0.3250

Unequal Variances:

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	Control	8			
2	Background	8	0.7898	14.8	0.3250

8503-128-019-086-093
Rana pipiens
Control vs. Background Survival

QA: AR02-0107
JAP 2/12/07

Title: 8503-128-019-086-093 Control vs Background Survival
File: 086csu Transform: NO TRANSFORMATION
Number of Groups: 2

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	1.0000	1.0000
1	Control	2	1.0000	1.0000
1	Control	3	1.0000	1.0000
1	Control	4	1.0000	1.0000
1	Control	5	1.0000	1.0000
1	Control	6	1.0000	1.0000
1	Control	7	0.8000	0.8000
1	Control	8	0.8000	0.8000
2	SDTBKSTA	1	1.0000	1.0000
2	SDTBKSTA	2	1.0000	1.0000
2	SDTBKSTA	3	1.0000	1.0000
2	SDTBKSTA	4	0.8000	0.8000
2	SDTBKSTA	5	1.0000	1.0000
2	SDTBKSTA	6	1.0000	1.0000
2	SDTBKSTA	7	0.4000	0.4000
2	SDTBKSTA	8	1.0000	1.0000

8503-128-019-086-093
Rana pipiens
Control vs. Background Survival

QA: AR02-01-07
DAP 2/12/07

File: 086csu Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Control	8	0.8000	1.0000	0.9500
2	SDTBKSTA	8	0.4000	1.0000	0.9000

File: 086csu Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	Control	0.0086	0.0926	0.0327	9.7455
2	SDTBKSTA	0.0457	0.2138	0.0756	23.7566

QA: AR02-0507

DAP 2/12/07

8503-128-019-086-093
Rana pipiens
Control vs. Background Survival

```
Title:  8503-128-019-086-093 Control vs Background Survival
File:            086csu              Transform:      ARC SINE(SQUARE ROOT(Y))

                  Shapiro - Wilk's Test for Normality
-----------------------------------------------------------------------------
     D =   0.4772
     W =   0.6623

     Critical W  = 0.8440 (alpha = 0.01 , N = 16)
              W  = 0.8870 (alpha = 0.05 , N = 16)
-----------------------------------------------------------------------------
Data FAIL normality test (alpha = 0.01). Try another transformation.

Warning   -  The F-test of homogeneity is sensitive to non-normality
             and should not be performed with this data as is.


Title:  8503-128-019-086-093 Control vs Background Survival
File:            086csu              Transform:      ARC SINE(SQUARE ROOT(Y))

Wilcoxon's Rank Sum Test w/ Bonferroni Adjustment     Ho: Control<Treatment
-----------------------------------------------------------------------------
                                TRANSFORMED    RANK     CRIT.           SIG
GROUP    IDENTIFICATION           MEAN         SUM     VALUE    REPS   0.05
------ --------------------     -----------   -------  ------   -----  ---
  1                Control       1.2858
  2               SDTBKSTA       1.2329        67.00     51       8
-----------------------------------------------------------------------------
Critical values are 1 tailed ( k = 1 )
```

8503-128-019-086-093
Rana pipiens
Background Sediment as Control Length

@A: MRD2-05-07
DAP 2/12/07

Title: 8503-128-019-086-093 Background as Control Length
File: 0861b Transform: NO TRANSFORMATION
Number of Groups: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Background	1	8.4000	8.4000
1	Background	2	8.6000	8.6000
1	Background	3	8.0000	8.0000
1	Background	4	6.2000	6.2000
1	Background	5	9.0000	9.0000
1	Background	6	8.0000	8.0000
1	Background	7	3.6000	3.6000
1	Background	8	8.0000	8.0000
2	M1	1	7.0000	7.0000
2	M1	2	8.2000	8.2000
2	M1	3	7.6000	7.6000
2	M1	4	6.8000	6.8000
2	M1	5	8.4000	8.4000
2	M1	6	8.0000	8.0000
2	M1	7	7.6000	7.6000
2	M1	8	6.2000	6.2000
3	M2	1	8.0000	8.0000
3	M2	2	7.0000	7.0000
3	M2	3	6.8000	6.8000
3	M2	4	8.0000	8.0000
3	M2	5	6.8000	6.8000
3	M2	6	8.0000	8.0000
3	M2	7	8.2000	8.2000
3	M2	8	6.0000	6.0000
4	M3	1	0.0000	0.0000
4	M3	2	2.0000	2.0000
4	M3	3	7.4000	7.4000
4	M3	4	2.0000	2.0000
4	M3	5	6.6000	6.6000
4	M3	6	7.8000	7.8000
4	M3	7	7.4000	7.4000
4	M3	8	6.0000	6.0000
5	SDT04DTA	1	7.2000	7.2000
5	SDT04DTA	2	6.8000	6.8000
5	SDT04DTA	3	6.6000	6.6000
5	SDT04DTA	4	6.8000	6.8000

QA: AR02-0507
DAP 2/12/07

8503-128-019-086-093
Rana pipiens
Background Sediment as Control Length

5	SDT04DTA	5	7.2000	7.2000
5	SDT04DTA	6	7.2000	7.2000
5	SDT04DTA	7	7.4000	7.4000
5	SDT04DTA	8	7.8000	7.8000
6	SST13DTA	1	8.2000	8.2000
6	SST13DTA	2	7.2000	7.2000
6	SST13DTA	3	6.2000	6.2000
6	SST13DTA	4	7.0000	7.0000
6	SST13DTA	5	6.8000	6.8000
6	SST13DTA	6	6.4000	6.4000
6	SST13DTA	7	7.2000	7.2000
6	SST13DTA	8	6.8000	6.8000
7	SDT14DTA	1	7.6000	7.6000
7	SDT14DTA	2	6.8000	6.8000
7	SDT14DTA	3	7.6000	7.6000
7	SDT14DTA	4	7.2000	7.2000
7	SDT14DTA	5	7.2000	7.2000
7	SDT14DTA	6	7.6000	7.6000
7	SDT14DTA	7	7.6000	7.6000
7	SDT14DTA	8	7.0000	7.0000

Title: 8503-128-019-086-093 Background as Control Length
File: 0861b Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Background	8	3.6000	9.0000	7.4750
2	M1	8	6.2000	8.4000	7.4750
3	M2	8	6.0000	8.2000	7.3500
4	M3	8	0.0000	7.8000	4.9000
5	SDT04DTA	8	6.6000	7.8000	7.1250
6	SST13DTA	8	6.2000	8.2000	6.9750
7	SDT14DTA	8	6.8000	7.6000	7.3250

8503-128-019-086-093
Rana pipiens
Background Sediment as Control Length

QA: AR02-05-07
DAP 2/12/07

Title: 8503-128-019-086-093 Background as Control Length
File: 0861b Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	Background	3.1307	1.7694	0.6256	23.6707
2	M1	0.5707	0.7555	0.2671	10.1064
3	M2	0.6486	0.8053	0.2847	10.9570
4	M3	9.4057	3.0669	1.0843	62.5893
5	SDT04DTA	0.1479	0.3845	0.1359	5.3968
6	SST13DTA	0.3707	0.6089	0.2153	8.7292
7	SDT14DTA	0.1021	0.3196	0.1130	4.3631

8503-128-019-086-093
Rana pipiens
Background Sediment as Control Length

QA: NR02-05-07
DRP 2/12/07

Title: 8503-128-019-086-093 Background as Control Length
File: 086lb Transform: NO TRANSFORMATION

Chi-Square Test for Normality

Actual and Expected Frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	3.7520	13.5520	21.3920	13.5520	3.7520
OBSERVED	5	13	17	19	2

Chi-Square = 4.3476 (p-value = 0.3610)

Critical Chi-Square = 13.277 (alpha = 0.01 , df = 4)
= 9.488 (alpha = 0.05 , df = 4)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: 8503-128-019-086-093 Background as Control Length
File: 086lb Transform: NO TRANSFORMATION

Bartlett's Test for Homogeneity of Variance

Calculated B1 statistic = 52.0093 (p-value = 0.0000)

Data FAIL B1 homogeneity test at 0.01 level. Try another transformation.

Critical B = 16.8119 (alpha = 0.01, df = 6)
= 12.5916 (alpha = 0.05, df = 6)

8503-128-019-086-093
Rana pipiens
Poudre River Sediment as Control Length

A: AR02-05-07
DAP 2/12/07

Title: 8503-128-019-086-093 Poudre as Control length
File: 086lc Transform: NO TRANSFORMATION
Number of Groups: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	8.2000	8.2000
1	Control	2	8.2000	8.2000
1	Control	3	7.4000	7.4000
1	Control	4	8.0000	8.0000
1	Control	5	7.8000	7.8000
1	Control	6	8.4000	8.4000
1	Control	7	6.6000	6.6000
1	Control	8	6.4000	6.4000
2	M1	1	7.0000	7.0000
2	M1	2	8.2000	8.2000
2	M1	3	7.6000	7.6000
2	M1	4	6.8000	6.8000
2	M1	5	8.4000	8.4000
2	M1	6	8.0000	8.0000
2	M1	7	7.6000	7.6000
2	M1	8	6.2000	6.2000
3	M2	1	8.0000	8.0000
3	M2	2	7.0000	7.0000
3	M2	3	6.8000	6.8000
3	M2	4	8.0000	8.0000
3	M2	5	6.8000	6.8000
3	M2	6	8.0000	8.0000
3	M2	7	8.2000	8.2000
3	M2	8	6.0000	6.0000
4	M3	1	0.0000	0.0000
4	M3	2	2.0000	2.0000
4	M3	3	7.4000	7.4000
4	M3	4	2.0000	2.0000
4	M3	5	6.6000	6.6000
4	M3	6	7.8000	7.8000
4	M3	7	7.4000	7.4000
4	M3	8	6.0000	6.0000
5	SDT04DTA	1	7.2000	7.2000
5	SDT04DTA	2	6.8000	6.8000
5	SDT04DTA	3	6.6000	6.6000

8503-128-019-086-093
Rana pipiens
Poudre River Sediment as Control Length

QA: AR020507
DAP 2/12/07

5	SDT04DTA	4	6.8000	6.8000
5	SDT04DTA	5	7.2000	7.2000
5	SDT04DTA	6	7.2000	7.2000
5	SDT04DTA	7	7.4000	7.4000
5	SDT04DTA	8	7.8000	7.8000
6	SST13DTA	1	8.2000	8.2000
6	SST13DTA	2	7.2000	7.2000
6	SST13DTA	3	6.2000	6.2000
6	SST13DTA	4	7.0000	7.0000
6	SST13DTA	5	6.8000	6.8000
6	SST13DTA	6	6.4000	6.4000
6	SST13DTA	7	7.2000	7.2000
6	SST13DTA	8	6.8000	6.8000
7	SDT14DTA	1	7.6000	7.6000
7	SDT14DTA	2	6.8000	6.8000
7	SDT14DTA	3	7.6000	7.6000
7	SDT14DTA	4	7.2000	7.2000
7	SDT14DTA	5	7.2000	7.2000
7	SDT14DTA	6	7.6000	7.6000
7	SDT14DTA	7	7.6000	7.6000
7	SDT14DTA	8	7.0000	7.0000

Title: 8503-128-019-086-093 Poudre as Control length
File: 0861c Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Control	8	6.4000	8.4000	7.6250
2	M1	8	6.2000	8.4000	7.4750
3	M2	8	6.0000	8.2000	7.3500
4	M3	8	0.0000	7.8000	4.9000
5	SDT04DTA	8	6.6000	7.8000	7.1250
6	SST13DTA	8	6.2000	8.2000	6.9750
7	SDT14DTA	8	6.8000	7.6000	7.3250

QA: AR02-0507
DAP 2/12/07

8503-128-019-086-093
Rana pipiens
Poudre River Sediment as Control Length

Title: 8503-128-019-086-093 Poudre as Control length
File: 086lc Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	Control	0.5764	0.7592	0.2684	9.9571
2	M1	0.5707	0.7555	0.2671	10.1064
3	M2	0.6486	0.8053	0.2847	10.9570
4	M3	9.4057	3.0669	1.0843	62.5893
5	SDT04DTA	0.1479	0.3845	0.1359	5.3968
6	SST13DTA	0.3707	0.6089	0.2153	8.7292
7	SDT14DTA	0.1021	0.3196	0.1130	4.3631

8503-128-019-086-093
Rana pipiens
Poudre River Sediment as Control Length

QA: ARD2-05-07
DAP 2/12/07

Title: 8503-128-019-086-093 Poudre as Control length
File: 086lc Transform: NO TRANSFORMATION

Chi-Square Test for Normality

Actual and Expected Frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	3.7520	13.5520	21.3920	13.5520	3.7520
OBSERVED	5	13	17	19	2

Chi-Square = 4.3476 (p-value = 0.3610)

Critical Chi-Square = 13.277 (alpha = 0.01 , df = 4)
= 9.488 (alpha = 0.05 , df = 4)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: 8503-128-019-086-093 Poudre as Control length
File: 086lc Transform: NO TRANSFORMATION

Bartlett's Test for Homogeneity of Variance

Calculated B1 statistic = 54.1526 (p-value = 0.0000)

Data FAIL B1 homogeneity test at 0.01 level. Try another transformation.

Critical B = 16.8119 (alpha = 0.01, df = 6)
= 12.5916 (alpha = 0.05, df = 6)

DAP 2/12/07
QA: NRO2-05-07

8503-128-019-086-093
Rana pipiens
Poudre River Sediment as Control Length

Title: 8503-128-019-086-093 Poudre as Control length
File: 0861c Transform: NO TRANSFORMATION

Steel's Many-One Rank Test - Ho: Control<Treatment

GROUP	IDENTIFICATION	MEAN IN ORIGINAL UNITS	RANK SUM	CRIT. VALUE	DF	SIG 0.05
1	Control	7.6250				
2	M1	7.4750	64.00	46.00	8.00	
3	M2	7.3500	61.50	46.00	8.00	
4	M3	4.9000	46.00	46.00	8.00	*
5	SDT04DTA	7.1250	53.50	46.00	8.00	
6	SST13DTA	6.9750	52.50	46.00	8.00	
7	SDT14DTA	7.3250	56.00	46.00	8.00	

Critical values are 1 tailed (k = 6)

APPENDIX D

Analytical Data

PARAGON ANALYTICS
225 Commerce Drive ◆ Fort Collins, CO 80524 ◆ (800) 443-1511 ◆ (970) 490-1511 ◆ FAX (970) 490-1522

November 14, 2006

Mr. David Pillard
ENSR Consulting and Engineering
4303 W. Laporte
Fort Collins, CO 80521

Re: Paragon Workorder: 06-11-012
Client Project Name: ESTCP
Client Project Number: 09070-056-503

Dear Mr. Pillard:

Eleven soil samples were received from ENSR Consulting and Engineering on November 1, 2006. The samples were scheduled for Total Metals (pages 1-19) analysis.

The results for this analysis are contained in the enclosed reports.

Thank you for your confidence in Paragon Analytics. Should you have any questions, please call.

Sincerely,

Paragon Analytics
for Debbie Fazio
Project Manager

DJF/ja
Enclosure: Report

Paragon Analytics

Sample Number(s) Cross-Reference Table

Paragon OrderNum: 0611012
Client Name: ENSR Consulting and Engineering
Client Project Name: ESTCP
Client Project Number: 09070-056-503
Client PO Number: 2041736

Client Sample	Lab Sample Number	COC Number	Matrix	Date Collected	Time Collected
Sed Ab-ABK11	0611012-1		SOIL	31-Oct-06	
Sed Ab-A08	0611012-2		SOIL	31-Oct-06	
Sed Ab-A07	0611012-3		SOIL	31-Oct-06	
Sed Ab-A04	0611012-4		SOIL	31-Oct-06	
Sed Ab-A3A	0611012-5		SOIL	31-Oct-06	
SDT BK-STA	0611012-6		SOIL	31-Oct-06	
SDT 04-DTA	0611012-7		SOIL	31-Oct-06	
SST 04-DTA	0611012-8		SOIL	31-Oct-06	
SST 07-DTA	0611012-9		SOIL	31-Oct-06	
SDT 14-DTA	0611012-10		SOIL	31-Oct-06	
SST 13-DTA	0611012-11		SOIL	01-Nov-06	

ENSR INTERNATIONAL

CHAIN OF CUSTODY RECORD

0611012

Page ____ of ____

Client/Project Name: ESTCP

Project Location: ENSR / FCETC

Project Number: 09070-056-503

Field Logbook No.:

Sampler (Print Name)/(Affiliation): Patrick Odenbeck (ENSR)

Chain of Custody Tape No.:

Signature:

Send Results/Report to: Dave Pillard

Analysis Requested

Field Sample No./ Identification	Date	Time	Grab	Comp	Sample Container (Size/Mat'l)	Sample Type (Liquid, Sludge, Etc.)	Preservative	Field Filtered	UV	PB						Lab I.D.	Remarks
Sed Ab-ABK11	10/31/06				2 oz Amber	Sediment	None	NA	X	X							STANDARD 14 Day Turnaround
Sed Ab-A08																	
Sed Ab-A07																	
Sed Ab-A04																	
Sed Ab-A3A																	
SDT BK ~~[illegible]~~ STA																	
SDT 04-DTA																	
SST 04-DTA																	
SST 07-DTA																	
SDT14-DTA																	

Relinquished by: (Print Name)/(Affiliation)	Date/Time	Received by: (Print Name)/(Affiliation)	Date/Time
Patrick Odenbeck (ENSR) Signature:	Date: 11/1/06 Time: 1200	Sheri Lafferty Signature: Sheri Lafferty	Date: 11.1.06 Time: 1620
Relinquished by: (Print Name)/(Affiliation) Signature:	Date: Time:	Received by: (Print Name)/(Affiliation) Signature:	Date: Time:
Relinquished by: (Print Name)/(Affiliation) Signature:	Date: Time:	Received by: (Print Name)/(Affiliation) Signature:	Date: Time:

Analytical Laboratory (Destination):

PARAGON

~~ENSR Toxicology Lab~~
~~4303 W. Laporte Avenue~~
~~Fort Collins, CO 80521~~
~~(970) 416-0916~~
(970) 493-8935 (FAX)

Serial № 43430

FM01008b_902

DP 11/1/06

ENSR INTERNATIONAL

CHAIN OF CUSTODY RECORD

0611012

Page ____ of ____

Client/Project Name: ESTCP

Project Location: ENSR / FCETL

Project Number: 0907-056-503

Field Logbook No.:

Sampler (Print Name)/(Affiliation): Patrick Odenbeck (ENSR)

Chain of Custody Tape No.:

Signature:

Send Results/Report to: Dave Pillard

Analysis Requested

Field Sample No./ Identification	Date	Time	Grab	Comp	Sample Container (Size/Mat'l)	Sample Type (Liquid, Sludge, Etc.)	Preservative	Field Filtered	U5	R6							Lab I.D.	Remarks
SST 13 PTA	11/1/06				2oz Amber	Sediment	None	NA	X	X								STANDARD 14 Day Turnaround

Relinquished by: (Print Name)/(Affiliation)	Date	Received by: (Print Name)/(Affiliation)	Date
Patrick Odenbeck (ENSR) Signature:	11/1/06 Time: 1200	Signature: Sheri Lafferty	11-1-06 Time: 1620
Relinquished by: (Print Name)/(Affiliation) Signature:	Date: Time:	Received by: (Print Name)/(Affiliation) Signature:	Date: Time:
Relinquished by: (Print Name)/(Affiliation) Signature:	Date: Time:	Received by: (Print Name)/(Affiliation) Signature:	Date: Time:

Analytical Laboratory (Destination):

PARAGON

ENSR Toxicology Lab
4303 W. Laporte Avenue
Fort Collins, CO 80521
(970) 416-0916
(970) 493-8935 (FAX)

Serial No. 43429

010060_902

CONDITION OF SAMPLE UPON RECEIPT FORM **Paragon Analytics**

Client: ENSR Workorder No: 0611012

Project Manager: DF Initials: SL Date: 11·1·06

1. Does this project require any **special handling** in addition to standard Paragon procedures?		YES	NO
2. Are custody **seals** on **shipping containers** intact?	NONE	YES	NO
3. Are Custody seals on **sample containers** intact?	NONE	YES	NO
4. Is there a **COC (Chain-of-Custody) present** or other representative documents?		YES	NO
5. Are the **COC and bottle labels complete and legible**?		YES	NO
6. Is the **COC in agreement** with samples received? (IDs, dates, times, no. of samples, no. of containers, matrix, requested analyses, etc.)		YES	NO
7. Were **airbills / shipping documents** present and/or removable?	DROP OFF	YES	NO
8. Are all aqueous **samples requiring preservation preserved correctly?** (excluding volatiles)	N/A	YES	NO
9. Are all aqueous **non-preserved samples pH 4-9**?	N/A	YES	NO
10. Is there **sufficient sample** for the requested analyses?		YES	NO
11. Were all samples placed in the **proper containers** for the requested analyses?		YES	NO
12. Are all samples within **holding times** for the requested analyses?		YES	NO
13. Were all sample containers received **intact**? (not broken or leaking, etc.)		YES	NO
14. Are all samples requiring no headspace (VOC, GRO, Rx CN/S, radon), headspace free? **Size of bubble:** ____ < green pea ____ > green pea	N/A	YES	NO
15. Were samples checked for and free from the presence of **residual chlorine?** (Applicable when PM has indicated samples are from a chlorinated water source; note if field preservation with sodium thiosulfate was not observed.)	N/A	YES	NO
16. Were the samples **shipped on ice**?		YES	NO
17. Were cooler temperatures measured at 0.1-6.0°C? **IR gun used*:** #2 #4	RAD ONLY	YES	NO

Cooler #:	1							
Temperature (°C):	5.8							
No. of custody seals on cooler:	NA							
DOT Survey/ Acceptance Information — External µR/hr reading:	NA							
Background µR/hr reading:	12							

Were external µR/hr readings ≤ two times background and within DOT acceptance criteria? YES / NO / NA (If no, see Form 008.)

Additional Information: PROVIDE DETAILS BELOW FOR A NO RESPONSE TO ANY QUESTION ABOVE, EXCEPT #1 AND #16.

* No collection times listed on coc or bottles

If applicable, was the client contacted? YES / NO / NA Contact: _____ Date/Time: _____

Project Manager Signature / Date: Debbie Fazio 11/2/06

*IR Gun #2: Oakton, SN 29922500201-0066 *IR Gun #4: Oakton, SN 2372220101-0002

Paragon Analytics

TOTAL METALS CASE NARRATIVE

ENSR Consulting and Engineering

ESTCP -- 09070-056-503

Order Number - 0611012

1. This report consists of 11 soil samples.
2. The samples were received cool and intact on 11/01/06.
3. The samples were prepared for analysis based on SW-846, 3rd Edition procedures.

   For analysis by Trace ICP2, the samples were digested following method 3050B and PA SOP 806 Rev. 12.
4. The samples were analyzed following SW-846, 3rd Edition procedures.

   Analysis by Trace ICP2 followed method 6010B and PA SOP 834 Rev. 5.

   The relationship between intensity and concentration for each element is established using at least four standards, one of which is a blank solution.

   During sample analysis concentrations are computed by the software and the results are printed in mg/L. The instrument software does not provide a printout which gives both intensity and concentration. The validity of the calibration equation is tested by analyzing the following solutions: a blank, a low level check solution with concentrations near the reporting limit, an Initial Calibration Verification (ICV) standard from a 2nd source standard solution with concentrations near the middle of the analytical range, a Continuing Calibration Verification (CCV) standard with concentrations at two times those in the ICV, and a readback of the highest calibration standard.

   These solutions provide verification that the calibration equations are functioning properly throughout the analytical range of the instrument. During sample analysis dilutions are made for analytes found at concentrations above the highest calibration standard. No results are taken from extrapolations beyond the highest standard.
5. All standards and solutions are NIST traceable and were used within their recommended shelf life.
6. The samples were prepared and analyzed within the established hold times.

All in house quality control procedures were followed, as described below.

7. General quality control procedures.

   - A preparation (method) blank and laboratory control sample were digested and analyzed with the samples in this digestion batch. There were not more than 20 samples in the digestion batch.
   - The preparation (method) blank associated with this digestion batch was below the practical quantitation limit for each requested analyte.
   - The laboratory control sample associated with this digestion batch was within the acceptance limits. This indicates complete digestion according to the method.
   - All initial and continuing calibration blanks associated with this analytical batch were below the practical quantitation limits for the requested analytes.
   - All initial and continuing calibration verifications associated with this analytical batch were within the acceptance criteria for the requested analytes. This indicates a valid calibration and stable instrument conditions.
   - The interference check samples and high standard readbacks associated with Method 6010B analyses were within acceptance criteria.

8. Matrix specific quality control procedures.

   Sample 0611012-11 was designated as the quality control sample for this analysis.

   - A matrix spike and matrix spike duplicate were digested and analyzed with this batch. All acceptance criteria for accuracy were met.
   - Matrix spike recoveries could not be evaluated for the following analyte:

Analyte	Sample ID
Lead	0611012-11

     The concentration of this analyte in the native sample was greater than four times the concentration of matrix spike added during the digestion. When sample concentration is that much greater than the spike added, spike recoveries may not be accurate. The laboratory control sample indicates that the digestion and analysis were in control.

   - A sample duplicate and matrix spike duplicate were digested and analyzed with this batch. All acceptance criteria for precision were met.
   - A serial dilution was analyzed with the ICP batch. All acceptance criteria were met.

9. Sample IDs 0611012-5, -7, -8, -9, -10, and -11 required dilutions to bring iron and/or lead into the analytical range of the Trace ICP. Accurate quantitation of iron is necessary to correct for spectral interferences on lead. The lead result was determined from the diluted samples.

The data contained in the following report have been reviewed and approved by the personnel listed below. In addition, Paragon Analytics certifies that the analyses reported herein are true, complete and correct within the limits of the methods employed.

Rhonda Miller — 11/14/06

Rhonda Miller
Data Reporting Specialist

Date

RF — 11/13/06

Reviewer's Initials

Date

Paragon Analytics

Sample Number(s) Cross-Reference Table

Paragon OrderNum: 0611012
Client Name: ENSR Consulting and Engineering
Client Project Name: ESTCP
Client Project Number: 09070-056-503
Client PO Number: 2041736

Client Sample	Lab Sample Number	COC Number	Matrix	Date Collected	Time Collected
Sed Ab-ABK11	0611012-1		SOIL	31-Oct-06	
Sed Ab-A08	0611012-2		SOIL	31-Oct-06	
Sed Ab-A07	0611012-3		SOIL	31-Oct-06	
Sed Ab-A04	0611012-4		SOIL	31-Oct-06	
Sed Ab-A3A	0611012-5		SOIL	31-Oct-06	
SDT BK-STA	0611012-6		SOIL	31-Oct-06	
SDT 04-DTA	0611012-7		SOIL	31-Oct-06	
SST 04-DTA	0611012-8		SOIL	31-Oct-06	
SST 07-DTA	0611012-9		SOIL	31-Oct-06	
SDT 14-DTA	0611012-10		SOIL	31-Oct-06	
SST 13-DTA	0611012-11		SOIL	01-Nov-06	

Inorganic Data Reporting Qualifiers

The following qualifiers are used by the laboratory when reporting results of inorganic analyses.

- Result qualifier -- If the analyte was analyzed for but not detected a "U" is entered.

- QC qualifier -- Specified entries and their meanings are as follows:

  E - The reported value is estimated because of the presence of interference. An explanatory note may be included in the narrative.

  M - Duplicate injection precision was not met.

  N - Spiked sample recovery not within control limits. A post spike is analyzed for all 6010B analyses when the matrix spike and or spike duplicate fail and the native sample concentration is less than four times the spike added concentration.

  Z - Spiked recovery not within control limits. An explanatory note may be included in the narrative.

  * - Duplicate analysis (relative percent difference) not within control limits.

Total ICP Metals

Method SW6010B

Sample Results

Lab Name: Paragon Analytics
Work Order Number: 0611012
Client Name: ENSR Consulting and Engineering
ClientProject ID: ESTCP 09070-056-503

Field ID: Sed Ab-ABK11
Lab ID: 0611012-1

Sample Matrix: SOIL
% Moisture: 21.5
Date Collected: 31-Oct-06
Date Extracted: 02-Nov-06
Date Analyzed: 03-Nov-06
Prep Method: SW3050 Rev B

Prep Batch: IP061102-1
QCBatchID: IP061102-1-1
Run ID: it061103-2a2
Cleanup: NONE
Basis: Dry Weight

Sample Aliquot: 1.04 g
Final Volume: 100 ml
Result Units: mg/kg
Clean DF: 1
File Name: 061103a.

CASNO	Target Analyte	Dilution Factor	Result	Reporting Limit	Result Qualifier	EPA Qualifier
7440-50-8	COPPER	1	7.7	1.2		
7439-92-1	LEAD	1	26	0.37		

Data Package ID: *it0611012-1*

Total ICP Metals

Method SW6010B

Sample Results

Lab Name: Paragon Analytics
Work Order Number: 0611012
Client Name: ENSR Consulting and Engineering
ClientProject ID: ESTCP 09070-056-503

Field ID: Sed Ab-A08
Lab ID: 0611012-2

Sample Matrix: SOIL
% Moisture: 8.5
Date Collected: 31-Oct-06
Date Extracted: 02-Nov-06
Date Analyzed: 03-Nov-06
Prep Method: SW3050 Rev B

Prep Batch: IP061102-1
QCBatchID: IP061102-1-1
Run ID: it061103-2a2
Cleanup: NONE
Basis: Dry Weight

Sample Aliquot: 1.06 g
Final Volume: 100 ml
Result Units: mg/kg
Clean DF: 1
File Name: 061103a.

CASNO	Target Analyte	Dilution Factor	Result	Reporting Limit	Result Qualifier	EPA Qualifier
7440-50-8	COPPER	1	16	1		
7439-92-1	LEAD	1	170	0.31		

Data Package ID: *it0611012-1*

Total ICP Metals

Method SW6010B

Sample Results

Lab Name: Paragon Analytics
Work Order Number: 0611012
Client Name: ENSR Consulting and Engineering
ClientProject ID: ESTCP 09070-056-503

Field ID: Sed Ab-A07
Lab ID: 0611012-3

Sample Matrix: SOIL
% Moisture: 30.9
Date Collected: 31-Oct-06
Date Extracted: 02-Nov-06
Date Analyzed: 03-Nov-06
Prep Method: SW3050 Rev B

Prep Batch: IP061102-1
QCBatchID: IP061102-1-1
Run ID: it061103-2a2
Cleanup: NONE
Basis: Dry Weight

Sample Aliquot: 1.01 g
Final Volume: 100 ml
Result Units: mg/kg
Clean DF: 1
File Name: 061103a.

CASNO	Target Analyte	Dilution Factor	Result	Reporting Limit	Result Qualifier	EPA Qualifier
7440-50-8	COPPER	1	37	1.4		
7439-92-1	LEAD	1	410	0.43		

Data Package ID: *it0611012-1*

Total ICP Metals

Method SW6010B

Sample Results

Lab Name: Paragon Analytics
Work Order Number: 0611012
Client Name: ENSR Consulting and Engineering
ClientProject ID: ESTCP 09070-056-503

Field ID: Sed Ab-A04
Lab ID: 0611012-4

Sample Matrix: SOIL
% Moisture: 14.7
Date Collected: 31-Oct-06
Date Extracted: 02-Nov-06
Date Analyzed: 03-Nov-06
Prep Method: SW3050 Rev B

Prep Batch: IP061102-1
QCBatchID: IP061102-1-1
Run ID: it061103-2a2
Cleanup: NONE
Basis: Dry Weight

Sample Aliquot: 1.04 g
Final Volume: 100 ml
Result Units: mg/kg
Clean DF: 1
File Name: 061103a.

CASNO	Target Analyte	Dilution Factor	Result	Reporting Limit	Result Qualifier	EPA Qualifier
7440-50-8	COPPER	1	140	1.1		
7439-92-1	LEAD	1	960	0.34		

Data Package ID: *it0611012-1*

Total ICP Metals

Method SW6010B

Sample Results

Lab Name: Paragon Analytics
Work Order Number: 0611012
Client Name: ENSR Consulting and Engineering
ClientProject ID: ESTCP 09070-056-503

Field ID: Sed Ab-A3A
Lab ID: 0611012-5

Sample Matrix: SOIL
% Moisture: 34.4
Date Collected: 31-Oct-06
Date Extracted: 02-Nov-06
Date Analyzed: 03-Nov-06
Prep Method: SW3050 Rev B

Prep Batch: IP061102-1
QCBatchID: IP061102-1-1
Run ID: it061103-2a2
Cleanup: NONE
Basis: Dry Weight

Sample Aliquot: 1.04 g
Final Volume: 100 ml
Result Units: mg/kg
Clean DF: 1
File Name: 061103a.

CASNO	Target Analyte	Dilution Factor	Result	Reporting Limit	Result Qualifier	EPA Qualifier
7440-50-8	COPPER	1	1200	1.5		
7439-92-1	LEAD	50	17000	22		

Data Package ID: *it0611012-1*

Total ICP Metals

Method SW6010B

Sample Results

Lab Name: Paragon Analytics
Work Order Number: 0611012
Client Name: ENSR Consulting and Engineering
ClientProject ID: ESTCP 09070-056-503

Field ID: SDT BK-STA
Lab ID: 0611012-6

Sample Matrix: SOIL
% Moisture: 25.5
Date Collected: 31-Oct-06
Date Extracted: 02-Nov-06
Date Analyzed: 03-Nov-06
Prep Method: SW3050 Rev B

Prep Batch: IP061102-1
QCBatchID: IP061102-1-1
Run ID: it061103-2a2
Cleanup: NONE
Basis: Dry Weight

Sample Aliquot: 1.05 g
Final Volume: 100 ml
Result Units: mg/kg
Clean DF: 1
File Name: 061103a.

CASNO	Target Analyte	Dilution Factor	Result	Reporting Limit	Result Qualifier	EPA Qualifier
7440-50-8	COPPER	1	12	1.3		
7439-92-1	LEAD	1	15	0.38		

Data Package ID: *it0611012-1*

Total ICP Metals

Method SW6010B

Sample Results

Lab Name: Paragon Analytics
Work Order Number: 0611012
Client Name: ENSR Consulting and Engineering
ClientProject ID: ESTCP 09070-056-503

Field ID: SDT 04-DTA
Lab ID: 0611012-7

Sample Matrix: SOIL
% Moisture: 29.5
Date Collected: 31-Oct-06
Date Extracted: 02-Nov-06
Date Analyzed: 03-Nov-06
Prep Method: SW3050 Rev B

Prep Batch: IP061102-1
QCBatchID: IP061102-1-1
Run ID: it061103-2a2
Cleanup: NONE
Basis: Dry Weight

Sample Aliquot: 1 g
Final Volume: 100 ml
Result Units: mg/kg
Clean DF: 1
File Name: 061103a.

CASNO	Target Analyte	Dilution Factor	Result	Reporting Limit	Result Qualifier	EPA Qualifier
7440-50-8	COPPER	1	19	1.4		
7439-92-1	LEAD	10	1700	4.3		

Data Package ID: *it0611012-1*

Total ICP Metals

Method SW6010B

Sample Results

Lab Name: Paragon Analytics
Work Order Number: 0611012
Client Name: ENSR Consulting and Engineering
ClientProject ID: ESTCP 09070-056-503

Field ID: SST 04-DTA
Lab ID: 0611012-8

Sample Matrix: SOIL
% Moisture: 24.5
Date Collected: 31-Oct-06
Date Extracted: 02-Nov-06
Date Analyzed: 03-Nov-06
Prep Method: SW3050 Rev B

Prep Batch: IP061102-1
QCBatchID: IP061102-1-1
Run ID: it061103-2a2
Cleanup: NONE
Basis: Dry Weight

Sample Aliquot: 1.08 g
Final Volume: 100 ml
Result Units: mg/kg
Clean DF: 1
File Name: 061103a.

CASNO	Target Analyte	Dilution Factor	Result	Reporting Limit	Result Qualifier	EPA Qualifier
7440-50-8	COPPER	1	23	1.2		
7439-92-1	LEAD	50	30000	18		

Data Package ID: *it0611012-1*

Total ICP Metals

Method SW6010B

Sample Results

Lab Name: Paragon Analytics
Work Order Number: 0611012
Client Name: ENSR Consulting and Engineering
ClientProject ID: ESTCP 09070-056-503

Field ID: SST 07-DTA
Lab ID: 0611012-9

Sample Matrix: SOIL
% Moisture: 30.0
Date Collected: 31-Oct-06
Date Extracted: 02-Nov-06
Date Analyzed: 03-Nov-06
Prep Method: SW3050 Rev B

Prep Batch: IP061102-1
QCBatchID: IP061102-1-1
Run ID: it061103-2a2
Cleanup: NONE
Basis: Dry Weight

Sample Aliquot: 1.03 g
Final Volume: 100 ml
Result Units: mg/kg
Clean DF: 1
File Name: 061103a.

CASNO	Target Analyte	Dilution Factor	Result	Reporting Limit	Result Qualifier	EPA Qualifier
7440-50-8	COPPER	1	17	1.4		
7439-92-1	LEAD	10	2100	4.2		

Data Package ID: *it0611012-1*

Total ICP Metals

Method SW6010B

Sample Results

Lab Name: Paragon Analytics
Work Order Number: 0611012
Client Name: ENSR Consulting and Engineering
ClientProject ID: ESTCP 09070-056-503

Field ID: SDT 14-DTA
Lab ID: 0611012-10

Sample Matrix: SOIL
% Moisture: 30.1
Date Collected: 31-Oct-06
Date Extracted: 02-Nov-06
Date Analyzed: 03-Nov-06
Prep Method: SW3050 Rev B

Prep Batch: IP061102-1
QCBatchID: IP061102-1-1
Run ID: it061103-2a2
Cleanup: NONE
Basis: Dry Weight

Sample Aliquot: 1.01 g
Final Volume: 100 ml
Result Units: mg/kg
Clean DF: 1
File Name: 061103a.

CASNO	Target Analyte	Dilution Factor	Result	Reporting Limit	Result Qualifier	EPA Qualifier
7440-50-8	COPPER	1	21	1.4		
7439-92-1	LEAD	10	2800	4.2		

Data Package ID: *it0611012-1*

Total ICP Metals

Method SW6010B

Sample Results

Lab Name: Paragon Analytics
Work Order Number: 0611012
Client Name: ENSR Consulting and Engineering
ClientProject ID: ESTCP 09070-056-503

Field ID: SST 13-DTA
Lab ID: 0611012-11

Sample Matrix: SOIL
% Moisture: 2.2
Date Collected: 01-Nov-06
Date Extracted: 02-Nov-06
Date Analyzed: 03-Nov-06
Prep Method: SW3050 Rev B

Prep Batch: IP061102-1
QCBatchID: IP061102-1-1
Run ID: it061103-2a2
Cleanup: NONE
Basis: Dry Weight

Sample Aliquot: 1.04 g
Final Volume: 100 ml
Result Units: mg/kg
Clean DF: 1
File Name: 061103a.

CASNO	Target Analyte	Dilution Factor	Result	Reporting Limit	Result Qualifier	EPA Qualifier
7440-50-8	COPPER	1	15	0.98		
7439-92-1	LEAD	50	3700	15		

Data Package ID: *it0611012-1*

ICP Metals

Method SW6010B

Method Blank

Lab Name: Paragon Analytics
Work Order Number: 0611012
Client Name: ENSR Consulting and Engineering
ClientProject ID: ESTCP 09070-056-503

Lab ID: IP061102-1MB

Sample Matrix: SOIL
% Moisture: N/A
Date Collected: N/A
Date Extracted: 02-Nov-06
Date Analyzed: 03-Nov-06
Prep Method: SW3050 Rev B

Prep Batch: IP061102-1
QCBatchID: IP061102-1-1
Run ID: it061103-2a2
Cleanup: NONE
Basis: N/A

Sample Aliquot: 1 g
Final Volume: 100 ml
Result Units: mg/kg
Clean DF: 1
File Name: 061103a.

CASNO	Target Analyte	DF	Result	Reporting Limit	Result Qualifier	EPA Qualifier
7440-50-8	COPPER	1	1	1	U	
7439-92-1	LEAD	1	0.3	0.3	U	

Data Package ID: *it0611012-1*

ICP Metals

Method SW6010B

Laboratory Control Sample

Lab Name: Paragon Analytics
Work Order Number: 0611012
Client Name: ENSR Consulting and Engineering
ClientProject ID: ESTCP 09070-056-503

Lab ID: IP061102-1LCS

Sample Matrix: SOIL
% Moisture: N/A
Date Collected: N/A
Date Extracted: 11/02/2006
Date Analyzed: 11/03/2006
Prep Method: SW3050B

Prep Batch: IP061102-1
QCBatchID: IP061102-1-1
Run ID: it061103-2a2
Cleanup: NONE
Basis: N/A

Sample Aliquot: 1 g
Final Volume: 100 ml
Result Units: mg/kg
Clean DF: 1
File Name: 061103a.

CASNO	Target Analyte	Spike Added	LCS Result	Reporting Limit	Result Qualifier	LCS % Rec.	Control Limits
7440-50-8	COPPER	25	24.1	1		97	80 - 120%
7439-92-1	LEAD	50	47.2	0.3		94	80 - 120%

Data Package ID: *it0611012-1*

ICP Metals

Method SW6010B

Matrix Spike And Matrix Spike Duplicate

Lab Name: Paragon Analytics
Work Order Number: 0611012
Client Name: ENSR Consulting and Engineering
ClientProject ID: ESTCP 09070-056-503

Field ID: SST 13-DTA
LabID: 0611012-11MS

Sample Matrix: SOIL
% Moisture: 2.2
Date Collected: 01-Nov-06
Date Extracted: 02-Nov-06
Date Analyzed: 03-Nov-06
Prep Method: SW3050 Rev B

Prep Batch: IP061102-1
QCBatchID: IP061102-1-1
Run ID: it061103-2a2
Cleanup: NONE
Basis: Dry Weight

Sample Aliquot: 1 g
Final Volume: 100 ml
Result Units: mg/kg
File Name: 061103a.

CASNO	Target Analyte	Sample Result	Samp Qual	MS Result	MS Qual	Reporting Limit	Spike Added	MS % Rec.	Control Limits
7440-50-8	COPPER	15		41.9		1.02	25.6	105	80 - 120%
7439-92-1	LEAD	3700		3750		15.3	51.1	19	80 - 120%

Field ID: SST 13-DTA
LabID: 0611012-11MSD

Sample Matrix: SOIL
% Moisture: 2.2
Date Collected: 01-Nov-06
Date Extracted: 02-Nov-06
Date Analyzed: 03-Nov-06
Prep Method: SW3050 Rev B

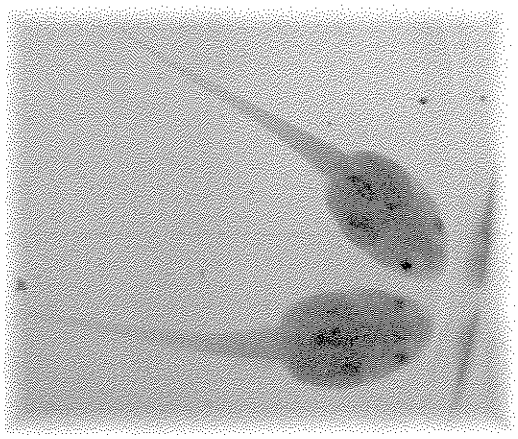
Prep Batch: IP061102-1
QCBatchID: IP061102-1-1
Run ID: it061103-2a2
Cleanup: NONE
Basis: Dry Weight

Sample Aliquot: 1.01 g
Final Volume: 100 ml
Result Units: mg/kg
File Name: 061103a.

CASNO	Target Analyte	MSD Result	MSD Qual	Spike Added	MSD % Rec.	Reporting Limit	RPD Limit	RPD
7440-50-8	COPPER	41.3		25.3	104	1.01	20	1
7439-92-1	LEAD	3680		50.6	-121	15.2	20	2

Data Package ID: *it0611012-1*

ENSR
Westford, MA
(09070-056)



Report of Short-Term Toxicity of Whole Sediment to *Rana pipiens*
(Sediments collected from Aberdeen Proving Ground)

Prepared by

ENSR | AECOM

ENSR
Environmental Toxicology
Fort Collins, CO

Laboratory Project 08503-128-019-(078-085)
ENSR Project 09070-056
December 2006

ENSR | AECOM

Report of Short-Term Toxicity of Whole Sediment to Rana pipiens

Project ID: 08503-128-019-(078-085)
December 2006

Sponsor and Laboratory Information

Sponsor	ENSR 2 Technology Park Drive Westford, MA 01886-3140 (978) 589-3000
ENSR Project Officer	John Bleiler
Testing Facility	ENSR Fort Collins Environmental Toxicology Laboratory 4303 West LaPorte Ave. Fort Collins, CO 80521 Fax: (970) 490-2963 State of Florida NELAP Laboratory ID E87972
Study Director	David A. Pillard, Ph.D.

Test Information

Test	Short-term chronic screening toxicity test of sediment	
Basis	NAVFAC (2004)	
Test Protocol	RP3ESTCP.TIE019.001	
Test Period	December 18, 2006 @ 1700 to December 28, 2006 @ 1235	
Test Length	10 days	
Species	*Rana pipiens*	
Test Material	Whole sediment	
Sediment ID	Sample (Field) ID	ENSR Laboratory ID
	SedAB-A08	19427
	SedAB-A07	19426
	SedAB-A04	19424
	M1	(19422+19423)
	M2	(19422+19423)
	M3	(19422+19423)
	SedAB-A03A	19423
Background (Reference) Sediment	SedAB-BK11	19422
Control Sediment	Cache la Poudre River Sediment	
Overlying water	Laboratory process water from Horsetooth Reservoir	
Test Concentrations	0 (control), SedAB-BK11, SedAB-A08, SedAB-A07, Sed-A04 and SedAB-A03A 100% of each test sediment. M1 = (17% SedAB-A03A and 83% SedAB-BK11) M2 = (25% SedAB-A03A and 75% SedAB-BK11) M2 = (50% SedAB-A03A and 50% SedAB-BK11)	

Sediment Collection and Receipt

Sample ID	Collection Date and Time	ENSR No.	Date of Receipt	Cooler Air Temp. at Arrival (°C)
SedAB-A08	4/12/06 @ 1410	19427	4/14/06	9
SedAB-A07	4/12/06 @ 1344	19426	4/14/06	9
SedAB-A04	4/12/06 @ 1215	19424	4/14/06	9
SedAB-A03A	4/12/06 @ 1200	19423	4/14/06	9
SedAB-BK11[a]	4/12/06 @ 1327	19422	4/14/06	9

[a] Background sample
Note: See Appendix A for copies of chain of custody records

Control Sediment

The control sediment was from the Cache la Poudre River northwest of Fort Collins, CO. The river sediment was washed through a No. 60 sieve with laboratory process water from Horsetooth Reservoir until the water ran clear. It was then air-dried before use.

Test Sediment Preparation

Sample ID	Date Homogenized	Time Homogenized
SedAB-A08	12/18/06	1145-1148
SedAB-A07		1232-1236
SedAB-A04		1207-1212
M1		1116-1120
M2		1116-1120
M3		1138-1145
SedAB-A03A		1209-1213
SedAB-BK11		1229-1232

Test Conditions

Test Type	Static sediment with continuous replacement of overlying water for 10 days
Overlying Water Delivery System	Continuous renewal (flow-through) [a]
Test Endpoints	Survival, Body Width and Length
Test Chambers	500-ml glass beakers
Test Sediment Volume	100 ml
Overlying Water Volume	175 ml
Replicates per Treatment	8
Organisms per Replicate	5
Test Temperature	23 ± 1°C; see Protocol Deviations
Lighting	Fluorescent, 16 hours light:8 hours dark
Chamber Placement	Randomized
Test Sediment Renewal	None
Test Overlying Water Renewal	Approximately two volume additions per test chamber per day

[a] Continuous replacement via a drip system
Note: See Appendix B for the Test Protocol

Test Organism

One the day of test initiation, five *Rana pipiens* were taken from the lot used in the test and examined to determine developmental stage. Based on gill development, tail length, and ventral appearance, it was determined that tadpoles were at stage 20 to 21, which is immediately post-hatch.

Species and Lot Number	*Rana pipiens*, Lot 06-056
Age	Stage 20-21 (Gosner 1960)
Source	Carolina Biological, Burlington, NC
Feeding	Approx. 3 mg TetraMin daily in each chamber, after reaching stage 25; see Protocol Deviations
Reference Toxicant Testing	Initiated December 18, 2006 using sodium chloride (NaCl)

TEST RESULTS

Biological Data – Mean Survival and Body Width and Length per Original Organism

Sample ID	Mean Percent Survival	Mean Body Width (mm)	Mean Body Length (mm)
Poudre River Sediment Control	100	5.4	9.0
SedAB-A08	98	5.7	9.8
SedAB-A07	100	5.8	9.9
SedAB-A04	75	3.8[a,b]	6.0[a,b]
M1	30[a,b]	1.3[c]	2.1[c]
M2	35[a,b]	1.5[c]	2.4[c]
M3	12[a,b]	0.4[c]	0.6[c]
SedAB-A03A	15[a,b]	0.4[c]	0.7[c]
SedAB-BK11	95	5.6	9.7
Control Performance	Acceptable	N/A	N/A

[a] Significantly (α=0.05) less than the Poudre River Control
[b] Significantly (α=0.05) less than the Background Sediment (SedAB-BK11)
[c] Excluded form statistical analysis because survival was significantly reduced
Note: See Appendix C for test data sheets

Data Analysis

Biological Endpoint	Comparison[a]	Procedure
Survival	Normality	Chi-Square Test (α=0.01)
	Homogeneity of Variance	Bartlett's Test (α=0.01)
	Significant Reduction Relative to the Poudre River Sediment Control or SedAB-BK11	Steel's Many-One Rank Test (α=0.05)
Body Width	Normality	Chi-Square Test (α=0.01)
	Homogeneity of Variance	Bartlett's Test (α=0.01)
	Significant Reduction Relative to the Poudre River Sediment Control or SedAB-BK11	Steel's Many-One Rank Test (α=0.05)
Body Length	Normality	Chi-Square Test (α=0.01)
	Homogeneity of Variance	Bartlett's Test (α=0.01)
	Significant Reduction Relative to the Poudre River Sediment Control or SedAB-BK11	Steel's Many-One Rank Test (α=0.05)

[a] Using Toxstat Version 3.5 (WEST, Inc. and Gulley 1996)

Physical and Chemical Data

Sample ID	pH (units)	DO (mg/L)	Conductivity (µS/cm)	Temperature (°C)[a]	Ammonia as N (mg/L)	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)
Poudre R. Control	7.4-7.9	5.3-6.6	107-110	22-23	<1.0-1.5	42-44	27-28
SedAB-A08	6.1-7.5	4.3-6.5	52-95	22-23	<1.0-1.9	18-34	13-33
SedAB-A07	6.4-7.2	4.4-6.1	58-89	22-23	2.0-2.4	16-32	6-29
SedAB-A04	6.6-7.6	4.5-6.4	68-89	22-23	<1.0-1.1	18-34	7-31
M1	6.2-7.6	4.0-6.5	45-92	22-23	<1.0-1.4	16-36	7-34
M2	6.3-7.6	4.5-6.4	44-96	22-23	<1.0-1.7	12-36	12-34
M3	6.6-7.6	5.2-6.4	59-85	22-23	<1.0-1.2	18-34	16-31
SedAB-A03A	7.0-7.7	4.8-6.9	78-81	22-23	<1.0-1.0	26-32	29-32
SedAB-BK11	5.5-7.3	4.5-6.4	31-83	22-23	<1.0-1.4	14-32	3-28

[a] Temperature in test chambers

Concentration of Copper and Lead in Test Sediments

Sample ID	Copper (mg/kg dry weight)	Lead (mg/kg dry weight)
Poudre R. Control	7.2	4.5
SedAB-A08	16	170
SedAB-A07	37	410
SedAB-A04	140	960
M1	210[a]	2912[a]
M2	306[a]	4270[a]
M3	604[a]	8513[a]
SedAB-A03A	1200	17000
SedAB-BK11	7.7	26

[a] Expected concentrations calculated using known concentrations of SedAB–BK11 and SedAB-A03A
Note: See Appendix D for raw analytical data from Paragon Analytics

Reference Toxicant Test Results for *R. pipiens*

Organism Lot Number	Test Date	96-Hour LC_{50} (mg/L Cl^-)	ENSR/FCETL Historical 95% Control Limits	
			Low	High
06-056	December 18, 2006	3502	NA	NA

Note: There are less than five data points in the database, therefore historical limits are not reported

Protocol Deviations

According to the continuous temperature monitoring system, which records temperature in the water bath every half hour, at some point during the time between approximately 0800 on day 9 and 0800 on day 10, the temperature in the water bath fell to 17.4°C. However, the daily mean temperature for the water bath during this time period was 22.2°C (acceptable) and all temperatures measured in overlying water in the test chambers were 23±1°C. The temperature excursion to 17.4°C was likely short-term and had no effect on the test outcome.

The amount of Tetrafin added to each test chamber varied from the approximate value of 3 mg/day suggested in the protocol. On days 3, 4, 5 and 6 approximately 4 mg of Tetrafin were used per chamber; on days 7 and 8 the amount was increased to approximately 8 mg of Tetrafin. On day 9 approximately 6 mg of Tetrafin was added to each chamber. As the amount of food added was the same in all chambers, this deviation probably did not affect test outcome.

In addition to 100% sediment as specified in the protocol, three mixtures of SedAB-BK11 and Sed AB-A03A were tested.

Ammonia was not measured in each treatment on days 3 and 7 as specified in the protocol. Instead, it was measured only on days 0 and 10. This deviation had no adverse effect on the biological data.

References

Gosner, K. L. 1960. A Simplified Table for Staging Anuran Embryos and Larvae with Notes on Identification. Herpetologica, 16:183-190.

NAVFAC. 2004. Development of a Standardized Approach for Assessing Potential Risks to Amphibians Exposed to Sediment and Hydric Soils. Prepared for Naval Facilities Engineering Service Center (NFESC), Port Hueneme, CA.

WEST, Inc. and D.D. Gulley. 1996. Toxstat Version 3.5. Western EcoSystems Technology, Inc., Cheyenne, WY.

Statement of Procedural Compliance

I certify that this document and all attachments were prepared under my direction or supervision in accordance with a system designed to assure that qualified personnel properly gather and evaluate the information submitted. Based on my inquiry of the person or persons who manage the system, or those persons directly responsible for gathering the information, the information submitted is, to the best of my knowledge, accurate and complete.

David A. Pillard 16 Jan 2007

David A. Pillard, Ph.D. Date
Study Director

Statement of Quality Assurance

The test data were reviewed by the Quality Assurance Unit to assure that the study was performed in accordance with the protocol and standard operating procedures, and that the resulting data and report meet the requirements of the NELAC standards. This report is an accurate reflection of the raw data.

Anita Rehner January 16, 2007

Quality Assurance Unit Date

APPENDIX A

Chain of Custody

ENSR INTERNATIONAL

CHAIN OF CUSTODY RECORD

Client/Project Name: ESTCP

Project Location: Aberdeen Proving Ground

Project Number: 09070-056-502

Field Logbook No.: 03061291

Sampler (Print Name)/(Affiliation): A. DesLetts / ENSR

Chain of Custody Tape No.: — 25320 + 25880

Signature: [signature]

Send Results/Report to: S. Bliler / ENSR

Analysis Requested: TOC

Field Sample No / Identification	Date	Time	Grab	Comp	Sample Container (Size/Mat'l)	Sample Type (Liquid, Sludge, Etc.)	Preservative	Field Filtered	TOC							Lab I.D.	Remarks
SedAb-A10	4/12/06	14:20	✓		1gal. pl.	Sed.	—	N	✓							194289 ①	
SedAb-A09	↓	1355	✓		1gal. pl.	↓	—	N	✓							19427~~7~~8 ①	
SedAb-A07	↓	1344	✓		1gal pl.	↓	—	N	✓							19428~~8~~6 ①	
SedAb-A08	↓	1410	✓		1gal. pl.	↓	—	N	✓							19428~~7~~7 ①	

Relinquished by: (Print Name)/(Affiliation)	Date / Time	Relinquished by: (Print Name)/(Affiliation)	Date / Time
A. DesLetts / ENSR Signature: [signature]	Date: 4/12/06 Time: 1200	~~Relinquished by~~ Received ② David S. Pillard Signature: [signature]	Date: 4-14-06 Time: 1330
Relinquished by: (Print Name)/(Affiliation) Signature:	Date: Time:	Relinquished by: (Print Name)/(Affiliation) Signature:	Date: Time:
Relinquished by: (Print Name)/(Affiliation) Signature:	Date: Time:	Relinquished by: (Print Name)/(Affiliation) Signature:	Date: Time:

Analytical Laboratory (Destination):

ENSR - Ft. Collins

Fed Ex

Cooler Air Temp = 9°C

Serial No. 38475

FM01008a_902

① DAP 4/14/06 WP ② DAP 4/14/06 E

ENSR INTERNATIONAL

CHAIN OF CUSTODY RECORD

Client/Project Name: ESTCP

Project Location: Aberdeen Proving Ground

Project Number: 09070-056-502

Field Logbook No.: 0306A291

Sampler (Print Name)/(Affiliation): A. Desilets / ENSR

Chain of Custody Tape No.: 27000 & 27500

Signature: [signature]

Send Results/Report to: J. Bleiler

Analysis Requested: TOC

Field Sample No./ Identification	Date	Time	Grab	Comp	Sample Container (Size/Mat'l)	Sample Type (Liquid, Sludge, Etc.)	Preservative	Field Filtered	TOC							Lab I.D.	Remarks
SedAb-Bk11	4/12/06	13:27	✓		1gal. pl.	Sed.	—	N	✓							19422	
SedAb-A03A	↓	12:00	✓		↓	↓	—	N	✓							19423	Container Labeled: SedAb-A3A
SedAb-A04	↓	12:15	✓		↓	↓	—	N	✓							19424	
SedAb-A03B	↓	11:30	✓		↓	↓	—	N	✓							19425	

Relinquished by: (Print Name)/(Affiliation)	Date / Time	~~Relinquished~~ Received by: (Print Name)/(Affiliation)	Date / Time
A Desilets / ENSR Signature: [signature]	Date: 4/13/06 Time: 1200	David Pillard Signature: [signature]	Date: 4-14-06 Time: 1330
Relinquished by: (Print Name)/(Affiliation) Signature:	Date: Time:	Relinquished by: (Print Name)/(Affiliation) Signature:	Date: Time:
Relinquished by: (Print Name)/(Affiliation) Signature:	Date: Time:	Relinquished by: (Print Name)/(Affiliation) Signature:	Date: Time:

Analytical Laboratory (Destination): ENSR - Ft. Collins

Feb Ex

Cooler Air Temp = 9°C

Serial No. 38473

FM01008a_902

GDAD 4-14-06 E

APPENDIX B

Test Protocol

Title: Toxicity of Sediment to Amphibian Larvae, *Rana pipiens*

Study Sponsor:	ENSR 2 Technology Park Drive Westford, MA 01886-3140
Testing Facility	Fort Collins Environmental Toxicology Laboratory 4303 West LaPorte Avenue Fort Collins, Colorado 80521

1.0 INTRODUCTION

1.1 Objective

To determine the toxicity of sediment samples to the Northern Leopard Frog, *Rana pipiens*.

1.2 Experimental Approach

Sediment samples will be visually examined for the presence of endemic organisms, which will be removed. Tadpoles will be added to the overlying water and test chambers maintained under test conditions until test termination. Test chambers will be examined for surviving organisms, which will then be measured for body width and length.

1.3 Test Material

The test material will be a field-collected sediment. Samples will be stored under refrigeration (4°C) until used in testing. Each sample will be homogenized mechanically or by hand, prior to testing.

2.0 BASIS AND TEST ORGANISM

2.1 Basis

This protocol is based on USEPA (2002) and NAVFAC (2004), where appropriate.

2.2 Test Organism

1. Species – *Rana pipiens*
2. Age – Organisms used to initiate the tests will be recently hatched tadpoles at approximately Gosner stage 18 (Gosner 1960).
3. Source - Test organisms will be obtained from a commercial supplier.
4. Feeding – Tadpoles will be fed approximately 3 mg TetraMin per chamber daily after stage 25.

3.0 TEST SYSTEM

3.1 Artificial Sediment

If the laboratory control sediment is an artificial sediment, it will consist of washed natural sediment or washed silica sand. Control sediment may be amended with the addition of organic matter (e.g., peat).

3.2 Overlying Water

The overlying water used in the toxicity test will be uncontaminated surface water from Horsetooth Reservoir.

3.3 Test Temperature

Test temperature will be 23 ± 1°C. Testing will be conducted in an environmental chamber or a temperature controlled water bath.

3.4 Test Containers

Test containers will be 500-ml beakers containing 100 ml of sediment and 175 ml of overlying water.

3.5 Photoperiod

The photoperiod will be 16-hours light and 8-hours dark.

3.6 Dissolved Oxygen Concentrations

Dissolved oxygen concentrations in the overlying water will be maintained ≥ 3.0 mg/L. If the dissolved oxygen concentration in the overlying water approaches this level, all test chambers will be gently aerated throughout the remainder of the test. If aeration is initiated, the aeration pipette will be appropriately positioned so as to avoid disturbance of the sediment.

3.7 Reference Toxicant Testing

In addition to the test material exposures, reference toxicant tests will be conducted using sodium chloride (NaCl) or copper chloride ($CuCl_2$) to determine the sensitivity range of the test organisms. Reference toxicant exposures will be conducted at the time of test initiation for commercially-supplied organisms.

4.0 TEST DESIGN

4.1 Test Treatments

The test concentration will be 100 percent of each test sediment. A 100 percent laboratory control sediment (see section 4.3) exposure will be conducted concurrently. A reference sediment collected at the study site may also be included.

4.2 Sediment/Water Mixture

Sediment will be placed in each test chamber at least one day (day –1) before test initiation. Because of uncertainty in the availability of the amphibian embryos and hatching time, sediment may remain in the chambers for up to three days prior to test initiation. After addition of sediment, the overlying water will be carefully poured into each beaker. The beakers will be left static and unaerated overnight to allow sediment to settle and to reduce turbidity prior to addition of test organisms.

4.3 Reference/Control Sediment

In addition to the field-collected samples, at least one control sediment will be tested concurrently. Control sediment will be either an artificial laboratory sediment (silica sand, see section 3.1) or natural sediment collected from the Cache la Poudre River (northwest of Fort Collins, CO).

4.4 Number of Test Organisms

Forty tadpoles will be exposed to each treatment. Five organisms will be assigned to each test chamber and eight replicates will be tested per treatment.

4.5 Analytical Sampling

Aliquots of sediment for chemical analysis will be removed by the study sponsor prior to shipping samples to ENSR's laboratory. No analytical sampling of the test chambers themselves is planned.

4.6 Test Initiation/Renewal Frequency

Testing will be initiated by addition of the test organisms after the overnight sediment settling period. Renewal of overlying water will be accomplished either by a flow-through system or with a renewal box. Each chamber will be renewed with a minimum of 2 volume additions per day.

4.7 Chemical and Physical Monitoring

At a minimum, the following measurements will be made:

1. Dissolved oxygen, temperature, and pH will be measured in the overlying water of each treatment and the control each day of testing.

2. Hardness, alkalinity, conductivity, and ammonia will be measured in the overlying water of each treatment on day 0.

3. Hardness, alkalinity, conductivity, and ammonia will be measured in overlying water from each treatment at test initiation (just prior to renewal on day 0 or 1) and at test termination.

4. Ammonia will also be measured in each treatment on days 3 and 7.

4.8 Biological Monitoring

After ten days of exposure, sediment from each test chamber will be removed and sieved or sorted (if needed) to recover living test organisms. Organisms not recovered at test termination will be presumed dead. At test termination, the surviving organisms in each test chamber will be counted and the width of the body (at the widest point) and length of the body (snout to base of tail) will be determined.

4.9 Test Duration

The test duration will be 10 days.

4.10 Calculations

Survival data will be transformed by arcsine squareroot. Normality and homogeneity assumptions for survival data will be evaluated by the Shapiro-Wilk's (or Chi-Square) test and Bartlett's test, respectively (α = 0.01). Data will then be evaluated (α = 0.05) using either parametric or nonparametric methods depending upon the outcome of the normality and homogeneity assessments.

Organism width and length will be statistically compared in treatments not having significantly reduced survival. Analysis will occur in the same manner as for survival, although the width and length measurements will not be transformed using arcsine squareroot.

4.11 Quality Criterion

The test will not be considered acceptable if mortality in the control sediment exceeds 20 percent.

5.0 TEST REPORT

The report will be a typed document describing the results of the test and will be signed by the Study Director and Quality Assurance Unit. The report will include, but not be limited to, the following:

- A copy of all raw data.
- Name of test, Study Director, and laboratory, and date test was begun.
- Time and date of sediment collection.
- The source of the overlying water and a description of any pretreatment.
- Test organism scientific name and age.
- A description of the experimental design, manner in which the test was initiated, the number of organisms per treatment, replicates per treatment and the lighting.
- A description of any aeration performed on test solutions before or during the test.
- Definition of the criterion used to determine the effect and a summary of general observations on other effects or symptoms.
- Percentage of organisms that died or showed an effect.
- The minimum dissolved oxygen concentration, range in tests temperature and pH, all visual observations.
- Any deviations from the protocol.

6.0 LITERATURE CITED

Gosner, K.L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*. 16:183-190.

NAVFAC. 2004. Development of a Standardized Approach for Assessing Potential Risks to Amphibians Exposed to Sediment and Hydric Soils.

USEPA. 2002. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. Fourth Edition. EPA-821-R-02-013.

7.0 PROCEDURAL COMPLIANCE

All test procedures, documentation, records, and reports will comply with USEPA (2002) general guidance on quality assurance related to effluent toxicity testing. To this end, random audits of the test may be scheduled while the test is in progress. The raw data will be checked and compared to protocol requirements and Standard Operating Procedures, and the final report will be audited for accuracy and signed, if satisfactory, by both the Study Director and an individual from the Quality Assurance Unit.

8.0 PROTOCOL AMENDMENTS AND DEVIATIONS

All changes (i.e., amendments, deviations, and final report revisions) of the approved protocol plus the reasons for the changes must be documented in writing. The changes will be signed and dated by the Study Director and maintained with the protocol. All amendments must be authorized in advance by the Sponsor.

9.0 SPONSOR AND STUDY DIRECTOR APPROVAL

Study Director: David A. Pillard	Date: 10 Jan 2007

APPENDIX C

Data Sheets

FCETL QA Form No. 051
Revision 4
Effective 1/96

DAP 1/16/07
QA: AR01-09-07

TOXICITY DATA PACKAGE COVER SHEET

Test Type: 10 Day Short-term Chronic

Project Number: 8503-128-019- 078-085

Test Substance: Sediment

Species: *Rana pipiens*

~~Dilution~~ Overlying Water Type: HT tempered

Organism Lot or Batch Number: 06-056

Concurrent Control Water Type: N/A

Age: Stage 21 (<24 hrs) Supplier: Carolina Biol.

Date and Time Test Began: 12/18/06 @ 1700

Date and Time Test Ended: 12/28/06 @ 1235

Protocol Number: ~~NA~~ ① RP3ESTCP.TIE019.001

Investigator(s): DAP / CW / HP / MM

Background Information

Type of Test:

pH Control?: Yes (No) If Yes, give % CO_2: N/A

Test Temperature: 23±1°C

Env. Chmbr/Bath #: Bath #3

Sediment volume: 100 ml

Test Chambers: 500 ml glass beakers

Test Solution Vol.: 175 ml

Number of Replicates per Treatment: 8

Length of Test: 10 days

Number of Organisms per Replicate: 5

Type of Food and Quantity per Chamber:

Feeding Frequency: 3 mg Tetramin per chamber after stage 25

Test Substance Characterization Parameters and Frequency: Hardness: Alkalinity:

NH_3: pH: Conductivity: TRC:

Test Concentrations (Volume:Volume): Control, SedAB-BK11, SedAB-A08, SedAB-A07, SedAB-A04, M1, M2, M3, SedAB-A3A

Agency Summary Sheet(s)?: NA

Reference Toxicant Data: Test Dates: 12/18/06 to 12/22/06 LC_{50} or IC_{25} (Circle): 3502 mg Cl/L

Hist. 95% Control Limits: N/A to N/A Method for Determining Ref. Tox. Value: T-S-K

Special Procedures and Considerations:

Tadpoles at Stages 20-21 (most 21) @ initiation (DAP)

Study Director Initials: DAP Date: 12/18/06

① INC for DAP01-09-07 CF

FCETL QA Form 014
Revision 0
Effective 1/96
DZP 1/16/07
QA: MC01-0907

TEST SUBSTANCE USAGE LOG

Project Number 8503-128-019- 078 to 085

	Sample 1	Sample 2	Sample 3	Sample 4
Test Substance Number	19423			
Test Substance Collection Date and Time	From: 4/12/06 @ 1200	From: @	From: @	From: @
	To: @ NA	To: @	To: @	To: @
Sample Type (GrAb or Comp)	Grab			
Date Test Substance Received	4-14-06			
Dilution ~~Water~~ Sediment Number RW# or ~~FCETL#, circle one~~	19422			
Concurrent Control Water RW#	NA			
Date(s) Used	12/14/06			

PREPARATION OF TEST SOLUTIONS

	Test Substance % Conc.	Test Substance ~~Volume (ml)~~ Sediment (g)	Dilution ~~Water Volume (ml)~~ Sediment (g)	Total ~~Volume (ml)~~ (g)	Test Substance Volume (ml)	Dilution Water Volume (ml)	Total Volume (ml)	Test Substance Volume (ml)	Dilution Water Volume (ml)	Total Volume (ml)
	Control	NA	NA	NA						
M1	17%	340	1660	2000						
M2	25%	500	1500	2000						
M3	50%	1000	1000	2000						
	Total									

Initials/Date	12/14/06		
Initials/Date			
Initials/Date			
Initials/Date			
Initials/Date			
Initials/Date			
Initials/Date			
Initials/Date			

D.P. 12/14/06 CF

FCETL QA Form No. 060
Revision 2
Effective 1/96

DAP 1/16/07
QA: AROH1007

Pickerel Frog (*Rana pipiens*)
CHRONIC BIOLOGICAL DATA

Project Number: 8503-128-019-078 to 085

Conc.	Test Replicate	Number of Surviving Organisms								
		Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Remarks
Control	A	5	X	X	X	X	X	5	X	
	B	5	X	X	X	X	X	5	X	
	C	5	X	X	X	X	X	5	X	
	D	5	X	X	X	X	X	5	X	
	E	5	X	X	X	X	X	5	X	
	F	5	X	X	X	X	X	5	X	
	G	5	X	X	X	X	X	5	X	
	H	5	X	X	X	X	X	5	X	
BK11	A	5	X	X	X	X	X	5	X	
	B	5	X	X	X	X	X	5	X	
	C	5	X	X	X	X	X	5	X	
	D	5	X	X	X	X	X	5	X	
	E	5	X	X	X	X	X	5	X	
	F	5	X	X	X	X	X	5	X	
	G	5	X	X	X	X	X	5	X	
	H	5	X	X	X	X	4	4	X	
A08	A	5	X	X	X	X	X	5	X	
	B	5	X	X	X	X	X	5	X	
	C	5	X	X	X	X	X	5	X	
	D	5	X	X	X	X	X	5	X	
	E	5	X	X	X	X	X	5	X	
	F	5	X	X	X	X	X	5	X	
	G	5	X	X	X	X	X	5	X	
	H	5	X	X	X	X	X	5	X	
	Date:	12/15/06	12/19/06	12/20/06	12/21/06	12/22/06	12/23/06	12/26/06	12/27/06	
	Time:	1700	1735	1350	1452	1625	1250	1015	1000	
	Initials:	DAP	DW	DAP	DAP	DAP	TP	TP	DAP	

note: X = no observations made

FCETL QA Form No. 060
Revision 2
Effective 1/96

DAP 1/16/07
QA: AR01-1007

Pickerel Frog (*Rana pipiens*)
CHRONIC BIOLOGICAL DATA

Project Number: 8503-128-019-~~065~~ 078 to

Conc.	Test Replicate	Number of Surviving Organisms								
		Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Remarks
A07	A	5	X	X	X	X	X	5	X	
	B	5	X	X	X	X	X	5	X	
	C	5	X	X	X	X	X	5	X	
	D	5	X	X	X	X	X	5	X	
	E	5	X	X	X	X	~~4~~ 5	5	X	
	F	5	X	X	X	X	X	5	X	
	G	5	X	X	X	X	X	5	X	
	H	5	X	X	X	X	X	5	X	
A04	A	5	X	X	X	X	X	5	X	
	B	5	X	X	X	X	4	4	X	
	C	5	X	X	X	X	X	5	X	
	D	5	X	X	X	X	X	5	X	
	E	5	X	X	X	X	X	5	X	
	F	5	X	X	X	X	X	5	X	
	G	5	X	X	X	X	X	5	X	
	H	5	X	X	X	X	X	5	X	
M1	A	5	X	X	X	5	X	5	X	
	B	5	X	X	X	4	3	3	X	
	C	5	X	X	X	5	~~4~~ 5	5	X	
	D	5	X	X	X	4	4	4	X	
	E	5	X	X	X	2	1	1	X	
	F	5	X	X	X	3	3	2	X	
	G	5	X	X	X	5	4	4	X	
	H	5	X	X	X	2	2	2	X	
	Date:	12/18/06	12/19/06	12/20/06	12/21/06	12/22/06	12/23/06	12/24/06	12/25/06	
	Time:	1200	1125	1350	1452	1625	1250	1015	1000	
	Initials:	DAP	AW	DAP	DAP	DAP	TD	TD	DAP	

note: X= no observations made DAP 12/22/06

FCETL QA Form No. 060
Revision 2
Effective 1/96

DAP 1/16/07
QA: AR01-1007

Pickerel Frog (*Rana pipiens*)
CHRONIC BIOLOGICAL DATA

Project Number: 8503-128-019-~~018~~ 085

Conc.	Test Replicate	Number of Surviving Organisms								Remarks
		Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	
M2	A	5	X	5	X	5	5	5	X	
	B	5	X	5	X	5	5	5	X	
	C	5	X	0	–	–	–	–	–	
	D	5	X	5	X	3	2	2	X	
	E	5	X	5	X	5	5	4	X	
	F	5	X	5	X	2	2	2	X	
	G	5	X	3	X	0	–	–	–	
	H	5	X	5	X	0	–	–	–	
M3	A	5	X	1	1	1	1	1	X	
	B	5	X	0	0	–	–	–	–	
	C	5	X	0	–	–	–	–	–	
	D	5	X	0	–	–	–	–	–	
	E	5	X	1	0	–	–	–	–	
	F	5	X	5	5	5	5	5	X	
	G	5	X	0	0	–	–	–	–	
	H	5	X	0	0	–	–	–	–	
A3A	A	5	X	0	–	–	–	–	–	
	B	5	X	0	–	–	–	–	–	
	C	5	X	5	5	4	3	3	X	
	D	5	X	0	–	–	–	–	–	
	E	5	X	3	3	3	X	3	X	
	F	5	X	0	–	–	–	–	–	
	G	5	X	0	–	–	–	–	–	
	H	5	X	0	–	–	–	–	–	
	Date:	12/18/06	12/19/06	12/20/06	12/21/06	12/22/06	12/23/06	12/24/06	12/25/06	
	Time:	1200	1125	1350	1452	1625	1250	1015	1000	
	Initials:	DAP	AW	DAP	DAP	DAP	TD	TD	DAP	

NOTE: X = No observations made.

FCETL QA Form No. 060
Revision 2
Effective 1/96

DDP 1/16/07
QA: ARC 01-10-07

Pickerel Frog (*Rana pipiens*)
CHRONIC BIOLOGICAL DATA

Project Number: 8503-128-019-~~018~~ 085

Conc.	Test Replicate	Number of Surviving Organisms								
		Day 8	Day 9	Day 10						Remarks
Control	A	5	X	5						100%
	B	5	X	5						
	C	5	X	5						
	D	5	X	5						
	E	5	X	5						~~97.5%~~
	F	5	X	5						
	G	5	X	5						
	H	5	X	5						
BK11	A	5	X	5						~~97.5%~~ 95%
	B	5	X	5						
	C	5	X	4						
	D	5	X	5						
	E	5	X	5						
	F	5	X	5						
	G	5	X	5						
	H	4	X	4						
A08	A	5	X	5						97.5%
	B	5	X	5						
	C	5	X	5						
	D	5	X	5						
	E	5	X	5						
	F	5	X	5						
	G	5	X	5						
	H	5	X	4						
	Date:	12/26/06	12/27/06	12/28/06						
	Time:	1145	1145	1235						
	Initials:	MM	MM	MM						

Note: X = no observations made

09 1/31/07 WY E

FCETL QA Form No. 060
Revision 2
Effective 1/96

DAP 1/16/07
QA: AR01-10-07

Pickerel Frog (*Rana pipiens*)
CHRONIC BIOLOGICAL DATA

Project Number: 8503-128-019-~~078~~ 085

Conc.	Test Replicate	Number of Surviving Organisms								
		Day 8	Day 9	Day 10						Remarks
A07	A	5	X	5						100%
	B	5	X	5						
	C	5	X	5						
	D	5	X	5						
	E	5	X	5						① ~~75%~~
	F	5	X	5						
	G	5	X	5						
	H	5	X	5						
A04	A	4	X	4						75%
	B	2	X	2						
	C	5	X	0						
	D	5	X	4						
	E	5	X	5						
	F	5	X	5						
	G	5	X	5						
	H	5	X	5						
M1	A	4	X	4						30%
	B	3	X	1						
	C	3	X	1						
	D	4	X	0						
	E	1	X	0						
	F	2	X	1						
	G	4	3	3						
	H	2	X	2						
	Date:	12/26/06	12/27/06	12/28/06						
	Time:	1145	1145	1235						
	Initials:	mm	mm	mm						

note: X = no observation made. ① ~~75%~~ 1/9/2007

FCETL QA Form No. 060
Revision 2
Effective 1/96

DAP 1/16/07
QA: AR01-10-07

Pickerel Frog (*Rana pipiens*)
CHRONIC BIOLOGICAL DATA

Project Number: 8503-128-019-078 to 085

Conc.	Test Replicate	Number of Surviving Organisms								
		Day 8	Day 9	Day 10						Remarks
M2	A	5	X	5						35%
	B	5	X	4						
	C	—	—	0						
	D	2	X	0						
	E	4	X	3						
	F	2	X	2						
	G	—	—	0						
	H	—	—	0						
M3	A	1	X	0						12.5%
	B	—	—	0						
	C	—	—	0						
	D	—	—	0						
	E	—	—	0						
	F	5	X	5						
	G	—	—	0						
	H	—	—	0						
A3A	A	—	—	0						15%
	B	—	—	0						
	C	3	X	3						
	D	—	—	0						
	E	3	X	3						
	F	—	—	0						
	G	—	—	0						
	H	—	—	0						
	Date:	12/26/06	12/27/06	12/28/06						
	Time:	1145	1145	1235						
	Initials:	MM	MM	MM						

note: X = no observations made

FCETL QA Form No. 059
Revision 2
Effective 1/96

DAP 1/16/07
QA: MR01-10-07

CHRONIC CHEMICAL DATA (FINAL)

Project Number: 8503-128-019-~~018~~ to 085

Test Species (Circle): *C. dubia* *D. magna* *D. pulex* *P. promelas* Other (Specify): ***Rana pipiens***

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Meter #	Remarks
Conc.: Control									All Conc.	
pH	7.8	7.7	7.9	7.6	7.5	7.7	7.8	7.5	16	
D.O. (mg/L)	6.0	6.2	6.1	6.0	5.5	6.0	6.0	5.3	6	
Temp. (°C)	22	23	23	22	23	22	23	23	10A	
Conc.: BK11										
pH	6.8	① ~~7.6~~ 6.9	② ~~7.3~~ 7.2	7.3	6.9	7.1	7.3	7.3		
D.O. (mg/L)	5.8	6.0	5.8	6.1	4.9	4.9	5.0	5.0		
Temp. (°C)	23	23	23	22	23	22	23	23		
Conc.: A08										
pH	6.1	6.8	7.3	7.1	6.6	7.2	7.5	7.3		
D.O. (mg/L)	5.8	5.2	5.5	5.4	4.3	5.2	6.1	5.3		
Temp. (°C)	23	23	23	22	23	23	23	23		
Conc.: A07										
pH	6.8	6.4	6.9	6.7	7.0	7.2	6.9	6.7		
D.O. (mg/L)	5.8	6.1	5.8	4.4	5.2	5.6	5.0	4.8		
Temp. (°C)	23	23	23	22	23	23	23	23		
Conc.: A04										
pH	6.8	6.6	6.9	6.6	7.6	7.5	7.6	7.3		
D.O. (mg/L)	6.0	6.2	5.8	4.5	6.2	6.2	6.2	5.3		
Temp. (°C)	23	23	23	22	22	22	23	23		
Conc.: M1										
pH	6.6	7.1	7.0	6.4	6.9	7.6	7.3	7.0		
D.O. (mg/L)	6.0	6.2	5.6	4.3	5.0	6.4	5.6	4.5		
Temp. (°C)	23	23	23	22	22	22	23	23		
Conc.: M2										
pH	6.8	6.5	6.8	6.3	7.0	7.5	7.6	7.3		
D.O. (mg/L)	5.8	6.4	5.8	4.5	5.0	6.2	6.3	5.2		
Temp. (°C)	23	23	23	22	22	22	23	23		
Date:	12/19/06	12/20/06	12/21/06	12/22/06	12/23/06	12/24/06	12/25/06	12/26/06		
Time:	1715	1320	1435	1610	1245	1010	1000	1200		
Initials:	AW	DAP	DAP	AW	AW	TD	DAP	MM		

① DAP 12/20/06 F ② DAP 12/21/06 F

FCETL QA Form No. 059
Revision 2
Effective 1/96

DAP 1/16/07
QA: AR01-1007

CHRONIC CHEMICAL DATA (FINAL)

Project Number: 8503-128-019-078 to 085

Test Species (Circle): *C. dubia* *D. magna* *D. pulex* *P. promelas* Other (Specify): ***Rana pipiens***

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Meter #	Remarks
Conc.: M3							*		All Conc.	
pH	6.8	6.6	7.0	7.1	7.4	7.5	7.6	7.5		
D.O. (mg/L)	5.8	6.1	5.2	5.9	6.0	6.3	6.4	5.8		
Temp. (°C)	23	23	23	22	22	22	23	23		
Conc.: A3A										
pH	7.1	7.0	7.0	7.0	7.6	7.6	7.7	7.5		
D.O. (mg/L)	5.8	6.1	4.8	5.0	6.2	6.9	6.5	5.9		
Temp. (°C)	23	23	23	22	22	23	23	23		
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Conc.:							*			
pH										
D.O. (mg/L)										
Temp. (°C)										
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Date:	12/19/06	12/20/06	12/21/06	12/22/06	12/23/06	12/24/06	12/25/06	12/26/06		
Time:	1715	1320	1435	1610	1245	1610	1000	1200		
Initials:	AW	DAP	DAP	[illegible]	[illegible]	TD	[illegible]	[illegible]		

FCETL QA Form No. 059
Revision 2
Effective 1/96

QA: 01-10-07

CHRONIC CHEMICAL DATA (FINAL)

Project Number: 8503-128-019-078 to 085

Test Species (Circle): *C. dubia* *D. magna* *D. pulex* *P. promelas* Other (Specify): ***Rana pipiens***

	Day 9	Day 10							Meter #	Remarks
Conc.: Control							*		All Conc.	
pH	7.4									
D.O. (mg/L)	6.6									
Temp. (°C)	22									
Conc.: BK11										
pH	7.1									
D.O. (mg/L)	4.5									
Temp. (°C)	23									
Conc.: A08										
pH	7.2									
D.O. (mg/L)	5.4									
Temp. (°C)	23									
Conc.: A07										
pH	6.8									
D.O. (mg/L)	4.9									
Temp. (°C)	23									
Conc.: A04										
pH	7.3									
D.O. (mg/L)	6.1									
Temp. (°C)	23									
Conc.: M1							*			
pH	7.0									
D.O. (mg/L)	4.0									
Temp. (°C)	23									
Conc.: M2										
~~Conc.:~~ pH	7.3									
~~pH~~ D.O.	5.8									
~~D.O. (mg/L)~~ Temp	23									
Date:	12/21/06									
Time:	1450									
Initials:	TD									

FCETL QA Form No. 059
Revision 2
Effective 1/96 DAP 1/16/07
QA: AR01-1007

CHRONIC CHEMICAL DATA (FINAL)

Project Number: 8503-128-019-~~0810~~ 085

Test Species (Circle): *C. dubia* *D. magna* *D. pulex* *P. promelas* Other (Specify): ***Rana pipiens***

	Day 9	Day 10							Meter #	Remarks
Conc.: M3							*		All Conc.	
pH	7.4									
D.O. (mg/L)	5.8									
Temp. (°C)	22									
Conc.: A3A										
pH	7.5									
D.O. (mg/L)	6.2									
Temp. (°C)	23									
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Conc.:							*			
pH										
D.O. (mg/L)										
Temp. (°C)										
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Date:	12/27/06									
Time:	1150									
Initials:	TP									

FCETL Form No. 058
Revision 3
Effective 3/98
DAP 1/16/07
QA: MR01-1007

CHRONIC CHEMICAL DATA (INITIAL) Day 0
(FINAL) Day 10

Project Number: 8503-128-019-~~078~~ 085

Test Species (Circle): *C. dubia* *D. magna* *D. pulex* *P. promelas* Other (Specify): ***Rana pipiens***

	Day 0	Day 10							Meter #	Remarks
Conc.: Control									All Conc.	
pH	7.6	7.6							16	
D.O. (mg/L) ①	~~6.4~~ 6.5	5.8							5	
Temp. (°C)	22	23							D34	
Cond. (µS/cm)	110	107							15	
Hard. (mg/L)	44	42							titr	
Alk. (mg/L)	27	28							titr	
TRC (mg/L)									19	
NH_3 (mg/L) (*)	<1.0	1.5 (**)							3	
Conc.: BK11										
pH	5.5	7.3								
D.O. (mg/L)	6.4	5.5								
Temp. (°C)	22	23								
Cond. (µS/cm)	31	83								
Hard. (mg/L)	14	32								
Alk. (mg/L)	3	28								
TRC (mg/L)										
NH_3 (mg/L) (*)	<1.0	1.4 (**)								
Conc.: A08										
pH	6.8	7.3								
D.O. (mg/L)	6.5	5.3								
Temp. (°C)	22	23								
Cond. (µS/cm)	52	95								
Hard. (mg/L)	18	34								
Alk. (mg/L)	13	33								
TRC (mg/L)										
NH_3 (mg/L) (*)	<1.0	1.9 (**)								
Date:	12/18/06	12/28/06								
Time:	1120	1140								
Initials:	AW	DAP								

NOTE: Hardness, alkalinity, TRC, and NH_3 data appearing on this page have been transcribed from the wet chemistry log, FCETL QA Form No. 084. Dilution/control water and effluent were brought to 25°C prior to mixing the dilution series. The temperature of resulting effluent dilutions is assumed to also be 25°C.

① AR for AW 01-10-07E

(*) measured on 12/19/06 on preserved samples for day 0 NH_3-N

(**) measured on 01/02/07 on preserved samples for day 10 NH_3-N

FCETL QA Form No. 058
Revision 3
Effective 3/98

DAP 1/16/07
QA: MR01-1007

CHRONIC CHEMICAL DATA (INITIAL) DAY 0 (FWAL) DAY 10

Project Number: 8503-128-019-085 (DT&O)

Test Species (Circle): *C. dubia* *D. magna* *D. pulex* P. promelas Other (Specify): ***Rana pipiens***

		Day 0	Day 10							Meter #	Remarks
Conc.:	A07										
pH		6.7	7.1								
D.O. (mg/L)		5.6	5.4								
Temp. (°C)		22	23								
Cond. (µS/cm)		58	89								
Hard. (mg/L)		16	32								
Alk. (mg/L)		6	29								
TRC (mg/L)											
NH_3 (mg/L) (*)		2.4	2.0	(**)							
Conc.:	A04										
pH		6.8	7.2								
D.O. (mg/L)		6.4	5.6								
Temp. (°C)		22	23								
Cond. (µS/cm)		68	89								
Hard. (mg/L)		18	34								
Alk. (mg/L)		7	31								
TRC (mg/L)											
NH_3 (mg/L) (*)		<1.0	1.1	(**)							
Conc.:	M1										
pH		6.2	7.3								
D.O. (mg/L)		6.5	5.6								
Temp. (°C)		22	23								
Cond. (µS/cm)		45	92								
Hard. (mg/L)		16	36								
Alk. (mg/L)		7	34								
TRC (mg/L)											
NH_3 (mg/L) (*)		<1.0	1.4	(**)							
	Date:	12/19/06	12/28/06								
	Time:	1320	1140								
	Initials:	QW	DAP								

NOTE: Hardness, alkalinity, TRC, and NH_3 data appearing on this page have been transcribed from the wet chemistry log, FCETL QA Form No. 084. Dilution/control water and effluent were brought to 25°C prior to mixing the dilution series. The temperature of resulting effluent dilutions is assumed to also be 25°C.

(*) measured on 12/19/06 on preserved samples for day 0 NH_3-N
(**) measured on 01/02/07 on preserved samples for day 10 NH_3-N

FCETL QA Form No. 058
Revision 3
Effective 3/98

DAP 1/16/07
QA: AC 01-10-07

CHRONIC CHEMICAL DATA (INITIAL) Day 0
(FINAL) Day 10

Project Number: 8503-128-019-085 (078-10)

Test Species (Circle): *C. dubia* *D. magna* *D. pulex* P. promelas Other (Specify): ***Rana pipiens***

		Day 0	Day 10							Meter #	Remarks
Conc.:	M2										
pH		6.5	7.3								
D.O. (mg/L)		6.3	5.3								
Temp. (°C)		22	23								
Cond. (:µS/cm)		44	96								
Hard. (mg/L)		12	36								
Alk. (mg/L)		12	34								
TRC (mg/L)											
NH_3 (mg/L)	(*)	<1.0	1.7	(**)							
Conc.:	M3										
pH		6.8	7.4								
D.O. (mg/L)		5.7	5.7								
Temp. (°C)		22	23								
Cond. (:µS/cm)		59	85								
Hard. (mg/L)		18	34								
Alk. (mg/L)		16	31								
TRC (mg/L)											
NH_3 (mg/L)	(*)	1.2	<1.0	(**)							
Conc.:	A3A										
pH		7.3	7.4								
D.O. (mg/L)		6.3	6.0								
Temp. (°C)		22	23								
Cond. (:µS/cm)		78	81								
Hard. (mg/L)		26	32								
Alk. (mg/L)		29	32								
TRC (mg/L)											
NH_3 (mg/L)	(*)	1.0	<1.0	(**)							
	Date:	12/19/06	12/28/06								
	Time:	1720	1140								
	Initials:	AW	DAP								

NOTE: Hardness, alkalinity, TRC, and NH_3 data appearing on this page have been transcribed from the wet chemistry log, FCETL QA Form No. 084. Dilution/control water and effluent were brought to 25°C prior to mixing the dilution series. The temperature of resulting effluent dilutions is assumed to also be 25°C.

(*) measured on 12/19/06 on preserved samples for day 0 NH_3-N

(**) measured on 01-02-07 on preserved samples for day 10 NH_3-N

FCETL QA Form No. 055
Revision 2
Effective 1/94

DAP 1/16/07
QA: AR01-1007

DAILY TOXICITY TEST LOG

Project Number: 8503-128-019-078 to 085

Test Species (Circle): *C. dubia* *D. magna* *D. pulex* P. promelas *O. mykiss* Other (Specify): ***Rana pipiens***

General Comments		Feeding	Initials/Date
Test Day 0	Test Solution Mixed at: on 12/17/06 Fr. 1116-1245 Test Organisms Added at: 1700 on 12/18/06	None	DAP 12/18/06
Test Day 1	CT= 23.2°C Range= 23.0 - 23.6 °C	None	JG 12/19/06
Test Day 2	CT= 23.2°C Range= 23.0 - 24.0 °C	None	DAP 12/20/06
Test Day 3	CT= 23.4°C Range= 23.0 - 24.0 °C	* ~1/8 Smidgen @ 1435	DAP 12/21/06
Test Day 4	CT= 23.2°C Range= 23.0 - 23.6°C	* ~1/8 Smidgen @ 1650	DAP 12/22/06
Test Day 5	CT= 23.2°C Range= 23.0 - 23.6°C	* ~1/8 smidgen @ 1300	TD 12/23/06
Test Day 6	CT= 23.2°C Range= 23.0 - 23.6°C	* ~1/8 Smidgen @ 1020	TD 12/24/06
Test Day 7	CT= 23.0°C Range= 23.0 - 23.2°C	⊛ ~1/4 Smidgen @ 1015	DAP 12/25/06
Test Day 8	CT= 23.2°C Range= 23.0 - 23.6 °C	⊛ ~1/4 smidgen @ 1200	MM 12/26/06

* 1/8 smidgen = ~4mg Tetrafin ⊛
⊛ 1/4 smidgen = ~8 mg Tetrafin ⊛

FCETL QA Form No. 055
Revision 2
Effective 1/94

DAP 1/16/07
QA: MRO1-1007

DAILY TOXICITY TEST LOG

Project Number: 8503-128-019-078 to 085

Test Species (Circle): *C. dubia* *D. magna* *D. pulex* P. promelas *O. mykiss* Other (Specify): ***Rana pipiens***

General Comments			Feeding	Initials/Date
Test Day 9	CT= 23.4 °C	Range= 23.0 - 24.0 °C	*Fed 1/6 smidgen	MM 12/27/06
Test Day 10	CT= 22.2 °C	Range= 17.4 - 23.6 °C	None	MM 12/28/06

DP 1/8/07

~~* 1/6 smidgen tetra-fin = ~4mg~~
* 1/6 smidgen = ~ 6 mg

NINE TREATMENTS EIGHT REPLICATES
RANDOM CHAMBER LOCATION "Aeon"

DAP 1/16/07
QA: AR01-1007

Project No. 8503-128-019-078 to 085

	A	B	C	D	E	F	G	H
1	6E	3F	6H	4H	8F	5F	7D	7C
2	1D	3A	2D	5C	2E	2F	1B	2G
3	9E	5E	4C	8C	3E	5H	2H	1G
4	2A	5D	1A	7G	5G	8D	7E	1E
5	7A	4A	2C	1C	3D	4F	8A	5B
6	8E	1H	6G	4D	6D	1F	9A	7B
7	2B	6C	9H	6F	3B	3H	9G	7H
8	9C	9D	7F	6B	5A	6A	4G	4E
9	4B	8H	3G	3C	8B	9B	8G	9F

1 = Control
2 = SedAB-BK11
3 = SedAB-A08
4 = SedAB-A07
5 = SedAB-A04
6 = ~~SedAB-~~ M1
7 = M2
8 = M3
9 = SedAB-A3A

DP 12/14/06 F

SEDIMENT/SOIL PREPARATION

DAP 1/16/07
QA: AR01-1007

Artificial soil	
Constituent/source	Amount added (units)

Notes:

8503-128-019-(078 to -085)

Soil/sediment	FCETL#	Homogenization			
		Date	From	To	Analyst
M1	NA	12/17/06	1116	1120	EJP
M2	NA	12/17/06	1116	1120	JCP
M3	NA	12/17/06	1138	1145	EJP
AB-A08	19427	12/17/06	1145	1148	JCP
AB-A04	19424	12/17/06	1207	1212	EJP
AB-A3A	19423	12/17/06	1209	1213	JCP
AB-A07	19426	12/17/06	1232	1236	EJP
AB-ABK11	19422	12/17/06	1229	1232	JCP
Control(PR)	NA	12/17/06	1245	1249	DJP

OSP 1/16/07
QA AR01-1707

Tadpole Sublethal Measurements

Test Number: 8503-128-019 ~~085~~ 078 to-085 ①	Test Description: 10 day short-term chronic
Test Dates: 12/18/06 - 12/28/06	Test Species: Rana pipiens

Treat/ Concn	Rep	Body Width (mm)	Body Length (S-V lngth) (mm)	Eye Width (mm)	Comments
Control	A	5	9		
	A	5	8		
	A	5	8		
	A	5	8		
	A	6	10		
	B	5	9		
	B	6	10		
	B	5	9		
	B	6	10		
	B	6	9		
	C	5	9		
	C	5	8		
	C	5	9		
	C	6	10		
	C	6	10		
	D	5	10		
	D	6	10		
	D	5	9		
	D	6	10		
	D	6	10		
Date		1/2/07	1/2/07		
Time		1130	1130		
Inits		MM	MM		

① AR 01-10 07E

Tadpole Sublethal Measurements

DAP 1/16/07
QA: AR01-1707

Test Number: 8503-128-019-078 to -085	Test Description: 10 day short-term chronic
Test Dates: 12/18/06 - 12/28/06	Test Species: Rana pipiens

Treat/ Concn	Rep	Body Width (mm)	Body Length (S-V lngth) (mm)	Eye Width (mm)	Comments
Control	E	5	9		
	E	5	8		
	E	5	8		
	E	6	9		
	E	5	8		
	F	5	9		
	F	5	8		
	F	5	8		
	F	5	8		
	F	5	8		
	G	5	9		
	G	5	9		
	G	5	9		
	G	5	8		
	G	4	7		
	H	6	11		
	H	6	10		
	H	7	11		
	H	6	10		
	H	6	10		
Date		1/2/07	1/2/07		
Time		1130	1130		
Inits		MM	MM		

Tadpole Sublethal Measurements

DAP 1/16/07
QA: AE01-1707

Test Number:	8503-128-019-078 to -085	Test Description:	10 day short-term chronic
Test Dates:	12/18/06 - 12/28/06	Test Species:	Rana pipiens

Treat/ Concn	Rep	Body Width (mm)	Body Length (S-V lngth) (mm)	Eye Width (mm)	Comments
BK11	A	6	10		
	A	6	11		
	A	7	12		
	A	5	10		
	A	5	9		
	B	6	10		
	B	6	11		
	B	6	10		
	B	5	9		
	B	4	8		
	C	5	9		
	C	6	11		
	C	6	10		
	C	6	10		
	C	—	—		
	D	6	11		
	D	6	11		
	D	6	10		
	D	6	9		
	D	5	9		
Date		1/2/07	1/2/07		
Time		1150	1150		
Inits		mm	mm		

Tadpole Sublethal Measurements

DAP 1/16/07
QA: AR01-1707

Test Number:	8503 - 128 - ~~012~~ 019-078 to-085	Test Description:	10 day short-term chronic
Test Dates:	12/18/06 - 12/28/06	Test Species:	Rana pipiens

Treat/ Concn	Rep	Body Width (mm)	Body Length (S-V lngth) (mm)	Eye Width (mm)	Comments
BK11	E	7	11		
	E	6	10		
	E	5	10		
	E	6	10		
	E	6	10		
	F	5	10		
	F	7	11		
	F	6	10		
	F	6	11		
	F	6	11		
	G	5	8		
	G	5	10		
	G	6	10		
	G	5	8		
	G	6	9		
	H	7	12		
	H	7	12		
	H	7	12		
	H	7	12		
	H	—	—		
Date		1/2/07	1/2/07		
Time		1150	1150		
Inits		MM	MM		

OMM 1/12/07 e

DRP 1/16/07
QA: AR017707

Tadpole Sublethal Measurements

Test Number:	8503 - 128 - 019 - 078 to-085	Test Description:	10 day short-term chronic
Test Dates:	12/18/06 - 12/28/06	Test Species:	Rana pipiens

Treat/ Concn	Rep	Body Width (mm)	Body Length (S-V lngth) (mm)	Eye Width (mm)	Comments
A08	A	5	9		
	A	6	10		
	A	6	10		
	A	5	9		
	A	5	9		
	B	6	10		
	B	6	10		
	B	6	10		
	B	5	9		
	B	6	10		
	C	6	10		
	C	6	11		
	C	6	11		
	C	6	10		
	C	6	11		
	D	6	10		
	D	6	10		
	D	6	10		
	D	5	9		
	D	5	9		
Date		1/2/07	1/2/07		
Time		1305	1305		
Inits		MM	MM		

OMM 1/2/07 CF

DAP 1/2 ② 1/16/07
QA: AR01-1707

Tadpole Sublethal Measurements

Test Number:	8503-128-019-078 to -085	Test Description:	10 day short-term chronic
Test Dates:	12/18/06 - 12/28/06	Test Species:	Rana pipiens

Treat/ Concn	Rep	Body Width (mm)	Body Length (S-V lngth) (mm)	Eye Width (mm)	Comments
A08	E	6	11		
	E	6	10		
	E	6	10		
	E	6	10		
	E	6	10		
	F	6	10		
	F	6	10		
	F	6	10		
	F	7	11		
	F	6	10		
	G	6	11		
	G	6	11		
	G	7	11		
	G	6	11		
	G	6	11		
	H	5	10		
	H	6	11		
	H	7	11		
	H	3	5		
	H	–	–		
Date		1/2/08 ①	1/2/08 ①		
Time		1305	1305		
Inits		MM	MM		

① MM 1/2/07 cF ② DAP 1/16/07 E

DAP 1/16/07
QA: AR01-1707

Tadpole Sublethal Measurements

Test Number:	8503-128-019-078 to-085	Test Description:	10 day short-term chronic
Test Dates:	12/18/06 - 12/28/06	Test Species:	Rana pipiens

Treat/ Concn	Rep	Body Width (mm)	Body Length (S-V lngth) (mm)	Eye Width (mm)	Comments
A07	A	6	10		
	A	6	10		
	A	6	10		
	A	6	10		
	A	5	8		
	B	5	10		
	B	6	10		
	B	6	10		
	B	5	9		
	B	5	9		
	C	5	9		
	C	5	9		
	C	6	10		
	C	5	9		
	C	5	10		
	D	7	12		
	D	6	11		
	D	6	11		
	D	6	11		
	D	5	10		
Date		1/2/07	1/2/07		
Time		1325	1325		
Inits		MM	MM		

QMM 1/2/07 cf

DAP 1/16/07
QA: AR01-1707

Tadpole Sublethal Measurements

Test Number:	8503-128-019-078 to -085	Test Description:	10 day short-term chronic
Test Dates:	12/18/06 - 12/28/06	Test Species:	Rana pipiens

Treat/ Concn	Rep	Body Width (mm)	Body Length (S-V lngth) (mm)	Eye Width (mm)	Comments
A07	E	5	9		
	E	6	10		
	E	6	10		
	E	7	10		
	E	6	10		
	F	6	10		
	F	6	10		
	F	6	10		
	F	6	10		
	F	6	10		
	G	6	10		
	G	6	10		
	G	6	10		
	G	6	10		
	G	6	10		
	H	6	10		
	H	6	10		
	H	6	10		
	H	7	10		
	H	6	10		
Date		1/2/07	1/2/07		
Time		1325	1325		
Inits		mm	mm		

DAP 1/16/07
QA: AROM1707

Tadpole Sublethal Measurements

Test Number:	8503-128-019-078 to-085	Test Description:	10 day short-term chronic
Test Dates:	12/18/06 - 12/28/06	Test Species:	Rana pipiens

Treat/ Concn	Rep	Body Width (mm)	Body Length (S-V lngth) (mm)	Eye Width (mm)	Comments
A04	A	5	8		
	A	5	8		
	A	5	8		
	A	5	8		
	A	—	—		
	B	5	8		
	B	5	8		
	B	—	—		
	B	—	—		
	B	—	—		
	C	—	—		
	C	—	—		
	C	—	—		
	C	—	—		
	C	—	—		
	D	5	8		
	D	4	7		
	D	5	8		
	D	5	8		
	D	—	—		
Date		1/2/07	1/2/07		
Time		1345	1345		
Inits		MA	MA		

DAP 1/16/07
QA: AR017707

Tadpole Sublethal Measurements

Test Number:	8503-128-019-078 to-085	Test Description:	10 day chart-term chronic
Test Dates:	12/18/06 - 12/28/06	Test Species:	Rana pipiens

Treat/ Concn	Rep	Body Width (mm)	Body Length (S-V lngth) (mm)	Eye Width (mm)	Comments
A04	E	5	8		
	E	5	8		
	E	6	9		
	E	5	8		
	E	5	8		
	F	6	9		
	F	6	8		
	F	5	8		
	F	6	9		
	F	5	8		
	G	5	7		
	G	5	8		
	G	4	7		
	G	5	8		
	G	5	7		
	H	5	8		
	H	5	8		
	H	5	8		
	H	5	8		
	H	4	7		
Date		1/2/07	1/2/07		
Time		1345	1345		
Inits		MM	MM		

DAP 1/16/07
QA-AR017707

Tadpole Sublethal Measurements

Test Number: 8503-128-019-078 to-085	Test Description: 10 day short-term chronic
Test Dates: 12/18/06 - 12/28/06	Test Species: Rana pipiens

D, E all dead

Treat/ Concn	Rep	Body Width (mm)	Body Length (S-V lngth) (mm)	Eye Width (mm)	Comments
M1	A	5	8		
	A	5	8		
	A	5	7		
	A	4	7		
	A	—	—		
	B	6	9		
	B	—	—		
	B	—	—		
	B	—	—		
	B	—	—		
	C	4	7		
	C	—	—		
	C	—	—		
	C	—	—		
	C	—	—		
	F	5	8		
	F	—	—		
	F	—	—		
	F	—	—		
	F	—	—		
Date		1/2/07	1/2/07		
Time		1400	1400		
Inits		MM	MM		

DAP 1/16/07
QA: A201-17-07

Tadpole Sublethal Measurements

Test Number:	8503-128-019-078 to -085	Test Description:	10 day short term chronic
Test Dates:	12/18/06 - 12/28/06	Test Species:	Rana pipiens

Treat/ Concn	Rep	Body Width (mm)	Body Length (S-V lngth) (mm)	Eye Width (mm)	Comments
M1	G	4	7		
	G	5	8		
	G	4	7		
	G	—	—		
	G	—	—		
	H	2	4		
	H	2	4		
	H	—	—		
	H	—	—		
	H	—	—		
Date		1/2/07	1/2/07		
Time		1400	1400		
Inits		MM	MM		

DAP 1/16/07
QA: R01-1707

Tadpole Sublethal Measurements

Test Number: 8503-128-019-078 to-085	Test Description: 10 day Short-term chronic
Test Dates: 12/18/06 - 12/28/06	Test Species: Rana pipiens

C, D, G, H all dead

Treat/ Concn	Rep	Body Width (mm)	Body Length (S-V lngth) (mm)	Eye Width (mm)	Comments
M2	A	4	7		
	A	4	7		
	A	4	7		
	A	4	7		
	A	3	6		
	B	5	8		
	B	5	8		
	B	6	9		
	B	5	7		
	B	—	—		
	E	4	6		
	E	5	7		
	E	4	7		
	E	—	—		
	E	—	—		
	F	3	5		
	F	3	5		
	F	—	—		
	F	—	—		
	F	—	—		
Date		1/2/07	1/2/07		
Time		1410	1410		
Inits		MM	MM		

DAP 1/16/07

QA: AR01-1707

Tadpole Sublethal Measurements

Test Number: 8503- 128-019-078 to -085	Test Description: 10 day short-term chronic
Test Dates: 12/18/06 - 12/28/06	Test Species: Rana pipiens

* A-E, G, H all dead

Treat/ Concn	Rep	Body Width (mm)	Body Length (S-V lngth) (mm)	Eye Width (mm)	Comments
M3	F	4	6		
	F	4	6		
	F	3	4		
	F	3	4		
	F	3	5		
Date		1/2/07	1/2/07		
Time		1415	1415		
Inits		MM	MM		

DAP 1/16/07
QA: AR014707

Tadpole Sublethal Measurements

Test Number:	8503-128-019-078 to -085	Test Description:	10 day short-term chronic
Test Dates:	12/18/06 - 12/28/06	Test Species:	Rana pipiens

A, B, D, F-H all dead

Treat/ Concn	Rep	Body Width (mm)	Body Length (S-V lngth) (mm)	Eye Width (mm)	Comments
A3A	C	3	4		
	C	4	6		
	C	3	5		
	C	–	–		
	C	–	–		
	E	3	4		
	E	2	3		
	E	3	5		
	E	–	–		
	E	–	–		
Date		1/2/07	1/2/07		
Time		1420	1420		
Inits		MM	MM		

DAP 1/16/07
QA: AR 01-16-07

08503-128-019-078-085 Rana pipiens Survival and Sublethal Results

Sediment	Replicate	Survival	Body Width	Body Length	Mean Survival	Replicate Mean Width per Original	Replicate Mean Length per Original
Control	A	1	5	9	100	5.2	8.6
Control	A	1	5	8			
Control	A	1	5	8			
Control	A	1	5	8			
Control	A	1	6	10			
Control	B	1	5	9	100	5.6	9.4
Control	B	1	6	10			
Control	B	1	5	9			
Control	B	1	6	10			
Control	B	1	6	9			
Control	C	1	5	9	100	5.4	9.2
Control	C	1	5	8			
Control	C	1	5	9			
Control	C	1	6	10			
Control	C	1	6	10			
Control	D	1	5	10	100	5.6	9.8
Control	D	1	6	10			
Control	D	1	5	9			
Control	D	1	6	10			
Control	D	1	6	10			
Control	E	1	5	9	100	5.2	8.4
Control	E	1	5	8			
Control	E	1	5	8			
Control	E	1	6	9			
Control	E	1	5	8			
Control	F	1	5	9	100	5	8.2
Control	F	1	5	8			
Control	F	1	5	8			
Control	F	1	5	8			
Control	F	1	5	8			
Control	G	1	5	9	100	4.8	8.4
Control	G	1	5	9			
Control	G	1	5	9			
Control	G	1	5	8			
Control	G	1	4	7			
Control	H	1	6	11	100	6.2	10.4

DAP 1/16/07
QA: AR 01-16-07

08503-128-019-078-085 Rana pipiens Survival and Sublethal Results

Sediment	Replicate	Survival	Body Width	Body Length	Mean Survival	Replicate Mean Width per Original	Replicate Mean Length per Original
Control	H	1	6	10			
Control	H	1	7	11			
Control	H	1	6	10			
Control	H	1	6	10			
SedAB-BK11	A	1	6	10	100	5.8	10.4
SedAB-BK11	A	1	6	11			
SedAB-BK11	A	1	7	12			
SedAB-BK11	A	1	5	10			
SedAB-BK11	A	1	5	9			
SedAB-BK11	B	1	6	10	100	5.4	9.6
SedAB-BK11	B	1	6	11			
SedAB-BK11	B	1	6	10			
SedAB-BK11	B	1	5	9			
SedAB-BK11	B	1	4	8			
SedAB-BK11	C	1	5	9	80	4.6	8
SedAB-BK11	C	1	6	11			
SedAB-BK11	C	1	6	10			
SedAB-BK11	C	1	6	10			
SedAB-BK11	C	0	0	0			
SedAB-BK11	D	1	6	11	100	5.8	10
SedAB-BK11	D	1	6	11			
SedAB-BK11	D	1	6	10			
SedAB-BK11	D	1	6	9			
SedAB-BK11	D	1	5	9			
SedAB-BK11	E	1	7	11	100	6	10.2
SedAB-BK11	E	1	6	10			
SedAB-BK11	E	1	5	10			
SedAB-BK11	E	1	6	10			
SedAB-BK11	E	1	6	10			
SedAB-BK11	F	1	5	10	100	6	10.6
SedAB-BK11	F	1	7	11			
SedAB-BK11	F	1	6	10			
SedAB-BK11	F	1	6	11			
SedAB-BK11	F	1	6	11			
SedAB-BK11	G	1	5	8	100	5.4	9
SedAB-BK11	G	1	5	10			

DAP 1/16/07
QA: AR 01-16-07

08503-128-019-078-085 Rana pipiens Survival and Sublethal Results

Sediment	Replicate	Survival	Body Width	Body Length	Mean Survival	Replicate Mean Width per Original	Replicate Mean Length per Original
SedAB-BK11	G	1	6	10			
SedAB-BK11	G	1	5	8			
SedAB-BK11	G	1	6	9			
SedAB-BK11	H	1	7	12	80	5.6	9.6
SedAB-BK11	H	1	7	12			
SedAB-BK11	H	1	7	12			
SedAB-BK11	H	1	7	12			
SedAB-BK11	H	0	0	0			
SedAB-A08	A	1	5	9	100	5.4	9.4
SedAB-A08	A	1	6	10			
SedAB-A08	A	1	6	10			
SedAB-A08	A	1	5	9			
SedAB-A08	A	1	5	9			
SedAB-A08	B	1	6	10	100	5.8	9.8
SedAB-A08	B	1	6	10			
SedAB-A08	B	1	6	10			
SedAB-A08	B	1	5	9			
SedAB-A08	B	1	6	10			
SedAB-A08	C	1	6	10	100	6	10.6
SedAB-A08	C	1	6	11			
SedAB-A08	C	1	6	11			
SedAB-A08	C	1	6	10			
SedAB-A08	C	1	6	11			
SedAB-A08	D	1	6	10	100	5.6	9.6
SedAB-A08	D	1	6	10			
SedAB-A08	D	1	6	10			
SedAB-A08	D	1	5	9			
SedAB-A08	D	1	5	9			
SedAB-A08	E	1	6	11	100	6	10.2
SedAB-A08	E	1	6	10			
SedAB-A08	E	1	6	10			
SedAB-A08	E	1	6	10			
SedAB-A08	E	1	6	10			
SedAB-A08	F	1	6	10	100	6.2	10.2
SedAB-A08	F	1	6	10			
SedAB-A08	F	1	6	10			

DAP 1/16/07
QA: AR01-16-07

08503-128-019-078-085 Rana pipiens Survival and Sublethal Results

Sediment	Replicate	Survival	Body Width	Body Length	Mean Survival	Replicate Mean Width per Original	Replicate Mean Length per Original
SedAB-A08	F	1	7	11			
SedAB-A08	F	1	6	10			
SedAB-A08	G	1	6	11	100	6.2	11
SedAB-A08	G	1	6	11			
SedAB-A08	G	1	7	11			
SedAB-A08	G	1	6	11			
SedAB-A08	G	1	6	11			
SedAB-A08	H	1	5	10	80	4.2	7.4
SedAB-A08	H	1	6	11			
SedAB-A08	H	1	7	11			
SedAB-A08	H	1	3	5			
SedAB-A08	H	0	0	0			
SedAB-A07	A	1	6	10	100	5.8	9.6
SedAB-A07	A	1	6	10			
SedAB-A07	A	1	6	10			
SedAB-A07	A	1	6	10			
SedAB-A07	A	1	5	8			
SedAB-A07	B	1	5	10	100	5.4	9.6
SedAB-A07	B	1	6	10			
SedAB-A07	B	1	6	10			
SedAB-A07	B	1	5	9			
SedAB-A07	B	1	5	9			
SedAB-A07	C	1	5	9	100	5.2	9.4
SedAB-A07	C	1	5	9			
SedAB-A07	C	1	6	10			
SedAB-A07	C	1	5	9			
SedAB-A07	C	1	5	10			
SedAB-A07	D	1	7	12	100	6	11
SedAB-A07	D	1	6	11			
SedAB-A07	D	1	6	11			
SedAB-A07	D	1	6	11			
SedAB-A07	D	1	5	10			
SedAB-A07	E	1	5	9	100	6	9.8
SedAB-A07	E	1	6	10			
SedAB-A07	E	1	6	10			
SedAB-A07	E	1	7	10			

DAP 1/16/07
QA: AR 01-16-07

08503-128-019-078-085 Rana pipiens Survival and Sublethal Results

Sediment	Replicate	Survival	Body Width	Body Length	Mean Survival	Replicate Mean Width per Original	Replicate Mean Length per Original
SedAB-A07	E	1	6	10			
SedAB-A07	F	1	6	10	100	6	10
SedAB-A07	F	1	6	10			
SedAB-A07	F	1	6	10			
SedAB-A07	F	1	6	10			
SedAB-A07	F	1	6	10			
SedAB-A07	G	1	6	10	100	6	10
SedAB-A07	G	1	6	10			
SedAB-A07	G	1	6	10			
SedAB-A07	G	1	6	10			
SedAB-A07	G	1	6	10			
SedAB-A07	H	1	6	10	100	6.2	10
SedAB-A07	H	1	6	10			
SedAB-A07	H	1	6	10			
SedAB-A07	H	1	7	10			
SedAB-A07	H	1	6	10			
SedAB-A04	A	1	5	8	80	4	6.4
SedAB-A04	A	1	5	8			
SedAB-A04	A	1	5	8			
SedAB-A04	A	1	5	8			
SedAB-A04	A	0	0	0			
SedAB-A04	B	1	5	8	40	2	3.2
SedAB-A04	B	1	5	8			
SedAB-A04	B	0	0	0			
SedAB-A04	B	0	0	0			
SedAB-A04	B	0	0	0			
SedAB-A04	C	0	0	0	0	0	0
SedAB-A04	C	0	0	0			
SedAB-A04	C	0	0	0			
SedAB-A04	C	0	0	0			
SedAB-A04	C	0	0	0			
SedAB-A04	D	1	5	8	80	3.8	6.2
SedAB-A04	D	1	4	7			
SedAB-A04	D	1	5	8			
SedAB-A04	D	1	5	8			
SedAB-A04	D	0	0	0			

DAP 1/16/07
QA: AR 01-16-07

08503-128-019-078-085 Rana pipiens Survival and Sublethal Results

Sediment	Replicate	Survival	Body Width	Body Length	Mean Survival	Replicate Mean Width per Original	Replicate Mean Length per Original
SedAB-A04	E	1	5	8	100	5.2	8.2
SedAB-A04	E	1	5	8			
SedAB-A04	E	1	6	9			
SedAB-A04	E	1	5	8			
SedAB-A04	E	1	5	8			
SedAB-A04	F	1	6	9	100	5.6	8.4
SedAB-A04	F	1	6	8			
SedAB-A04	F	1	5	8			
SedAB-A04	F	1	6	9			
SedAB-A04	F	1	5	8			
SedAB-A04	G	1	5	7	100	4.8	7.4
SedAB-A04	G	1	5	8			
SedAB-A04	G	1	4	7			
SedAB-A04	G	1	5	8			
SedAB-A04	G	1	5	7			
SedAB-A04	H	1	5	8	100	4.8	7.8
SedAB-A04	H	1	5	8			
SedAB-A04	H	1	5	8			
SedAB-A04	H	1	5	8			
SedAB-A04	H	1	4	7			
M1	A	1	5	8	80	3.8	6
M1	A	1	5	8			
M1	A	1	5	7			
M1	A	1	4	7			
M1	A	0	0	0			
M1	B	1	6	9	20	1.2	1.8
M1	B	0	0	0			
M1	B	0	0	0			
M1	B	0	0	0			
M1	B	0	0	0			
M1	C	1	4	7	20	0.8	1.4
M1	C	0	0	0			
M1	C	0	0	0			
M1	C	0	0	0			
M1	C	0	0	0			
M1	D	0	0	0	0	0	0

DAP 1/16/07
QA: AR 01-16-07

08503-128-019-078-085 Rana pipiens Survival and Sublethal Results

Sediment	Replicate	Survival	Body Width	Body Length	Mean Survival	Replicate Mean Width per Original	Replicate Mean Length per Original
M1	D	0	0	0			
M1	D	0	0	0			
M1	D	0	0	0			
M1	D	0	0	0			
M1	E	0	0	0	0	0	0
M1	E	0	0	0			
M1	E	0	0	0			
M1	E	0	0	0			
M1	E	0	0	0			
M1	F	1	5	8	20	1	1.6
M1	F	0	0	0			
M1	F	0	0	0			
M1	F	0	0	0			
M1	F	0	0	0			
M1	G	1	4	7	60	2.6	4.4
M1	G	1	5	8			
M1	G	1	4	7			
M1	G	0	0	0			
M1	G	0	0	0			
M1	H	1	2	4	40	0.8	1.6
M1	H	1	2	4			
M1	H	0	0	0			
M1	H	0	0	0			
M1	H	0	0	0			
M2	A	1	4	7	100	3.8	6.8
M2	A	1	4	7			
M2	A	1	4	7			
M2	A	1	4	7			
M2	A	1	3	6			
M2	B	1	5	8	80	4.2	6.4
M2	B	1	5	8			
M2	B	1	6	9			
M2	B	1	5	7			
M2	B	0	0	0			
M2	C	0	0	0	0	0	0
M2	C	0	0	0			

DAP 1/16/07
QA: AR01-16-07

08503-128-019-078-085 Rana pipiens Survival and Sublethal Results

Sediment	Replicate	Survival	Body Width	Body Length	Mean Survival	Replicate Mean Width per Original	Replicate Mean Length per Original
M2	C	0	0	0			
M2	C	0	0	0			
M2	C	0	0	0			
M2	D	0	0	0	0	0	0
M2	D	0	0	0			
M2	D	0	0	0			
M2	D	0	0	0			
M2	D	0	0	0			
M2	E	1	4	6	60	2.6	4
M2	E	1	5	7			
M2	E	1	4	7			
M2	E	0	0	0			
M2	E	0	0	0			
M2	F	1	3	5	40	1.2	2
M2	F	1	3	5			
M2	F	0	0	0			
M2	F	0	0	0			
M2	F	0	0	0			
M2	G	0	0	0	0	0	0
M2	G	0	0	0			
M2	G	0	0	0			
M2	G	0	0	0			
M2	G	0	0	0			
M2	H	0	0	0	0	0	0
M2	H	0	0	0			
M2	H	0	0	0			
M2	H	0	0	0			
M2	H	0	0	0			
M3	A	0	0	0	0	0	0
M3	A	0	0	0			
M3	A	0	0	0			
M3	A	0	0	0			
M3	A	0	0	0			
M3	B	0	0	0	0	0	0
M3	B	0	0	0			
M3	B	0	0	0			

DAP 1/16/07
QA: AR 01-16-07

08503-128-019-078-085 Rana pipiens Survival and Sublethal Results

Sediment	Replicate	Survival	Body Width	Body Length	Mean Survival	Replicate Mean Width per Original	Replicate Mean Length per Original
M3	B	0	0	0			
M3	B	0	0	0			
M3	C	0	0	0	0	0	0
M3	C	0	0	0			
M3	C	0	0	0			
M3	C	0	0	0			
M3	C	0	0	0			
M3	D	0	0	0	0	0	0
M3	D	0	0	0			
M3	D	0	0	0			
M3	D	0	0	0			
M3	D	0	0	0			
M3	E	0	0	0	0	0	0
M3	E	0	0	0			
M3	E	0	0	0			
M3	E	0	0	0			
M3	E	0	0	0			
M3	F	1	4	6	100	3.4	5
M3	F	1	4	6			
M3	F	1	3	4			
M3	F	1	3	4			
M3	F	1	3	5			
M3	G	0	0	0	0	0	0
M3	G	0	0	0			
M3	G	0	0	0			
M3	G	0	0	0			
M3	G	0	0	0			
M3	H	0	0	0	0	0	0
M3	H	0	0	0			
M3	H	0	0	0			
M3	H	0	0	0			
M3	H	0	0	0			
SedAB-A3A	A	0	0	0	0	0	0
SedAB-A3A	A	0	0	0			
SedAB-A3A	A	0	0	0			
SedAB-A3A	A	0	0	0			

DAP 1/16/07

QA: AR 01-16-07

08503-128-019-078-085 Rana pipiens Survival and Sublethal Results

Sediment	Replicate	Survival	Body Width	Body Length	Mean Survival	Replicate Mean Width per Original	Replicate Mean Length per Original
SedAB-A3A	A	0	0	0			
SedAB-A3A	B	0	0	0	0	0	0
SedAB-A3A	B	0	0	0			
SedAB-A3A	B	0	0	0			
SedAB-A3A	B	0	0	0			
SedAB-A3A	B	0	0	0			
SedAB-A3A	C	1	3	4	60	2	3
SedAB-A3A	C	1	4	6			
SedAB-A3A	C	1	3	5			
SedAB-A3A	C	0	0	0			
SedAB-A3A	C	0	0	0			
SedAB-A3A	D	0	0	0	0	0	0
SedAB-A3A	D	0	0	0			
SedAB-A3A	D	0	0	0			
SedAB-A3A	D	0	0	0			
SedAB-A3A	D	0	0	0			
SedAB-A3A	E	1	3	4	60	1.6	2.4
SedAB-A3A	E	1	2	3			
SedAB-A3A	E	1	3	5			
SedAB-A3A	E	0	0	0			
SedAB-A3A	E	0	0	0			
SedAB-A3A	F	0	0	0	0	0	0
SedAB-A3A	F	0	0	0			
SedAB-A3A	F	0	0	0			
SedAB-A3A	F	0	0	0			
SedAB-A3A	F	0	0	0			
SedAB-A3A	G	0	0	0	0	0	0
SedAB-A3A	G	0	0	0			
SedAB-A3A	G	0	0	0			
SedAB-A3A	G	0	0	0			
SedAB-A3A	G	0	0	0			
SedAB-A3A	H	0	0	0	0	0	0
SedAB-A3A	H	0	0	0			
SedAB-A3A	H	0	0	0			
SedAB-A3A	H	0	0	0			
SedAB-A3A	H	0	0	0			

8503-128-019-078-085
Rana pipiens
Survival Poudre River Sediment as Control

DAP 1/16/07
QA: AR01-1207

Title: 8503-128-019-078-085 Rana pipiens Survival Control
File: 078surc Transform: NO TRANSFORMATION
Number of Groups: 8

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	1.0000	1.0000
1	Control	2	1.0000	1.0000
1	Control	3	1.0000	1.0000
1	Control	4	1.0000	1.0000
1	Control	5	1.0000	1.0000
1	Control	6	1.0000	1.0000
1	Control	7	1.0000	1.0000
1	Control	8	1.0000	1.0000
2	SedAB-A08	1	1.0000	1.0000
2	SedAB-A08	2	1.0000	1.0000
2	SedAB-A08	3	1.0000	1.0000
2	SedAB-A08	4	1.0000	1.0000
2	SedAB-A08	5	1.0000	1.0000
2	SedAB-A08	6	1.0000	1.0000
2	SedAB-A08	7	1.0000	1.0000
2	SedAB-A08	8	0.8000	0.8000
3	SedAB-A07	1	1.0000	1.0000
3	SedAB-A07	2	1.0000	1.0000
3	SedAB-A07	3	1.0000	1.0000
3	SedAB-A07	4	1.0000	1.0000
3	SedAB-A07	5	1.0000	1.0000
3	SedAB-A07	6	1.0000	1.0000
3	SedAB-A07	7	1.0000	1.0000
3	SedAB-A07	8	1.0000	1.0000
4	SedAB-A04	1	0.8000	0.8000
4	SedAB-A04	2	0.4000	0.4000
4	SedAB-A04	3	0.0000	0.0000
4	SedAB-A04	4	0.8000	0.8000
4	SedAB-A04	5	1.0000	1.0000
4	SedAB-A04	6	1.0000	1.0000
4	SedAB-A04	7	1.0000	1.0000
4	SedAB-A04	8	1.0000	1.0000

DAP 1/16/07
QA: AR01-1207

8503-128-019-078-085
Rana pipiens
Survival Poudre River Sediment as Control

5	M1	1	0.8000	0.8000
5	M1	2	0.2000	0.2000
5	M1	3	0.2000	0.2000
5	M1	4	0.0000	0.0000
5	M1	5	0.0000	0.0000
5	M1	6	0.2000	0.2000
5	M1	7	0.6000	0.6000
5	M1	8	0.4000	0.4000
6	M2	1	1.0000	1.0000
6	M2	2	0.8000	0.8000
6	M2	3	0.0000	0.0000
6	M2	4	0.0000	0.0000
6	M2	5	0.6000	0.6000
6	M2	6	0.4000	0.4000
6	M2	7	0.0000	0.0000
6	M2	8	0.0000	0.0000
7	M3	1	0.0000	0.0000
7	M3	2	0.0000	0.0000
7	M3	3	0.0000	0.0000
7	M3	4	0.0000	0.0000
7	M3	5	0.0000	0.0000
7	M3	6	1.0000	1.0000
7	M3	7	0.0000	0.0000
7	M3	8	0.0000	0.0000
8	SedAB-A3A	1	0.0000	0.0000
8	SedAB-A3A	2	0.0000	0.0000
8	SedAB-A3A	3	0.6000	0.6000
8	SedAB-A3A	4	0.0000	0.0000
8	SedAB-A3A	5	0.6000	0.6000
8	SedAB-A3A	6	0.0000	0.0000
8	SedAB-A3A	7	0.0000	0.0000
8	SedAB-A3A	8	0.0000	0.0000

8503-128-019-078-085
Rana pipiens
Survival Poudre River Sediment as Control

DAP 1/16/07
QA: AR01-12-07

Title: 8503-128-019-078-085 Rana pipiens Survival Control
File: 078surc Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Control	8	1.0000	1.0000	1.0000
2	SedAB-A08	8	0.8000	1.0000	0.9750
3	SedAB-A07	8	1.0000	1.0000	1.0000
4	SedAB-A04	8	0.0000	1.0000	0.7500
5	M1	8	0.0000	0.8000	0.3000
6	M2	8	0.0000	1.0000	0.3500
7	M3	8	0.0000	1.0000	0.1250
8	SedAB-A3A	8	0.0000	0.6000	0.1500

Title: 8503-128-019-078-085 Rana pipiens Survival Control
File: 078surc Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	Control	0.0000	0.0000	0.0000	0.0000
2	SedAB-A08	0.0050	0.0707	0.0250	7.2524
3	SedAB-A07	0.0000	0.0000	0.0000	0.0000
4	SedAB-A04	0.1343	0.3665	0.1296	48.8600
5	M1	0.0800	0.2828	0.1000	94.2809
6	M2	0.1686	0.4106	0.1452	117.3070
7	M3	0.1250	0.3536	0.1250	282.8427
8	SedAB-A3A	0.0771	0.2777	0.0982	185.1640

DSP 1/16/07
GA: AR011207

8503-128-019-078-085
Rana pipiens
Survival Poudre River Sediment as Control

Title: 8503-128-019-078-085 Rana pipiens Survival Control
File: 078surc Transform: ARC SINE(SQUARE ROOT(Y))

Chi-Square Test for Normality

Actual and Expected Frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	4.2880	15.4880	24.4480	15.4880	4.2880
OBSERVED	2	13	37	7	5

Chi-Square = 12.8349 (p-value = 0.0121)

Critical Chi-Square = 13.277 (alpha = 0.01 , df = 4)
= 9.488 (alpha = 0.05 , df = 4)

Data PASS normality test (alpha = 0.01). Continue analysis.

File: 078surc Transform: ARC SINE(SQUARE ROOT(Y))

Hartley's Test for Homogeneity of Variance
Bartlett's Test for Homogeneity of Variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

DAP 1/16/07
QA: MR 01-12-07

8503-128-019-078-085
Rana pipiens
Survival Poudre River Sediment as Control

Title: 8503-128-019-078-085 Rana pipiens Survival Control
File: 078surc Transform: ARC SINE(SQUARE ROOT(Y))

Steel's Many-One Rank Test Ho: Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	DF	SIG 0.05
1	Control	1.3453				
2	SedAB-A08	1.3155	64.00	45.00	8.00	
3	SedAB-A07	1.3453	68.00	45.00	8.00	
4	SedAB-A04	1.0632	52.00	45.00	8.00	
5	M1	0.5650	36.00	45.00	8.00	*
6	M2	0.6157	40.00	45.00	8.00	*
7	M3	0.3655	40.00	45.00	8.00	*
8	SedAB-A3A	0.3907	36.00	45.00	8.00	*

Critical values are 1 tailed (k = 7)

DAP 1/16/07
QA: AR 01-12-07

8503-128-019-078-085
Rana pipiens
Survival Background Sediment as Control

Title: 8503-128-019-078-085 Rana pipiens Survival Background
File: 078surbk Transform: NO TRANSFORMATION
Number of Groups: 8

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	SedAB-BK11	1	1.0000	1.0000
1	SedAB-BK11	2	1.0000	1.0000
1	SedAB-BK11	3	0.8000	0.8000
1	SedAB-BK11	4	1.0000	1.0000
1	SedAB-BK11	5	1.0000	1.0000
1	SedAB-BK11	6	1.0000	1.0000
1	SedAB-BK11	7	1.0000	1.0000
1	SedAB-BK11	8	0.8000	0.8000
2	SedAB-A08	1	1.0000	1.0000
2	SedAB-A08	2	1.0000	1.0000
2	SedAB-A08	3	1.0000	1.0000
2	SedAB-A08	4	1.0000	1.0000
2	SedAB-A08	5	1.0000	1.0000
2	SedAB-A08	6	1.0000	1.0000
2	SedAB-A08	7	1.0000	1.0000
2	SedAB-A08	8	0.8000	0.8000
3	SedAB-A07	1	1.0000	1.0000
3	SedAB-A07	2	1.0000	1.0000
3	SedAB-A07	3	1.0000	1.0000
3	SedAB-A07	4	1.0000	1.0000
3	SedAB-A07	5	1.0000	1.0000
3	SedAB-A07	6	1.0000	1.0000
3	SedAB-A07	7	1.0000	1.0000
3	SedAB-A07	8	1.0000	1.0000
4	SedAB-A04	1	0.8000	0.8000
4	SedAB-A04	2	0.4000	0.4000
4	SedAB-A04	3	0.0000	0.0000
4	SedAB-A04	4	0.8000	0.8000
4	SedAB-A04	5	1.0000	1.0000
4	SedAB-A04	6	1.0000	1.0000
4	SedAB-A04	7	1.0000	1.0000
4	SedAB-A04	8	1.0000	1.0000

DAP 1/16/07
QA: AR01-12-07

8503-128-019-078-085
Rana pipiens
Survival Background Sediment as Control

5	M1	1	0.8000	0.8000
5	M1	2	0.2000	0.2000
5	M1	3	0.2000	0.2000
5	M1	4	0.0000	0.0000
5	M1	5	0.0000	0.0000
5	M1	6	0.2000	0.2000
5	M1	7	0.6000	0.6000
5	M1	8	0.4000	0.4000
6	M2	1	1.0000	1.0000
6	M2	2	0.8000	0.8000
6	M2	3	0.0000	0.0000
6	M2	4	0.0000	0.0000
6	M2	5	0.6000	0.6000
6	M2	6	0.4000	0.4000
6	M2	7	0.0000	0.0000
6	M2	8	0.0000	0.0000
7	M3	1	0.0000	0.0000
7	M3	2	0.0000	0.0000
7	M3	3	0.0000	0.0000
7	M3	4	0.0000	0.0000
7	M3	5	0.0000	0.0000
7	M3	6	1.0000	1.0000
7	M3	7	0.0000	0.0000
7	M3	8	0.0000	0.0000
8	SedAB-A3A	1	0.0000	0.0000
8	SedAB-A3A	2	0.0000	0.0000
8	SedAB-A3A	3	0.6000	0.6000
8	SedAB-A3A	4	0.0000	0.0000
8	SedAB-A3A	5	0.6000	0.6000
8	SedAB-A3A	6	0.0000	0.0000
8	SedAB-A3A	7	0.0000	0.0000
8	SedAB-A3A	8	0.0000	0.0000

DAP 1/16/07
QA: AR01-1207

8503-128-019-078-085
Rana pipiens
Survival Background Sediment as Control

Title: 8503-128-019-078-085 Rana pipiens Survival Background
File: 078surbk Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	SedAB-BK11	8	0.8000	1.0000	0.9500
2	SedAB-A08	8	0.8000	1.0000	0.9750
3	SedAB-A07	8	1.0000	1.0000	1.0000
4	SedAB-A04	8	0.0000	1.0000	0.7500
5	M1	8	0.0000	0.8000	0.3000
6	M2	8	0.0000	1.0000	0.3500
7	M3	8	0.0000	1.0000	0.1250
8	SedAB-A3A	8	0.0000	0.6000	0.1500

Title: 8503-128-019-078-085 Rana pipiens Survival Background
File: 078surbk Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	SedAB-BK11	0.0086	0.0926	0.0327	9.7455
2	SedAB-A08	0.0050	0.0707	0.0250	7.2524
3	SedAB-A07	0.0000	0.0000	0.0000	0.0000
4	SedAB-A04	0.1343	0.3665	0.1296	48.8600
5	M1	0.0800	0.2828	0.1000	94.2809
6	M2	0.1686	0.4106	0.1452	117.3070
7	M3	0.1250	0.3536	0.1250	282.8427
8	SedAB-A3A	0.0771	0.2777	0.0982	185.1640

DMP 1/16/07
QM: ARCH-1207

8503-128-019-078-085
Rana pipiens
Survival Background Sediment as Control

Title: 8503-128-019-078-085 Rana pipiens Survival Background
File: 078surbk Transform: ARC SINE(SQUARE ROOT(Y))

Chi-Square Test for Normality

Actual and Expected Frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	4.2880	15.4880	24.4480	15.4880	4.2880
OBSERVED	4	13	29	13	5

Chi-Square = 1.7845 (p-value = 0.7753)

Critical Chi-Square = 13.277 (alpha = 0.01 , df = 4)
= 9.488 (alpha = 0.05 , df = 4)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: 8503-128-019-078-085 Rana pipiens Survival Background
File: 078surbk Transform: ARC SINE(SQUARE ROOT(Y))

Hartley's Test for Homogeneity of Variance
Bartlett's Test for Homogeneity of Variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

DAP 1/16/07
QA: AR01-12-07

8503-128-019-078-085
Rana pipiens
Survival Background Sediment as Control

File: 078surbk Transform: ARC SINE(SQUARE ROOT(Y))

Steel's Many-One Rank Test - Ho: Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	DF	SIG 0.05
1	SedAB-BK11	1.2858				
2	SedAB-A08	1.3155	72.00	45.00	8.00	
3	SedAB-A07	1.3453	76.00	45.00	8.00	
4	SedAB-A04	1.0632	58.00	45.00	8.00	
5	M1	0.5650	37.00	45.00	8.00	*
6	M2	0.6157	42.00	45.00	8.00	*
7	M3	0.3655	41.00	45.00	8.00	*
8	SedAB-A3A	0.3907	36.00	45.00	8.00	*

Critical values are 1 tailed (k = 7)

DAP 1/16/07
QA: AR 01-10-07

8503-128-019-078-085
Rana pipiens
Body Width Poudre River Sediment as Control

Title: 8503-128-019-078-085 Rana pipiens Body Width ① ~~Background~~
File: 078wc Transform: NO TRANSFORMATION

Chi-Square Test for Normality

Actual and Expected Frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	2.1440	7.7440	12.2240	7.7440	2.1440
OBSERVED	3	4	11	13	1

Chi-Square = 6.4522 (p-value = 0.1678)

Critical Chi-Square = 13.277 (alpha = 0.01 , df = 4)
= 9.488 (alpha = 0.05 , df = 4)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: 8503-128-019-078-085 Rana pipiens Body Width Background
File: 078wc Transform: NO TRANSFORMATION

Bartlett's Test for Homogeneity of Variance

Calculated B1 statistic = 24.1392 (p-value = 0.0000)

Data FAIL B1 homogeneity test at 0.01 level. Try another transformation.

Critical B = 11.3449 (alpha = 0.01, df = 3)
= 7.8147 (alpha = 0.05, df = 3)

① AR for PO 01-10-07

DAP 1/16/07
QA: AR01-10-07

8503-128-019-078-085
Rana pipiens
Body Width Poudre River Sediment as Control

Title: 8503-128-019-078-085 Rana pipiens Body Width ~~Background~~ ①
File: 078wc Transform: NO TRANSFORMATION
Number of Groups: 4

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	5.2000	5.2000
1	Control	2	5.6000	5.6000
1	Control	3	5.4000	5.4000
1	Control	4	5.6000	5.6000
1	Control	5	5.2000	5.2000
1	Control	6	5.0000	5.0000
1	Control	7	4.8000	4.8000
1	Control	8	6.2000	6.2000
2	SedAB-A08	1	5.4000	5.4000
2	SedAB-A08	2	5.8000	5.8000
2	SedAB-A08	3	6.0000	6.0000
2	SedAB-A08	4	5.6000	5.6000
2	SedAB-A08	5	6.0000	6.0000
2	SedAB-A08	6	6.2000	6.2000
2	SedAB-A08	7	6.2000	6.2000
2	SedAB-A08	8	4.2000	4.2000
3	SedAB-A07	1	5.8000	5.8000
3	SedAB-A07	2	5.4000	5.4000
3	SedAB-A07	3	5.2000	5.2000
3	SedAB-A07	4	6.0000	6.0000
3	SedAB-A07	5	6.0000	6.0000
3	SedAB-A07	6	6.0000	6.0000
3	SedAB-A07	7	6.0000	6.0000
3	SedAB-A07	8	6.2000	6.2000
4	SedAB-A04	1	4.0000	4.0000
4	SedAB-A04	2	2.0000	2.0000
4	SedAB-A04	3	0.0000	0.0000
4	SedAB-A04	4	3.8000	3.8000
4	SedAB-A04	5	5.2000	5.2000
4	SedAB-A04	6	5.6000	5.6000
4	SedAB-A04	7	4.8000	4.8000
4	SedAB-A04	8	4.8000	4.8000

① Ref for PO 01-10-07 E

DAP 1/16/07
QA: AR 01-10-07

8503-128-019-078-085
Rana pipiens
Body Width Poudre River Sediment as Control

Title: 8503-128-019-078-085 Rana pipiens Body Width ~~Background~~ ①
File: 078wc Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Control	8	4.8000	6.2000	5.3750
2	SedAB-A08	8	4.2000	6.2000	5.6750
3	SedAB-A07	8	5.2000	6.2000	5.8250
4	SedAB-A04	8	0.0000	5.6000	3.7750

Title: 8503-128-019-078-085 Rana pipiens Body Width Background
File: 078wc Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	Control	0.1879	0.4334	0.1532	8.0637
2	SedAB-A08	0.4336	0.6585	0.2328	11.6028
3	SedAB-A07	0.1193	0.3454	0.1221	5.9292
4	SedAB-A04	3.5593	1.8866	0.6670	49.9763

Title: 8503-128-019-078-085 Rana pipiens Body Width Background
File: 078wc Transform: NO TRANSFORMATION

Steel's Many-One Rank Test - Ho: Control<Treatment

GROUP	IDENTIFICATION	MEAN IN ORIGINAL UNITS	RANK SUM	CRIT. VALUE	DF	SIG 0.05
1	Control	5.3750				
2	SedAB-A08	5.6750	82.50	48.00	8.00	
3	SedAB-A07	5.8250	86.00	48.00	8.00	
4	SedAB-A04	3.7750	46.00	48.00	8.00	*

Critical values are 1 tailed (k = 3)

① AR for RO 01-10-07 E

DAP 1/16/07
QA: AR01-10-07

8503-128-019-078-085
Rana pipiens
Body Width Background Sediment as Control

Title: 8503-128-019-078-085 Rana pipiens Body Width Background
File: 078wbk Transform: NO TRANSFORMATION

Chi-Square Test for Normality

Actual and Expected Frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	2.1440	7.7440	12.2240	7.7440	2.1440
OBSERVED	4	2	13	13	0

Chi-Square = 11.6278 (p-value = 0.0203)

Critical Chi-Square = 13.277 (alpha = 0.01 , df = 4)
= 9.488 (alpha = 0.05 , df = 4)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: 8503-128-019-078-085 Rana pipiens Body Width Background
File: 078wbk Transform: NO TRANSFORMATION

Bartlett's Test for Homogeneity of Variance

Calculated B1 statistic = 23.5207 (p-value = 0.0000)

Data FAIL B1 homogeneity test at 0.01 level. Try another transformation.

Critical B = 11.3449 (alpha = 0.01, df = 3)
= 7.8147 (alpha = 0.05, df = 3)

DAP 1/16/07
QA: A2011-10-07

8503-128-019-078-085
Rana pipiens
Body Width Background Sediment as Control

Title: 8503-128-019-078-085 Rana pipiens Body Width Background
File: 078wbk Transform: NO TRANSFORMATION
Number of Groups: 4

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	SedAB-BK11	1	5.8000	5.8000
1	SedAB-BK11	2	5.4000	5.4000
1	SedAB-BK11	3	4.6000	4.6000
1	SedAB-BK11	4	5.8000	5.8000
1	SedAB-BK11	5	6.0000	6.0000
1	SedAB-BK11	6	6.0000	6.0000
1	SedAB-BK11	7	5.4000	5.4000
1	SedAB-BK11	8	5.6000	5.6000
2	SedAB-A08	1	5.4000	5.4000
2	SedAB-A08	2	5.8000	5.8000
2	SedAB-A08	3	6.0000	6.0000
2	SedAB-A08	4	5.6000	5.6000
2	SedAB-A08	5	6.0000	6.0000
2	SedAB-A08	6	6.2000	6.2000
2	SedAB-A08	7	6.2000	6.2000
2	SedAB-A08	8	4.2000	4.2000
3	SedAB-A07	1	5.8000	5.8000
3	SedAB-A07	2	5.4000	5.4000
3	SedAB-A07	3	5.2000	5.2000
3	SedAB-A07	4	6.0000	6.0000
3	SedAB-A07	5	6.0000	6.0000
3	SedAB-A07	6	6.0000	6.0000
3	SedAB-A07	7	6.0000	6.0000
3	SedAB-A07	8	6.2000	6.2000
4	SedAB-A04	1	4.0000	4.0000
4	SedAB-A04	2	2.0000	2.0000
4	SedAB-A04	3	0.0000	0.0000
4	SedAB-A04	4	3.8000	3.8000
4	SedAB-A04	5	5.2000	5.2000
4	SedAB-A04	6	5.6000	5.6000
4	SedAB-A04	7	4.8000	4.8000
4	SedAB-A04	8	4.8000	4.8000

DAP 1/16/07
QA: AR01-10-07

8503-128-019-078-085
Rana pipiens
Body Width Background Sediment as Control

Title: 8503-128-019-078-085 Rana pipiens Body Width Background
File: 078wbk Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	SedAB-BK11	8	4.6000	6.0000	5.5750
2	SedAB-A08	8	4.2000	6.2000	5.6750
3	SedAB-A07	8	5.2000	6.2000	5.8250
4	SedAB-A04	8	0.0000	5.6000	3.7750

Title: 8503-128-019-078-085 Rana pipiens Body Width Background
File: 078wbk Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	SedAB-BK11	0.2107	0.4590	0.1623	8.2338
2	SedAB-A08	0.4336	0.6585	0.2328	11.6028
3	SedAB-A07	0.1193	0.3454	0.1221	5.9292
4	SedAB-A04	3.5593	1.8866	0.6670	49.9763

Title: 8503-128-019-078-085 Rana pipiens Body Width Background
File: 078wbk Transform: NO TRANSFORMATION

Steel's Many-One Rank Test - Ho: Control<Treatment

GROUP	IDENTIFICATION	MEAN IN ORIGINAL UNITS	RANK SUM	CRIT. VALUE	DF	SIG 0.05
1	SedAB-BK11	5.5750				
2	SedAB-A08	5.6750	76.50	48.00	8.00	
3	SedAB-A07	5.8250	80.00	48.00	8.00	
4	SedAB-A04	3.7750	42.50	48.00	8.00	*

Critical values are 1 tailed (k = 3)

8503-128-019-078-085
Rana pipiens
Body Length Poudre River Sediment as Control

DAP 1/16/07
QA: AR01-1007

Title: 8503-128-019-078-085 Rana pipiens Body Length Control
File: 078lc Transform: NO TRANSFORMATION

Chi-Square Test for Normality

Actual and Expected Frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	2.1440	7.7440	12.2240	7.7440	2.1440
OBSERVED	2	8	14	6	2

Chi-Square = 0.6786 (p-value = 0.9539)

Critical Chi-Square = 13.277 (alpha = 0.01 , df = 4)
= 9.488 (alpha = 0.05 , df = 4)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: 8503-128-019-078-085 Rana pipiens Body Length Control
File: 078lc Transform: NO TRANSFORMATION

Bartlett's Test for Homogeneity of Variance

Calculated B1 statistic = 23.0612 (p-value = 0.0000)

Data FAIL B1 homogeneity test at 0.01 level. Try another transformation.

Critical B = 11.3449 (alpha = 0.01, df = 3)
= 7.8147 (alpha = 0.05, df = 3)

DSP 1/16/07

QA: NR01-10-07

8503-128-019-078-085
Rana pipiens
Body Length Poudre River Sediment as Control

Title: 8503-128-019-078-085 Rana pipiens Body Length Control
File: 078lc Transform: NO TRANSFORMATION
Number of Groups: 4

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	8.6000	8.6000
1	Control	2	9.4000	9.4000
1	Control	3	9.2000	9.2000
1	Control	4	9.8000	9.8000
1	Control	5	8.4000	8.4000
1	Control	6	8.2000	8.2000
1	Control	7	8.4000	8.4000
1	Control	8	10.4000	10.4000
2	SedAB-A08	1	9.4000	9.4000
2	SedAB-A08	2	9.8000	9.8000
2	SedAB-A08	3	10.6000	10.6000
2	SedAB-A08	4	9.6000	9.6000
2	SedAB-A08	5	10.2000	10.2000
2	SedAB-A08	6	10.2000	10.2000
2	SedAB-A08	7	11.0000	11.0000
2	SedAB-A08	8	7.4000	7.4000
3	SedAB-A07	1	9.6000	9.6000
3	SedAB-A07	2	9.6000	9.6000
3	SedAB-A07	3	9.4000	9.4000
3	SedAB-A07	4	11.0000	11.0000
3	SedAB-A07	5	9.8000	9.8000
3	SedAB-A07	6	10.0000	10.0000
3	SedAB-A07	7	10.0000	10.0000
3	SedAB-A07	8	10.0000	10.0000
4	SedAB-A04	1	6.4000	6.4000
4	SedAB-A04	2	3.2000	3.2000
4	SedAB-A04	3	0.0000	0.0000
4	SedAB-A04	4	6.2000	6.2000
4	SedAB-A04	5	8.2000	8.2000
4	SedAB-A04	6	8.4000	8.4000
4	SedAB-A04	7	7.4000	7.4000
4	SedAB-A04	8	7.8000	7.8000

DAP 1/16/07
QA: AR201-10-07

8503-128-019-078-085
Rana pipiens
Body Length Poudre River Sediment as Control

Title: 8503-128-019-078-085 Rana pipiens Body Length Control
File: 078lc Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Control	8	8.2000	10.4000	9.0500
2	SedAB-A08	8	7.4000	11.0000	9.7750
3	SedAB-A07	8	9.4000	11.0000	9.9250
4	SedAB-A04	8	0.0000	8.4000	5.9500

Title: 8503-128-019-078-085 Rana pipiens Body Length Control
File: 078lc Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	Control	0.6143	0.7838	0.2771	8.6604
2	SedAB-A08	1.1936	1.0925	0.3863	11.1765
3	SedAB-A07	0.2393	0.4892	0.1729	4.9286
4	SedAB-A04	8.5457	2.9233	1.0335	49.1312

Title: 8503-128-019-078-085 Rana pipiens Body Length Control
File: 078lc Transform: NO TRANSFORMATION

Steel's Many-One Rank Test - Ho: Control<Treatment

GROUP	IDENTIFICATION	MEAN IN ORIGINAL UNITS	RANK SUM	CRIT. VALUE	DF	SIG 0.05
1	Control	9.0500				
2	SedAB-A08	9.7750	84.00	48.00	8.00	
3	SedAB-A07	9.9250	89.00	48.00	8.00	
4	SedAB-A04	5.9500	38.50	48.00	8.00	*

Critical values are 1 tailed (k = 3)

DSP 1/16/07
QA: AR01-10-07

8503-128-019-078-085
Rana pipiens
Body Length Background as Control

Title: 8503-128-019-078-085 Rana pipiens Body Length Bckground
File: 078lbk Transform: NO TRANSFORMATION

Chi-Square Test for Normality

Actual and Expected Frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	2.1440	7.7440	12.2240	7.7440	2.1440
OBSERVED	3	5	15	8	1

Chi-Square = 2.5634 (p-value = 0.6333)

Critical Chi-Square = 13.277 (alpha = 0.01 , df = 4)
= 9.488 (alpha = 0.05 , df = 4)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: 8503-128-019-078-085 Rana pipiens Body Length Bckground
File: 078lbk Transform: NO TRANSFORMATION

Bartlett's Test for Homogeneity of Variance

Calculated B1 statistic = 22.2793 (p-value = 0.0001)

Data FAIL B1 homogeneity test at 0.01 level. Try another transformation.

Critical B = 11.3449 (alpha = 0.01, df = 3)
= 7.8147 (alpha = 0.05, df = 3)

DAP 1/16/07
QA: AR01-10-07

8503-128-019-078-085
Rana pipiens
Body Length Background as Control

Title: 8503-128-019-078-085 Rana pipiens Body Length Bckground
File: 0781bk Transform: NO TRANSFORMATION
Number of Groups: 4

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	SedAB-BK11	1	10.4000	10.4000
1	SedAB-BK11	2	9.6000	9.6000
1	SedAB-BK11	3	8.0000	8.0000
1	SedAB-BK11	4	10.0000	10.0000
1	SedAB-BK11	5	10.2000	10.2000
1	SedAB-BK11	6	10.6000	10.6000
1	SedAB-BK11	7	9.0000	9.0000
1	SedAB-BK11	8	9.6000	9.6000
2	SedAB-A08	1	9.4000	9.4000
2	SedAB-A08	2	9.8000	9.8000
2	SedAB-A08	3	10.6000	10.6000
2	SedAB-A08	4	9.6000	9.6000
2	SedAB-A08	5	10.2000	10.2000
2	SedAB-A08	6	10.2000	10.2000
2	SedAB-A08	7	11.0000	11.0000
2	SedAB-A08	8	7.4000	7.4000
3	SedAB-A07	1	9.6000	9.6000
3	SedAB-A07	2	9.6000	9.6000
3	SedAB-A07	3	9.4000	9.4000
3	SedAB-A07	4	11.0000	11.0000
3	SedAB-A07	5	9.8000	9.8000
3	SedAB-A07	6	10.0000	10.0000
3	SedAB-A07	7	10.0000	10.0000
3	SedAB-A07	8	10.0000	10.0000
4	SedAB-A04	1	6.4000	6.4000
4	SedAB-A04	2	3.2000	3.2000
4	SedAB-A04	3	0.0000	0.0000
4	SedAB-A04	4	6.2000	6.2000
4	SedAB-A04	5	8.2000	8.2000
4	SedAB-A04	6	8.4000	8.4000
4	SedAB-A04	7	7.4000	7.4000
4	SedAB-A04	8	7.8000	7.8000

DAP 1/16/07
QA: AR01-10-07

8503-128-019-078-085
Rana pipiens
Body Length Background as Control

Title: 8503-128-019-078-085 Rana pipiens Body Length Bckground
File: 078lbk Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	SedAB-BK11	8	8.0000	10.6000	9.6750
2	SedAB-A08	8	7.4000	11.0000	9.7750
3	SedAB-A07	8	9.4000	11.0000	9.9250
4	SedAB-A04	8	0.0000	8.4000	5.9500

Title: 8503-128-019-078-085 Rana pipiens Body Length Bckground
File: 078lbk Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	SedAB-BK11	0.7193	0.8481	0.2999	8.7660
2	SedAB-A08	1.1936	1.0925	0.3863	11.1765
3	SedAB-A07	0.2393	0.4892	0.1729	4.9286
4	SedAB-A04	8.5457	2.9233	1.0335	49.1312

Title: 8503-128-019-078-085 Rana pipiens Body Length Bckground
File: 078lbk Transform: NO TRANSFORMATION

Steel's Many-One Rank Test - Ho: Control<Treatment

GROUP	IDENTIFICATION	MEAN IN ORIGINAL UNITS	RANK SUM	CRIT. VALUE	DF	SIG 0.05
1	SedAB-BK11	9.6750				
2	SedAB-A08	9.7750	71.50	48.00	8.00	
3	SedAB-A07	9.9250	69.50	48.00	8.00	
4	SedAB-A04	5.9500	38.00	48.00	8.00	*

Critical values are 1 tailed (k = 3)

APPENDIX D

Analytical Data

PARAGON ANALYTICS
225 Commerce Drive ◆ Fort Collins, CO 80524 ◆ (800) 443-1511 ◆ (970) 490-1511 ◆ FAX (970) 490-1522

November 14, 2006

Mr. David Pillard
ENSR Consulting and Engineering
4303 W. Laporte
Fort Collins, CO 80521

Re: Paragon Workorder: 06-11-012
Client Project Name: ESTCP
Client Project Number: 09070-056-503

Dear Mr. Pillard:

Eleven soil samples were received from ENSR Consulting and Engineering on November 1, 2006. The samples were scheduled for Total Metals (pages 1-19) analysis.

The results for this analysis are contained in the enclosed reports.

Thank you for your confidence in Paragon Analytics. Should you have any questions, please call.

Sincerely,

Paragon Analytics
for Debbie Fazio
Project Manager

DJF/ja
Enclosure: Report

Paragon Analytics

Sample Number(s) Cross-Reference Table

Paragon OrderNum: 0611012
Client Name: ENSR Consulting and Engineering
Client Project Name: ESTCP
Client Project Number: 09070-056-503
Client PO Number: 2041736

Client Sample	Lab Sample Number	COC Number	Matrix	Date Collected	Time Collected
Sed Ab-ABK11	0611012-1		SOIL	31-Oct-06	
Sed Ab-A08	0611012-2		SOIL	31-Oct-06	
Sed Ab-A07	0611012-3		SOIL	31-Oct-06	
Sed Ab-A04	0611012-4		SOIL	31-Oct-06	
Sed Ab-A3A	0611012-5		SOIL	31-Oct-06	
SDT BK-STA	0611012-6		SOIL	31-Oct-06	
SDT 04-DTA	0611012-7		SOIL	31-Oct-06	
SST 04-DTA	0611012-8		SOIL	31-Oct-06	
SST 07-DTA	0611012-9		SOIL	31-Oct-06	
SDT 14-DTA	0611012-10		SOIL	31-Oct-06	
SST 13-DTA	0611012-11		SOIL	01-Nov-06	

ENSR INTERNATIONAL

CHAIN OF CUSTODY RECORD

0611012

Page ____ of ____

Client/Project Name: ESTCP

Project Location: ENSR / FCETC

Project Number: 09070-056-503

Field Logbook No.:

Sampler (Print Name)/(Affiliation): Patrick Ochsenbeck (ENSR)

Chain of Custody Tape No.:

Signature:

Send Results/Report to: Dave Pillard

Analysis Requested

Field Sample No./ Identification	Date	Time	Grab	Comp	Sample Container (Size/Mat'l)	Sample Type (Liquid, Sludge, Etc.)	Preservative	Field Filtered	Cu	Pb						Lab I.D.	Remarks
Sed Ab-ABK11	10/31/06				2 oz Amber	Sediment	None	NA	X	X							STANDARD 14 Day Turnaround
Sed Ab-A08																	
Sed Ab-A07																	
Sed Ab-A04																	
Sed Ab-A3A																	
SDT BK ~~[illegible]~~ STA																	
SDT 04-DTA																	
SST 04-DTA																	
SST 07-DTA																	
SDT14-DTA																	

Relinquished by: (Print Name)/(Affiliation)	Date	Received by: (Print Name)/(Affiliation)	Date
Patrick Ochsenbeck (ENSR) Signature:	11/1/06 Time: 1200	Sheri Lafferty Signature: Sheri Lafferty	11.1.06 Time: 1620
Relinquished by: (Print Name)/(Affiliation) Signature:	Date: Time:	Received by: (Print Name)/(Affiliation) Signature:	Date: Time:
Relinquished by: (Print Name)/(Affiliation) Signature:	Date: Time:	Received by: (Print Name)/(Affiliation) Signature:	Date: Time:

Analytical Laboratory (Destination):

PARAGON

~~ENSR Toxicology Lab~~
~~4303 W. Laporte Avenue~~
~~Fort Collins, CO 80521~~
~~(970) 416-0916~~
(970) 493-8935 (FAX)

Serial No. 43430

FM01008b_902

09 11/01/05

ENSR INTERNATIONAL

CHAIN OF CUSTODY RECORD

061101 2

Page ____ of ____

Client/Project Name: ESTCP

Project Location: ENSR / FCETL

Project Number: 0907-056-503

Field Logbook No.:

Sampler (Print Name)/(Affiliation): Patrick Odenbeck (ENSR)

Chain of Custody Tape No.:

Signature:

Send Results/Report to: Dave Pillard

Analysis Requested

Field Sample No./ Identification	Date	Time	Grab	Comp	Sample Container (Size/Mat'l)	Sample Type (Liquid, Sludge, Etc.)	Preservative	Field Filtered	U5	R6							Lab I.D.	Remarks
SST 13 PTA	11/1/06				2 oz Amber	Sediment	None	NA	X	X								STANDARD 14 Day Turnaround

Relinquished by: (Print Name)/(Affiliation)	Date	Received by: (Print Name)/(Affiliation)	Date
Patrick Odenbeck (ENSR) Signature:	11/1/06 Time: 1200	Signature: Sheri Lafferty	11-1-06 Time: 1620
Relinquished by: (Print Name)/(Affiliation) Signature:	Date: Time:	Received by: (Print Name)/(Affiliation) Signature:	Date: Time:
Relinquished by: (Print Name)/(Affiliation) Signature:	Date: Time:	Received by: (Print Name)/(Affiliation) Signature:	Date: Time:

Analytical Laboratory (Destination):

PARAGON

ENSR Toxicology Lab
4303 W. Laporte Avenue
Fort Collins, CO 80521
(970) 416-0916
(970) 493-8935 (FAX)

Serial No. 43429

FM01008b_902

CONDITION OF SAMPLE UPON RECEIPT FORM **Paragon Analytics**

Client: ENSR Workorder No: 0611012

Project Manager: DF Initials: SL Date: 11.1.06

1. Does this project require any **special handling** in addition to standard Paragon procedures?		YES	(NO)
2. Are custody **seals** on **shipping containers** intact?	(NONE)	YES	NO
3. Are Custody seals on **sample containers** intact?	(NONE)	YES	NO
4. Is there a **COC (Chain-of-Custody) present** or other representative documents?		(YES)	NO
5. Are the **COC and bottle labels complete and legible**?		YES	(NO)
6. Is the **COC in agreement** with samples received? (IDs, dates, times, no. of samples, no. of containers, matrix, requested analyses, etc.)		(YES)	NO
7. Were **airbills / shipping documents** present and/or removable?	(DROP OFF)	YES	NO
8. Are all aqueous **samples requiring preservation preserved correctly?** (excluding volatiles)	(N/A)	YES	NO
9. Are all aqueous **non-preserved samples pH 4-9**?	(N/A)	YES	NO
10. Is there **sufficient sample** for the requested analyses?		(YES)	NO
11. Were all samples placed in the **proper containers** for the requested analyses?		(YES)	NO
12. Are all samples within **holding times** for the requested analyses?		(YES)	NO
13. Were all sample containers received **intact**? (not broken or leaking, etc.)		(YES)	NO
14. Are all samples requiring no headspace (VOC, GRO, Rx CN/S, radon), headspace free? **Size of bubble:** ____ < green pea ____ > green pea	(N/A)	YES	NO
15. Were samples checked for and free from the presence of **residual chlorine?** (Applicable when PM has indicated samples are from a chlorinated water source; note if field preservation with sodium thiosulfate was not observed.)	(N/A)	YES	NO
16. Were the samples **shipped on ice**?		(YES)	NO
17. Were cooler temperatures measured at 0.1-6.0°C? **IR gun used*:** (#2) #4	RAD ONLY	(YES)	NO

Cooler #:	1							
Temperature (°C):	5.8							
No. of custody seals on cooler:	NA							
DOT Survey/ Acceptance Information — External µR/hr reading:	NA							
Background µR/hr reading:	12							

Were external µR/hr readings ≤ two times background and within DOT acceptance criteria? (YES) / NO / NA (If no, see Form 008.)

Additional Information: PROVIDE DETAILS BELOW FOR A NO RESPONSE TO ANY QUESTION ABOVE, EXCEPT #1 AND #16.

* No collection times listed on coc or bottles

If applicable, was the client contacted? YES / NO / NA Contact: _____ Date/Time: _____

Project Manager Signature / Date: Debbie Fazio 11/2/06

*IR Gun #2: Oakton, SN 29922500201-0066 *IR Gun #4: Oakton, SN 2372220101-0002

Paragon Analytics

TOTAL METALS CASE NARRATIVE

ENSR Consulting and Engineering

ESTCP -- 09070-056-503

Order Number - 0611012

1. This report consists of 11 soil samples.
2. The samples were received cool and intact on 11/01/06.
3. The samples were prepared for analysis based on SW-846, 3rd Edition procedures.

   For analysis by Trace ICP2, the samples were digested following method 3050B and PA SOP 806 Rev. 12.
4. The samples were analyzed following SW-846, 3rd Edition procedures.

   Analysis by Trace ICP2 followed method 6010B and PA SOP 834 Rev. 5.

   The relationship between intensity and concentration for each element is established using at least four standards, one of which is a blank solution.

   During sample analysis concentrations are computed by the software and the results are printed in mg/L. The instrument software does not provide a printout which gives both intensity and concentration. The validity of the calibration equation is tested by analyzing the following solutions: a blank, a low level check solution with concentrations near the reporting limit, an Initial Calibration Verification (ICV) standard from a 2nd source standard solution with concentrations near the middle of the analytical range, a Continuing Calibration Verification (CCV) standard with concentrations at two times those in the ICV, and a readback of the highest calibration standard.

   These solutions provide verification that the calibration equations are functioning properly throughout the analytical range of the instrument. During sample analysis dilutions are made for analytes found at concentrations above the highest calibration standard. No results are taken from extrapolations beyond the highest standard.
5. All standards and solutions are NIST traceable and were used within their recommended shelf life.
6. The samples were prepared and analyzed within the established hold times.

All in house quality control procedures were followed, as described below.

7. General quality control procedures.

   - A preparation (method) blank and laboratory control sample were digested and analyzed with the samples in this digestion batch. There were not more than 20 samples in the digestion batch.
   - The preparation (method) blank associated with this digestion batch was below the practical quantitation limit for each requested analyte.
   - The laboratory control sample associated with this digestion batch was within the acceptance limits. This indicates complete digestion according to the method.
   - All initial and continuing calibration blanks associated with this analytical batch were below the practical quantitation limits for the requested analytes.
   - All initial and continuing calibration verifications associated with this analytical batch were within the acceptance criteria for the requested analytes. This indicates a valid calibration and stable instrument conditions.
   - The interference check samples and high standard readbacks associated with Method 6010B analyses were within acceptance criteria.

8. Matrix specific quality control procedures.

   Sample 0611012-11 was designated as the quality control sample for this analysis.

   - A matrix spike and matrix spike duplicate were digested and analyzed with this batch. All acceptance criteria for accuracy were met.
   - Matrix spike recoveries could not be evaluated for the following analyte:

Analyte	Sample ID
Lead	0611012-11

     The concentration of this analyte in the native sample was greater than four times the concentration of matrix spike added during the digestion. When sample concentration is that much greater than the spike added, spike recoveries may not be accurate. The laboratory control sample indicates that the digestion and analysis were in control.

   - A sample duplicate and matrix spike duplicate were digested and analyzed with this batch. All acceptance criteria for precision were met.
   - A serial dilution was analyzed with the ICP batch. All acceptance criteria were met.

9. Sample IDs 0611012-5, -7, -8, -9, -10, and -11 required dilutions to bring iron and/or lead into the analytical range of the Trace ICP. Accurate quantitation of iron is necessary to correct for spectral interferences on lead. The lead result was determined from the diluted samples.

The data contained in the following report have been reviewed and approved by the personnel listed below. In addition, Paragon Analytics certifies that the analyses reported herein are true, complete and correct within the limits of the methods employed.

Rhonda Miller
Data Reporting Specialist

11/14/06
Date

Reviewer's Initials

11/13/06
Date

Paragon Analytics

Sample Number(s) Cross-Reference Table

Paragon OrderNum: 0611012
Client Name: ENSR Consulting and Engineering
Client Project Name: ESTCP
Client Project Number: 09070-056-503
Client PO Number: 2041736

Client Sample	Lab Sample Number	COC Number	Matrix	Date Collected	Time Collected
Sed Ab-ABK11	0611012-1		SOIL	31-Oct-06	
Sed Ab-A08	0611012-2		SOIL	31-Oct-06	
Sed Ab-A07	0611012-3		SOIL	31-Oct-06	
Sed Ab-A04	0611012-4		SOIL	31-Oct-06	
Sed Ab-A3A	0611012-5		SOIL	31-Oct-06	
SDT BK-STA	0611012-6		SOIL	31-Oct-06	
SDT 04-DTA	0611012-7		SOIL	31-Oct-06	
SST 04-DTA	0611012-8		SOIL	31-Oct-06	
SST 07-DTA	0611012-9		SOIL	31-Oct-06	
SDT 14-DTA	0611012-10		SOIL	31-Oct-06	
SST 13-DTA	0611012-11		SOIL	01-Nov-06	

Inorganic Data Reporting Qualifiers

The following qualifiers are used by the laboratory when reporting results of inorganic analyses.

- Result qualifier -- If the analyte was analyzed for but not detected a "U" is entered.

- QC qualifier -- Specified entries and their meanings are as follows:

  E - The reported value is estimated because of the presence of interference. An explanatory note may be included in the narrative.

  M - Duplicate injection precision was not met.

  N - Spiked sample recovery not within control limits. A post spike is analyzed for all 6010B analyses when the matrix spike and or spike duplicate fail and the native sample concentration is less than four times the spike added concentration.

  Z - Spiked recovery not within control limits. An explanatory note may be included in the narrative.

  * - Duplicate analysis (relative percent difference) not within control limits.

Total ICP Metals

Method SW6010B

Sample Results

Lab Name: Paragon Analytics
Work Order Number: 0611012
Client Name: ENSR Consulting and Engineering
ClientProject ID: ESTCP 09070-056-503

Field ID: Sed Ab-ABK11
Lab ID: 0611012-1

Sample Matrix: SOIL
% Moisture: 21.5
Date Collected: 31-Oct-06
Date Extracted: 02-Nov-06
Date Analyzed: 03-Nov-06
Prep Method: SW3050 Rev B

Prep Batch: IP061102-1
QCBatchID: IP061102-1-1
Run ID: it061103-2a2
Cleanup: NONE
Basis: Dry Weight

Sample Aliquot: 1.04 g
Final Volume: 100 ml
Result Units: mg/kg
Clean DF: 1
File Name: 061103a.

CASNO	Target Analyte	Dilution Factor	Result	Reporting Limit	Result Qualifier	EPA Qualifier
7440-50-8	COPPER	1	7.7	1.2		
7439-92-1	LEAD	1	26	0.37		

Data Package ID: *it0611012-1*

Total ICP Metals

Method SW6010B

Sample Results

Lab Name: Paragon Analytics
Work Order Number: 0611012
Client Name: ENSR Consulting and Engineering
ClientProject ID: ESTCP 09070-056-503

Field ID: Sed Ab-A08
Lab ID: 0611012-2

Sample Matrix: SOIL
% Moisture: 8.5
Date Collected: 31-Oct-06
Date Extracted: 02-Nov-06
Date Analyzed: 03-Nov-06
Prep Method: SW3050 Rev B

Prep Batch: IP061102-1
QCBatchID: IP061102-1-1
Run ID: it061103-2a2
Cleanup: NONE
Basis: Dry Weight

Sample Aliquot: 1.06 g
Final Volume: 100 ml
Result Units: mg/kg
Clean DF: 1
File Name: 061103a.

CASNO	Target Analyte	Dilution Factor	Result	Reporting Limit	Result Qualifier	EPA Qualifier
7440-50-8	COPPER	1	16	1		
7439-92-1	LEAD	1	170	0.31		

Data Package ID: *it0611012-1*

Total ICP Metals

Method SW6010B

Sample Results

Lab Name: Paragon Analytics
Work Order Number: 0611012
Client Name: ENSR Consulting and Engineering
ClientProject ID: ESTCP 09070-056-503

Field ID: Sed Ab-A07
Lab ID: 0611012-3

Sample Matrix: SOIL
% Moisture: 30.9
Date Collected: 31-Oct-06
Date Extracted: 02-Nov-06
Date Analyzed: 03-Nov-06
Prep Method: SW3050 Rev B

Prep Batch: IP061102-1
QCBatchID: IP061102-1-1
Run ID: it061103-2a2
Cleanup: NONE
Basis: Dry Weight

Sample Aliquot: 1.01 g
Final Volume: 100 ml
Result Units: mg/kg
Clean DF: 1
File Name: 061103a.

CASNO	Target Analyte	Dilution Factor	Result	Reporting Limit	Result Qualifier	EPA Qualifier
7440-50-8	COPPER	1	37	1.4		
7439-92-1	LEAD	1	410	0.43		

Data Package ID: *it0611012-1*

Total ICP Metals

Method SW6010B

Sample Results

Lab Name: Paragon Analytics
Work Order Number: 0611012
Client Name: ENSR Consulting and Engineering
ClientProject ID: ESTCP 09070-056-503

Field ID: Sed Ab-A04
Lab ID: 0611012-4

Sample Matrix: SOIL
% Moisture: 14.7
Date Collected: 31-Oct-06
Date Extracted: 02-Nov-06
Date Analyzed: 03-Nov-06
Prep Method: SW3050 Rev B

Prep Batch: IP061102-1
QCBatchID: IP061102-1-1
Run ID: it061103-2a2
Cleanup: NONE
Basis: Dry Weight

Sample Aliquot: 1.04 g
Final Volume: 100 ml
Result Units: mg/kg
Clean DF: 1
File Name: 061103a.

CASNO	Target Analyte	Dilution Factor	Result	Reporting Limit	Result Qualifier	EPA Qualifier
7440-50-8	COPPER	1	140	1.1		
7439-92-1	LEAD	1	960	0.34		

Data Package ID: *it0611012-1*

Total ICP Metals

Method SW6010B

Sample Results

Lab Name: Paragon Analytics
Work Order Number: 0611012
Client Name: ENSR Consulting and Engineering
ClientProject ID: ESTCP 09070-056-503

Field ID: Sed Ab-A3A
Lab ID: 0611012-5

Sample Matrix: SOIL
% Moisture: 34.4
Date Collected: 31-Oct-06
Date Extracted: 02-Nov-06
Date Analyzed: 03-Nov-06
Prep Method: SW3050 Rev B

Prep Batch: IP061102-1
QCBatchID: IP061102-1-1
Run ID: it061103-2a2
Cleanup: NONE
Basis: Dry Weight

Sample Aliquot: 1.04 g
Final Volume: 100 ml
Result Units: mg/kg
Clean DF: 1
File Name: 061103a.

CASNO	Target Analyte	Dilution Factor	Result	Reporting Limit	Result Qualifier	EPA Qualifier
7440-50-8	COPPER	1	1200	1.5		
7439-92-1	LEAD	50	17000	22		

Data Package ID: *it0611012-1*

Total ICP Metals

Method SW6010B

Sample Results

Lab Name: Paragon Analytics
Work Order Number: 0611012
Client Name: ENSR Consulting and Engineering
ClientProject ID: ESTCP 09070-056-503

Field ID: SDT BK-STA
Lab ID: 0611012-6

Sample Matrix: SOIL
% Moisture: 25.5
Date Collected: 31-Oct-06
Date Extracted: 02-Nov-06
Date Analyzed: 03-Nov-06
Prep Method: SW3050 Rev B

Prep Batch: IP061102-1
QCBatchID: IP061102-1-1
Run ID: it061103-2a2
Cleanup: NONE
Basis: Dry Weight

Sample Aliquot: 1.05 g
Final Volume: 100 ml
Result Units: mg/kg
Clean DF: 1
File Name: 061103a.

CASNO	Target Analyte	Dilution Factor	Result	Reporting Limit	Result Qualifier	EPA Qualifier
7440-50-8	COPPER	1	12	1.3		
7439-92-1	LEAD	1	15	0.38		

Data Package ID: *it0611012-1*

Total ICP Metals

Method SW6010B

Sample Results

Lab Name: Paragon Analytics
Work Order Number: 0611012
Client Name: ENSR Consulting and Engineering
ClientProject ID: ESTCP 09070-056-503

Field ID: SDT 04-DTA
Lab ID: 0611012-7

Sample Matrix: SOIL
% Moisture: 29.5
Date Collected: 31-Oct-06
Date Extracted: 02-Nov-06
Date Analyzed: 03-Nov-06
Prep Method: SW3050 Rev B

Prep Batch: IP061102-1
QCBatchID: IP061102-1-1
Run ID: it061103-2a2
Cleanup: NONE
Basis: Dry Weight

Sample Aliquot: 1 g
Final Volume: 100 ml
Result Units: mg/kg
Clean DF: 1
File Name: 061103a.

CASNO	Target Analyte	Dilution Factor	Result	Reporting Limit	Result Qualifier	EPA Qualifier
7440-50-8	COPPER	1	19	1.4		
7439-92-1	LEAD	10	1700	4.3		

Data Package ID: *it0611012-1*

Total ICP Metals

Method SW6010B

Sample Results

Lab Name: Paragon Analytics
Work Order Number: 0611012
Client Name: ENSR Consulting and Engineering
ClientProject ID: ESTCP 09070-056-503

Field ID: SST 04-DTA
Lab ID: 0611012-8

Sample Matrix: SOIL
% Moisture: 24.5
Date Collected: 31-Oct-06
Date Extracted: 02-Nov-06
Date Analyzed: 03-Nov-06
Prep Method: SW3050 Rev B

Prep Batch: IP061102-1
QCBatchID: IP061102-1-1
Run ID: it061103-2a2
Cleanup: NONE
Basis: Dry Weight

Sample Aliquot: 1.08 g
Final Volume: 100 ml
Result Units: mg/kg
Clean DF: 1
File Name: 061103a.

CASNO	Target Analyte	Dilution Factor	Result	Reporting Limit	Result Qualifier	EPA Qualifier
7440-50-8	COPPER	1	23	1.2		
7439-92-1	LEAD	50	30000	18		

Data Package ID: *it0611012-1*

Total ICP Metals

Method SW6010B

Sample Results

Lab Name: Paragon Analytics
Work Order Number: 0611012
Client Name: ENSR Consulting and Engineering
ClientProject ID: ESTCP 09070-056-503

Field ID: SST 07-DTA
Lab ID: 0611012-9

Sample Matrix: SOIL
% Moisture: 30.0
Date Collected: 31-Oct-06
Date Extracted: 02-Nov-06
Date Analyzed: 03-Nov-06
Prep Method: SW3050 Rev B

Prep Batch: IP061102-1
QCBatchID: IP061102-1-1
Run ID: it061103-2a2
Cleanup: NONE
Basis: Dry Weight

Sample Aliquot: 1.03 g
Final Volume: 100 ml
Result Units: mg/kg
Clean DF: 1
File Name: 061103a.

CASNO	Target Analyte	Dilution Factor	Result	Reporting Limit	Result Qualifier	EPA Qualifier
7440-50-8	COPPER	1	17	1.4		
7439-92-1	LEAD	10	2100	4.2		

Data Package ID: *it0611012-1*

Total ICP Metals

Method SW6010B

Sample Results

Lab Name: Paragon Analytics
Work Order Number: 0611012
Client Name: ENSR Consulting and Engineering
ClientProject ID: ESTCP 09070-056-503

Field ID: SDT 14-DTA
Lab ID: 0611012-10

Sample Matrix: SOIL
% Moisture: 30.1
Date Collected: 31-Oct-06
Date Extracted: 02-Nov-06
Date Analyzed: 03-Nov-06
Prep Method: SW3050 Rev B

Prep Batch: IP061102-1
QCBatchID: IP061102-1-1
Run ID: it061103-2a2
Cleanup: NONE
Basis: Dry Weight

Sample Aliquot: 1.01 g
Final Volume: 100 ml
Result Units: mg/kg
Clean DF: 1
File Name: 061103a.

CASNO	Target Analyte	Dilution Factor	Result	Reporting Limit	Result Qualifier	EPA Qualifier
7440-50-8	COPPER	1	21	1.4		
7439-92-1	LEAD	10	2800	4.2		

Data Package ID: *it0611012-1*

Total ICP Metals

Method SW6010B

Sample Results

Lab Name: Paragon Analytics
Work Order Number: 0611012
Client Name: ENSR Consulting and Engineering
ClientProject ID: ESTCP 09070-056-503

Field ID: SST 13-DTA
Lab ID: 0611012-11

Sample Matrix: SOIL
% Moisture: 2.2
Date Collected: 01-Nov-06
Date Extracted: 02-Nov-06
Date Analyzed: 03-Nov-06
Prep Method: SW3050 Rev B

Prep Batch: IP061102-1
QCBatchID: IP061102-1-1
Run ID: it061103-2a2
Cleanup: NONE
Basis: Dry Weight

Sample Aliquot: 1.04 g
Final Volume: 100 ml
Result Units: mg/kg
Clean DF: 1
File Name: 061103a.

CASNO	Target Analyte	Dilution Factor	Result	Reporting Limit	Result Qualifier	EPA Qualifier
7440-50-8	COPPER	1	15	0.98		
7439-92-1	LEAD	50	3700	15		

Data Package ID: *it0611012-1*

ICP Metals

Method SW6010B

Method Blank

Lab Name: Paragon Analytics
Work Order Number: 0611012
Client Name: ENSR Consulting and Engineering
ClientProject ID: ESTCP 09070-056-503

Lab ID: IP061102-1MB

Sample Matrix: SOIL
% Moisture: N/A
Date Collected: N/A
Date Extracted: 02-Nov-06
Date Analyzed: 03-Nov-06
Prep Method: SW3050 Rev B

Prep Batch: IP061102-1
QCBatchID: IP061102-1-1
Run ID: it061103-2a2
Cleanup: NONE
Basis: N/A

Sample Aliquot: 1 g
Final Volume: 100 ml
Result Units: mg/kg
Clean DF: 1
File Name: 061103a.

CASNO	Target Analyte	DF	Result	Reporting Limit	Result Qualifier	EPA Qualifier
7440-50-8	COPPER	1	1	1	U	
7439-92-1	LEAD	1	0.3	0.3	U	

Data Package ID: *it0611012-1*

ICP Metals

Method SW6010B

Laboratory Control Sample

Lab Name: Paragon Analytics
Work Order Number: 0611012
Client Name: ENSR Consulting and Engineering
ClientProject ID: ESTCP 09070-056-503

Lab ID: IP061102-1LCS

Sample Matrix: SOIL
% Moisture: N/A
Date Collected: N/A
Date Extracted: 11/02/2006
Date Analyzed: 11/03/2006
Prep Method: SW3050B

Prep Batch: IP061102-1
QCBatchID: IP061102-1-1
Run ID: it061103-2a2
Cleanup: NONE
Basis: N/A

Sample Aliquot: 1 g
Final Volume: 100 ml
Result Units: mg/kg
Clean DF: 1
File Name: 061103a.

CASNO	Target Analyte	Spike Added	LCS Result	Reporting Limit	Result Qualifier	LCS % Rec.	Control Limits
7440-50-8	COPPER	25	24.1	1		97	80 - 120%
7439-92-1	LEAD	50	47.2	0.3		94	80 - 120%

Data Package ID: *it0611012-1*

ICP Metals

Method SW6010B

Matrix Spike And Matrix Spike Duplicate

Lab Name: Paragon Analytics
Work Order Number: 0611012
Client Name: ENSR Consulting and Engineering
ClientProject ID: ESTCP 09070-056-503

Field ID: SST 13-DTA
LabID: 0611012-11MS

Sample Matrix: SOIL
% Moisture: 2.2
Date Collected: 01-Nov-06
Date Extracted: 02-Nov-06
Date Analyzed: 03-Nov-06
Prep Method: SW3050 Rev B

Prep Batch: IP061102-1
QCBatchID: IP061102-1-1
Run ID: it061103-2a2
Cleanup: NONE
Basis: Dry Weight

Sample Aliquot: 1 g
Final Volume: 100 ml
Result Units: mg/kg
File Name: 061103a.

CASNO	Target Analyte	Sample Result	Samp Qual	MS Result	MS Qual	Reporting Limit	Spike Added	MS % Rec.	Control Limits
7440-50-8	COPPER	15		41.9		1.02	25.6	105	80 - 120%
7439-92-1	LEAD	3700		3750		15.3	51.1	19	80 - 120%

Field ID: SST 13-DTA
LabID: 0611012-11MSD

Sample Matrix: SOIL
% Moisture: 2.2
Date Collected: 01-Nov-06
Date Extracted: 02-Nov-06
Date Analyzed: 03-Nov-06
Prep Method: SW3050 Rev B

Prep Batch: IP061102-1
QCBatchID: IP061102-1-1
Run ID: it061103-2a2
Cleanup: NONE
Basis: Dry Weight

Sample Aliquot: 1.01 g
Final Volume: 100 ml
Result Units: mg/kg
File Name: 061103a.

CASNO	Target Analyte	MSD Result	MSD Qual	Spike Added	MSD % Rec.	Reporting Limit	RPD Limit	RPD
7440-50-8	COPPER	41.3		25.3	104	1.01	20	1
7439-92-1	LEAD	3680		50.6	-121	15.2	20	2

Data Package ID: *it0611012-1*

Appendix D

ASTM Standard Guide

Designation: E 2591 – 07

Standard Guide for Conducting Whole Sediment Toxicity Tests with Amphibians[1]

This standard is issued under the fixed designation E 2591; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ϵ) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This standard covers procedures for obtaining laboratory data concerning the toxicity of test material (for example, sediment or hydric soil (that is, a soil that is saturated, flooded, or ponded long enough during the growing season to develop anaerobic (oxygen-lacking) conditions that favor the growth and regeneration of hydrophytic vegetation)) to amphibians. This test procedure uses larvae of the northern leopard frog (*Rana pipiens*). Other anuran species (for example, the green frog (*Rana clamitans*), the wood frog (*Rana sylvatica*), the American toad (*Bufo americanus*)) may be used if sufficient data on handling, feeding, and sensitivity are available. Test material may be sediments or hydric soil collected from the field or spiked with compounds in the laboratory.

1.2 The test procedure describes a 10-d whole sediment toxicity test with an assessment of mortality and selected sublethal endpoints (that is, body width, body length). The toxicity tests are conducted in 300 to 500-mL chambers containing 100 mL of sediment and 175 mL of overlying water. Overlying water is renewed daily and larval amphibians are fed during the toxicity test once they reach Gosner stage 25 (operculum closure over gills). The test procedure is designed to assess freshwater sediments, however, *R. pipiens* can tolerate mildly saline water (not exceeding about 2500 mg Cl^-/L, equivalent to a salinity of about 4.1 when $Na^=$ is the cation) in 10-d tests, although such tests should always include a concurrent freshwater control. Alternative test durations and sublethal endpoints may be considered based on site-specific needs. Statistical evaluations are conducted to determine whether test materials are significantly more toxic than the laboratory control sediment or a field-collected reference sample(s).

1.3 Where appropriate, this standard has been designed to be consistent with previously developed methods for assessing sediment toxicity to invertebrates (for example, *Hyalella azteca* and *Chironomus dilutus* toxicity tests) described in the United States Environmental Protection Agency (USEPA, (**1**))[2] freshwater sediment testing guidance, Test Methods E 1367 and E 1706, and Guides E 1391, E 1525, E 1611, and E 1688. Tests extending to 10 d or beyond, and including sublethal measurements such as growth, are considered more effective in identifying chronic toxicity and thus delineating areas of moderate contamination (**1-3**).

1.4 Many historical amphibian studies, both water and sediment exposure, have used tests of shorter duration (5 days or less) (for example, **4-7**) and, although both survival and sublethal endpoints were often assessed, there is substantive evidence that tests of longer duration are likely to be more sensitive to some contaminants (**8, 9**). Research performed to develop and validate this test protocol included long-term (through metamorphosis) investigations and other researchers have also conducted long-duration tests with anurans (**7-11**). In the development of these procedures, an attempt was made to balance the needs of a practical assessment with the importance of assessing longer-term effects so that the results will demonstrate the needed accuracy and precision. The most recent sediment toxicity testing protocols for invertebrates have encompassed longer duration studies which allow the measurement of reproductive endpoints (**1, 12**). Such tests, because of increased sensitivity of the sublethal endpoints, may also be helpful in evaluating toxicity. Full life-cycle studies with anurans (including reproduction) are usually not feasible from either a technical or monetary standpoint. However, if site-specific information indicates that the contaminants present are likely to affect other endpoints (including teratogenicity), then the duration of the toxicity test may be increased through metamorphosis or additional sublethal endpoints may be measured (for example, impaired behavior, deformities, time-to-metamorphosis). The possible inclusion of these endpoints and extension of test length should be considered during development of the project or study plan (see 8.1.1).

1.5 The methodology presented in this standard was developed under Department of Defense (DoD) a research program and presented in a guidance manual for risk assessment staff

[1] This guide is under the jurisdiction of ASTM Committee E47 on Biological Effects and Environmental Fate and is the direct responsibility of Subcommittee E47.03 on Sediment Assessment and Toxicology.

Current edition approved Nov. 15, 2007. Published December 2007.

[2] The boldface numbers in parentheses refer to the list of references at the end of this standard.

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and state/federal regulators involved in the review and approval of risk assessment work plans and reports (**13**). To develop this method, a number of tests with spiked sediment tests were conducted (**13, 14**). Since development of the methodology it has been used operationally to evaluate field-collected sediments from several state and federal environmental sites (**15, 16**). For most of these studies the preferred test organisms, *Rana pipiens*, was used. At a lead-contaminated state-led site, operated by the Massachusetts Highway Department, *Xenopus laevis* was used in the sediment test system because of availability problems with *Rana pipiens* (**17**), The test method was also used to evaluate sediment toxicity at a cadmium-contaminated USEPA Region 4-led site in Tennessee (**18**). The methodology was used to help characterize potential effects of contaminants on amphibians and to help develop preliminary remedial goals, if warranted. All tests evaluated survival and growth effects after 10 d of exposure in accordance with the methods presented in this standard.

1.6 The use of larval amphibians to assess environmental toxicity is not novel. Researchers have used tadpoles to examine toxicity of metals and organic compounds. Most of these studies have been through water exposure, usually in a manner similar to fish or invertebrate exposure as described in Guide E 729 (**19-29**). Fewer studies have focused on exposure of anuran larvae to sediments, and the methods employed vary widely, from *in situ* enclosures (**30**) to laboratory tests using variable exposure conditions and organism ages (**4, 8, 31-33**). No studies were identified that used the same test conditions as described in this standard. However, several laboratory-based evaluations of sediment effects on amphibians are described in the following subsections.

1.6.1 Sediment toxicity tests conducted in the laboratory with amphibians were performed over a range of test durations from 4 d (**4**, **31**, Guide E 1439-98 Appendix X2) to 12 d (**33**) and through metamorphosis (**8, 32**). Sediment toxicity tests with anurans native to North America were started with larval tadpoles between Gosner stages 23 and 25 (**8, 32, 33**). Test temperatures were between 21 and 23°C and feeding began after tadpoles reached Gosner stage 25. Food sources were Tetramin™ (**8**), boiled romaine lettuce (**32**), or boiled romaine lettuce and dissipated rabbit food pellets (**33**). Tests were conducted in static renewal mode with water replacements conducted at varying rates (daily (**31, 33**), weekly (**8**), every 3 to 5 d (**32**)). Test design (number of replicates, test vessel size, number of organisms per replicate) varied depending on the objective of the study with several tests conducted in aquaria (**32**), large bins (**8**), or swimming pools (**33**). Endpoints evaluated at test termination included survival (**4, 8, 31-33**), growth (**8, 31-33**), bioaccumulation of metals (**8**), developmental rates (**8, 32**), deformities (**31, 32**), swimming speed (**33**) and foraging activity levels (**32**).

1.6.2 To assess the effect of direct contact with the sediments containing PCBs, Savage et al. (**32**) exposed larval tadpoles (Gosner stage 23 to 25; wood frogs (*R. sylvatica*)) to field-collected sediments under conditions that allowed both direct contact with the sediment and separation from the sediment with a 500 µm mesh barrier. The study found that lethal and sublethal effects on tadpoles observed through metamorphosis were more pronounced when direct contact with the sediment was allowed. The test conditions described in this standard allow tadpoles to maintain direct contact with the sediment.

1.6.3 Sediment toxicity testing with the African clawed frog (*Xenopus laevis*) has focused on evaluating the developmental effects of sediment extracts, as opposed to whole sediments, on frog embryos. Methods have been developed which expose blastula stage embryos to sediment by enclosing the embryos in a Teflon mesh insert that rests over the top of the sediment in the sediment–water interface region (**31**, Guide E 1439-98 Appendix X2). These studies are conducted evaluate survival, growth, and physical malformations of the embryos after a 4-d exposure period. The test conditions described in this standard allow more direct contact with the sediment, using older test organisms, and a longer exposure duration.

1.7 Sediment toxicity tests are an effective means for evaluating the impact of sediment contamination on amphibians in a multiple lines of evidence paradigm. The evaluation is most powerful when toxicity testing sampling stations are co-located with sediment analytical chemistry samples and ecological surveys, allowing for a detailed evaluation of the co-occurring data in the ecological risk assessment. The spatial and temporal co-location of toxicity testing and analytical samples is particularly important for establishing contaminant-specific effects and assessing contaminant bioavailability.

1.8 In order for a sediment toxicity test to be sensitive it must be of sufficient duration to measure potential toxicity and it must be conducted during the appropriate developmental stage of the test organism's life cycle. Using recently hatched tadpoles and conducting the sediment exposure test for 10 d to allow the evaluation of growth endpoints meets both of these sensitivity requirements.

1.9 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:* [3]

D 4447 Guide for Disposal of Laboratory Chemicals and Samples

E 177 Practice for Use of the Terms Precision and Bias in ASTM Test Methods

E 691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method

E 729 Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates, and Amphibians

E 943 Terminology Relating to Biological Effects and Environmental Fate

E 1367 Test Method for Measuring the Toxicity of

[3] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

Sediment-Associated Contaminants with Estuarine and Marine Invertebrates

E 1391 Guide for Collection, Storage, Characterization, and Manipulation of Sediments for Toxicological Testing and for Selection of Samplers Used to Collect Benthic Invertebrates

E 1439 Guide for Conducting the Frog Embryo Teratogenesis Assay-Xenopus (FETAX)

E 1525 Guide for Designing Biological Tests with Sediments

E 1611 Guide for Conducting Sediment Toxicity Tests with Polychaetous Annelids

E 1688 Guide for Determination of the Bioaccumulation of Sediment-Associated Contaminants by Benthic Invertebrates

E 1706 Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Freshwater Invertebrates

3. Terminology

3.1 The words "must", "should", "may", "can" and "might" have very specific meanings in this standard. "Must" is used to express an absolute requirement, that is, to state that the design of a test ought to be in a manner that satisfies the specified conditions, unless project goals dictate needed alterations in order to address the study hypotheses. "Should" is used to state that the specified condition is recommended and ought to be met if possible. Although the violation of one "should" is rarely a serious matter, violation of several could render the results questionable. Terms such as "is desirable", "is often desirable" and "might be desirable" are used in association with less important factors, the alteration of which will probably not have substantive effects on test outcome. "May" means "is (are) allowed to," "can" means "is (are) able to" and "might" means "could possibly." In this manner, the classic distinction between "may" and "can" is preserved and "might" is never used as a synonym for either "may" or "can."

3.2 *Definitions*—For definitions of general terms related to toxicity testing and used in this guide, refer to Guide E 943.

3.3 *Definitions of Terms Specific to This Standard:*

3.3.1 *IC25 (25 % inhibition concentration)*, *n*—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.

3.3.2 *overlying water*, *n*—water that is placed over the sediment for the duration of the study. Overlying water may be surface water collected from the project site or from a clean lake or reservoir, or may be reconstituted water prepared in the laboratory (for example, moderately hard water; (**34**)).

3.3.3 *reference-toxicant test*, *n*—a test conducted with a reagent-grade reference chemical to assess the sensitivity of the test organisms. Deviations outside an established normal range may indicate a change in the sensitivity of the test organism population. Reference-toxicity tests are most often performed in the absence of sediment.

3.3.4 *test sediment or test material*, *n*—sediment that may contain contaminants, which is being evaluated using this test procedure.

4. Summary of Guide

4.1 Each test consists of eight replicates of the test material (for example, field-collected sediment or spiked sediment) and overlying water with five test organisms (recently-hatched tadpoles) per replicate. A laboratory control sediment (sometimes called a negative control) is used to provide *(1)* a measure of the acceptability of the test by indicating the quality of tadpoles, test conditions and handling procedures, and *(2)* a basis for interpreting data from other treatments. The test duration is ten days with an assessment of mortality and selected sublethal endpoints (that is, body width, body length) at the end of the test. Assessments of mortality can be made daily during the test and dead organisms removed. However, similar coloration of the tadpoles and sediment may make it difficult to see the organisms and sediment disturbance should be kept to a minimum. Alternative test durations and sublethal endpoints may be considered based on site-specific needs. The objective of the test is to evaluate whether test materials (spiked or field-collected sediments) are significantly more toxic than the laboratory control or reference sediment(s). Additional evaluations may be performed if an exposure gradient is tested. Statistical evaluations may be conducted to determine whether test materials are significantly more toxic than the laboratory control sediment or field-collected reference sample(s). If the test material is sediment spiked with a known concentration of a chemical stressor or if field-collected sediment contains a measured gradient of a particular chemical of concern, then point estimates (for example, median lethal concentrations (LC50s), 25 % inhibition concentrations (IC25s), or 50 % inhibition concentrations (IC50s)) may be calculated. Field-collected sediments often contain more than one potential chemical stressor and therefore calculating chemical-specific point estimates should only be done with caution. A reference-toxicant test should be run concurrently with a sediment test whenever a new batch or lot of organisms is used.

5. Significance and Use

5.1 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. Even where USEPA recommended Water Quality Criteria (WQC, (**35**)) are not exceeded by water-borne concentrations, organisms that live in or near the sediment may still be adversely affected (**36**). Therefore, simply measuring the concentration of a chemical in the sediment or in the water is often insufficient to evaluate its actual environmental toxicity. Concentrations of contaminants in sediment may be much higher than concentrations in overlying water; this is especially true of hydrophobic organic compounds as well as inorganic ions that have a strong affinity for organic ligands and negatively-charged surfaces. Higher chemical concentrations in sediment do not, however, always translate to greater toxicity or bioaccumulation (**37**), although research also suggests that amending sediment with organic matter actually increases the bioaccumulation of contaminant particles (**38, 39**). Other factors that can potentially influence

TABLE 1 Advantages and Disadvantages for Use of Sediment Tests (Modified from Test Method E 1706)

Advantages
Measure bioavailable fraction of contaminant(s).
Provide a direct measure of effects on sediment-associated receptors (benthos, larval amphibians), assuming no field adaptation or amelioration of effects.
Limited special equipment is required.
Methods are rapid and inexpensive.
Legal and scientific precedence exist for use; USEPA and ASTM standard methods and guides are available.
Measure unique information relative to chemical analyses or community analyses.
Tests with spiked chemicals provide data on cause-effect relationships.
Sediment-toxicity tests can be applied to all chemicals of concern.
Tests applied to field samples reflect cumulative effects of contaminants and contaminant interactions.
Toxicity tests are amenable to confirmation with natural populations (invertebrate or amphibian surveys).
Disadvantages
Sediment collection, handling, and storage may alter bioavailability.
Spiked sediment may not be representative of field contaminated sediment.
Natural geochemical characteristics of sediment may affect the response of test organisms.
Indigenous animals may be present in field-collected sediments.
Route of exposure may be uncertain and data generated in sediment toxicity tests may be difficult to interpret if factors controlling the bioavailability of contaminants in sediment are unknown.
Tests applied to field samples may not discriminate effects of individual chemicals.
Few comparisons have been made of methods or species.
Only a few chronic methods for measuring sublethal effects have been developed or extensively evaluated.
Laboratory tests have inherent limitations in predicting ecological effects.
Tests do not directly address human health effects.
Motile organisms may be able to avoid prolonged exposure to contaminated media so tests may overestimate actual exposure.
Species used in toxicity testing programs are typically chosen to be representative and protective of the organisms found on-site, but the use of surrogate species cannot precisely predict the health of ecological communities on-site.
Toxicity to organisms in situ may be dependent upon physical characteristics and equilibrium partitioning that are not readily replicated under laboratory conditions.

sediment bioaccumulation and toxicity include pH mineralogical composition, acid-volatile sulfide (AVS) and grain size (**40, 41**). Laboratory toxicity tests provide a direct and effective way to evaluate the effects of sediment contamination on environmental receptors while providing empirical consideration of all of the physical, chemical and biological parameters that may influence toxicity.

5.2 Amphibians are often a major ecosystem component of wetlands around the world, however limited data are available regarding the effects of sediment-bound contaminants to amphibians (**30-32, 41-43**). Laboratory studies such as the procedure described in this standard are one means of directly assessing sediment toxicity to amphibians in order to evaluate potential ecological risks in wetlands.

5.3 Results from sediment testing with this procedure may be useful in developing sediment screening values for amphibians.

5.4 Sediment toxicity test can be used to demonstrate the reaction of test organisms to the specific combination of physical and chemical characteristics in an environmental medium. The bioavailability of chemicals is dependent on a number of factors, which are both site-specific and medium-specific. Although many of these factors can be estimated using equilibrium partitioning techniques, it is difficult to account for all the physical and chemical properties which could potentially affect bioavailability. Sediment toxicity tests may be particularly applicable to evaluating hydrophobic compounds which may not readily partition into the water column. See Table 1 for a summary of advantages and disadvantages associated with sediment toxicity tests.

6. Interferences

6.1 *General Interferences*:

6.1.1 An interference is a characteristic of a sediment or a test system that can potentially affect test organism response aside from those related to sediment-associated contaminants. These interferences can potentially confound interpretation of test results in two ways: *(1)* toxicity is observed in the test sediment when contamination is low or there is more toxicity than expected, and *(2)* no toxicity is observed when contaminants are present at elevated concentrations or there is less toxicity than expected.

6.1.2 These general interferences may include: potential changes in contaminant bioavailability due to manipulation of field-collected sediments during collection, shipping, and storage; the influence of natural physico-chemical characteristics such as sediment texture, grain size, and organic carbon on the response of test organisms; tests conducted with field-collected samples usually cannot discriminate between effects of multiple contaminants. See Guide E 1706 Section 6 for a detailed discussion of several general interferences that pertain to sediment toxicity testing.

6.1.3 Some interferences, such as the presence of indigenous organisms in field-collected sediments, may have less of an impact on toxicity tests conducted with larval amphibians than on tests conducted with sediment invertebrates.

6.2 *Species-Specific Interferences*:

6.2.1 Particular characteristics of individual species that were tested during the development of this method will probably not act as substantial interferences to completion of successful tests. Those species include *Rana pipiens*, *Bufo americanus*, *Rana clamitans*, *Rana palustris*, *Rana sylvatica*, *Hyla chrysoscelis* and *Xenopus laevis*. However, because the sensitivity of these species to all potential sediment-associated contaminants is unknown, use of test organisms for which more toxicity data are available is recommended.

7. Facilities, Equipment, and Supplies

7.1 *Facilities*—While larval amphibians can be acclimated and held for short periods of time in static or static-renewal systems, continuous-renewal/flow-through conditions are preferable shortly after hatching. Tadpoles grow rapidly and, once feeding begins at about Gosner Stage 25 (**44**), ammonia concentrations are likely to increase and oxygen levels may be depressed, making flow-through conditions desirable. Culture/holding tanks and test chambers should be held at a constant temperature, either in an environmental chamber or temperature-controlled water bath. Addition of overlying water in a flow-through system should be gravity-fed from a water source that may be replaced via pumps. Overlying water should be near culture/test temperature although small temperature deviations should have little impact upon test water temperature at the slow rate of water replacement. Low dissolved oxygen concentrations may be remedied by increasing water replacement rates in small increments. If aeration is necessary, air should be free of contaminants including oil, dust and water; a filtration system may be desirable to remove bacterial contaminants. Lighting should be maintained at a 16-h light and 8-h dark cycle unless the test-specific protocol calls for an alternative photoperiod.

7.2 *Special Requirements*—Amphibian eggs and tadpoles can be highly sensitive to alterations in temperature, oxygen deprivation and handling. If eggs are received from an out-of-laboratory source, attention should be paid to how embryos are packed for shipment, shipment time and handling at the laboratory. Shipping containers should be durable, insulated and water tight. Embryos may be contained in large plastic bags sealed with rubber bands. Double bagging is recommended for added security. Oxygenation of the water containing the embryos is recommended before sealing the bags for shipment. Coolers containing embryos should be firmly taped shut before shipment. The use of ice packs or additional insulation in the shipping containers may be needed when outdoor temperatures are elevated or reduced. It is recommended that temperatures be monitored during shipment, if possible, or upon receipt at the laboratory. Upon receipt at the laboratory, eggs should be allowed to hatch with minimal disturbance.

7.3 *Equipment and Supplies*—All equipment used to prepare test sediments or reagents, transfer sediments or organisms and conduct tests, should be decontaminated as outlined below. Table 2 provides a list of the general equipment needed to conduct testing. Glass is the preferable material in which to conduct tests, however, alternative materials such as stainless steel, high-density polyethylene (HDPE), polycarbonate and fluorocarbon plastics may be appropriate, depending upon the contaminants of concern that might be present in the sediment. Used equipment should not be used if there is a possibility of residual contamination that cannot be removed via the washing process. In some cases, test substances present in field-collected sediments or introduced into spiked sediments may not be thoroughly washed from the test vessels. In these cases the test vessels should not be re-used. All new and used equipment needs to be washed in detergent and should be rinsed with dilute acid and deionized water. Rinsing with an organic solvent (for example, acetone) should also be considered for those materials that will not be damaged by the solvent (for example, some plastics) (see Test Method E 1706 section 9.3.6 for a step-by-step cleaning procedure). Materials that should not contact overlying water include copper, cast iron, brass, lead, galvanized metal (that may contain zinc) and natural rubber.

TABLE 2 General Equipment Required for Conducting a 10-d Sediment Toxicity Test with *Rana pipiens*

Stainless steel bowls and spoons or auger to homogenize sediment
Testing chambers (usually 300 to 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
Dissecting microscopes
Dissolved oxygen meter and probe
Conductivity meter and probe
pH meter/selection ion meter and probe
Ammonia meter and probe
Reagents and equipment for hardness and alkalinity determinations
Temperature-controlled water bath or environmental chamber capable of controlling to 23 ± 1ºC
Flow-through water delivery system
Buffered 3-aminobenzoic acid ethyl ester, methanesulfonate salt (MS-222 anesthetic) solution.
Food source (TetraMin™)
Appropriate data forms
Metric ruler
Forceps
Statistical software

8. Test Material Collection and Processing

8.1 *Collection*:

8.1.1 Before field collection and preparation of sediments, a sampling/processing procedure should be established that outlines the site- or project-specific steps to be followed. The statistical analyses that will be applied to the data should be considered during the development of the sampling/processing procedure. See Guide E 1391 for additional detail regarding methods for collecting, storing, and characterizing sediment samples.

8.1.2 Sediment should be collected with as little disturbance as possible. It may be desirable to collect sediments from a boat (even if wading is possible) to minimize sediment disruption.

8.1.3 Since the distribution of contaminants in sediment matrices can demonstrate a great deal of spatial variability (**45**), it is desirable to collect multiple replicates from within the delineated study area. At a minimum, multiple samples should be collected and thoroughly composited in the field so the sample better represents environmental conditions.

8.1.4 Large pieces of plant material and other debris, such as large rocks and glass, should be removed and discarded in the field. Alternatively, these materials can be removed in the laboratory prior to test setup.

8.1.5 In general, unless project specific conditions dictate otherwise, sediment should be collected from the top 15 cm of the native horizon, which generally represents the maximum bioactive zone and area of most probable exposure.

8.1.6 The exact collection procedures will depend upon study design. In deeper water where a boat is used, a benthic

grab, dredge or corer should be used (Guide E 1391). At locations where the water is very shallow, including saturated hydric soils, these devices can also be used or a clean trowel or shovel can be used. Whatever collection method is selected, all cleaning and decontamination protocols need to be followed to minimize sample contamination.

8.1.7 The testing procedure described in this standard requires a minimum of about one liter of sediment. Since this amount does not allow for accidental loss, spillage, analytical chemistry, or test reruns, collection of a minimum of two liters is recommended.

8.1.8 The most convenient sample containers are wide-mouth, high-density polyethylene (HDPE) bottles with a screw-on cap. Glass jars may be desirable for some studies where adsorption to plastic surfaces is of concern. However, glass containers require greater care in handling and packing for shipment and are generally more expensive than plastic jars.

8.2 *Storage*:

8.2.1 Light and heat can stimulate and accelerate chemical and biological reactions that may alter chemical composition, promote degradation of potential toxicants, and affect bioavailability. Samples, therefore, should be kept out of sunlight and stored in the dark under refrigeration. Samples should be cooled before shipping, unless the ambient temperature is already <10ºC. Target cooling temperature for sediments is about 4°C (Test Method E 1367). Ice or blue ice should be included with the samples when they are shipped. Samples should not be frozen as freezing can alter sediment characteristics.

8.2.2 For additional information on sediment collection and shipment see Guide E 1391.

8.2.3 It is desirable to initiate tests as soon as possible following field collection of sediments (Test Method E 1706). Several studies have addressed the question of storage time for sediments, and the conclusions reached in these studies vary considerably. Where the potential chemical stressors are known to recalcitrant, storage under the conditions described in 7.9 should allow the sample to remain stable for longer periods. However, some labile chemicals (for example, ammonia and volatile organics) can degrade or volatize during storage. For these labile materials, a maximum holding time of two weeks (from the time of sample collection to test initiation) is recommended **(46)**. However, more stable sediments can be stored for much longer periods of time with little change in toxicity.

8.2.4 During even short periods of storage, density differences will results in settling in samples, resulting in a heterogeneous mixture. Therefore, prior to test initiation, the sediment should be homogenized again, even if it was already mixed in the field. In most situations, overlying water should not be drained off the sample, but should be remixed with solid material. If, after 24 hours of undisturbed settling, >75 % of the sample volume can still be considered standing water, it may be desirable to remove some or all of that water so as to ensure that the test material will be a solid matrix.

8.3 *Manipulation*:

8.3.1 *Homogenization*:

8.3.1.1 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. Additional large debris should be removed at this time. Sieving of samples is not recommended, however, indigenous organisms can be removed by hand during the mixing process. Special attention should be paid to any predaceous organisms that might be present in the collected sample. Augers, spoons, and any other equipment that comes in contact with the sediment during homogenization must be washed and decontaminated between samples.

8.3.2 *Sediment Spiking*:

8.3.2.1 Test sediment can be prepared by manipulating the properties of a control sediment (Test Method E 1706). Mixing time **(45)** and aging **(47)** of spike sediment can affect bioavailability of chemicals. If tests are initiated within only a few days of spiking a sediment, the spiked chemicals may not be at equilibrium with the sediment. There are not, however, specified equilibrium intervals for all chemicals that might be spiked into sediment. Such specifications would not be reasonable since sediment characteristics will play a major role in time to equilibration as well as equilibration concentrations. For a series of spiked sediment studies, where results will be compared, spiking methods should be consistent and the amount of time between spiking and test initiation should also be consistent.

8.3.2.2 The test material(s) should be at least reagent grade, unless a test using a formulated commercial product, technical-grade or use-grade material is specifically needed. Before a test is initiated, the following should be known about the test material (not all of this information may be available): *(1)* the identity and concentration of major ingredients and impurities, *(2)* solubility in test water and water used to prepare any stock solutions, *(3)* log K_{ow}, BCF, persistence, hydrolysis and photolysis rates, *(4)* estimated toxicity to the test organism, *(5)* toxicity to humans and potential handling hazards, *(6)* if and when analytical samples will be collected, how much material will be needed to obtain the needed resolution and preservation methods, and *(7)* recommended handling and disposal methods.

8.3.2.3 Different sediment spiking methods are available. Sediment spiking techniques used during development and validation of the amphibian sediment test method **(13)** were previously employed for incorporation of both inorganic contaminants and organic chemicals into sediment **(42)**. The procedure included: *(1)* place appropriate (considering testing and analytical needs) amount of sediment in a mixing jar, *(2)* if sediment is dry, wet it with deionized water to ensure holes in the sediment will remain open, *(3)* using a 10-mL or 5-mL pipet, punch at least five holes into the sediment to different depths, *(4)* distribute equally to each hole the volume of the stock solution needed to achieve the desired target concentration of test material. The stock solution may be an inorganic salt dissolved in water (for example, copper as $CuCl_2$). If a hydrophobic chemical is to be tested, it may first be dissolved

into a stock solution using a carrier solvent (for example, acetone or methanol). A surfactant should not be used in the preparation of a stock solution because it might affect the bioavailability, form or toxicity of the test material. If a carrier solvent is used, a solvent control must also be prepared which contains the solvent but not the contaminant to be tested. See USEPA (**48**), Guide E 1391, and Test Method E 1706 for additional details regarding sediment spiking techniques.

8.3.2.4 Once spiked, the sediments need to be thoroughly mixed to incorporate the chemical into the sediment and create a homogenized matrix. Homogenization methods include roller mixers, end-over-end mixers stainless steel kitchen mixers, mixing manually with a spoon or a combination of these. Mixing times, speeds and temperatures should be consistent among treatments, replicates and tests.

8.3.3 *Test Concentration(s) for Laboratory-Spiked Sediments*:

8.3.3.1 If a test is intended to generate an LC50, IC50 or IC25 of a test chemical, a concentration series should be created that will bracket that effect concentration. If mortality is one of the desired endpoints, at least one test concentration should produce greater than 50 % mortality and there should be two or more concentrations with partial mortality. Determining the concentration(s) that will result in desired lethal or sublethal effects can be difficult if *(1)* the environmental toxicity of the test material is unknown and/or *(2)* the impact(s) of sediment characteristics is/are unknown. The latter can be particularly important since there are many factors that can significantly affect toxicity (**37-41**). It may be desirable to conduct a range-finding test in which the organisms are exposed to a control and three or more concentrations of the test material that differ by a factor of ten. For example, test concentrations in a range-finding test may include the control, 10, 100 and 1000 mg/kg.

8.4 *Sediment Characterization*:

8.4.1 It is recommended that a subsample of each field-collected or spiked sediment be analyzed for at least the following parameters: pH, total organic carbon (TOC), particle size distribution (percent sand, silt, clay). Similar analyses should also be conducted on laboratory control sediment and reference sediment(s).

8.4.2 Further characterization may be warranted depending on the objectives of the study. This may include chemical analyses of inorganic and organic compounds of interest, ammonia, pore water chemistry, chemical oxygen demand, sediment oxygen demand, oxidation-reduction potential (Eh), acid volatile sulfides (AVS), and simultaneously extracted metals (SEM), or other analyses depending on the program.

8.4.3 Chemical and physical data should be obtained using appropriate standard methods whenever possible. For those measurements for which standard methods do not exist or are not sensitive enough, methods should be obtained from other reliable sources.

8.4.4 Sediment characterization helps to evaluate sediment homogenization and accuracy of sediment-spiking, and identifies potential chemical or physical stressors for test organisms.

9. Test Organisms

9.1 *Species*—Test organisms are recently hatched tadpoles of small North American anurans. The preferred species is the Northern Leopard Frog, *R. pipiens*. Sediment toxicity testing conducted with both *R. pipiens* and the American toad, *B. americanus*, during the development of this standard indicated that *R. pipiens* was generally more sensitive to spiked sediments containing metals (cadmium, copper, lead, or zinc) than was *B. americanus* (**13**). A review of amphibian data presented in U.S. EPA ambient water quality criteria documents for cadmium, copper, and zinc (**13**) and relative sensitivity data evaluating amphibian aquatic LC50s (**49**) indicate that *R. pipiens* is considered to be sensitive to metals, relative to other frog, toad, and salamander species. Other ranid species (*R. catesbeiana*, *R. palustris*) were also sensitive to the metals reviewed (**13, 49**). The potential for field-collection of *R. pipiens* eggs with minimal impact to local communities was also a consideration in the selection of this species as the preferred test species. Other species may be used for testing if handling and holding conditions are known.

9.2 *Sources*—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 *R. pipiens* can be collected in the wild during the spring. Since it may be difficult to distinguish between the eggs of related anuran species, collectors should be well-trained in species' habitats and identification. Collectors should comply with all state and federal regulations and be in possession of current collecting permits, if required. If possible, adult animals should also be collected for identification in the same area that eggs are being collected.

9.2.1 Eggs of *R. pipiens* can be obtained from commercial suppliers or be field collected from about November until April. Eggs that are produced and fertilized in the laboratory are preferable since the taxonomy is known. Researchers are encouraged to use available resources to find suppliers.

9.3 *Care and Handling*—Eggs received from commercial suppliers or collected in the wild should be subjected to a minimum of handling. Suppliers generally package and ship eggs in sealed bags or other containers that have been injected with oxygen (dissolved oxygen levels should be maintained above 4 mg/L to avoid stressing the test organisms). Hatching success is higher if handling of eggs is minimized; if possible eggs should left in the original shipping package until development is verified and organisms are near hatching stage. Upon receipt, bags containing eggs should be allowed to slowly rise (no more than 3°C per hour) to test temperature (avoid rapid temperature changes). If eggs arrive in containers that have not been injected with oxygen or otherwise cannot be left intact, organisms should be transferred to an aquarium or other holding container and slowly brought to test temperature.

9.3.1 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/early stage tadpoles placed in an aquarium or other large chamber.

9.3.2 Once the eggs/tadpoles are released for the shipping container to an aquarium or other chamber, shipping water

TABLE 3 Developmental Stages of Anuran Embryos (from Gosner (44) and Shumway (51))

Stage	Approximate Age at 18ºC (h) for Stages 1 through 25	Major Characteristics/Formations of the Stage
1	0	Prior to fertilization
2	1	Appearance of post-fertilization gray crescent
3	3.5	Two blastomeres
4	4.5	Four blastomeres
5	5.7	Eight blastomeres
6	6.5	Sixteen blastomeres
7	7.5	Thirty-two blastomeres
8	16	Mid-cleavage
9	21	Late cleavage
10	26	Appearance of dorsal lip of blastopore
11	34	Mid-gastrula, blastoporal lip invaginating along semicircle
12	42	Late gastrula, blastoporal lip invaginating around the circular yolk plug. Yolk plug diameter ~ ⅕ diameter of gastrula
13	50	Neural plate, blastopore forming slit
14	62	Neural folds
15	67	Rotation of embryo
16	72	Neural tube
17	84	Tail Bud
18	96	"Tadpole" shape becoming distinct; muscular response to stimulation
19	118	Heart beat; external gill buds; hatching begins
20	140	Complete hatching; swimming upon physical stimulation; capillary circulation in first gill
21	162	Mouth open; transparent cornea; tail length approximately equal to length of head and body
22	192	Transparent epidermis; capillary circulation in tail; asymmetrical appearance from dorsal aspect; left gills filaments more apparent
23	216	Opercular fold apparent; asymmetrical from ventral aspect
24	240	Operculum covering right external gills; external gills on left side still apparent; sucker represented by two small prominences
25	284	Operculum complete; no external gill filaments; Sucker represented by two pigmented patches; begin feeding; gut clearly visible
26–30		Hind limb buds appear and grow progressively larger; spiracle present on left side (most North American tadpoles)
31		Toes begin to develop on hind limbs
32–37		Toes on hind limbs grow progressively distinct; all five toes apparent at stage 37
38–40		Toes continue to lengthen; metatarsal and subarticular tubercles develop
41		Tail begins to shorten; cloacal tail piece disappears; skin over forelimbs becomes transparent; lateral forelimb "bulges" appear
42–45		Forelimbs break through membrane; Face shortens; mouth lengthens; posterior edge of mouth extends beyond posterior edge of eye; tail absorption continues
46		Metamorphosis complete; tail stub usually present; froglets must have physical platform to leave the water

should be slowly replaced with culture/overlying water. This should be done by initially adding culture/overlying water at a proportion of no more than 10 % for one hour. If organisms do not appear to be adversely affected, increase the amount of culture/overlying water by about 15 to 25 %/ hour for 4 to 5 hours.

9.3.3 Additional acclimation of test organisms should not be needed under most circumstances.

9.3.4 Low dissolved oxygen will increase organism stress and may cause mortality in the holding chamber or result in increased mortality during a test. Dissolved oxygen should not be allowed to fall below 3.0 mg/L. If needed, gentle aeration should be initiated using a small pipette and low bubble rate.

9.3.5 Always wear laboratory gloves (for example, latex; talc-free) when handling eggs. Direct contact with eggs or tadpoles should be avoided to minimize stress on the organisms. Transfer eggs and tadpoles gently and with minimal handling time.

9.4 Once embryos have reached a distinctive tadpole shape (about Gosner stage 19-20) they are far less prone to mortality from handling.

9.5 A sub-sample of specimens should be collected and preserved for species verification.

10. Hazards

10.1 Some test materials, as well as some materials used to preserve test organisms, may be inherently hazardous. Caution needs to be used when handling these materials. Guidelines for the handling and disposal of hazardous materials should be strictly followed (Guide D 4447). When working with any potentially hazardous materials, including those used for analytical measurements (for example, acid used during alkalinity titrations), users need to wear appropriate protective equipment (for example, safety glasses and gloves). Common laboratory protective wear should also be used to reduce exposure to potential biological hazards (for example *Salmonella*, *Vibrio* ssp.). All laboratory-specific health and safety considerations should be followed. (see Test Method E 1706 for additional detail).

11. Procedure

11.1 *Experimental Design*—Each test consists of eight replicates of the test material (e.g., field-collected sediment or spiked sediment) and overlying water with five test organisms (recently-hatched tadpoles) per replicate. It may be necessary to make modifications of the basic experimental design to

accommodate project-specific circumstances, including shortage of available test sediment (for example,, scarce depositional areas in riverine systems), bioaccumulation (need for extra tissue) or additional analytical measurements. A laboratory control sediment (negative control) must be included with all tests and reference sediment(s) may be included when field-collected sediments are tested.

11.1.1 A laboratory control sediment is a sediment that is essentially free of contaminants and is used to ensure that contamination is not introduced during the experimental set up and that test organisms are healthy. This sediment is not necessarily collected near the site of concern. A reference sediment is collected near an area of concern and is used to assess sediment conditions exclusive of material(s) of interest. Testing a reference sediment provides a site-specific basis for evaluating toxicity.

11.2 *Initiating a Test*:

11.2.1 *Adding Sediment to Test Chambers*—The day before the test is to start (Day -1) sediment should be thoroughly homogenized and 100 mL of sediment is added to each test chamber. Overlying water (175 mL) is added to each test chamber in a manner that minimizes disturbance of the sediment. This can most easily be accomplished by pouring against the inside of the chamber. The sediment should be left undisturbed overnight.

11.2.1.1 On the day of test setup (Day 0), withdraw an adequate amount of overlying water from each treatment to conduct all necessary chemical characterizations and analyses. Removal of water should be done with as little sediment disturbance as possible. At a minimum, dissolved oxygen, temperature, pH, conductivity, hardness, alkalinity and ammonia should be measured in each treatment. If samples are collected for other parameters, such as metals, then proper handling and preservatives should be used (see Guide E 1391 for additional detail).

11.2.1.2 Overlying water should be renewed during a test, unless nonrenewal is a fundamental part of the test design. Renewal may be done continuously through a water-delivery system, including diluters or drip-manifolds, or by static replacement. In either case, the volume of water addition in a 24-hour period should not exceed 2 to 3 volumes of overlying water (about 350 to 525 mL). A water-delivery system should be calibrated at test initiation and examined on a daily basis so all test chambers receive about the same amount of water. If manual water addition is conducted, no more than 80 % of the overlying water should be removed at any one time and sediment disturbance should be minimized. The toxicity test is designed to include both sediment and water column exposure to contaminants so it is important to maintain the indicated renewal rates in order to avoid excessive dilution of water column constituents that could lead to an underestimation of sediment toxicity.

11.2.2 *Addition of Test Organisms*—Test organisms should be handled as little as possible. Organisms should be added to the overlying water using a pipette with a large enough bore to prevent constriction and damage to the animals. The animals should be gently released just below the water's surface. The developmental stage (Gosner stage) of the tadpoles should be documented by examining a subset of at least 10 organisms.

11.2.2.1 Development stage should be determined in accordance with descriptions provided by Gosner (**44**). Table 3 provides a summary of the major characteristics of each stage between fertilization and metamorphosis.

11.3 *Monitoring a Test*—All chambers should be checked daily for dead organisms and behavior. Tadpole coloration often makes it difficult to see them against sediment, however, if dead organisms are found, they should be removed with a pipette. Animals that die during a test need only be kept if sublethal observations are to be made or tissue will be analyzed for chemicals of concern. Organisms need to be preserved appropriately for the analyses (see Guide E 1688 for additional detail). The overlying water renewal system should be checked daily to ensure adequate flow and an acceptable addition rate. Screens on the outside of test chambers should be checked

TABLE 4 Test Conditions for Conducting a 10-d Sediment Toxicity Test with *Rana pipiens*

Parameter	Conditions
1. Test type:	Whole-sediment toxicity test with renewal of overlying water
2. Temperature:	23 ± 1°C
3. Light quality:	Wide-spectrum fluorescent lights
4. Illuminance:	About 100 to 1000 lux
5. Photoperiod:	16L:8D
6. Test chamber:	400 to 500-mL glass or plastic beaker or chamber with drainage system
7. Sediment volume:	100 mL
8. Overlying water volume:	175 mL
9. Renewal of overlying water:	Continuous flow-through of overlying water or daily static water addition (not to exceed 2 to 3 volume additions/day)
10. Age of organisms:	≤72 hours, 24 hours or less preferred at the start of the test
11. Number of organisms/chamber:	5
12. Number of replicate chambers/treatment:	Depends on the objective of the test. Eight replicates are recommended for routine testing (see 11.1)
13. Feeding:	4 mg of ground TetraMin™ per vessel daily after tadpoles reach stage 25; reduced proportionally with mortality
14. Aeration:	None, unless dissolved oxygen in overlying water drops below 3.0 mg/L.
15. Overlying water:	Site water, site water match (hardness and alkalinity), natural lake or groundwater, or reconstituted laboratory water (for example, U.S. EPA moderately hard **(5)**)
16. Test chamber cleaning:	If screens become clogged during a test, gently brush the *outside* of the screen
17. Overlying water quality:	Hardness, alkalinity, conductivity, pH, dissolved oxygen, and ammonia at the beginning and end of a test. Temperature and dissolved oxygen daily. Ammonia may also be measured periodically (Days 1, 3, and 7).
18. Test duration:	10 d
19. Endpoints:	Survival and growth
20. Test acceptability:	Minimum mean control survival of 80 %; mean body width of at least 4 mm and body length of at least 7 mm for test organisms in the control sediment. See Table 6 for additional performance-based criteria.

daily to ensure that water is adequately draining. Clogged screens can be brushed to remove impinged debris; cleaning and brushing should only be done with a small, clean brush, cleaning tool or gloved finger. Test conditions are summarized in Table 4 and a list of daily activities is presented in Table 5.

11.3.1 *Monitoring of Overlying Water Characteristics*—Conductivity, hardness, alkalinity, pH and dissolved oxygen must be measured in all treatments at the beginning and end of the test. Dissolved oxygen should also be measured daily. Temperature should be measured continuously in the environmental chamber or water bath and periodically in each treatment (for example, days 3, 6 and 9). If continuous temperature monitoring is not available then instantaneous temperature in each treatment should be measured daily. In any test chamber where mortality has occurred, dissolved oxygen and pH should be measured on the day when mortality was observed.

11.3.1.1 If dissolved oxygen in any one chamber of a treatment is less than 3.0 mg/L, then dissolved oxygen in other chambers within that treatment should be checked. The flow rate (drip rate if a continuous drip manifold is used) in any one chamber can be increased slightly to increase dissolved oxygen. All test chambers should be treated the same relative to test condition modifications (for example, increase in water delivery rate). If after one hour, dissolved oxygen is still <3.0 mg/L, then all of the test chambers within that treatment should be aerated. Set aeration tubes or pipettes so that the narrow tip is submerged not more than 0.5 cm. Bubble rate should be slow and should not disturb the sediment or overly agitate the water's surface to avoid the release of volatile substances. Occasional dissolved oxygen measurements of <3.0 mg/L during a test is not sufficient reason to discard test data, although evidence of extended oxygen depression should be considered with regard to possible adverse affects.

11.3.1.2 Ammonia should be measured in the overlying water on Day 0, at test termination and periodically during the test, for example, days 1, 3 and 7. If ammonia concentrations are >5.0 mg/L (NH_3-N) in any treatment, than a second sample should be collected and measured from another replicate. Tadpoles are sensitive to elevated ammonia, although *R. pipiens* has been found to be less sensitive than some other anurans (**7**, **48**). Elevated ammonia concentrations may be a reflection of sediment characteristics and should be taken into consideration when interpreting test results. Test specifications are listed in Table 4.

11.3.1.3 *Temperature*—Target test temperature is 23 ± 1ºC. Daily mean temperature (directly in the water bath or a surrogate test chamber in the water bath or environmental chamber) should be within 1ºC of 23ºC; instantaneous temperature should be 23 ± 3ºC. Continuous monitoring of bath or environmental chamber temperature is preferred.

11.3.2 *Feeding*—Feeding should begin when tadpoles reach Gosner stage 25 (**44**), that is, when an operculum develops and external gills disappear. About 3 to 4 mg of ground, dry TetraMin™ is added daily to each test chamber. Adding excess food should be avoided since it can cause dissolved oxygen depression and may also affect the toxicity of certain chemicals (**39**). Tadpoles in at least three chambers should be examined daily to determine if stage 25 has been reached (see Table 3 or (**44**)). Some toxicants may delay development; feeding of organisms may start on different days for different treatments. It takes about 3 to 5 days for newly-hatched tadpoles to reach stage 25. If older organisms were used, feeding will begin sooner. The amount of food added to each chamber should be decreased if some animals have died. In general, follow the USEPA (**34**) procedures for conducting short-term chronic tests with fathead minnows, *Pimephales promelas*. That is, if 50 % or more of the test organisms have died in a test chamber, reduce the amount of food by 50 %.

11.4 *Ending a Test*—Final water characterization measurements should be made and live organisms should be removed from each chamber with a pipette. All live organisms from a replicate chamber should be placed into a separate, small glass or plastic beaker or cup containing 10 to 20 mL of clean (unchlorinated) water (for example, USEPA Moderately Hard Water (see (**5**) or Guide E 729)). All chambers should be carefully examined for any missing organisms. Dead tadpoles will decompose rapidly and may easily blend into sediment. Unaccounted-for organisms should be considered mortalities.

11.4.1 *Sublethal Measurements*—Live tadpoles should be anesthetized or euthanized before sublethal measurements are

TABLE 5 General Activity Schedule for Conducting a 10-d Sediment Toxicity Test with *Rana pipiens*

Day	Activity
-1	Add homogenized sediment into each test chamber, place chambers into exposure system, and add overlying water.
0	Begin flow through system or conduct first water replacement if using static renewal. After at least one hour collect overlying water for initial water characterization (hardness, alkalinity, conductivity, pH, dissolved oxygen, and ammonia, and total residual chlorine). Add 5 tadpoles to each test chamber. Release organisms under the surface of the water. Archive and preserve 5 to 10 organisms for possible examination of metamorphic stage.
1 to 9	Measure temperature, dissolved oxygen. Measure ammonia periodically in each treatment during the toxicity test (for example, Days 1, 3, and 7). Observe behavior and metamorphic stage of test organisms. Remove dead organisms. Feed 4 mg of ground, dry TetraMin™ per chamber daily after tadpoles reach Gosner stage 25.
10	Measure temperature, dissolved oxygen, pH, conductivity. Collect samples for final water quality measurements (for example, hardness, alkalinity, ammonia), as indicated in project requirements. Remove and count live organisms from each test chamber and transfer them to small beakers (glass or plastic) containing 10 to 20 mL of clean (unchlorinated) water. Euthanize or anesthetize test organisms prior to making sublethal measurements. Measure the maximum body width and body length (snout-to-vent length).

made. The use of a buffered 3-aminobenzoic acid ethyl ester (MS-222) solution is recommended. To each of the small beakers or cups containing live tadpoles, add about 1 mL of a MS-222 stock solution (2 g/L) buffered to about pH 7 using an appropriate buffer medium (for example, sodium bicarbonate). If organisms continue to move after several minutes, add a few more drops of the MS-222. Tadpoles should not be left for an extended period of time in the MS-222 solution as it may cause disintegration of tissue.

11.4.1.1 Using a metric ruler, measure the maximum body length along the center line of the body, excluding the tail (snout–to-vent length). Also, measure the maximum body width. Do not push down on the tadpole body as that will distort these measurements.

11.4.2 Digital photographs and digitizing software may also be used to quantify sublethal measurements.

11.4.3 Statistical evaluations for lethal and sublethal endpoints may be conducted using comparisons to results from the laboratory control or a field-collected reference sample(s). If the test was one in which sediment was spiked with a hydrophobic test material dissolved in a solvent carrier and a solvent control was included in addition to a laboratory control sediment, then survival and growth should be compared between the two controls. If a statistically significant difference is detected between the controls, then only the solvent control may be used for meeting the acceptability of the test and as the basis for calculation of results. The laboratory control may provide additional information on the general health of the test organisms. If no statistically significant difference is detected between the controls, the data from both controls may be pooled and used as a basis for meeting acceptability criteria and as a basis for calculation of results. If the solvent control is markedly different from the laboratory control, it is possible that the data are compromised by experimental artifacts and may not accurately reflect the toxicity of the test material in natural sediments. In such circumstances, the test may need to be repeated or alternative means of test material introduction explored. A discussion of possible statistical evaluations is presented in Appendix X2 but may be modified based on project-specific requirements.

11.5 *Studies Conducted Beyond Ten Days*—If site-specific information indicates that longer duration toxicity tests should be conducted, the daily activities described previously should be followed until test termination.

11.5.1 Activities conducted at test termination will be similar to those conducted for the 10-d toxicity test but may also include inspection for deformities, observations of impaired behavior (prior to anesthetizing), or developmental stage. Feeding should be increased in proportion to the increase in body size of the test organisms. If growth is not affected, the amount of food can be increased by about 2 mg per chamber every five days; not to exceed 12 mg per chamber. If the growth of organisms is diminished, feeding levels should remain unchanged or be increased at a slower rate. Excess food on the surface, sediment or sides of the test chambers indicates that too much is being added and the amount of food should be reduced. At metamorphosis, most larval anurans stop eating as their internal and external physiology undergoes substantial alterations in the shift from a fully aquatic tadpole to an amphibious adult (**43**). As the organisms within a replicate approach late-stage metamorphosis, the amount of food consumed will drop substantially and feeding amounts should proportionally decrease to initial levels or less. At some point, if no feeding behavior is observed and unconsumed food is present, feeding may be stopped within a particular replicate.

11.5.2 If the toxicity test is to be conducted through metamorphosis, some modifications would need to be made in the test system. At complete metamorphosis (about Gosner stages 45 and 46) froglets crawl out of the water. Failure to provide a means of leaving the water will result in tadpole death. Providing an "emergence platform" may be difficult if the original test chambers were beakers or similar vessels. Sediment, water and organisms can be transferred to a vessel with a larger surface area that provides better access for the researcher (for example, a 12 by 25 cm plastic chamber). The emergence platform can be constructed in several ways, but the froglet will need to be able to crawl from the water to air. Possible emergence structures include inclined glass or plexiglass, bricks or stones, sponges and arched pieces of heavy, nylon netting. Any material used as an emergence structure needs to be decontaminated as outlined in 7.3 and should not block water circulation or prevent tadpoles from moving freely about the test chamber.

11.6 *Reference Toxicant Testing*—Reference toxicant tests involve exposing organisms that are used to start a sediment study to known concentrations of a specific reagent-grade reference chemical in water-only exposures in order to assess their sensitivity to a toxicant challenge. Organisms of a given species should demonstrate a consistent response to a reference toxicant. Deviations outside an established normal range may indicate a change in the sensitivity of the test organism. A reference toxicant test must be conducted with each new lot or batch of test organisms that are used to initiate a test. Test conditions for conducting reference-toxicity tests with *R. pipiens* are outlined in Table 6. The procedures can also be used for conducting reference-toxicity tests with the test organisms outlined in Appendix X1.

11.6.1 There are several chemicals that are used as reference toxicants. Copper chloride ($CuCl_2$) has been found to produce consistent responses from the test organisms when organism age and test water are held constant. Other possible reference toxicants include salts such as NaCl and KCl. A reference-toxicant concentration series should be selected that will provide partial mortalities at two or more concentrations of the test chemical in order to allow calculation of appropriate point estimates (LC50, EC50).

11.6.2 A reference toxicant control chart should be prepared for each toxicant (if difference ones are used) that progressively illustrates reference toxicant test results. Results should be illustrated as the calculated value for a test, bracketed by the upper and lower control limits. The control chart should include the 20 most recent reference toxicant data points (**34**).

11.6.3 If the reference toxicity results from a given study fall outside the "expected" range (more than 2 standard deviations), the sensitivity of the organisms and the acceptability of the study may be in question. However, at a 0.05

TABLE 6 Recommended Test Conditions for Conducting Reference-Toxicity Tests

Parameter	Conditions
1. Test type:	Water-only test
2. Dilution series:	Control and at least 5 test concentrations (0.5 dilution factor)
3. Toxicant:	KCl, NaCl, or $CuCl_2$
4. Temperature:	23 ± 1°C
5. Light quality:	Wide-spectrum fluorescent lights
6. Illuminance:	About 100 to 1000 lux
7. Photoperiod:	16L:8D
8. Renewal of water:	At least every 48 hours
9. Age of organisms:	≤72 hours, ≤24 hours preferred
10. Test chamber:	250-500 mL glass or plastic beaker
11. Volume of water:	100 mL (minimum)
12. Number of organisms/chamber:	5
13. Number of replicate chambers/treatment:	3 minimum
14. Feeding:	4 mg/day to each test chamber when organisms reach Gosner stage 25
15. Substrate:	None
16. Aeration:	None, unless DO ≤ 3 mg/L
17. Dilution water:	Culture water, well water, surface water, site water, or reconstituted laboratory water (for example, USEPA moderately hard **(5)**)
18. Test chamber cleaning:	None
19. Water quality:	Hardness, alkalinity, conductivity, dissolved oxygen, and pH at the beginning and end of a test. Temperature daily.
20. Test duration:	7 d
21. Endpoint:	Survival (LC50) and growth (IC25)
22. Test acceptability:	80 % control survival

TABLE 7 Test Acceptability Requirements for a 10-d Sediment Toxicity Test with *Rana pipiens*

A. It is recommended for conducting a 10-d test with *Rana pipiens* that the following performance criteria be met:
1. Age of *R. pipiens* at the start of the test must be ≤72 hours.
2. Average survival of *R. pipiens* in the control sediment must be greater than or equal to 80 % at the end of the test. Growth of test organisms should be measurable in the control sediment at the end of the 10-d test (mean body width of at least 4 mm and body length of at least 7 mm for test organisms in the control sediment).
3. Hardness, alkalinity, and ammonia of overlying water typically should not vary by more than 50 % during the test, and dissolved oxygen should be maintained above 3.0 mg/L in the overlying water.

B. Performance-based criteria for maintaining *R. pipiens* include the following:
1. It may be desirable for laboratories to periodically perform water-only reference toxicity tests to assess the sensitivity of culture organisms (see 11.6). Data from these reference toxicity tests could be used to assess genetic strain or life-stage sensitivity of test organisms to select chemicals.
2. Laboratories should characterize and monitor background contamination and nutrient quality of food if problems are observed in culturing or testing organisms.
3. Physiological measurements such as lipid content might provide useful information regarding the health of the cultures.

C. Additional requirements:
1. All organisms in a test must be from the same source.
2. Storage of sediments collected from the field should follow guidance outlined in 8.2.
3. All test chambers should be identical and should contain the same amount of sediment and overlying water.
4. Negative-control sediment and appropriate solvent controls must be included in a test. The concentration of solvent used must not adversely affect test organisms.
5. Test organisms must be cultured and tested at 23°C (±1°C).
6. The daily mean test temperature must be within ±1°C of 23°C. The instantaneous temperature must always be within ±3°C of 23°C.
7. Natural physico-chemical characteristics of test sediment collected from the field should be within the tolerance limits of the test organisms.

probability level, it is expected that, by chance alone, one in 20 test results would fall outside the control limits. If more than one in 20 reference toxicant tests fall outside of the control limits, the laboratory should investigate possible sources of variability and take corrective action, if appropriate. If serious problems are not found, then associated test results may be considered acceptable.

12. Acceptability of Test

12.1 Acceptable survival in the test control is 80 % or greater. Control organisms (*R. pipiens*) should also have a mean body width of at least 4 mm and a body length (snout-to-vent) of 7 mm. If alternative test species are used , researchers may need to complete appropriate data gathering tests to determine acceptable size criteria prior to conducting the toxicity tests. If control performance does not meet these criteria, then the test data should be examined to determine if it is acceptable. Test acceptability criteria are presented in Table 7. Even if control performance does not meet these criteria, test data may still be valuable and yield important results. The following test data should be examined:

12.1.1 Survival in all test treatments. If survival in all test treatments is greater than in the control, then statistical evaluations of test sediments against the laboratory control do not need to be conducted. Statistical comparisons against the reference sediments may still be conducted.

12.1.1.1 If poor performance is observed in the laboratory control, such studies should be repeated to ensure accurate results. However, the scope or sampling associated with some studies may make it difficult or impossible to repeat a study. There may be cases where performance in the negative control is poor, but performance criteria are met in reference sediment included in the study design. In these cases, it might be reasonable to infer that other samples that demonstrate organism performance equivalent to, or better than, the reference

sediment are probably not toxic; however, any samples showing poor performance should not be judged to be toxic, since it is unknown whether the factors that caused poor control performance might have also caused poor performance in the test treatments.

12.1.2 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 (for example, low dissolved oxygen or elevated ammonia due to excess food in some chambers), variability in organism health, or differences in how chambers were treated (for example, different amounts of food or flow rates of overlying water).

12.2 There are no specific acceptability requirements for survival in test treatments collected from reference stations. If reference sediment was collected and if survival in the reference sediments is significantly reduced, then questions are raised as to the appropriateness of the reference site.

12.3 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.

13. Report

13.1 Report the following information:

13.1.1 Identity of the test material (for example, test sediments and reference sediment, if collected), investigator(s) name, location of laboratory, and dates of test initiation and termination.

13.1.2 Source of test material (if a specific chemical or compound), its lot number, composition (identities and purity), known physical and chemical properties and the identity and source of any solvent used.

13.1.3 Source of the laboratory control sediment and overlying water.

13.1.4 Chemical characteristics of test material, laboratory control sediment, and overlying water, if available.

13.1.5 Source of test organisms, scientific name (and subspecies, if appropriate), life stage, treatments, acclimation procedures and food.

13.1.6 Description of the experimental design, test chambers or compartments, amount of sediment and overlying water, replicates, organisms per replicate, lighting, food type and feeding rate.

13.1.7 Range of measured concentrations of dissolved oxygen, temperature, pH and conductivity of overlying water.

13.1.8 Chemical and biological monitoring information recorded on daily data sheets during the toxicity test.

13.1.9 A table that lists the percent mortality and mean sublethal results (that is, body width, body length) for each test material.

13.1.10 The names of the statistical tests employed, the alpha-levels of the tests, and some measure of the variability of the hypothesis tested.

13.1.11 Anything unusual about the test and any deviations from the test-specific protocol or procedures followed.

14. Precision and Bias

14.1 *Determining Precision and Bias*—Precision is a term that describes the degree to which data generated from replicate measurements differ and reflects the closeness of agreement between randomly selected test results. Bias is the difference between the value of the measured data and the true value and is the closeness of agreement between an observed value and an accepted reference value (Practices E 177 and E 691). Quantitative determination of precision and bias in sediment testing of aquatic organisms is difficult or may be impossible in some cases, as compared to analytical (chemical) determinations. This is due, in part, to the many unknown variables which affect organism response. For a detailed discussion of precision as it relates to sediment toxicity testing, see Section 17 in Test Method E 1706.

14.1.1 *Bias*—The bias of toxicity tests cannot be determined since there is no acceptable reference material. The bias of the reference-toxicity tests can only be evaluated by comparing test responses to control charts be evaluated by comparing test responses to control charts. For a detailed discussion of bias as it relates to sediment toxicity testing, see Section 17 in Test Method E 1706.

14.1.2 The sensitivity of a toxicity test will depend upon the number of replicates per concentration or treatment, the variability within that treatment (among replicates), the probability levels (alpha and beta) and the statistical test used. Tests with anuran larvae have demonstrated that variability may occur within a treatment. This is especially the case for sublethal growth parameters where particularly small or large organisms can occur within a single treatment. Such differences in size may represent natural physiological differences (that is, poor health) or behavioral differences in individuals that affect access and consumption to available food and subsequent lower growth rates. The presence of unusually small or large specimens within a replicate chamber is to be occasionally expected and is not reason to discard individual measurements as outliers, unless all or most individuals in a single replicate exhibit mortality or growth patterns that are substantially different from other replicates within a treatment. Such a situation may indicate poorly homogenized sediment, technician error at test initiation or the presence of a highly-consolidated particle containing a toxic substance that is not representative of the sediment as a whole. In such cases, an outlier test may be appropriate to determine whether the replicate should be excluded from analysis. Exclusion of replicates should be avoided, however and every effort should be made collect enough sediment for a full eight replicates, in order to increase the statistical power of the test and reduce the effects of replicate variability (**50**).

14.1.3 Intralaboratory precision data are routinely calculated for test organisms using water-only exposures to a reference toxicant, such as NaCl or KCl (as described in 11.6). Intralaboratory precision data should be tracked using a control chart. For reference toxicant tests with anurans, both survival and growth parameters should be tracked. Reference toxicant tests should be of a sufficient duration to achieve measurable growth (relative to the size of organisms at test initiation). For anurans, a minimum of seven days is recommended. Each

laboratory's reference-toxicant data will reflect conditions unique to that facility, including dilution water, culturing, and other variables. The conditions for the reference toxicant test, such as water type, test containers, organism age, feeding and concentration series, should remain the same. Altering test variables will introduce variation, wider confidence intervals and will compromise the integrity and usability of the reference toxicant data as a means of tracking intralaboratory precision.

14.1.4 Before conducting tests with potentially contaminated sediment, it is strongly recommended that the laboratory conduct the tests with control sediment(s) alone. Results of these preliminary studies should be used to determine if the use of the control sediment and other test conditions (that is, water quality) result in acceptable performance in the tests. If organism performance in the selected control sediment is inconsistent, an alternative sediment should be selected.

15. Keywords

15.1 amphibian; bioavailability; *Bufo* spp.; hydric soils; *Rana* spp.; *Rana pipiens*; sediment; toxicity; wetland

APPENDIXES

(Nonmandatory Information)

X1. LIST OF ALTERNATIVE SPECIES

X1.1 *Use of Alternative Species*—Although this procedure was developed with *R. pipiens*, it might be necessary to use alternative species when required by regulation or limited by seasonal availability of test organisms. Deviations from the procedures outlined in Table 4 should be recorded and it may be difficult to compare data between toxicity tests conducted with *R. pipiens* and alternative species.

X1.2 *Recommended Anurans*—Other members of the family Ranidae (for example, *R. sphenocephala*, *R. palustris*, or *R. catesbiena*) and Bufonidae (for example, *B. americanus* or *B. fowleri*) might be best suited for conducting a whole-sediment exposure toxicity test due to the commercial availability of eggs. High egg production, relevant geographical range, short hatching periods, and sensitivity to contaminants should be considered in selecting alternative species. *Xenopus laevis* may be considered as an alternative species due to the generally consistent availability of eggs; however, researchers should review existing data on the relative sensitivity to some contaminants (**49**).

X1.2.1 Standard E 1439-98 includes a methodology for exposing *X. laevis* to whole sediments (referred to as solid phase sample testing). This methodology is an alternative to FETAX studies conducted in aqueous solutions. Although *Xenopus* is not native to the United States, the standardized, FETAX testing protocol, the availability of test organisms, and ease of use of *Xenopus* in the laboratory has made it a popular test species for amphibian toxicity testing.

X1.2.1.1 The FETAX solid phase testing may be performed in 250 mL specimen bottles or similar capped vessels equipped with a 55 mL glass tube with Teflon mesh insert as the exposure chamber. For screening tests, 35 g of sediment (dry weight) should be placed in the bottom of the vessel, with the Teflon mesh insert added, and should be filled with 140 mL of FETAX Solution. Blastulae stage embryos are placed directly on the mesh insert that rests directly over the top of the soil or sediment in the sediment/water interface region. Four to six dilutions ranging from 0 to 100 % soil sample and a FETAX Solution control are typically tested. Each sample should be tested in triplicate. Solutions and soils or sediments should be changed every 24 hours of the four-day test. At the end of the four-day exposure period, surviving embryos should be preserved in 3 % (w/v) formalin (pH 7.0) and morphological characteristics evaluated using a dissecting scope. Growth may be determined using a digitizing software package.

X1.2.1.2 While the alternative FETAX methodology exposes young amphibians to sediments there are several differences relative to the test conditions presented in Table 4. Primarily these differences are related to test duration and the age of the test organisms. The FETAX test is a rapid test designed to identify developmental toxicants. It is conducted over a relatively short duration (4 d) with recently fertilized embryos (mid blastula to early gastrula) and evaluates malformations, in addition to mortality and growth. The test conditions presented in Table 4 indicate a longer test duration (10 d) with older test organisms (≤72 hours old). This methodology evaluates survival and growth of tadpoles exposed directly to sediment and overlying water. The FETAX methodology is conducted with an amphibian species that is not native to North America. Although *X. laevis* may be available with less seasonal variability, in some cases it may be preferable to conduct a toxicity test with a species that is native to the test site.

X2. DATA ANALYSIS

X2.1 *General*—Test Method E 1706 provides guidance on data analysis. The following sections briefly summarize this guidance. Mortality or apparent size reduction in any sediment treatment is not necessarily an indication of toxicity. Statistical analysis is used to determine if apparent differences are significant (**52-54**). Organism response to test sediments is typically compared to the control response. If a reference sediment (for example, upstream or independent of a study site) is also collected, then test sediment results may be statistically compared 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 (for example, weight or tissue burden) then those data can also be analyzed separately.

X2.2 *Forms of Evaluation*—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 field-collected sediment is tested, then data analysis will typically consist only of hypothesis testing. If however, a series of sediment dilutions were prepared (that is, mixing test sediment with control sediment at fixed percentages [6.25, 12.5, 25, 50]), or if spiked-sediment samples are prepared representing a true concentration gradient for chemical(s) of concern, then point estimates can be made. A point estimate, such as an LC50, is a concentration of test media at which a certain effect (for example, half the test organisms die) is determined to occur. General guidance for conducting these analyses is given in the following sections.

X2.2.1 *Hypothesis Testing*—Hypothesis testing should follow the same general structure as described by Test Method E 1706 and by U.S. Environmental Protection Agency (**1, 34**). 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 X2.1).

X2.2.1.1 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 (**50**).

TABLE X2.1 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)

X2.2.1.2 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).

X2.2.1.3 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 objective of the study may be 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.

X2.2.1.4 Sublethal effects are analyzed after mortality effects have been evaluated. Individual sublethal measurements are averaged to produce a mean width and length (per surviving organism) for each replicate. 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.

X2.2.2 *Point Estimates*—Point estimations for individual chemicals of concern are seldom used in sediment tests conducted with field-collected samples because there is generally not a single concentration gradient for the particular chemical of concern. In addition, field-collected sediments may contain multiple toxicants that could act independently or have synergistic, additive, or antagonistic effects. For example, if a sediment (for example, 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 about 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.

X2.2.2.1 Any of the point estimation procedures calculate a concentration (mass per volume or percent) at which a certain effect will occur. An LC50, for example, is the concentration at which 50 % of the organisms are expected to die while an IC25 is the concentration which causes a 25 % reduction in the endpoint of interest. The manner in which LC50s or other point estimates 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. LC50s 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 should be used. These methods are described in detail by U.S. Environmental Protection Agency (**55**). 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 LC50 procedures are available with a variety of computer software programs (**52-54**).

X2.2.2.2 LC50 models, by definition, are used to calculate point estimates for mortality endpoints, although the models can also be used to calculate point estimates for nonlethal endpoints (for example, median effects concentrations (EC50s)). 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 (**56**).

REFERENCES

(**1**) U.S. Environmental Protection Agency, "Methods for Measuring the Toxicity and Bioaccumulation of Sediment-Associated Contaminants with Freshwater Invertebrates–Second Edition," EPA/600/R-99/064, 2000.

(**2**) U.S. Environmental Protection Agency–U.S. Army Corps of Engineers, "Evaluation of Dredged Material Proposed for Ocean Disposal–Testing Manual," EPA 503/8-91/001, 1991.

(**3**) U.S. Environmental Protection Agency–U.S. Army Corps of Engineers, "Evaluation of Dredged Material Proposed for Discharge in Waters of the U.S.–Testing Manual," EPA 823/B-98/004, 1998.

(**4**) Francis, P. C., Birge, W. J., and Black, J. A., "Effects of Cadmium-Enriched Sediment on Fish and Amphibian Embryo-Larval Stages," *Ecotoxicology and Environmental Safety*, Vol 8, 1984, pp. 378–387.

(**5**) Berrill, M., Coulson, D., McGillivray, L., and Pauli, B., "Toxicity of Endosulfan to Aquatic Species of Anuran Amphibians," *Environmental Toxicology and Chemistry*, Vol 17, 1998, pp. 1738–1744.

(**6**) Edginton, A. N., Stephenson, G. R., Sheridan, P. M., Thompson, D. G., and Boermans, H. J., "Effect of pH and Release® on Two Life Stages of Four Anuran Amphibians," *Environmental Toxicology and Chemistry*, Vol 22, 2003, pp. 2673–2678.

(**7**) Jofre, M. B. and Karasov, W. H., "Direct Effect of Ammonia on Three Species of North American Anuran Amphibians," *Environmental Toxicology and Chemistry*, Vol 18, 1999, pp. 1806–1812.

(**8**) Snodgrass, J. W., Hopkins, W. A., Jackson, B. P., Baionno, J. A., and Broughton, J., "Influence of Larval Period on Responses of Overwintering Green Frog (*Rana clamitans*) Larvae Exposed to Contaminated Sediments," *Environmental Toxicology and Chemistry*, Vol 24, 2005, pp. 1508–1514.

(**9**) Leney, J. L., Drouillard, K. G., and Haffner, G. D., "Does Metamorphosis Increase the Susceptibility of Frogs to Highly Hydrophobic Contaminants?" *Environmental Science and Technology*, Vol 40, 2006, pp. 1491–1496.

(**10**) Ankley, G. T., Tietge, J. E., DeFoe, D. L., Jensen, K. M., Holcombe, G. W., Durhan, E. J., and Diamond, S. A., "Effects of Ultraviolet Light and Methoprene on Survival and Development of *Rana pipiens*," *Environmental Toxicology and Chemistry*, Vol 17, 1998, pp. 2530–2542.

(**11**) James, S. M. and Little, E. E., "The Effects of Chronic Cadmium Exposure on American Toad (*Bufo americanus*) Tadpoles," *Environmental Toxicology and Chemistry*, Vol 22, 2003, pp. 377–380.

(**12**) U.S. Environmental Protection Agency, "Method for Assessing the Chronic Toxicity of Marine and Estuarine Sediment-Associated Contaminants with the Amphipod *Leptocheirus plumulosus*," EPA/600/R-01/020, 2001.

(**13**) Naval Facilities Engineering Command (NAVFAC), "Development of a Standardized Approach for Assessing Potential Risks to Amphibians Exposed to Sediments and Hydric Soils," March, 2004.

(**14**) Naval Facilities Engineering Command (NAVFAC), "Laboratory Validation Plan for ESTCP Project CU-0514 Demonstration and Certification of Amphibian Ecological Risk Assessment Protocol," December 2005.

(**15**) Loveridge, A. R., Lavoie, D. R., Corl, W. E., Jackson, R., Speicher, J., Pillard, D. A., and Christopher, J., "Use of a New Standardized Test for Assessing Potential Risks to Amphibians Exposed to Whole Sediments," Poster presented at Annual Meeting of Society of Environmental Toxicology and Chemistry, Portland, OR. November 2004.

(**16**) Environmental Chemical Corporation, "Draft Ecological Risk Assessment Addendum–Area of Concern CS-10 Detail F, Massachusetts Military Reservations, Cape Cod, MA," October, 2004.

(**17**) ENSR, "RAM Completion Statement & RAO Class A-2 Statement, Wetlands Reclamation Project Adjacent to MassHighway Maintenance Depot, Marrett Road (Rte. 2A) Lexington, MA MADEP RTN: 3–20801," November, 2002.

(18) ENSR, "Draft Baseline Ecological Risk Assessment, Sixty-One Industrial Park Site Memphis, Tennessee," May, 2007.

(19) Lande, S. P. and Guttman, S. I., "The Effects of Copper Sulfate on the Growth and Mortality Rate of *Rana pipiens* Tadpoles," *Herpetologia*, Vol 29, 1973, pp. 22–27.

(20) Birge W. A. and Black, J. A., "Effects of Copper on Embryonic and Juvenile Stages of Aquatic Animals, " J. O. Nriago (ed.), *Copper in the Environment*, 1979, pp. 373–399.

(21) Khangarot, B. S., Sehgal, A., and Bhasin, M. K., "Man and Biosphere–Studies on the Sikkim Himalayas. Part 5: Acute Toxicity of Selected Heavy Metals on the Tadpoles of *Rana hexadactyla*," *Acta Hydrochimica et Hydrobiologica*, Vol 13, 1985, pp. 259–263.

(22) Mudgall, C. F. and Patil, S. S., "Toxicity of Lead and Mercury to Frogs *Rana cyamophlyotis* and *Rana tigerina*," *Environment and Ecology*, Vol 6, 1988, pp. 506–507.

(23) Taylor, D. H, Steele, C. W., and Strickler-Shaw, S., "Responses of Green Frog (*Rana clamitans*) Tadpoles to Lead-Polluted Water, " *Environmental Toxicology and Chemistry*, Vol 9, 1990, pp. 87–93.

(24) Horne, M. T. and Dunson, W. A., "Effects of Low pH, Metals and Water Hardness on Larval Amphibians," *Arch. Enviro. Contam. Toxicol*, Vol 29, 1995, pp. 500–505.

(25) Fioramonti, E., Semlitsch, R. D., Reyer, Heinz-Ulrich, and Fent, K., "Effects of Triphenyltin and pH on the Growth and Development of *Rana lessonae* and *Rana esculenta* Tadpoles," *Environmental Toxicology and Chemistry*, Vol 16, 1997, pp. 1940–1947.

(26) Hall Jr., L. W., Scott, M. C., and Killen, W. D., "Ecological Risk Assessment of Copper and Cadmium in Surface Waters of Chesapeake Bay Watershed," *Environmental Toxicology and Chemistry*, Vol 17, 1998, pp. 1172–1189.

(27) Howe, C. M., Berrill, M., Pauli, B. D., Helbing, C., Werry, K., and Veldhoen, N., "Toxicity of Glyphosate-based Pesticides to Four North American Frog Species," *Environmental Toxicology and Chemistry*, Vol 23, 2004, pp. 1928–1938.

(28) Jung, R. E. and Walker, M. K., "Effects of 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (TCDD) on Development of Anuran Amphibians," *Environmental Toxicology and Chemistry*, Vol 16, 1997, pp. 230–240.

(29) Allran, J. W. and Karasov, W. H., "Effects of Atrazine and Nitrate on Northern Leopard Frog (*Rana pipiens*) Larvae Exposed in the Laboratory from Posthatch through Metamorphosis," *Environmental Toxicology and Chemistry*, Vol 19, 2000, pp. 2850–2855.

(30) Wojtaszek, B. F., Buscarini, T. M., Chartrand, D. T., Stephenson, G. R., and Thompson, D. G., "Effect of Release® Herbicide on Mortality, Avoidance Response, and Growth of Amphibian Larvae in Two Forest Wetlands," *Environmental Toxicology and Chemistry*, Vol 24, 2005, pp. 2533–2544.

(31) Fort, D. J., Propst, T. L., Stover, E. L., Helgen, J. C., Levey, B., Gallagher, K., and Burkhart, J. G., "Effects of Pond Water, Sediment, and Sediment Extracts from Minnesota and Vermont, USA, on Early Development and Metamorphosis of *Xenopus*," *Environmental Toxicology and Chemistry*, Vol 18, 1999, pp. 2305–2315.

(32) Savage, W. K., Quimby, F. W., and DeCaprio, A. P., "Lethal and Sublethal Effects of Polychlorinated Biphenyls on *Rana sylvatica* Tadpoles," *Environmental Toxicology and Chemistry*, Vol 21, 2002, pp. 168–174.

(33) Widder, P. D. and Bidwell, J. R., "Cholinesterase Activity and Behavior in Chlorpyrifos-exposed *Rana sphenocephala* Tadpoles," *Environmental Toxicology and Chemistry*, Vol 25, 2006, pp. 2446–2454.

(34) U.S. Environmental Protection Agency, "Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms-Fourth Edition," EPA-821-R-02-013, 2002.

(35) U.S. Environmental Protection Agency, "National Recommended Water Quality Criteria," United States Environmental Protection Agency, 2006, p. 24.

(36) Chapman, P. M., "Current Approaches to Developing Sediment Quality Criteria," *Environmental Toxicology and Chemistry*, Vol 8, 1989, pp. 589–599.

(37) Di Toro, D. M., Zarba, C. S., Hansen, D. J., Berry, W. J., Swartz, R. C., Cowan, C. E., Pavlou, S. P., Allen, H. E., Thomas, N. A., and Paquin, P. R., "Technical Basis for Establishing Sediment Quality Criteria for Nonionic Organic Chemicals Using Equilibrium Partitioning," *Environmental Toxicology and Chemistry*, Vol 10, 1991, pp. 1541–1583.

(38) Maloney, J., "Influence of Organic Enrichment on the Partitioning and Bioavailability of Cadmium in a Microcosm Study," *Marine Ecology Progress Series*, Vol 144, 1996, pp. 147–161.

(39) Granberg, M. E. and Forbes, T. L., "Role of Sediment Organic Matter Quality and Feeding History in Dietary Absorption and Accumulation of Pyrene in the Mud Snail (*Hydrobia ulvae*)," *Environmental Toxicology and Chemistry*, Vol 25, 2006, pp. 995–1006.

(40) Ankley, G. T., Benoit, D. A., Balogh, J. C., Reynoldsen, T. B., Day, K. E., and Hoke, R. A., "Evaluation of Potential Confounding Factors in Sediment Toxicity Tests with Three Freshwater Benthic Invertebrates," *Environmental Toxicology and Chemistry*, Vol 13, 1994, pp. 627–635.

(41) Sibley, P. K., Ankley, G. T., Cotter, A. M. and Leonardi, E. N., "Predicting Chronic Toxicity of Sediments Spiked with Zinc: An Evaluation of the Acid Volatile Sulfide (AVS) Model Using a Lifecycle Test with the Midge (*Chironomus tentans*)," *Environmental Toxicology and Chemistry*, Vol 15, 1996, pp. 2102–2112.

(42) Ditsworth, G. R. and Schults, D. W., "Preparation of Benthic Substrates for Sediment Toxicity Testing," *Environmental Toxicology and Chemistry*, Vol 9, 1990, pp. 1523–1529.

(43) Hourdry, J., L'Hermite, A., and Ferrand, R., "Changes in the Digestive Tract and Feeding Behavior of Anuran Amphibians During Metamorphosis," *Physiological Zoology*, Vol 69, 1996, pp. 219–251.

(44) Gosner, K. L., "A Simplified Table for Staging Anuran Embryos and Larvae with Notes on Identification," *Herpetologica*, Vol 16, 1960, pp. 183–190.

(45) Stemmer, B. L., Burton Jr., G. A., and Sasson-Bricksen, G., "Effect of Sediment Spatial Variance and Collection Method on Cladoceran Toxicity and Indigenous Microbial Activity Determinations," *Environmental Toxicology and Chemistry*, Vol 9, 1990, pp. 1035–1044.

(46) Sarda, N. and Burton Jr., G. A., "Ammonia Variation in Sediments: Spatial, Temporal and Method-Related Effects," *Environmental Toxicology and Chemistry*, Vol 14, 1995, pp. 1499–1506.

(47) Landrum, P. F., Eadie, B. J., and Faust, W. R., "Variation in the Bioavailability of Polycyclic Aromatic Hydrocarbons to the Amphipod Diporeia with Sediment Aging," *Environmental Toxicology and Chemistry*, Vol 11, 1992, pp. 1197–1208.

(48) Karasov, W. H., Jung, R. E., Langenberg, S. V., and Bergeson, T. L. E., "Field Exposure of Frog Embryos and Tadpoles Along a Pollution Gradient in the Fox River and Green Bay Ecosystem in Wisconsin, USA," *Environmental Toxicology and Chemistry*, Vol 24, 2005, pp. 942–953.

(49) Birge, W. J., Westerman, A. G., and Spromberg, J. A., "Comparative Toxicology and Risk Assessment of Amphibians," *Ecotoxicology of Amphibians and Reptiles*, Sparling, Linder and Bishop, eds., Society of Environmental Toxicology and Chemistry, Pensacola, FL, 2000, pp. 727–791.

(50) Denton, D. L. and Norberg-King, T .J., "Whole Effluent Toxicity Statistics: A Regulatory Perspective," *Whole Effluent Toxicity Testing: An Evaluation of Methods and Prediction of Receiving System Impacts*, Grothe, Dickson, and Reed-Judkins, eds., Society of Environmental Toxicology and Chemistry, Pensacola, FL, 1996, pp. 83–102.

(51) Shumway, W., "Stages in the Normal Development of Rana pipiens," *The Anatomical Record*, Vol 78, 1940, pp. 139–148.

(52) WEST, Inc. and D. D. Gulley, "Toxstat Version 3.5," Western Ecosystems Technology, Inc. Cheyenne, WY, 1996.

(**53**) Analytical Software, "Statistix 7 for Windows," Analytical Software, Tallahassee, FL, 2000.

(**54**) U.S. Environmental Protection Agency, "U.S. EPA Toxicity Data Analysis Software. Version 1.5," Environmental Monitoring Systems Laboratory, Cincinnati, OH, 1994.

(**55**) U.S. Environmental Protection Agency, "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms–Fifth Edition," EPA-821-R-02-012, 2002.

(**56**) Norberg-King, T., "A Linear Interpolation Method for Sublethal Toxicity: The Inhibition Concentration (ICp) Approach (Version 2.0)," National Effluent Toxicity Assessment Center Technical Report 03-93, U.S. Environmental Protection Agency, Duluth, MN, 1993.

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Appendix E

Species Sensitivity Testing Results

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1.0 Introduction

The Department of Defense (DoD) Environmental Security Technology Certification Program (ESTCP) has funded the Naval Facilities Engineering Service Center (NFESC) and its DoD partners (U.S. Navy Engineering Facilities Engineering Command, Atlantic Division (LANTDIV), U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM) and Air Force Center for Environmental Excellence (AFCEE)), as well as their contractor ENSR Corporation (ENSR), to demonstrate and validate protocols for the evaluation of potential risks to amphibians in palustrine wetland environments. This work is described further in the following deliverables that have been produced as part of this ESTCP-funded effort (ESTCP ER-0514, *Demonstration and Certification of Amphibian Ecological Risk Assessment Protocol*):

- NAVFAC, 2005. Laboratory Validation Plan for ESTCP Project ER-0514 Demonstration and Certification of Amphibian Ecological Risk Assessment Protocol. December 2005.
- NAVFAC, 2006. Site Selection Memorandum for ESTCP Project ER-0514 Demonstration and Certification of Amphibian Ecological Risk Assessment Protocol. February 2006.
- NAVFAC, 2007a. Field Demonstration Plan for ESTCP Project ER-0514 Demonstration and Certification of Amphibian Ecological Risk Assessment Protocol. May 2007.
- NAVFAC, 2007b. Test Refinement Interim Report for ESTCP Project ER-0514 Demonstration and Certification of Amphibian Ecological Risk Assessment Protocol. June 2007.
- NAVFAC, 2008. Final Report for ESTCP Project ER -0514 Demonstration and Certification of Amphibian Ecological Risk Assessment Protocol. September 2008.

In response to November 2006 In-Progress Review (IPR) comments on the existing ESTCP project, additional studies are being conducted to investigate the differential sensitivities of larval amphibians to copper and lead. This element of the program is being conducted to assess, compare, and contrast the responses of multiple amphibian species to exposure to two chemical stressors (lead and copper) in hydric soils. The *Species Sensitivity Testing Work Plan* (NAVFAC, 2007c) presented the scope of work for the amphibian species sensitivity study.

This document provides an overview of the methodology, as well as the results of the testing conducted through July 2008.

1.1 Objectives of Species Sensitivity Testing Program

The ER-0514 program is being conducted to demonstrate and validate an innovative technique for the evaluation of potential risks to amphibians in palustrine wetland environments. During the course of this investigation, an additional phase of work was authorized to evaluate the differential sensitivities of various larval amphibians to chemical stressors. The 10-day sediment exposure bioassay used in the ER-0514 project is being used to evaluate the responses of different amphibian species exposed to sediments spiked with known concentrations of copper or lead.

The results of this study have been compiled and analyzed in order to predict concentrations of metals affecting a given proportion of the amphibian species in an ecological community. In practice, the use of a species sensitivity distribution or the individual test species data to predict amphibian toxicity would permit risk managers to select risk-based amphibian screening values based on site-specific circumstances. High value wetlands with sensitive taxa would presumably warrant further protection than lower value wetlands with relatively insensitive taxa.

2.0 Project Background

Amphibians are a front-line indicator of possible adverse impacts to wetland ecosystems. However, outside of the current ESTCP effort, few standardized procedures exist to evaluate the potential toxicity of sediments and hydric soils to amphibians, and no risk-based screening values, specific to amphibians, exist for use in screening level risk assessments. Under the ongoing ESTCP-funded project ER-0514, a multi-phase project is being conducted to demonstrate a standardized approach for assessing potential risks to amphibians at DoD facilities. The ongoing laboratory testing program includes a comparative evaluation of lead and copper toxicity to larval frogs and adult salamanders. These metals were selected as the constituents for this study since they are commonly co-located at military sites and ranges, and are often found at elevated concentrations in hydric soils at these sites.

Evaluating the variability in sensitivities between amphibian species was not an *a priori* goal of the ER-0514 project. However, during the course of this study (and under an earlier Navy-funded program) sufficient range-finding data were collected on several species of frogs and toads that allowed for some comparisons (see Table 2-1). In addition, a literature review was conducted to evaluate the potential differences in toxicity among taxa (this review was partially funded through the Navy's Pollution Abatement Ashore (Y0817) program).

Although these previous studies were not directly designed to evaluate species sensitivity distributions, the results of the YO817 and ESTCP work yielded several intriguing findings:

- Based on the toxicity testing conducted through the YO817 program and during the ER-0514 project, there clearly are substantial differences in early life stage sensitivities between the tested frogs and toads, and among the frogs (i.e., within the genus *Rana*).
- Based on the literature review, certain taxa appear to be more sensitive to metals, whereas others may be more tolerant of contaminant toxicity. In fact, some literature suggests that the primary species historically used in amphibian testing (*Xenopus laevis*) appears to generally be more tolerant of organic compounds than the majority of native species (Birge, et al, 2000).
- The African clawed frog (*X. laevis*) is an important test species for evaluation of potential constituent toxicity and has recently been used by several researchers (Goleman et al., 2002) to evaluate perchlorate toxicity. These researchers found that *X. laevis* development and metamorphosis were inhibited by environmentally relevant concentrations of perchlorate.
- Of the various amphibian species reported in the technical ecotoxicology literature, *X. laevis* is perhaps the most common. *X. laevis* eggs and larvae are readily available year-round, this species readily adapts to laboratory conditions, can complete metamorphosis in an entirely aquatic exposure system, and protocols for evaluation of this species are fairly standardized; it is therefore valuable to include this species in the proposed differential species toxicity testing. However, there are considerable uncertainties with basing environmental cleanups on testing with this species, given that it is very different

physiologically than native species (tetraploid genotype), is non-native, is totally aquatic, and perhaps most importantly, appears to be at the less sensitive end of species sensitive distribution for amphibians exposed to environmental toxicants (Birge, et al, 2000).

Obtaining additional information on amphibian species sensitivity is important from several additional perspectives:

- If amphibians are a species of concern at a DoD Site, it is important for risk managers to have information regarding the relative and differential sensitivity of various taxa;
- Due to limitations in amphibian availability, even experienced project managers may need to use alternative test organisms. Market conditions and climatic factors make it difficult to predict with any certainty which species of amphibians are available at any one time. Understanding the relative sensitivities of differing taxa may be necessary if the use of alternative test organisms is required; and
- There is a universally recognized scarcity of amphibian toxicological literature. Much of the data that are available are based on water-only, as opposed to sediment exposures which may be more ecologically relevant to amphibians.

The comparative data obtained from this study are anticipated to be readily publishable and should serve to help fill an important scientific data gap relative to basic amphibian toxicology and ecology. This information can be used by a number of different end users, including conservation professionals and natural resources managers within and outside of the DoD and federal services.

Table 2-1 Summary of Lowest Statistical Toxicity Endpoints from the Navy's Y0817 Amphibian Testing Program

	Lowest IC25		**Lowest LC50**	
Analyte (mg/kg)	***Rana***	***Bufo***	***Rana***	***Bufo***
Cadmium	230	540	700	> 580
Copper	> 64 [1]	NA	> 64	NA
Lead	3,490	NA	4,662	NA
Zinc	980	1,600	1,500	2,100

Test duration was 10 days.
NA = Not analyzed
IC = Inhibition Concentration
LC = Lethal Concentration
1 = Highest tested concentration used in study without detectable effect.

3.0 Species Sensitivity Evaluation Methodology

In order to evaluate the sensitivities of various larval amphibian species to copper and lead in sediment, 10-day toxicity tests were conducted using the sediment protocol refined under the current project (published in December 2007 by ASTM as E2591-07 *Standard Guide for Conducting Whole Sediment Toxicity Tests with Amphibians*). Amphibian eggs were either field-collected in Massachusetts or purchased from biological vendors. Biological vendors may field-collect amphibian eggs or eggs may be fertilized in the laboratory.

The goal of the project was to conduct tests with up to eight species of amphibians in order to determine the sensitivity of these organisms to sediment-associated contaminants. Severe drought conditions in the northeastern and southeastern U.S. in 2007 substantially limited the availability of test organisms. Therefore, additional testing was conducted in spring and summer 2008 when amphibian eggs became available again.

3.1 Larval Amphibian Procurement

Testing was conducted in phases depending on organism availability. Because younger organisms were found to generally be more sensitive than older organisms to metals, the sediment test protocol strongly recommends the use of <72-h old tadpoles. To account for shipping and sediment preparation time, therefore, it is preferable to receive organisms as eggs. 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. In order to test with a variety of amphibian species, amphibians were obtained from both commercial suppliers and field collection efforts. Table 3-1 presents the sources for the amphibian eggs used in the testing program.

3.1.1 Field Collection

Although obtaining amphibian eggs from biological vendors is preferable, availability is highly variable and some species must be field collected as they are never available from commercial suppliers. A scientific collection permit was provided by the Massachusetts Division of Fisheries and Wildlife to selected ENSR staff to allow the collection of amphibian egg masses for testing purposes (see Attachment A). Eggs were also collected from Navy property in Massachusetts during routine field efforts at the former Naval Air Station South Weymouth.

It was anticipated that eggs of several amphibian species could be collected in the wild during the spring. Typically, in the Northeast, these would include: pickerel frog (*R. palustris*), green frog (*R. clamitans*), wood frog (*R. sylvatica*), American toad (*Bufo americanus*), and spotted salamander (*Ambystoma maculatum*). However, due to the 2007 drought conditions, only wood frog (*R. sylvatica*) and spotted salamander (*A. maculatum*) eggs could be collected and tested.

Although pickerel frog (*R. palustris*) eggs could not be found in 2007 or 2008, testing had previously been conducted with field collected pickerel frog (*R. palustris*) eggs as part of the supporting investigations for this ER-0514 project so those results were evaluated as part of the

species sensitivity work. During the spring of 2008 American toad (*B. americanus*) eggs were field collected from the former Naval Air Station South Weymouth in Massachusetts and included in the testing program.

3.1.2 Commercial Suppliers

Egg availability from commercial suppliers was investigated during the preparation of the *Species Sensitivity Testing Work Plan* (NAVFAC, 2007c). Generally, eggs of the northern leopard frog (*R. pipiens)* can be obtained from 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.

Eggs of the African clawed frog (*X. laevis*) and its close relation the western clawed frog (*X. tropicalis*) are produced in the laboratory and generally available all year. *X. tropicalis* is a diploid species with a relatively short (<5 months) life cycle that has been used recently as a popular amphibian model for vertebrate development genetics (http://faculty.virginia.edu/xtropicalis).

Vendors indicated the likely availability of American toad (*B. americanus*), and spring peeper (*Pseudacris crucifer)* eggs from January through June, bullfrog (*R. catesbeiana)* eggs from April through August, and pickerel frog (*R. palustris),* green frog *(R. clamitans)*, gray treefrog (*Hyla chrysoscelis)*, western chorus frog (*P. triseriata)*, cricket frog (*Acris crepitans)*, and narrow-mouthed toad (*Gastrophryne carolinensis*) eggs during the months of March and April. Eggs for some of these species were also expected to be available through a good portion of the summer months. The species tiger salamander (*A. tigrinum)*, southern leopard frog (*R. sphenocephala)*, and spotted salamander (*A. maculatum)* were not expected to be commercially available until the fall. No commercial vendor was identified for the eggs of wood frog (*R. sylvatica).*

However, due to drought conditions, egg availability was limited during the 2007 testing period. Only four species were available from commercial suppliers in 2007 - gray treefrog (*H. chrysoscelis)*, green frog *(R. clamitans)*, African clawed frog (*X. laevis*), and western clawed frog (*X. tropicalis*).

As part of the supporting investigations for this ER-0514 project, testing had previously been conducted with the northern leopard frog (*R. pipiens)* and the green frog *(R. clamitans)* so those results were also evaluated as part of the species sensitivity work. During the spring of 2008 southern leopard frog (*R. sphenocephala*) and American bullfrog (*R. catesbeiana*) eggs were obtained from commercial vendors and included in the testing program.

3.2 Sediment Exposure Protocol

The sediment exposure protocol (ASTM, 2007) evaluates potential risks to early life stage frogs and toads from exposure to submerged sediments. The test evaluates effects on amphibian growth (i.e., weight, length) and survival following a 10-day laboratory exposure to contaminated sediments.

The toxicity testing program applied the sediment exposure protocol with one exception: rather than using a total of eight replicates/test treatment in each study, the tests included six replicates/treatment. This deviation was implemented primarily because of concerns over the availability of a sufficient number of test organisms. Six replicates still provide enough statistical power to collect meaningful data (particularly when using a homogeneous, spiked, laboratory sediment), while being sensitive to the concerns associated with the use of field-collected organisms in this study, and allowing for sufficient laboratory bench space to conduct the work.

3.2.1 Test Treatments

For each of the two metals being evaluated (copper and lead), the sediment test program included five to seven dilutions per test, plus a laboratory control.

The base sediment for all the toxicity investigations was collected from the Cache La Poudre River northwest of Fort Collins, Colorado. This sediment is used regularly by the laboratory and was used as the base sediment in the laboratory validation phase of this ESTCP project. The sediments are classified as poorly graded sand (99.6% sand and 0.4% silt and clay), with approximately 50% retained by a 0.42 mm sieve. The sediments are also characterized by low TOC (0.066%) and a near neutral pH (approximately 7.5). Sediment was collected from the river floodplain immediately adjacent to the river (submerged during high flow) and rinsed at the laboratory in sieves until the water ran clear according to published preparation methods (Walsh et al 1991; Harrahy and Clements 1997; Kemble et al. 1999). The rinsed sediment was then air-dried and kept in closed containers until used. Laboratory control replicates for the tests were unamended Poudre River sediment.

Copper and lead solutions were prepared in deionized (Milli-Q) water by adding $CuCl_2$ or $PbCl_2$ in appropriate amounts to yield nominal stock concentrations of 5,000, 10,000 or 100,000 mg/L as copper or 10,000 or 100,000 mg/L as lead. Multiple stock solutions of each metal were prepared to accommodate preparation of widely-spaced concentrations. The volume of copper or lead stock solution added to each sediment sample to generate the final tested material is not important *per se*, however, the goal is to have enough volume so that it could be 1) accurately measured and dispensed and 2) distributed evenly to the test sediment.

To prepare a spiked sediment, the base sediment was first wetted with Milli-Q water to cause the dry sediment particles to stick together. Using a 10-ml pipette, five vertical holes were made into the sediment mixture, which was contained in wide-mouth jars. The volume of copper or lead stock needed to make the target concentration was then added in roughly equal amounts to each of the five holes. Lids were replaced and the jars were placed in an end-over-end tumbler for homogenization. Just prior to filling test chambers, the sediment was mixed again by placing it into a clean, stainless steel bowl and mixing with a stainless steel spoon for a minimum of three minutes. When multiple tests were anticipated, a large batch of each test sediment was generated at the start of the testing program and used for several individual tests.

3.2.2 Test Methodology

The test methodology followed for the species sensitivity evaluation was consistent with that applied throughout the on-going ESTCP project and recently published by ASTM (ASTM, 2007). Young amphibians (<24 to 72 h old) were placed in beakers containing sediment and overlying water. The overlying water in each beaker was replaced continuously via a flow-through (drip) delivery system. The beakers were placed in a water bath that was held constant at 23 ± 1°C. Water chemistry (e.g., pH, dissolved oxygen (DO), temperature, etc.) was measured in the overlying water as specified in the ASTM guide (e.g., minimum daily measurement of DO; pH on days 3, 6, and 9). A small amount of ground TetraMin® was added daily as a food source once all external evidence of gills was gone.

Beakers were examined daily for live organisms. Because some of the larval amphibians tended to blend in with the test sediment, it was often difficult to make a complete count of each test beaker when they were in the water bath. Therefore, the daily observations were primarily to verify the ongoing presence of live organisms. If a cursory examination indicated possible mortality in any one beaker, then all of the beakers in that treatment were removed from the bath and examined for dead organisms. Dead test organisms were removed. Live test organisms were left in the chamber and it was placed back into the water bath.

At the end of the test, final overlying water chemistry samples were collected and measured. These parameters include, at a minimum, temperature, DO, pH, and conductivity, and, at the Study Director's discretion, hardness and alkalinity. All living organisms were counted and removed for sublethal (width and body length) measurements. Sediment, water and/or tissue were collected for chemical analysis, when necessary. Mortality and growth, as body length and body width, were evaluated at test termination (Day 10).

All test organisms were handled according to ENSR Standard Operating Procedure 5116, "Care of Vertebrate Test Animals during Department of Defense, or Similar, Studies" which was written in accordance with Army Regulation 70-18, "The Use of Animals in DOD Programs."

Test specifications for the species sensitivity testing program are listed in Table 3-2 and additional detail is provided in the *Standard Guide for Conducting Whole Sediment Toxicity Tests with Amphibians* (ASTM, 2007).

3.2.3 Chemical Analysis

In addition to collection of toxicological data, the testing program included collection of sediment and surface water chemistry during the course of the tests. To reduce analytical costs, samples were generally archived until tests were completed and a dose response was established. Selected samples within the relevant response range were analyzed for copper and lead in sediment. These measured concentrations were used in the statistical evaluations conducted for each test. Analytical methods were consistent with those described in previous ESTCP deliverables.

Table 3-3 presents a comparison of the nominal and measured concentrations for copper and lead in the sediment. Measured copper concentrations were similar to the nominal concentrations. Measured lead concentrations were well below the nominal lead concentrations, generally in the range of 50 to 60% of the nominal concentrations. Similar differences between the nominal and measured concentrations were also observed during the laboratory validation phase of this ESTCP project. Lower measured lead concentrations were expected due to the low solubility of the lead chloride (PbCl2) in the stock solutions used to spike the sediment. The difference between nominal and measured concentrations was usually more pronounced at higher concentrations where a relatively higher proportion of the lead did not go into solution. Since actual concentrations of both lead and copper were determined analytically, and these values were used to complete the statistical analyses, the discrepancy between actual and nominal lead concentrations does not impact the statistical evaluations.

3.3 Data Evaluation and Reporting

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. Once the testing program is completed, the organism responses to the test sediments are compared to the control response. Two types of data were obtained from the toxicity tests: lethal (mortality) and sub-lethal (width and length). Each data type was analyzed independently.

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. An alpha level of 0.05 was used to evaluate the statistical significance between the treatments and the associated control. In this study a series of sediment dilutions were prepared (see Section 3.2.1), and therefore point estimates could be made. A point estimate, such as a median lethal concentration (LC50), is a concentration of test media or chemical stressor (in this case, copper or lead) at which a certain effect (e.g., half the test organisms die) is determined to occur. Measured, not nominal, concentrations of copper and lead in the sediment were used in the statistical analyses.

The results of this study are presented on a per species basis, and as a species sensitivity distribution. This approach allows for the single-species data to be compiled in order to predict concentrations of metals affecting defined percentages of amphibian species in an ecological community.

Table 3-1 Amphibian Egg Sources

Test Dates	Species	Supplier
Dec 9 - 19, 2005	Northern leopard frog (*R. pipiens*)	Carolina Biological
May 13 - 23, 2006	Pickerel frog (*R. palustris*)	Field collected (Navy property; Massachusetts)
July 1 - 11, 2006	Green frog (*R. clamitans*)	Charles D. Sullivan Inc
Apr 15 - 25, 2007	Wood frog (*R. sylvatica*)	Field collected (Massachusetts)
May 2 - 12, 2007	Spotted salamander (*A. maculatum*)	Field collected (Massachusetts)
May 10 - 20, 2007	Gray treefrog (*H. chrysoscelis*)	Charles D. Sullivan Inc
May 12 - 22, 2007	Green frog (*R. clamitans*)	Charles D. Sullivan Inc
July 6 - 16, 2007	African clawed frog (*X. laevis*)	Xenopus1
Sept 28 - Oct 8, 2007	Western clawed frog (*X. tropicalis*)	Nasco
Feb 19 – 29, 2008	African clawed frog (*X. laevis*)	Carolina Biological
Mar 21 – Mar 31, 2008	Southern leopard frog (*R. sphenocephala*)	Charles D. Sullivan Inc
Apr 21 – May 1, 2008	American toad (*B. americanus*)	Field collected (Navy property; Massachusetts)
June 27 – July 6, 2008	American bullfrog (*R. catesbeiana*)	Carolina Biological

Table 3-2 Sediment Test Specifications

Test Organism	Larval amphibians; various species
Test Organism Age	≤72 hours
Test Duration	10 days
Test Chambers	500 ml beakers or chambers with an overflow drainage system
Vol. of Sediment	100 ml
Vol. of Overlying Water	175 ml
Replicates	Minimum of 6
Organisms/replicates	Minimum of 5
Control Sediment	Uncontaminated natural sediment from Cache La Poudre River in Colorado
Overlying Water	Natural lake water from Horsetooth Reservoir near Ft. Collins, Colorado
Test Temperature	23 ± 1°C
Dissolved Oxygen	≥3.0 mg/L
Solution Renewal	Continuous flow-through
Feeding	4 mg TetraMin per vessel daily after external evidence of gills is gone (i.e., when tadpoles reach stage 25)
Test Endpoints	Survival, body width, and body length
Acceptability	Mean control survival of at least 80%

Table 3-3 Comparison of Nominal and Measured Concentrations in Sediment

Nominal Concentration (mg/kg)	Range of Measured Concentrations (mg/kg)
Copper	
0	3.2 - 7.2
10	13 - 15
30	21 - 32
80	73 - 87
150	140 - 170
300	230 - 280
500	470 - 510
Lead	
0	1.9 - 4.5
50	27 - 40
100	52 - 77
200	95 - 140
500	250 - 340
1000	480 - 750
2000	1200 - 1300
2500	1300 - 1900
3000	1600
Measured concentrations were used in the statistical evaluations.	

4.0 Results

Between December 2005 and July 2008 24 toxicity tests were conducted according to the methodology described in Section 3. Daily observations were made during the 10 day tests and at test termination survival, body width, and body length were measured. Results of the toxicity tests were evaluated statistically relative to the copper and lead concentrations measured in the sediments. The results of the individual species results and the differences between species are presented in the sections below.

4.1 Overview of Tests and Data Treatment

Tables 4-1 and 4-2 present a summary of the amphibian toxicity tests that have been conducted with copper and lead spiked sediments, respectively. Daily survival results and test termination results are presented in Attachment B. Tables 4-3 and 4-4 present the statistical endpoints for the acceptable tests. Figures 4-1 through 4-44 present the mean survival and growth data obtained from each test for which statistics were calculated.

The Comprehensive Environmental Toxicity Information System (CETIS) toxicity data analysis application was used to evaluate the toxicity data from each test. Measured copper and lead concentrations in the sediments were used in the statistical evaluations. 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 modified Levene test (α =0.01). The 10-day median lethal concentration (LC50) was calculated for each test, provided there was >50% mortality in any concentration. LC50s 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. LC50 values were calculated for survival results on Days 2, 4, and 10. The LC50 represents the concentration of a substance that kills 50% of the test subjects.

Depending upon the number of test treatments and whether or not the data met the requirements for parametric analyses, analysis of variance was conducted (α =0.05) using the CETIS software. The software package selects the appropriate statistical method based on the data distribution. Tests used to evaluate these data included Dunnett's Test, t-Test, Steel's Many-One Rank Test, or the Wilcoxon Rank Sum Test with Bonferroni's Adjustment. The 25% Inhibition Concentrations (IC25s) were calculated by CETIS software using the linear interpolation method.

No observed adverse effect concentrations (NOAECs), lowest observed adverse effect concentrations (LOAECs), and Inhibition Concentration 25% (IC25s) were calculated for survival, body width and body length at test termination (Day 10). The NOAEC represents the highest tested concentration of a substance where no statistically significant effect to test organisms was found. The LOAEC is the lowest tested concentration of a substance where statistically significant effects were found. The IC25 is the concentration of a substance that causes a 25% reduction in an endpoint such as survival, growth, fecundity, or reproduction in the test population when compared

to the control population response. These statistical endpoints were based on the measured, not nominal, concentrations of copper and lead in the sediments.

4.1.1 Copper Testing

Twelve 10 day tests were conducted with copper-spiked sediments in order to evaluate the potential differences in responses between various amphibian species (Table 4-1). In three tests (Tests 107, 113, and 124) the laboratory control results did not meet test acceptability criteria of ≥80% survival at test termination. For each of these tests, the results of the other treatments within the copper test and the results of the concurrent lead laboratory control replicates were reviewed in order to determine whether the laboratory control failures indicated an issue with overall test organism health or whether the failure was an anomaly limited to the control treatment.

For Test 113 with the western clawed frog (*X. tropicalis*), survival was low in all treatments so statistics were not calculated for this test. In Tests 107 with the African clawed frog (*X. laevis*) and 124 with the American bullfrog (*R. catesbeiana*), although the laboratory controls were slightly below the 80% survival acceptability criteria, survival in several tested concentrations was above the acceptability criteria and the copper-spiked sediment treatments indicated a dose response curve. Toxicity statistics were calculated using these data and they are discussed below, recognizing that there may be some uncertainty in the results since the laboratory controls exhibited less than 80% survival.

4.1.2 Lead Testing

Twelve 10 day tests were conducted with lead-spiked sediments in order to evaluate the potential differences in responses between various amphibian species (Table 4-2). In two tests (Tests 108 and 119) the laboratory control results did not meet test acceptability criteria of ≥80% survival at test termination. For each of these tests, the results of the other treatments within the copper test and the results of the concurrent lead laboratory control replicates were reviewed in order to determine whether the laboratory control failures indicated an issue with overall test organism health or whether the failure was an anomaly limited to the control treatment.

For Test 119 with the African clawed frog (*X. laevis*), survival was low in all treatments so statistics were not calculated for this test. For Test 108, also with the African clawed frog (*X. laevis*), four treatments met the test acceptability criteria and the spiked lead sediment treatments indicated a dose response curve. Toxicity statistics were calculated using these data and they are discussed below, recognizing that there may be some uncertainty in the results since the laboratory controls exhibited less than 80% survival.

4.2 Summary of Toxicity Testing Results

Statistical endpoints for each test are presented in Tables 4-3 and 4-4. Table 4-3 presents a summary of the LC50 values calculated at three different durations during the testing: Day 2, Day 4, and Day 10. In some cases the LC50 values were similar at all three durations, indicating that toxicity occurred very quickly after test initiation, and in other cases the LC50 values decreased

over time, indicating a more gradual response to the stressor. Table 4-4 summarizes the NOAECs, LOAECs, and IC25s for the three measurements made at test termination: survival, body width, and body length. The results for each species are presented in the following sub-sections and survival and growth results at test termination are displayed in Figures 4-1 through 4-44.

4.2.1 Northern leopard frog (*R. pipiens*)

Tests 036 and 039 both met the test acceptability criteria of ≥80% survival in the laboratory controls.

Copper concentrations in Test 036 ranged from 7.2 mg/kg to 230 mg/kg and survival at test termination ranged from 78% to 100%. The LC50 values at all three durations were above the highest tested concentration (>230 mg/kg). At test termination, survival of tadpoles in 230 mg/kg copper was reduced, but not significantly so, resulting in a NOAEC of 230 mg/kg (Figure 4-1). Growth, however, was reduced at lower copper concentrations; the NOAEC for body width and body length was 21 mg/kg (Figure 4-2). At test termination the IC25 values were >230 mg/kg for survival, 95.1 mg/kg for body width, and 109 mg/kg for body length. In this evaluation, body width appeared to be the most sensitive endpoint.

Lead concentrations in Test 039 ranged from 4.5 mg/kg to 1,200 mg/kg and survival at test termination ranged from 70% to 100%. The LC50 values at all three durations were above the highest tested concentration (>1200 mg/kg). At test termination, survival of tadpoles was reduced in the highest treatment (1,200 mg/kg lead) resulting in a NOAEC of 680 mg/kg (Figure 4-3). Both body width and length, however, were significantly reduced resulting in NOAECs of 260 mg/kg for body width and body length (Figure 4-4). At test termination the IC25 values were 1,098 mg/kg for survival, 796 mg/kg for body width, and 731 mg/kg for body length. In this evaluation, body length appeared to be the most sensitive endpoint.

4.2.2 Pickerel frog (*R. palustris*)

Test 075 met the test acceptability criteria of ≥80% survival in the laboratory controls for both the copper and lead exposures.

Copper concentrations ranged from 4.2 mg/kg to 265 mg/kg and survival at test termination ranged from 0% to 100%. The LC50 values at all three durations were 46.8 mg/kg. At test termination, there was complete mortality in the 265 mg/kg, 150 mg/kg, and 80 mg/kg treatments resulting in a NOAEC of 29 mg/kg (Figure 4-5). Growth was reduced in all treatments relative to the control resulting in NOAECs of <29 mg/kg (the lowest tested treatment) for both body width and body length (Figure 4-6). At test termination the IC25 value was 35.9 mg/kg for survival. Due to the complete mortality in three of the four copper-spiked treatments, the IC25s for body length and body width could only be estimated as >29 mg/kg. In this evaluation, the sub-lethal endpoints appear to be equally sensitive.

Lead concentrations ranged from 2.6 mg/kg to 1,300 mg/kg and survival at test termination ranged from 0% to 100%. The LC50 values were 869 mg/kg at Day 2, 763 mg/kg at Day 4, and 348

mg/kg at Day 10 (test termination). At test termination, survival of tadpoles was reduced in the three highest treatments resulting in a NOAEC of 116 mg/kg (Figure 4-7). Both body width and length were also significantly reduced resulting in NOAECs of 116 mg/kg for body width and body length (Figure 4-8). At test termination the IC25 values were 254 mg/kg for survival, 188 mg/kg for body width, and 189 mg/kg for body length. In this evaluation, body width and body length exhibit a very similar response and are more sensitive than the survival endpoint.

4.2.3 Green frog (*R. clamitans*)

Two sets of tests were conducted with *R. clamitans* over the course of this ESTCP project. One set of tests was conducted in July 2006 when test organism availability for the field demonstration effort became limited and alternative species were being assessed. The second set of tests were conducted as part of the species sensitivity testing during May 2007. The results of both sets of tests are discussed below. All four tests met the test acceptability criteria of ≥80% survival in the laboratory controls for copper (Tests 076 and 103) and lead (Tests 077 and 104) exposures.

Copper concentrations in Tests 076 and 103 ranged from 3.2 mg/kg to 470 mg/kg. The LC50 values in Test 076 were approximately 78 mg/kg at all three durations, and in Test 103 were 55 mg/kg at Day 2, 51 mg/kg at Day 4, and 38 mg/kg at Day 10 (test termination). In both tests complete mortality was observed in treatments above approximately 70 mg/kg (Figures 4-9 and 4-10) resulting in survival NOAECs of 80 mg/kg for Test 076 and 31 mg/kg for Test 103. Growth was also significantly reduced resulting in NOAECs of 29 mg/kg for both body width and body length for Test 076 (Figure 4-11) and NOAECs of 31 mg/kg for body width and 13 mg/kg for body length for Test 103 (Figure 4-12). At test termination the IC25 values were 46 mg/kg for survival and approximately 44 mg/kg for body width and body length for Test 076 and approximately 32 mg/kg for both survival and body width and 27 mg/kg for Test 103. In this evaluation, body length was the most sensitive endpoint.

Lead concentrations in Tests 077 and 104 ranged from 1.9 mg/kg to 1,400 mg/kg. The LC50 values in Test 077 were 892 mg/kg at Days 2 and 4 and 785 mg/kg at Day 10. In Test 104 the LC50 values were 820 mg/kg at Day 2, 799 mg/kg at Day 4, and 677 mg/kg at Day 10. Only 20% mortality was observed in the highest tested concentration of Test 077 (635 mg/kg), so the lead concentrations were increased in Test 104. Complete mortality was only observed in the highest tested concentration of Test 104 (1,400 mg/kg). The resulting survival NOAECs were 298 mg/kg for Test 077 (Figure 4-13) and 480 mg/kg for Test 104 (Figure 4-14). Growth was also significantly reduced resulting in NOAECs of 298mg/kg for both body width and body length for Test 077 (Figure 4-15) and 250 mg/kg for body width and body length for Test 104 (Figure 4-16). At test termination the IC25 values were 670 mg/kg for survival, 441 mg/kg for body width, and 402 mg/kg for body length for Test 077 and 498 mg/kg for survival, 380 mg/kg for body width, and 327 mg/kg for body length for Test 104. In this evaluation, body length was the most sensitive endpoint for both tests.

These results show that different batches of the same species may have different responses to the same stressors. The test organisms used in Tests 103 and 104 were slightly more sensitive to both

copper and lead than the organisms used in Tests 076 and 077. The differences in the IC25s and LC50s were less than 40 mg/kg for copper and less than 200 mg/kg for lead.

4.2.4 Wood frog (*R. sylvatica*)

Tests 094 and 095 met the test acceptability criteria of ≥80% survival in the laboratory controls for both the copper and lead exposures.

Copper concentrations ranged from 3.2 mg/kg to 510 mg/kg and survival at test termination ranged from 0% to 100%. The LC50 values at all three durations were 111 mg/kg. At test termination, there was complete mortality in the 170 mg/kg, 280 mg/kg, and 510 mg/kg treatments resulting in a survival NOAEC of 72.7 mg/kg (Figure 4-17). Growth was reduced relative to the control in all but the lowest two tested treatments resulting in NOAECs of 32 mg/kg for both body width and body length (Figure 4-18). At test termination the IC25 value was 89.3 mg/kg for survival and IC25s for body length and body width were estimated at >72.7 mg/kg. In this evaluation it is likely that the sub-lethal endpoints are more sensitive endpoints than survival, but the truncated dose response curve for the growth endpoints results in uncertainty in the calculation of the IC25.

Lead concentrations ranged from 1.9 mg/kg to 1,600 mg/kg and survival at test termination ranged from 0% to 97%. The LC50 values were 509 mg/kg at Day 2, 446 mg/kg at Day 4, and 305 mg/kg at Day 10 (test termination). At test termination, survival of tadpoles was reduced in the four highest treatments resulting in a NOAEC of 130 mg/kg (Figure 4-19). Both body width and length were also significantly reduced resulting in NOAECs of 130 mg/kg for body width and body length (Figure 4-20). At test termination the IC25 values were 185 mg/kg for survival, 189 mg/kg for body width, and 192 mg/kg for body length. In this evaluation, all endpoints exhibit similar responses.

4.2.5 Spotted salamander (*A. maculatum*)

Only a small number of spotted salamander (*A. maculatum*) larvae were available for testing so only a modified copper test could be conducted (two replicates per treatment were tested instead of eight). Test 100 met the test acceptability criteria of ≥80% survival in the laboratory controls for the copper exposure.

Copper concentrations in Test 100 ranged from 3.2 mg/kg to 470 mg/kg and survival at test termination ranged from 0% to 100%. The LC50 values at all were 106 mg/kg at Days 2 and 4 and 98 mg/kg at Day 10. At test termination, there was complete mortality in the 470 mg/kg, 270 mg/kg, and 150 mg/kg treatments resulting in a NOAEC of 75 mg/kg (Figure 4-21). Body width was not measured in this test. Growth was reduced in two treatments relative to the control resulting in NOAECs of 75 mg/kg (the lowest tested treatment) for body length (Figure 4-22). At test termination the IC25 value was 84.2 mg/kg for survival. Due to the complete mortality in three of the five copper-spiked treatments, the IC25s for body length could only be estimated as >75 mg/kg. In this evaluation it is likely that body length is a more sensitive endpoint than survival, but the truncated dose response curve for the growth endpoints results in uncertainty in the calculation of the IC25.

4.2.6 Gray treefrog (*H. chrysoscelis*)

Tests 101 and 102 met the test acceptability criteria of ≥80% survival in the laboratory controls for both the copper and lead exposures.

Copper concentrations in Test 101 ranged from 3.2 mg/kg to 470 mg/kg and survival at test termination ranged from 0% to 100%. The LC50 values were 48.0 mg/kg at Day 2, 45.3 mg/kg at Day 4, and 43.8 mg/kg at Day 10. At test termination, there was complete mortality in the 75 mg/kg, 150 mg/kg, 270 mg/kg, and 470 mg/kg treatments resulting in a NOAEC of 30.7 mg/kg (Figure 4-23). Both body width and length were also significantly reduced resulting in NOAECs of 30.7 mg/kg for body width and body length (Figure 4-24). At test termination the IC25 value was 35.6 mg/kg for survival. Due to the complete mortality in four of the six copper-spiked treatments, the IC25s for body length and body width could only be estimated as >30.7 mg/kg. In this evaluation, the sub-lethal endpoints appear to be equally sensitive and are expected to be more sensitive endpoints than survival. However, the truncated dose response curve for the growth endpoints results in uncertainty in the calculation of the sub-lethal IC25s.

Lead concentrations in Test 102 ranged from 1.9 mg/kg to 1,400 mg/kg and survival at test termination ranged from 0% to 100%. The LC50 values were 820 mg/kg at Day 2, 612 mg/kg at Day 4, and 468 mg/kg at Day 10 (test termination). At test termination, survival of tadpoles was reduced in the two highest treatments resulting in a NOAEC of 250 mg/kg (Figure 4-25). Both body width and length were also significantly reduced resulting in NOAECs of 250 mg/kg for body width and body length (Figure 4-26). At test termination the IC25 values were 334 mg/kg for survival, 291 mg/kg for body width, and 320 mg/kg for body length. In this evaluation, body width was the most sensitive endpoint.

4.2.7 African clawed frog (*X. laevis*)

Three tests were initiated with African clawed frog (*X. laevis*) larvae obtained from two different vendors. None of the three tests (Tests 107, 108, and 119) met the test acceptability criteria of ≥80% survival in the laboratory controls. However, statistics were calculated for Tests 107 and 108 since some treatments met the test acceptability criteria and the spiked sediment treatments indicated a dose response curve for each test. There may be some uncertainty in the statistical results since the laboratory controls consistently exhibited less than 80% survival. Due to low survival in all treatments, no statistics were calculated for Test 119.

Copper concentrations in Test 107 ranged from 3.2 mg/kg to 500 mg/kg and survival at test termination ranged from 0% to 100%. The LC50 values were 57.7 mg/kg at Day 4 and 55.3 mg/kg at Day 10 (survival measurements were not made on Day 2). At test termination, there was complete mortality in the 500 mg/kg, 280 mg/kg, and 140 mg/kg treatments resulting in a NOAEC of 32 mg/kg (Figure 4-27). Growth was reduced in the highest four treatments relative to the control resulting in NOAECs of 32 mg/kg for both body width and body length (Figure 4-28). At test termination the IC25 value was 40.7 mg/kg for survival, 38.4 mg/kg for body width, and 42.4 mg/kg for body length. In this evaluation, body width appears to be the most sensitive endpoint.

Lead concentrations in Test 108 ranged from 1.9 mg/kg to 1,900 mg/kg and survival at test termination ranged from 0% to 90%. The LC50 values were 617 mg/kg at Day 4 and 718 mg/kg at Day 10 (survival measurements were not made on Day 2). At test termination, survival of tadpoles was reduced in the highest treatment, relative to the 50% survival observed in the laboratory control, resulting in a NOAEC of 750 mg/kg (Figure 4-29). Both body width and length were also significantly reduced in the highest test concentration resulting in NOAECs of 750 mg/kg for body width and body length (Figure 4-30). These NOAEC values should be viewed with caution due to the low survival in the laboratory control. The NOAECs would likely be 340 mg/kg if higher survival and growth were observed in the control. At test termination the IC25 values were 475 mg/kg for survival, 444 mg/kg for body width, and 447 mg/kg for body length. In this evaluation, body width and body length exhibit a very similar response and are more sensitive than the survival endpoint.

4.2.8 Western clawed frog (*X. tropicalis*)

Test 114 met the test acceptability criteria of ≥80% survival in the laboratory controls for the lead exposure. For Test 113 survival was low in all treatments so statistics were not calculated.

Lead concentrations in Test 114 ranged from 1.9 mg/kg to 1,900 mg/kg and survival at test termination ranged from 0% to 80%. The LC50 values were 226 mg/kg at Day 2, 229 mg/kg at Day 4, and 105 mg/kg at Day 10 (test termination). At test termination, survival of tadpoles was reduced in the four highest treatments resulting in a NOAEC of 77 mg/kg (Figure 4-31). Both body width and length were also significantly reduced resulting in NOAECs of 77 mg/kg for body width and body length (Figure 4-32). At test termination the IC25 values were 47.2 mg/kg for survival, 22.5 mg/kg for body width, and 24.5 mg/kg for body length. In this evaluation, body width was the most sensitive endpoint.

4.2.9 Southern leopard frog (*R. sphenocephala*)

Tests 120 and 121 met the test acceptability criteria of ≥80% survival in the laboratory controls for both the copper and lead exposures.

Copper concentrations in Test 120 ranged from 3.2 mg/kg to 500 mg/kg and survival at test termination ranged from 0% to 100%. The LC50 values were 179 mg/kg at Day 2, 159 mg/kg at Day 4, and 160 mg/kg at Day 10. At test termination, there was complete mortality in the 280 mg/kg and 500 mg/kg treatments resulting in a NOAEC of 140 mg/kg (Figure 4-33). Both body width and length were also significantly reduced resulting in NOAECs of 85 mg/kg for body width and 32 mg/kg for body length (Figure 4-34). At test termination the IC25 value was 120 mg/kg for survival. The IC25s for body length and body width were 96.8 mg/kg and 105 mg/kg, respectively. In this evaluation, body length was the most sensitive endpoint.

Lead concentrations in Test 121 ranged from 1.9 mg/kg to 1,900 mg/kg and survival at test termination ranged from 0% to 100%. The LC50 values were 1,194 mg/kg at Day 2, 654 mg/kg at Day 4, and 574 mg/kg at Day 10 (test termination). At test termination, survival of tadpoles was reduced in the two highest treatments resulting in a NOAEC of 340 mg/kg (Figure 4-35). Both

body width and length were also significantly reduced resulting in NOAECs of 140 mg/kg for body width and 340 mg/kg for body length (Figure 4-36). At test termination the IC25 values were 428 mg/kg for survival, 364 mg/kg for body width, and 375 mg/kg for body length. In this evaluation, body width was the most sensitive endpoint.

4.2.10 American toad (*B. americanus*)

Tests 122 and 123 met the test acceptability criteria of ≥80% survival in the laboratory controls for both the copper and lead exposures.

Copper concentrations in Test 122 ranged from 3.2 mg/kg to 500 mg/kg and survival at test termination ranged from 0% to 100%. The LC50 values were 75.4 mg/kg at Day 2 and 4, and 71.8 mg/kg at Day 10. At test termination, there was complete mortality in the 140 mg/kg, 280 mg/kg, and 500 mg/kg treatments and reduced survival in the 85 mg/kg treatment resulting in a NOAEC of 32 mg/kg (Figure 4-37). Both body width and length were also significantly reduced resulting in NOAECs of 85 mg/kg for body width and body length (Figure 4-38). At test termination the IC25 values were 49.4 mg/kg for survival, 37.8 mg/kg for body width, and 51.2 mg/kg for body length. In this evaluation, body width was the most sensitive endpoint.

Lead concentrations in Test 123 ranged from 1.9 mg/kg to 1,900 mg/kg and survival at test termination ranged from 3% to 100%. The LC50 values were 631 mg/kg at Day 2, 567 mg/kg at Day 4, and 512 mg/kg at Day 10. At test termination, survival of tadpoles was reduced in the two highest treatments resulting in a NOAEC of 340 mg/kg (Figure 4-39). Both body width and length were also significantly reduced resulting in NOAECs of 140 mg/kg for body width and body length (Figure 4-40). At test termination the IC25 values were 389 mg/kg for survival, 350 mg/kg for body width, and 352 mg/kg for body length. In this evaluation, body width and body length exhibit similar responses and are more sensitive than the survival endpoint.

4.2.11 American bullfrog (*R. catesbeiana*)

Test 125 exceeded the test acceptability criteria of ≥80% survival in the laboratory controls with 100% survival. However, Test 124, initiated with the same batch of organisms, only had 73.3% survival in the laboratory controls. Although the laboratory control did not meet test acceptability criteria, toxicity statistics were calculated for Test 124 because other treatments met test acceptability criteria and the spiked sediment treatments indicated a dose-response curve. There may be some uncertainty in the statistical results for Test 124 since the laboratory controls exhibited less than 80% survival

Copper concentrations in Test 124 ranged from 3.2 mg/kg to 500 mg/kg and survival at test termination ranged from 0% to 100%. The LC50 values were >500 mg/kg at Day 2 and 4, and 198 mg/kg at Day 10. At test termination, there was complete mortality in the 280 mg/kg and 500 mg/kg treatments resulting in a survival NOAEC of 140 mg/kg (Figure 4-41). Growth was also significantly reduced resulting in NOAECs of 140 mg/kg for body width and body length (Figure 4-42). At test termination the IC25 values were 167 mg/kg for survival, 153 mg/kg for body width, and 167 mg/kg for body length. In this evaluation, body width was the most sensitive endpoint.

Lead concentrations in Test 125 ranged from 1.9 mg/kg to 1,300 mg/kg and survival at test termination ranged from 0% to 100%. The LC50 values were >1300 mg/kg at Day 2 and 4, and 381 mg/kg at Day 10. At test termination, survival of tadpoles was reduced in the three highest treatments resulting in a NOAEC of 120 mg/kg (Figure 4-43). Both body width and length were also significantly reduced resulting in NOAECs of 120 mg/kg for body width and 270 mg/kg for body length (Figure 4-44). At test termination the IC25 values were 312 mg/kg for survival, 315 mg/kg for body width, and 341 mg/kg for body length. In this evaluation, survival was the most sensitive endpoint.

Table 4-1 Summary of Species Sensitivity Testing with Copper

Test #	Test Dates	Species	Control Survival (%)	Test Meets Acceptability Criteria?
COPPER TESTS				
036 [1]	Dec 9 - 19, 2005	Northern leopard frog (*R. pipiens*)	100	Yes
075	May 13 - 23, 2006	Pickerel frog (*R. palustris)*	100	Yes
076	July 1 - 11, 2006	Green frog (*R. clamitans)*	90	Yes
094	Apr 15 - 25, 2007	Wood frog (*R. sylvatica)*	100	Yes
100	May 2 - 12, 2007	Spotted salamander (*A. maculatum)*	100	Yes
101	May 10 - 20, 2007	Gray treefrog (*H. chrysoscelis)*	93.3	Yes
103	May 12 - 22, 2007	Green frog (*R. clamitans)*	100	Yes
107	July 6 - 16, 2007	African clawed frog (*X. laevis)*	76.7	No [2]
113	Sept 28 - Oct 8, 2007	Western clawed frog *(X. tropicalis)*	43.3	No [3]
120	Mar 21 – Mar 31, 2008	Southern leopard frog (*R. sphenocephala*)	96.7	Yes
122	Apr 21 – May 1, 2008	American toad (*B. americanus*)	100	Yes
124	June 27 – July 6, 2008	American bullfrog (*R. catesbeiana*)	73.3	No [2]

Test acceptability criteria = $\geq$ 80% survival in control.

1 – Testing conducted as part of laboratory validation phase of ER-0514 project.
2 – Although laboratory control did not meet test acceptability criteria, toxicity statistics were calculated because other treatments met test acceptability criteria and a dose response curve was observed. These statistical results should be used with caution.
3 – Test results excluded from statistical evaluation due to low survival in multiple treatments.

Table 4-2 Summary of Species Sensitivity Testing with Lead

Test #	Test Dates	Species	Control Survival (%)	Test Meets Acceptability Criteria?
LEAD TESTS				
039 [1]	Dec 9 - 19, 2005	Northern leopard frog (*R. pipiens*)	97.5	Yes
075	May 13 - 23, 2006	Pickerel frog (*R. palustris)*	100	Yes
077	July 1 - 11, 2006	Green frog (*R. clamitans)*	97.5	Yes
095	Apr 15 - 25, 2007	Wood frog (*R. sylvatica)*	96.7	Yes
102	May 10 - 20, 2007	Gray treefrog (*H. chrysoscelis)*	100	Yes
104	May 12 - 22, 2007	Green frog (*R. clamitans)*	96.7	Yes
108	July 6 –16, 2007	African clawed frog (*X. laevis)*	50	No [2]
114	Sept 28 - Oct 8, 2007	Western clawed frog *(X. tropicalis)*	80	Yes
119	Feb 19 – 29, 2008	African clawed frog (*X. laevis)*	36.7	No [3]
121	Mar 21 – Mar 31, 2008	Southern leopard frog (*R. sphenocephala*)	96.7	Yes
123	Apr 21 – May 1, 2008	American toad (*B. americanus*)	96.7	Yes
125	June 27 – July 6, 2008	American bullfrog (*R. catesbeiana*)	100	Yes

Test acceptability criteria = $\geq$ 80% survival in control.

1 – Tests conducted as part of laboratory validation phase of ER-0514 project.
2 – Although laboratory control did not meet test acceptability criteria, toxicity statistics were calculated because other treatments met test acceptability criteria and a dose response curve was observed. These statistical results should be used with caution.
3 – Test results excluded from statistical evaluation due to low survival in multiple treatments.

Table 4-3 Summary of LC50 Values at Various Test Durations

		LC50		
Test #	**Species**	**Day 2**	**Day 4**	**Day 10**
Copper				
036	Northern leopard frog (*R. pipiens*)	>230	>230	>230
075	Pickerel frog (*R. palustris)*	46.8	46.8	46.8
076	Green frog (*R. clamitans)*	79.9	77.8	76.8
094	Wood frog (*R. sylvatica)*	111	111	111
100	Spotted salamander (*A. maculatum)*	106	106	98.0
101	Gray treefrog (*H. chrysoscelis)*	48.0	45.3	43.8
103	Green frog (*R. clamitans)*	54.8	50.6	37.5
107	African clawed frog (*X. laevis)*	NA	57.7	55.3
120	Southern leopard frog (*R. sphenocephala*)	179	159	160
122	American toad (*B. americanus*)	75.4	75.4	71.8
124	American bullfrog (*R. catesbeiana*)	>500	>500	198
Lead				
039	Northern leopard frog (*R. pipiens*)	>1200	>1200	>1200
075	Pickerel frog (*R. palustris)*	869	763	348
077	Green frog (*R. clamitans)*	892	892	785
095	Wood frog (*R. sylvatica)*	509	446	305
102	Gray treefrog (*H. chrysoscelis)*	820	612	468
104	Green frog (*R. clamitans)*	820	799	677
108	African clawed frog (*X. laevis)*	NA	617	718
114	Western clawed frog (*X. tropicalis)*	226	229	105
121	Southern leopard frog (*R. sphenocephala*)	1194	654	574
123	American toad (*B. americanus*)	631	567	512
125	American bullfrog (*R. catesbeiana*)	>1300	>1300	381

NA – Not applicable (Counts not made on Day 2)
LC50s are based on measured copper or lead concentrations in sediment (as mg/kg) at test initiation.

Table 4-4 Summary of Test Termination Values

		Survival			Body Width			Body Length		
Test #	**Species**	**NOAEC**	**LOAEC**	**IC25**	**NOAEC**	**LOAEC**	**IC25**	**NOAEC**	**LOAEC**	**IC25**
Copper										
036	Northern leopard frog (*R. pipiens*)	230	>230	>230	21.0	87.0	95.1	21.0	87.0	108.8
075	Pickerel frog (*R. palustris)*	28.9	79.9	35.9	<28.9	28.9	>28.9	<28.9	28.9	>28.9
076	Green frog (*R. clamitans)*	79.9	150	46.2	28.9	79.9	43.5	28.9	79.9	43.7
094	Wood frog (*R. sylvatica)*	72.7	170	89.3	32.0	72.7	>72.7	32.0	72.7	>72.7
100	Spotted salamander (*A. maculatum)*	75.0	150	84.2	NA	NA	NA	75.0	>75.0	>75.0
101	Gray treefrog (*H. chrysoscelis)*	30.7	75.0	35.6	30.7	>30.7	>30.7	30.7	>30.7	>30.7
103	Green frog (*R. clamitans)*	30.7	75.0	32.6	30.7	75.0	31.6	13.0	30.7	26.7
107	African clawed frog (*X. laevis)*	32.0	85.0	40.7	32.0	85.0	38.4	32.0	85.0	42.4
120	Southern leopard frog (*R. sphenocephala*)	140	280	120	85.0	140	104.7	32.0	85.0	96.8
122	American toad (*B. americanus*)	32.0	85.0	49.4	85.0	140	37.8	85.0	140	51.2
124	American bullfrog (*R. catesbeiana*)	140	280	167	140	280	153	140	280	167
Lead										
039	Northern leopard frog (*R. pipiens*)	680	1200	1098	260	680	796	260	680	731
075	Pickerel frog (*R. palustris)*	116	298	254	116	298	188	116	298	189
077	Green frog (*R. clamitans)*	298	635	670	298	635	441	298	635	402
095	Wood frog (*R. sylvatica)*	130	340	185	130	340	189	130	340	192
102	Gray treefrog (*H. chrysoscelis)*	250	480	334	250	480	291	250	480	321
104	Green frog (*R. clamitans)*	480	1400	498	250	480	380	250	480	327
108	African clawed frog (*X. laevis)*	750	1900	475	750	>750	444	750	>750	447
114	Western clawed frog (*X. tropicalis)*	77.0	140	47.2	77.0	140	22.5	77.0	140	24.5
121	Southern leopard frog (*R. sphenocephala*)	340	750	428	140	340	364	340	750	375
123	American toad (*B. americanus*)	340	750	389	140	340	350	140	340	352
125	American bullfrog (*R. catesbeiana*)	120	270	312	120	270	315	270	570	341

NA – Not applicable (Body width not measured)
Statistical endpoints are based on measured copper or lead concentrations in sediment (as mg/kg) at test initiation.

Figure 4-1 Survival of *R. pipiens* Following 10-Day Exposure to Copper [Test 036]

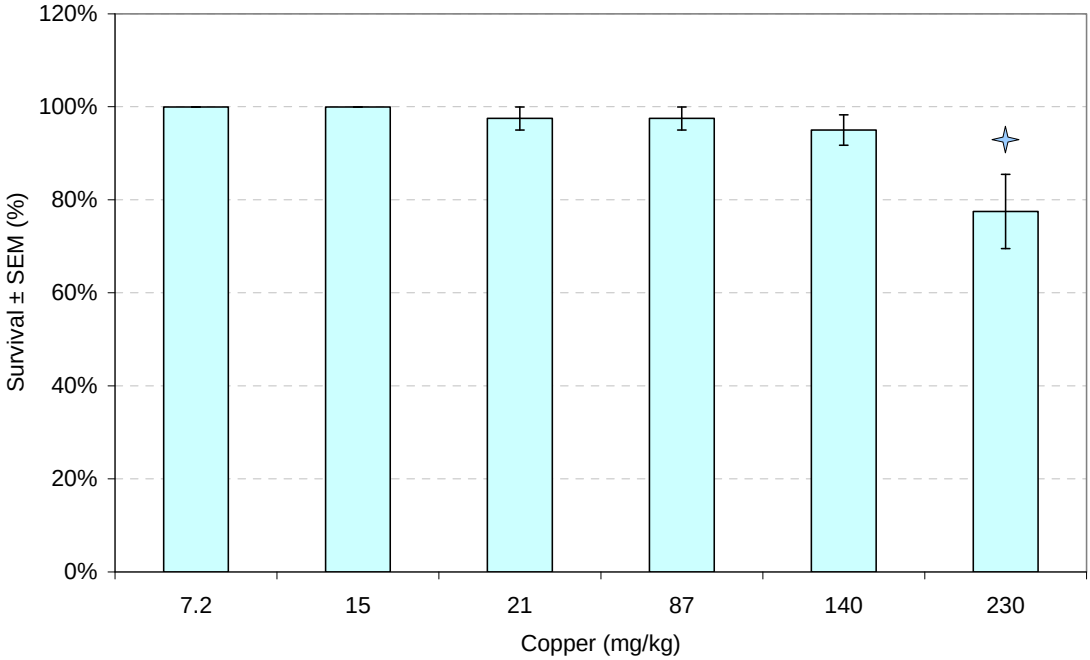
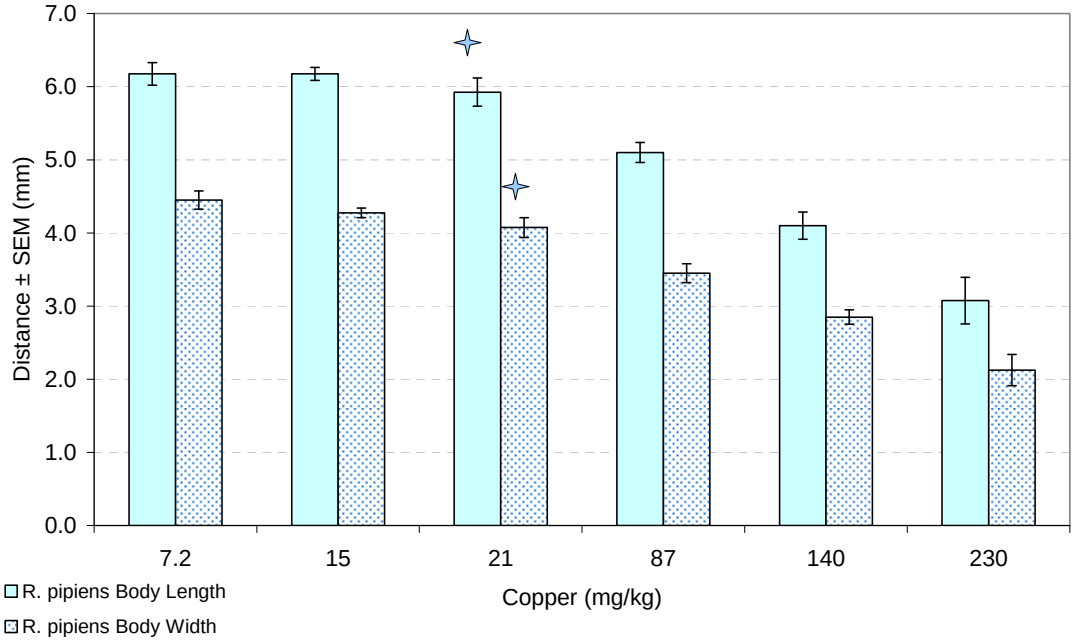


Figure 4-2 Growth of *R. pipiens* Following 10-Day Exposure to Copper [Test 036]



Bars represent mean survival and growth measurements at test termination.
Copper and lead concentrations represent measured values
SEM – Standard Error on the Mean
Body width and body length measured as distance (mm)

Error bars represent ±SEM
Indicates NOAEC

Figure 4-3 Survival of *R. pipiens* Following 10-Day Exposure to Lead [Test 039]

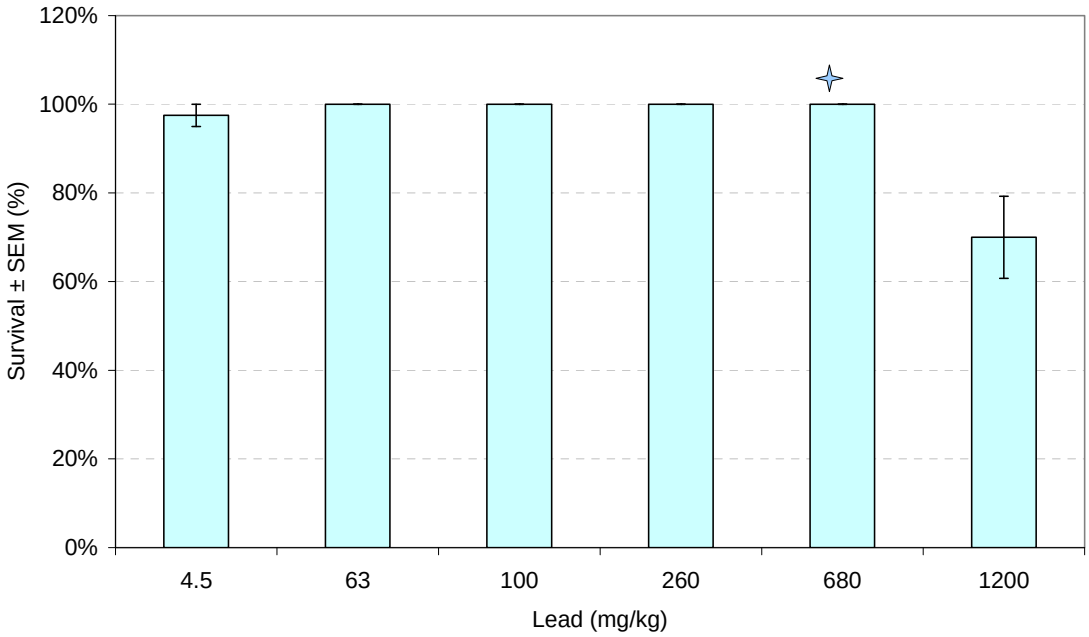
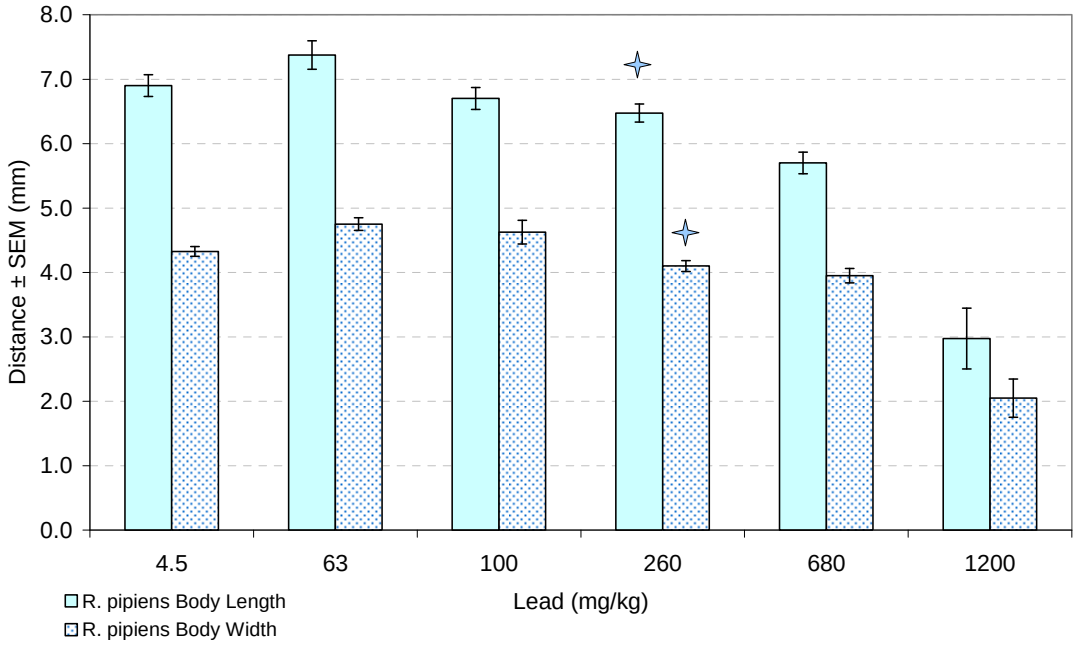


Figure 4-4 Growth of *R. pipiens* Following 10-Day Exposure to Lead [Test 039]



Bars represent mean survival and growth measurements at test termination.
Copper and lead concentrations represent measured values
SEM – Standard Error on the Mean
Body width and body length measured as distance (mm)

Error bars represent ±SEM
Indicates NOAEC

Figure 4-5 Survival of *R. palustris* Following 10-Day Exposure to Copper [Test 075]

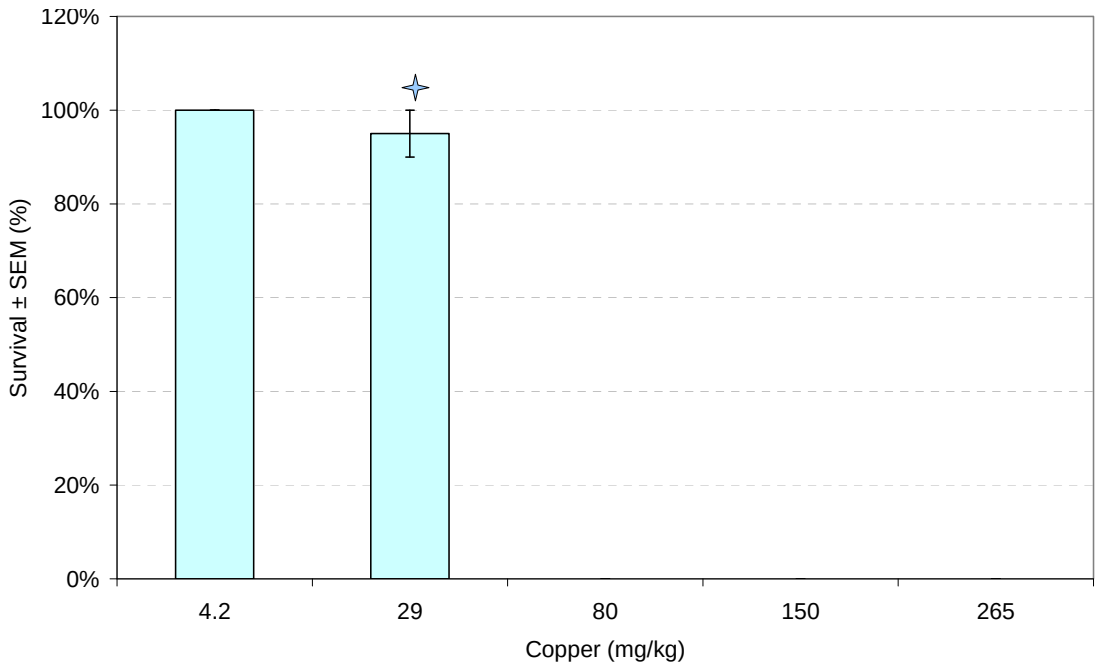
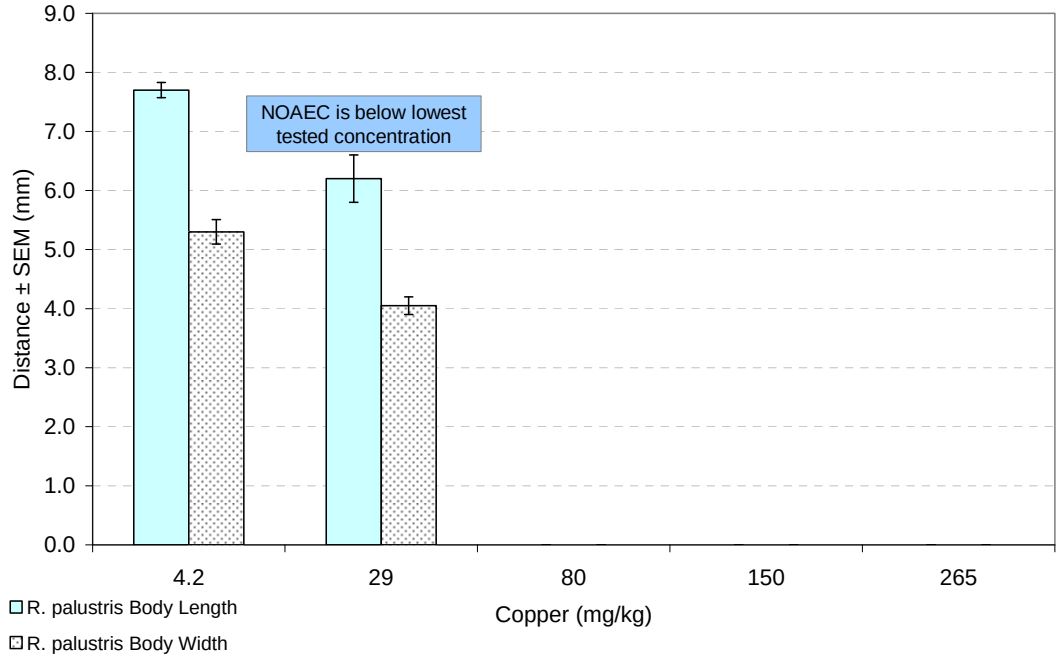


Figure 4-6 Growth of *R. palustris* Following 10-Day Exposure to Copper [Test 075]



Bars represent mean survival and growth measurements at test termination.
Copper and lead concentrations represent measured values
SEM – Standard Error on the Mean
Body width and body length measured as distance (mm)

Error bars represent ±SEM
Indicates NOAEC

Figure 4-7 Survival of *R. palustris* Following 10-Day Exposure to Lead [Test 075]

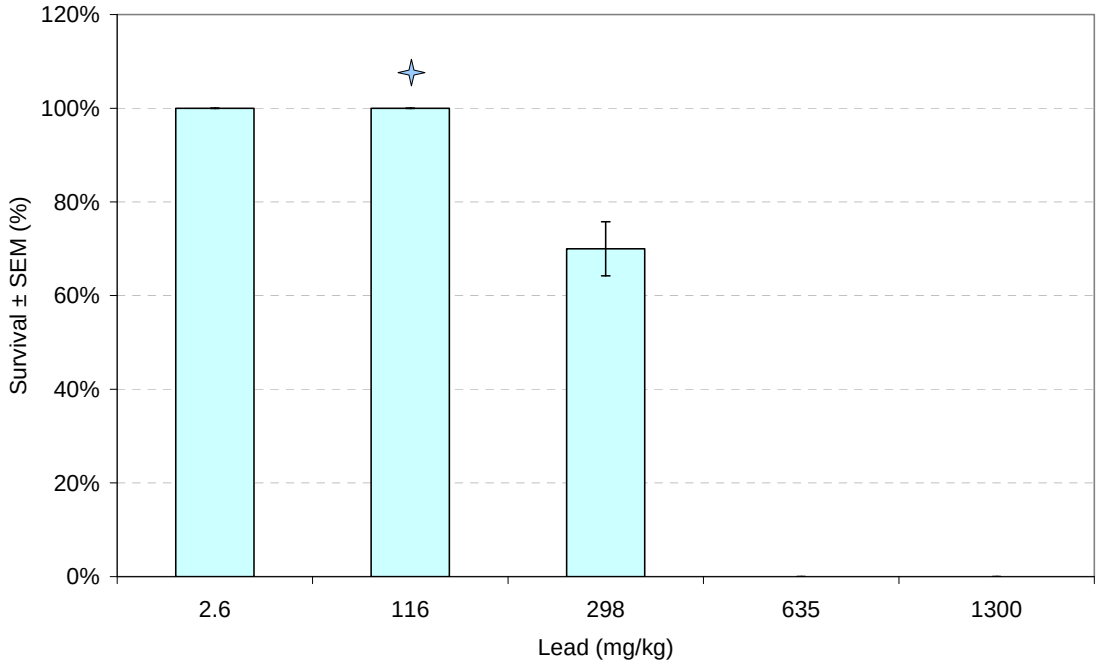
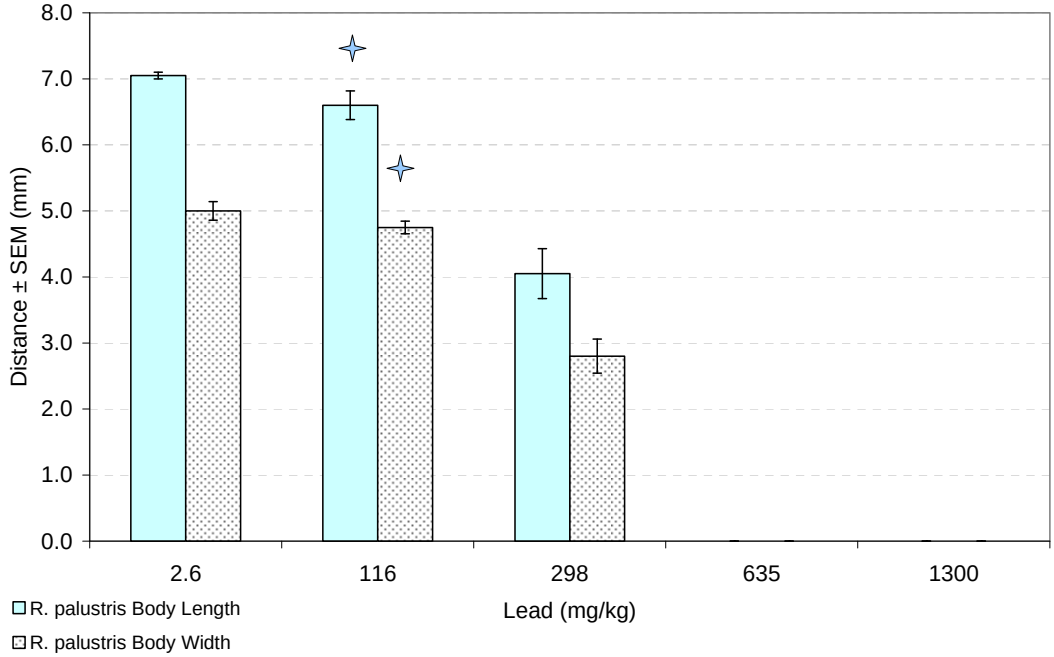


Figure 4-8 Growth of *R. palustris* Following 10-Day Exposure to Lead [Test 075]



Bars represent mean survival and growth measurements at test termination.
Copper and lead concentrations represent measured values
SEM – Standard Error on the Mean
Body width and body length measured as distance (mm)

Error bars represent ±SEM
Indicates NOAEC

Figure 4-9 Survival of *R. clamitans* Following 10-Day Exposure to Copper [Test 076]

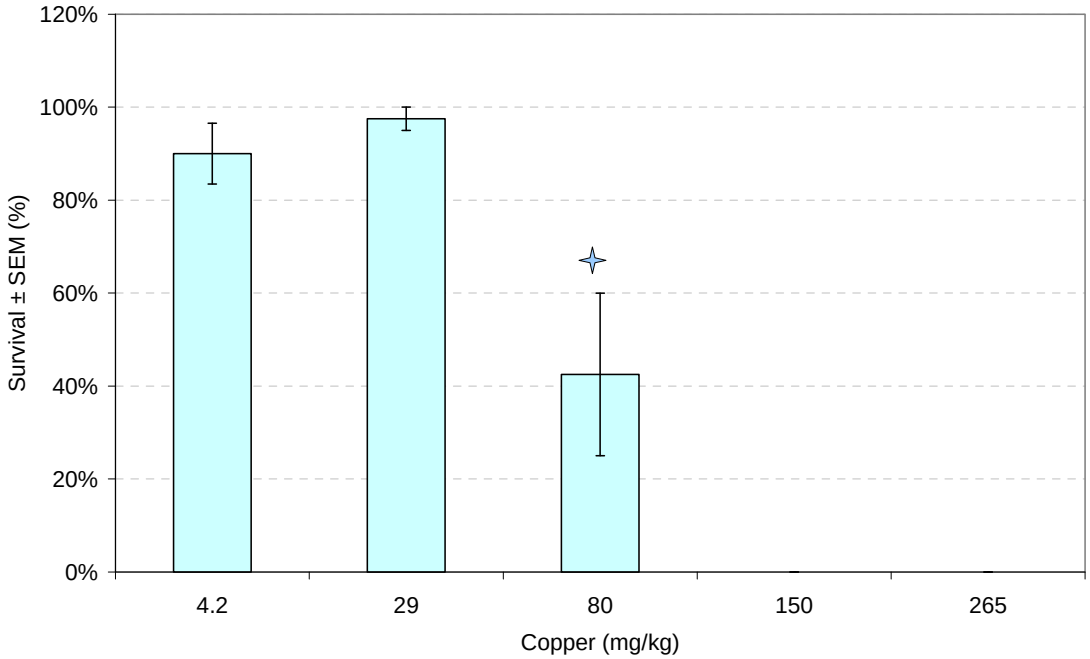
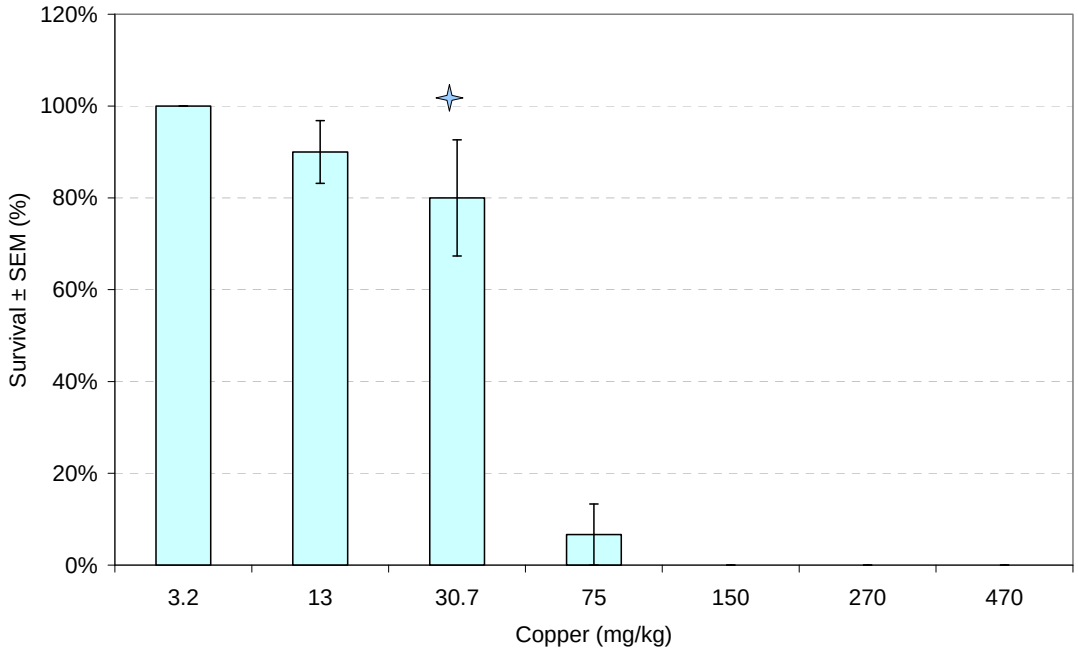


Figure 4-10 Survival of *R. clamitans* Following 10-Day Exposure to Copper [Test 103]



Bars represent mean survival and growth measurements at test termination.
Copper and lead concentrations represent measured values
SEM – Standard Error on the Mean
Body width and body length measured as distance (mm)

Error bars represent ±SEM
Indicates NOAEC

Figure 4-11 Growth of *R. clamitans* Following 10-Day Exposure to Copper [Test 076]

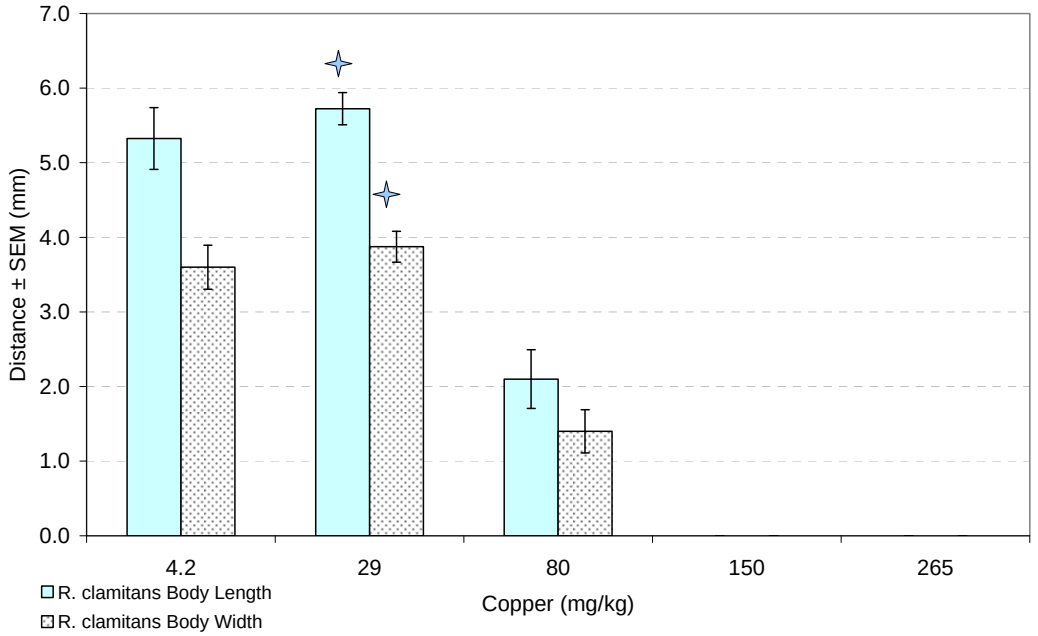
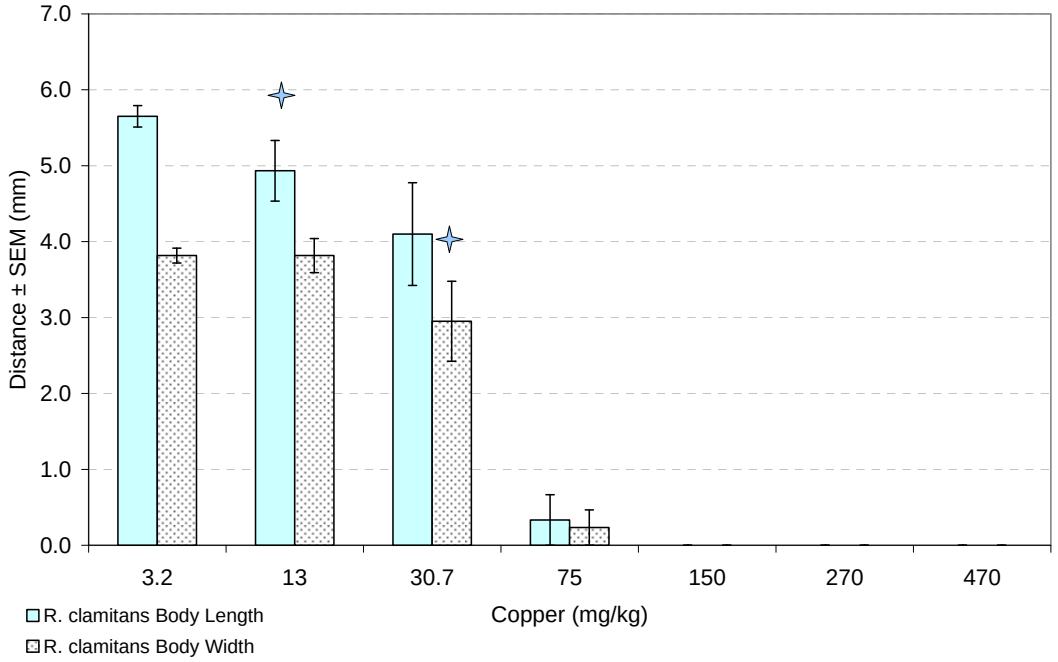


Figure 4-12 Growth of *R. clamitans* Following 10-Day Exposure to Copper [Test 103]



Bars represent mean survival and growth measurements at test termination.
Copper and lead concentrations represent measured values
SEM – Standard Error on the Mean
Body width and body length measured as distance (mm)

Error bars represent ±SEM
Indicates NOAEC

Figure 4-13 Survival of *R. clamitans* Following 10-Day Exposure to Lead [Test 077]

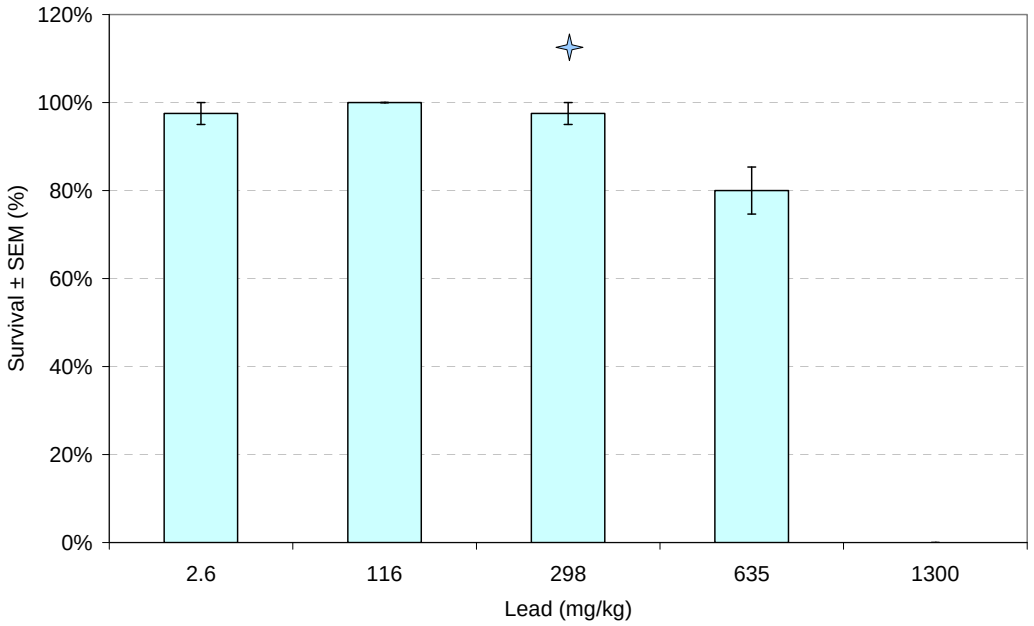
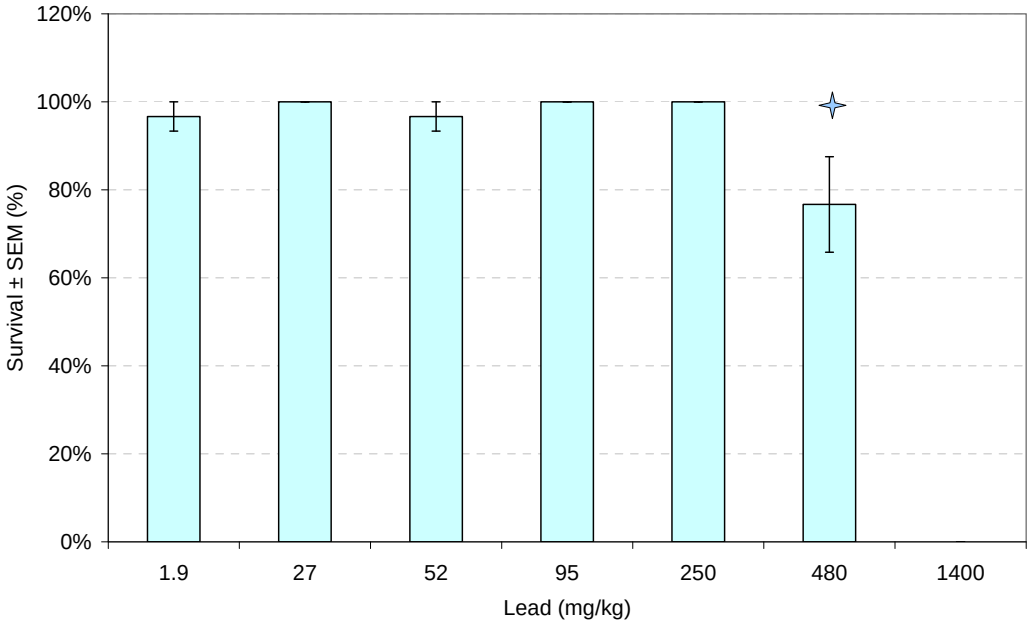


Figure 4-14 Survival of *R. clamitans* Following 10-Day Exposure to Lead [Test 104]



Bars represent mean survival and growth measurements at test termination.
Copper and lead concentrations represent measured values
SEM – Standard Error on the Mean
Body width and body length measured as distance (mm)

Error bars represent ±SEM
Indicates NOAEC

Figure 4-15 Growth of *R. clamitans* Following 10-Day Exposure to Lead [Test 077]

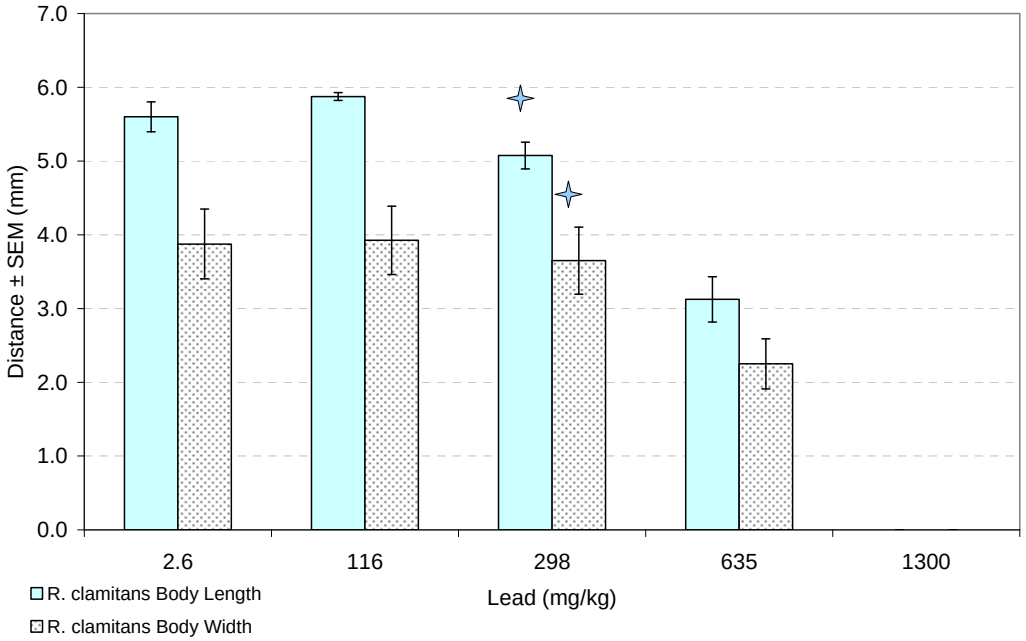
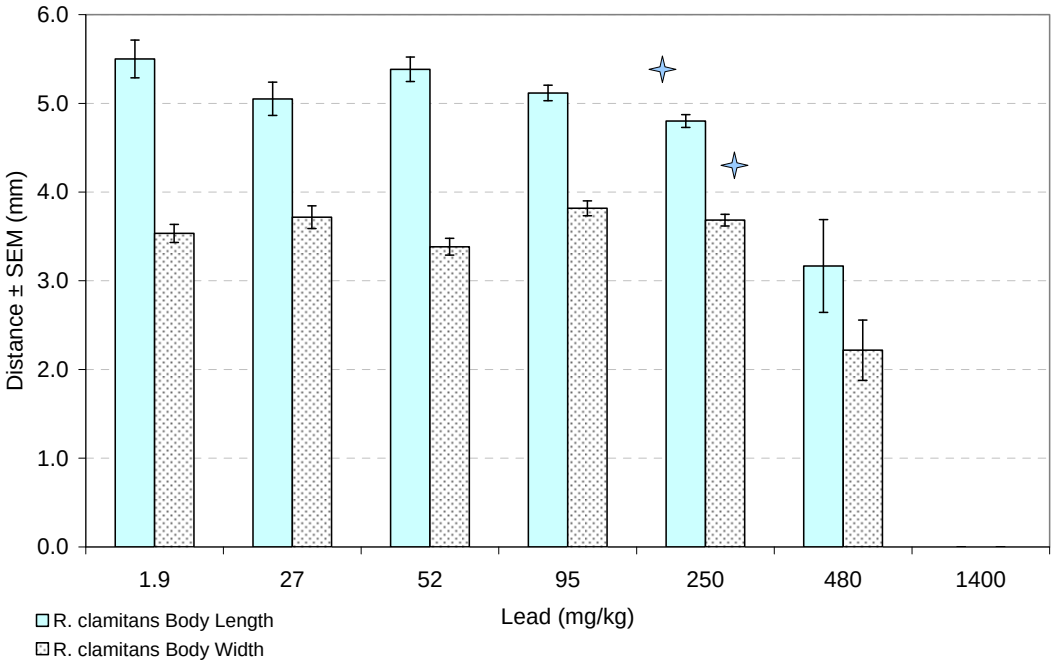


Figure 4-16 Growth of *R. clamitans* Following 10-Day Exposure to Lead [Test 104]



Bars represent mean survival and growth measurements at test termination.
Copper and lead concentrations represent measured values
SEM – Standard Error on the Mean
Body width and body length measured as distance (mm)

Error bars represent ±SEM
Indicates NOAEC

Figure 4-17 Survival of *R. sylvatica* Following 10-Day Exposure to Copper [Test 094]

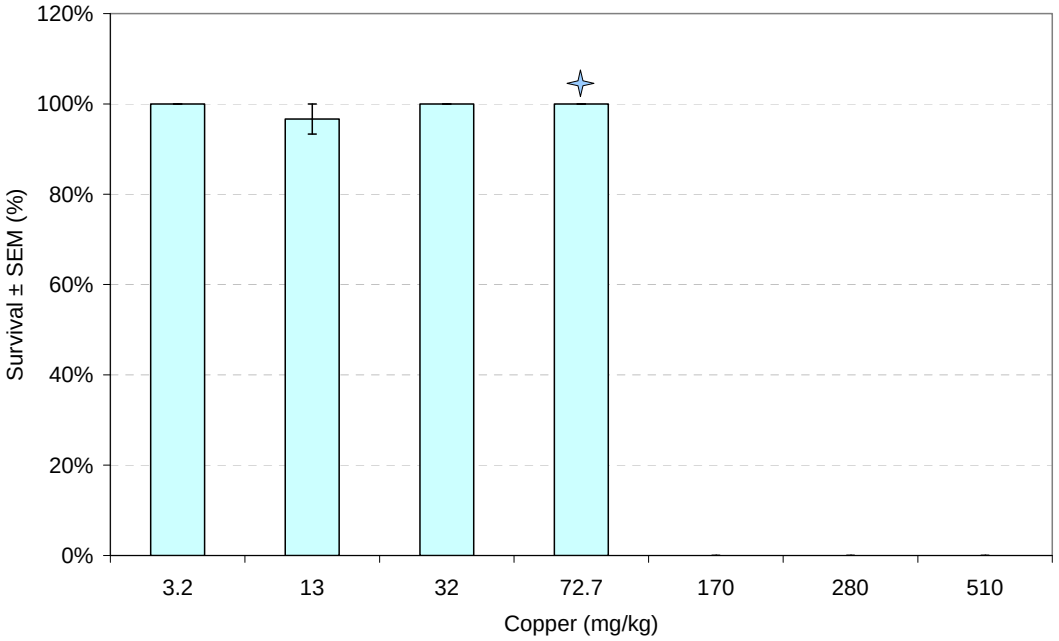
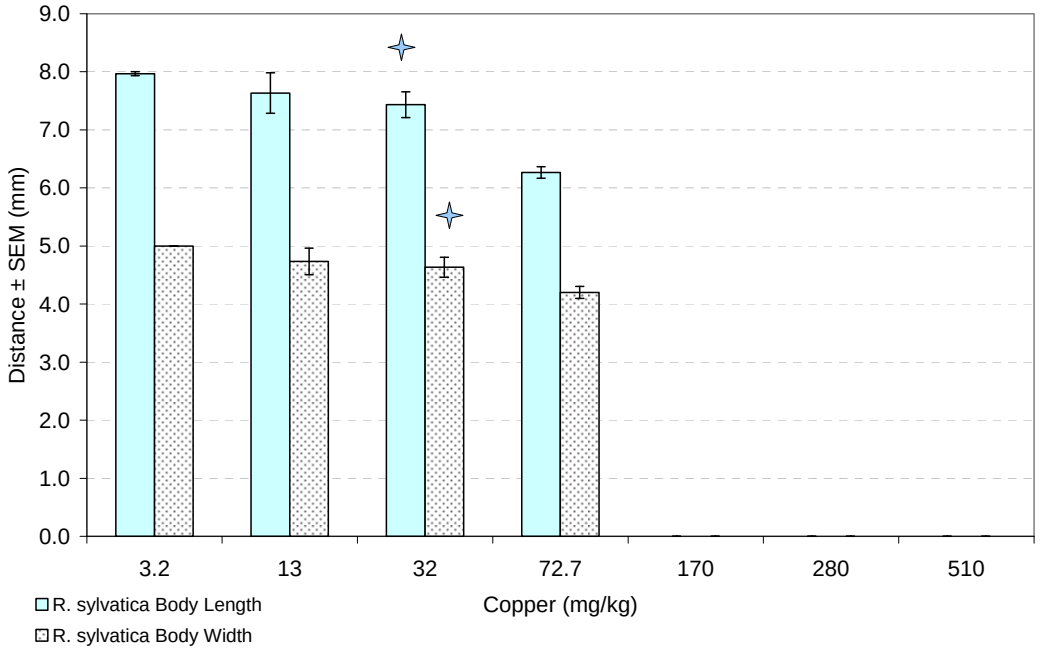


Figure 4-18 Growth of *R. sylvatica* Following 10-Day Exposure to Copper [Test 094]



Bars represent mean survival and growth measurements at test termination.
Copper and lead concentrations represent measured values
SEM – Standard Error on the Mean
Body width and body length measured as distance (mm)

Error bars represent ±SEM
Indicates NOAEC

Figure 4-19 Survival of *R. sylvatica* Following 10-Day Exposure to Lead [Test 095]

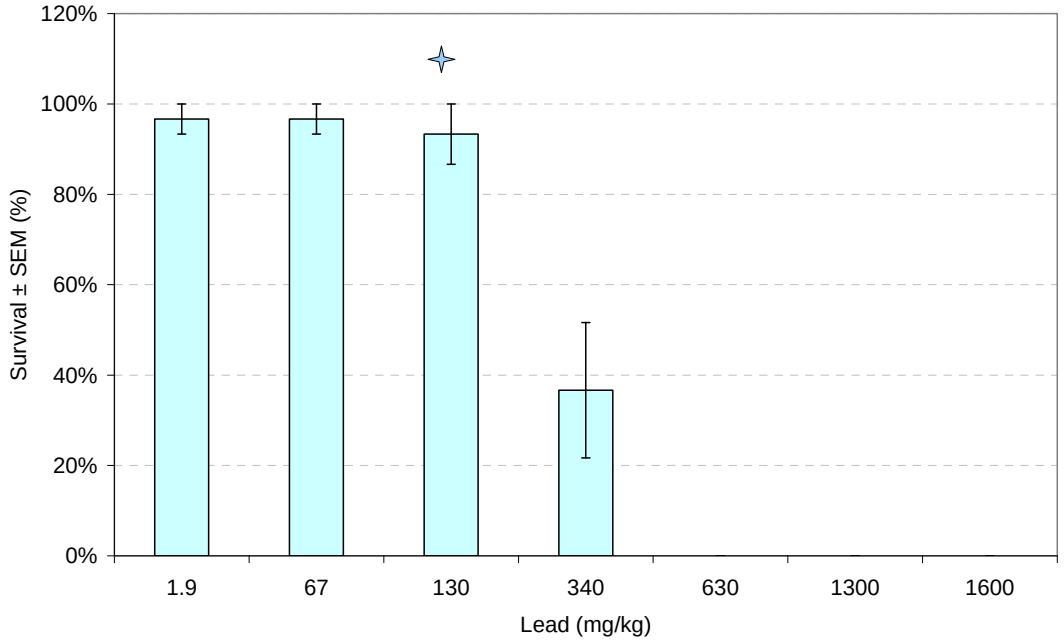
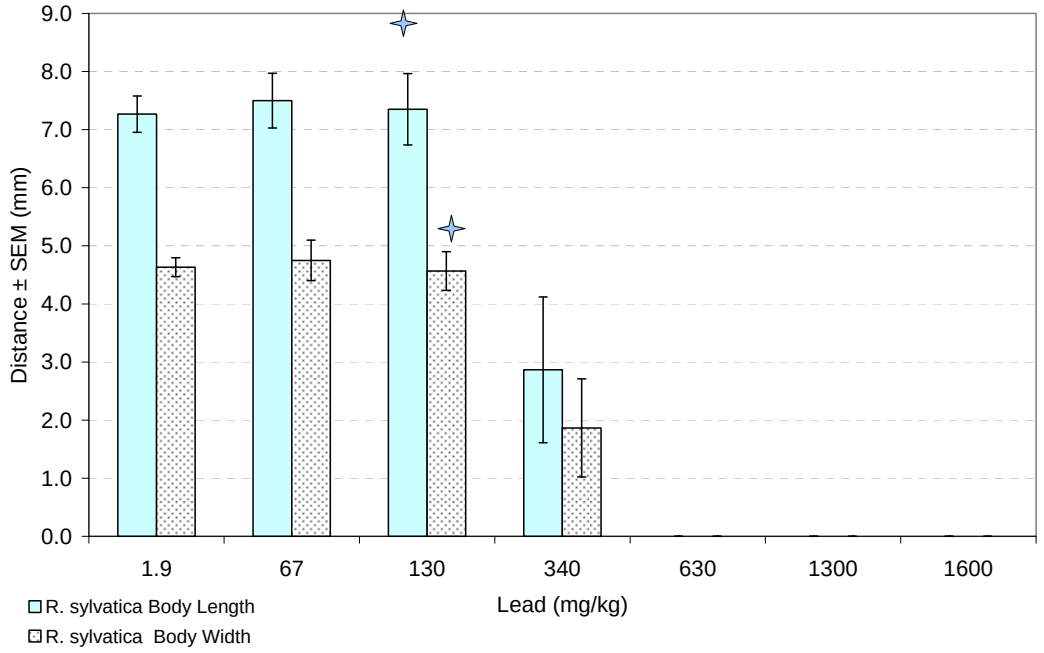


Figure 4-20 Growth of *R. sylvatica* Following 10-Day Exposure to Lead [Test 095]



Bars represent mean survival and growth measurements at test termination.
Copper and lead concentrations represent measured values
SEM – Standard Error on the Mean
Body width and body length measured as distance (mm)

Error bars represent ±SEM
Indicates NOAEC

Figure 4-21 Survival of *A. maculatum* Following 10-Day Exposure to Copper [Test 100]

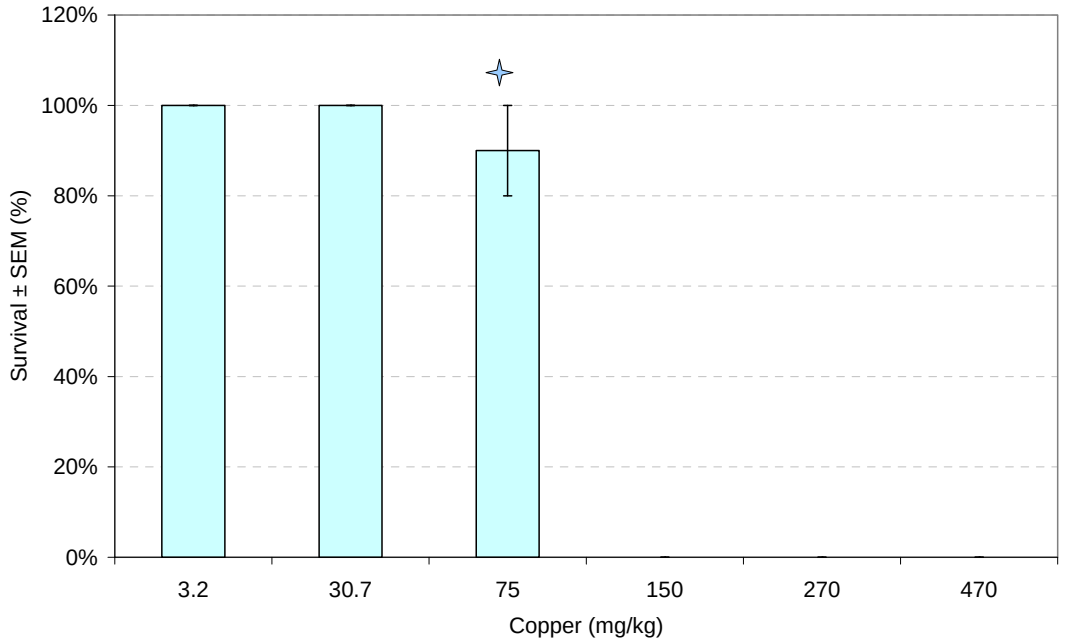
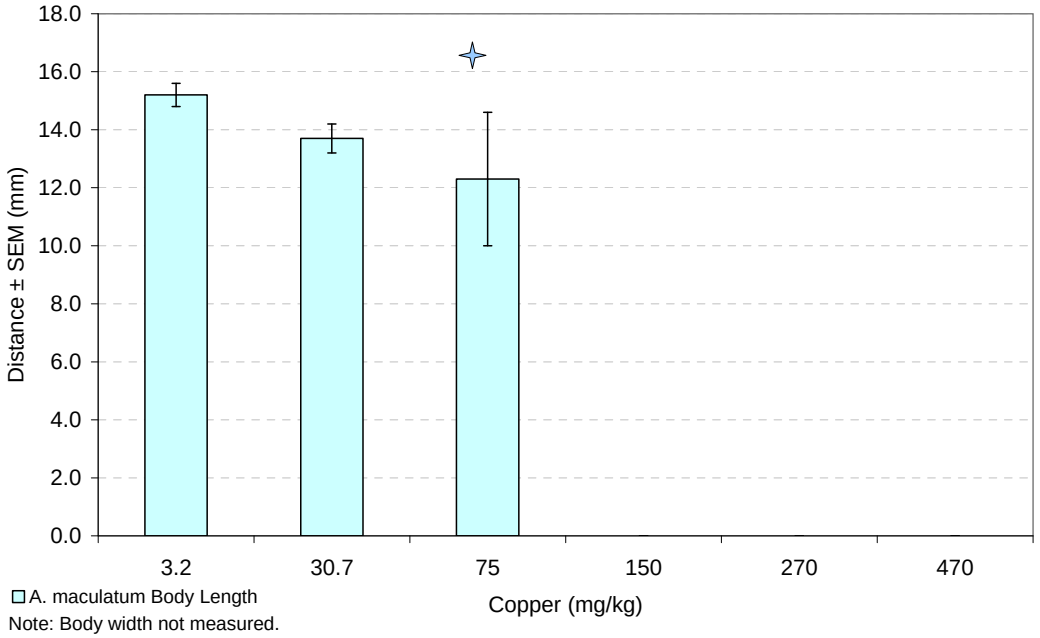


Figure 4-22 Growth of *A. maculatum* Following 10-Day Exposure to Copper [Test 100]



Bars represent mean survival and growth measurements at test termination.
Copper and lead concentrations represent measured values
SEM – Standard Error on the Mean
Body width and body length measured as distance (mm)

Error bars represent ±SEM
Indicates NOAEC

Figure 4-23 Survival of *H. chrysoscelis* Following 10-Day Exposure to Copper [Test 101]

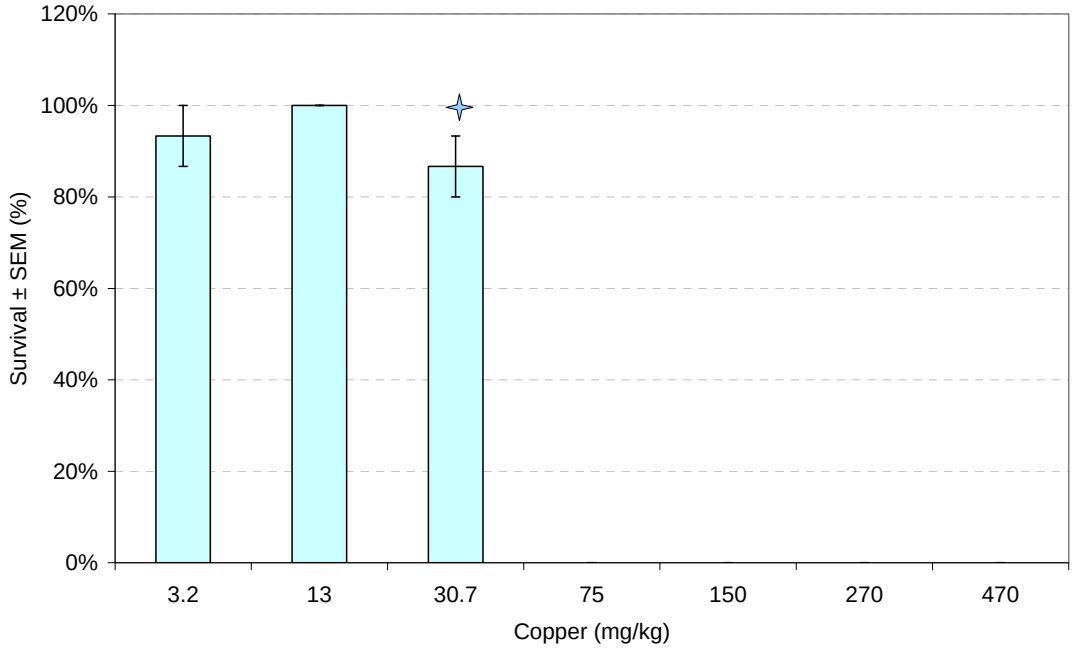
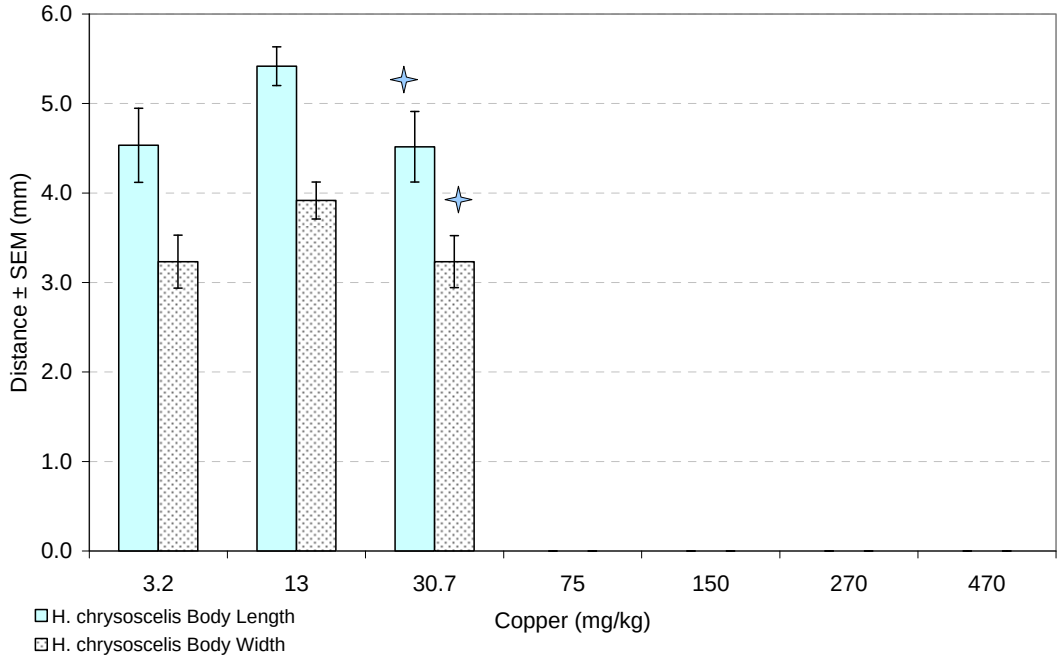


Figure 4-24 Growth of *H. chrysoscelis* Following 10-Day Exposure to Copper [Test 101]



Bars represent mean survival and growth measurements at test termination.
Copper and lead concentrations represent measured values
SEM – Standard Error on the Mean
Body width and body length measured as distance (mm)

Error bars represent ±SEM
Indicates NOAEC

Figure 4-25 Survival of *H. chrysoscelis* Following 10-Day Exposure to Lead [Test 102]

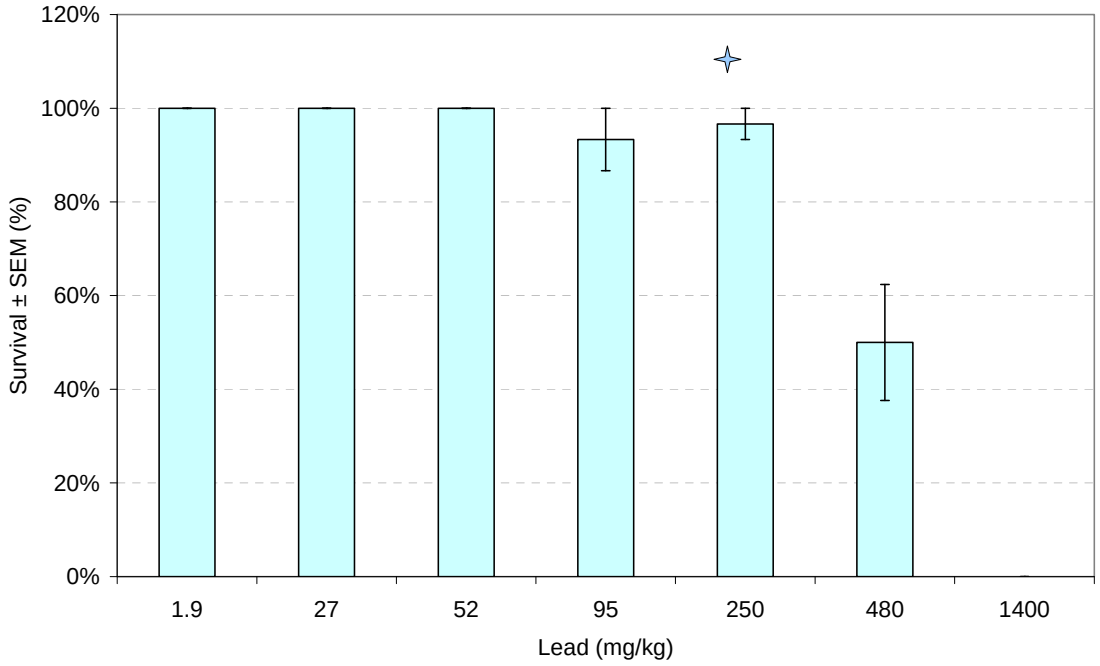
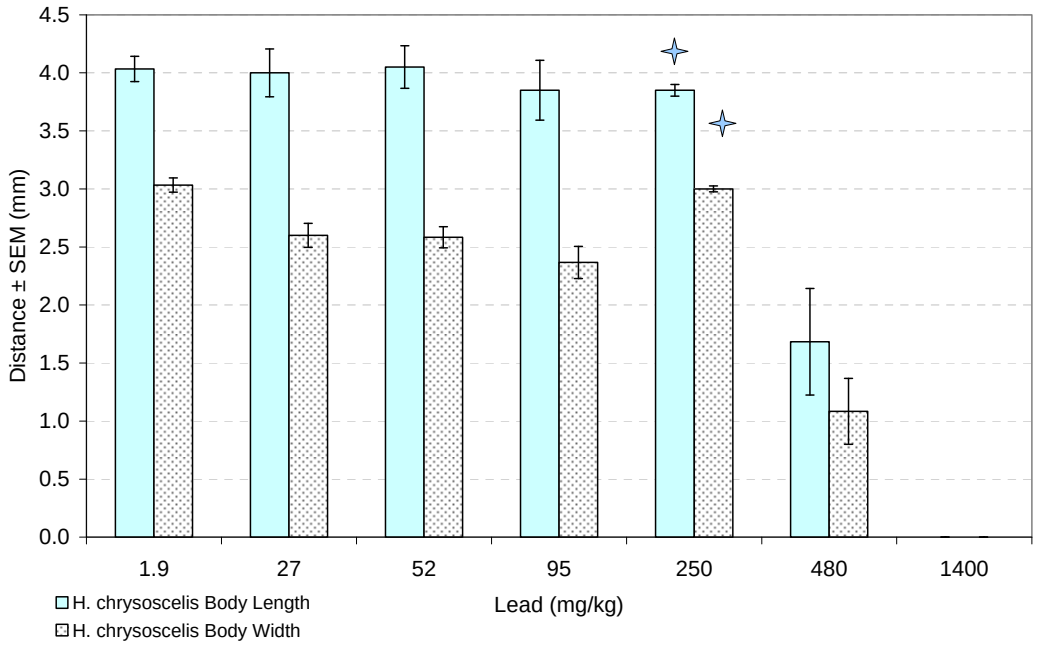


Figure 4-26 Growth of *H. chrysoscelis* Following 10-Day Exposure to Lead [Test 102]



Bars represent mean survival and growth measurements at test termination.
Copper and lead concentrations represent measured values
SEM – Standard Error on the Mean
Body width and body length measured as distance (mm)

Error bars represent ±SEM
Indicates NOAEC

Figure 4-27 Survival of *X. laevis* Following 10-Day Exposure to Copper [Test 107]

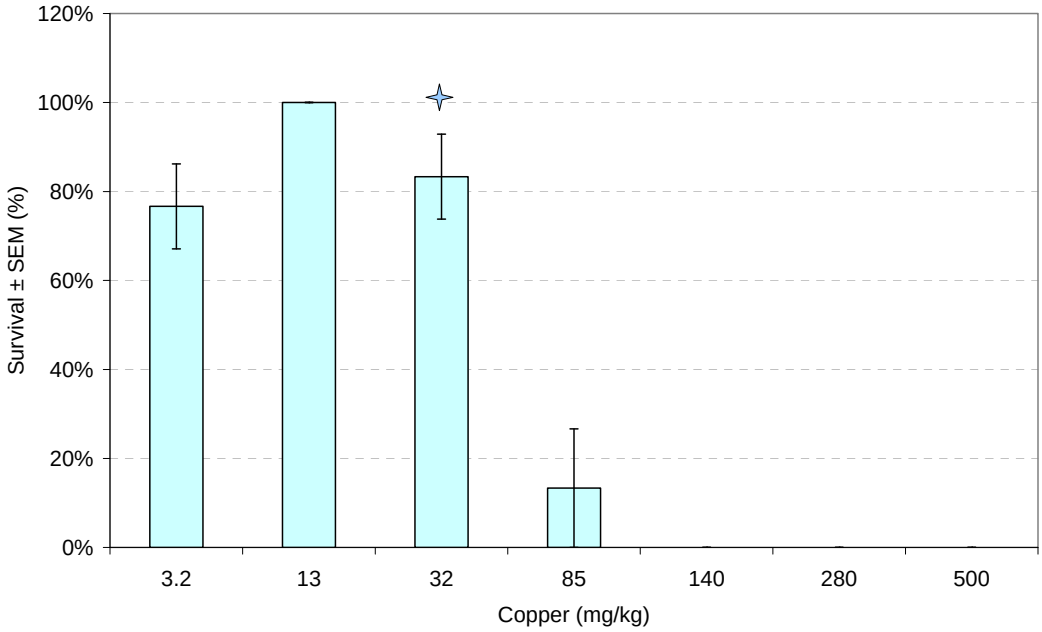
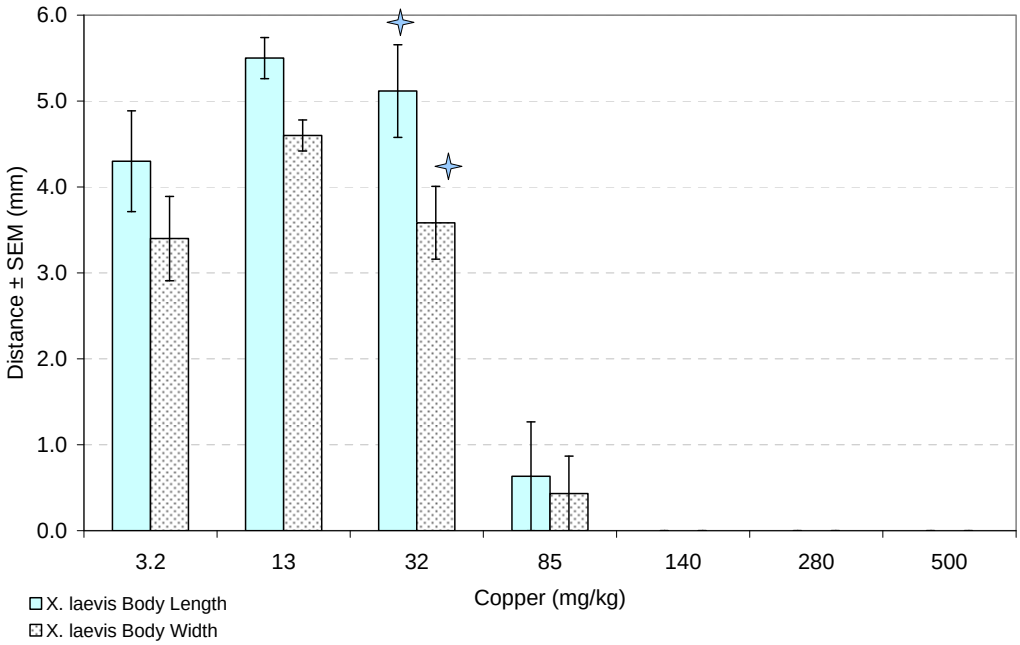


Figure 4-28 Growth of *X. laevis* Following 10-Day Exposure to Copper [Test 107]



Lab control did not meet test acceptability criteria of ≥80% survival. Results should be interpreted with caution.

Bars represent mean survival and growth measurements at test termination.
Copper and lead concentrations represent measured values
SEM – Standard Error on the Mean
Body width and body length measured as distance (mm)

Error bars represent ±SEM
Indicates NOAEC

Figure 4-29 Survival of *X. laevis* Following 10-Day Exposure to Lead [Test 108]

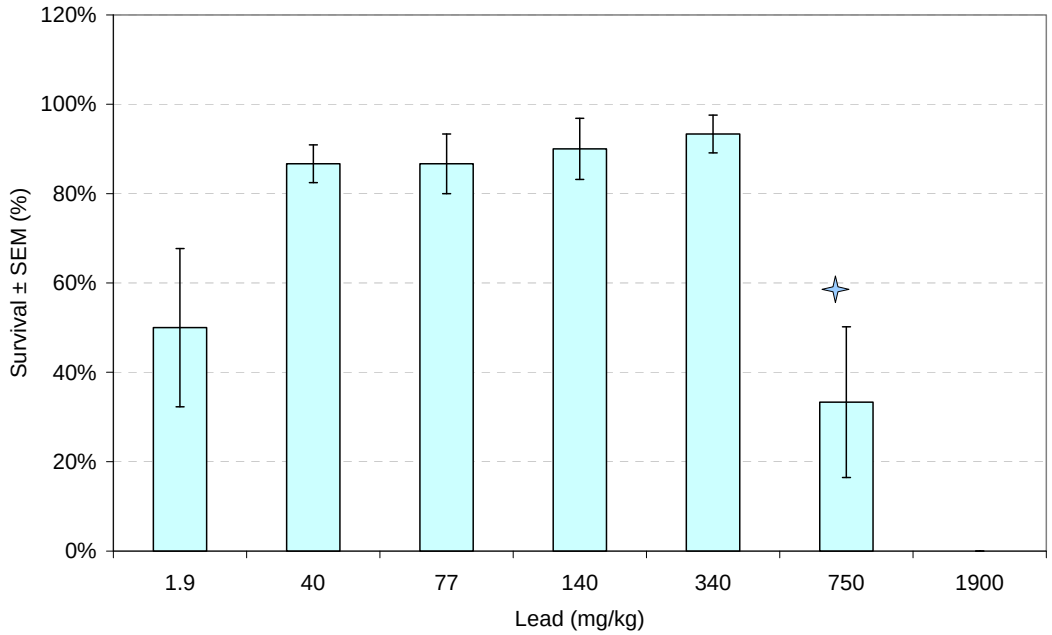
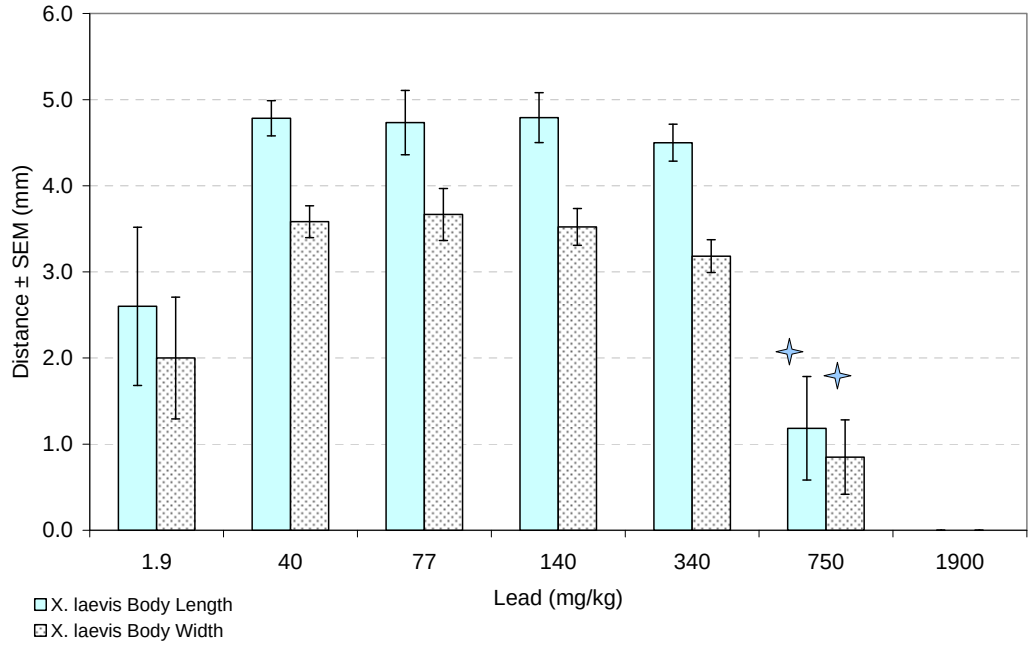


Figure 4-30 Growth of *X. laevis* Following 10-Day Exposure to Lead [Test 108]



Lab control did not meet test acceptability criteria of ≥80% survival. Results should be interpreted with caution.

Bars represent mean survival and growth measurements at test termination.
Copper and lead concentrations represent measured values
SEM – Standard Error on the Mean
Body width and body length measured as distance (mm)

Error bars represent ±SEM
Indicates NOAEC

Figure 4-31 Survival of *X. tropicalis* Following 10-Day Exposure to Lead [Test 114]

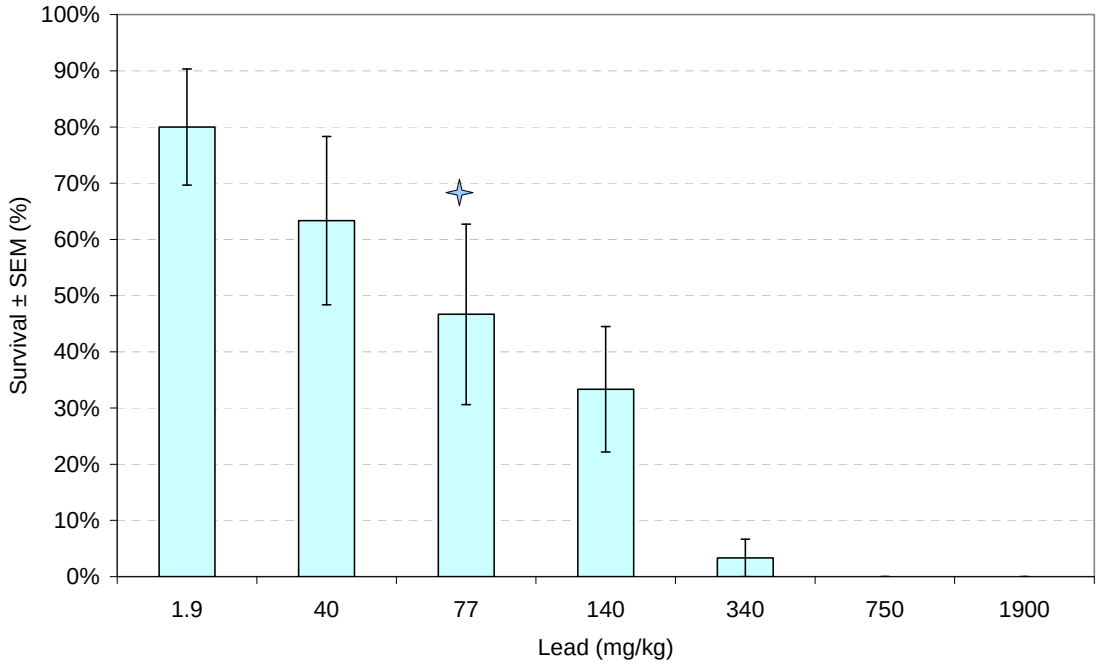
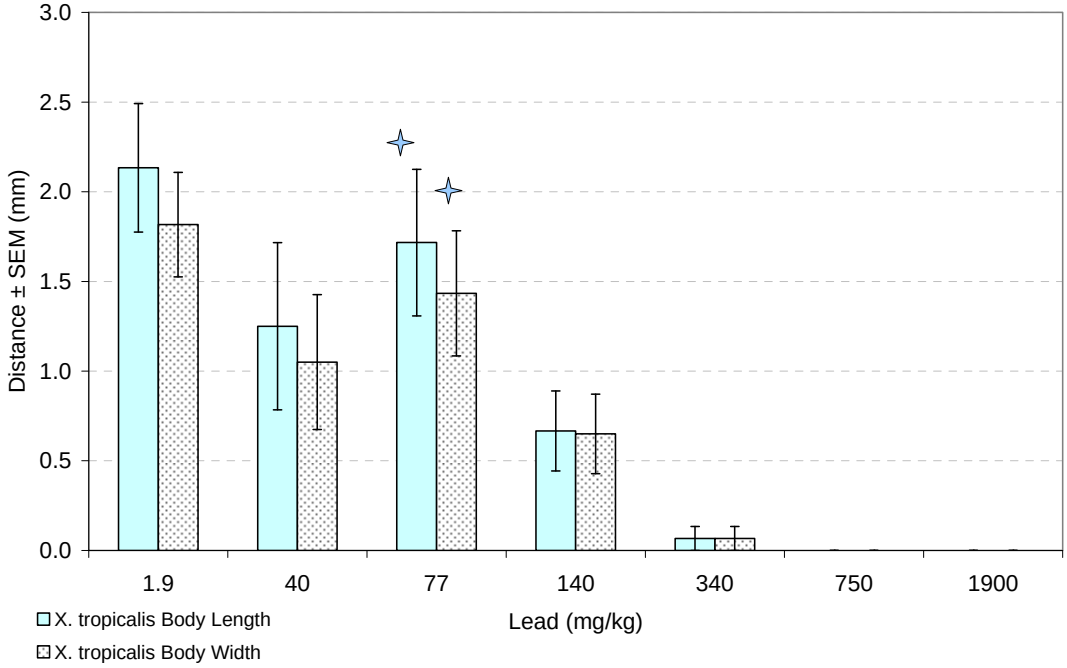


Figure 4-32 Growth of *X. tropicalis* Following 10-Day Exposure to Lead [Test 114]



Bars represent mean survival and growth measurements at test termination.
Copper and lead concentrations represent measured values
SEM – Standard Error on the Mean
Body width and body length measured as distance (mm)

Error bars represent ±SEM
Indicates NOAEC

Figure 4-33 Survival of *R. sphenocephala* Following 10-Day Exposure to Copper [Test 120]

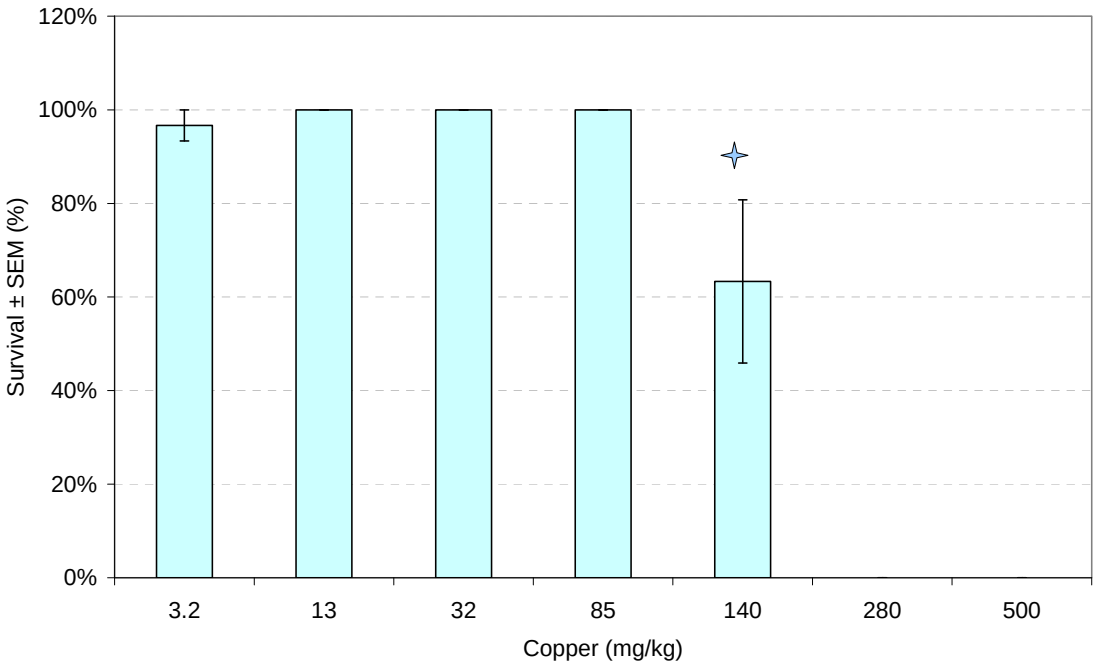
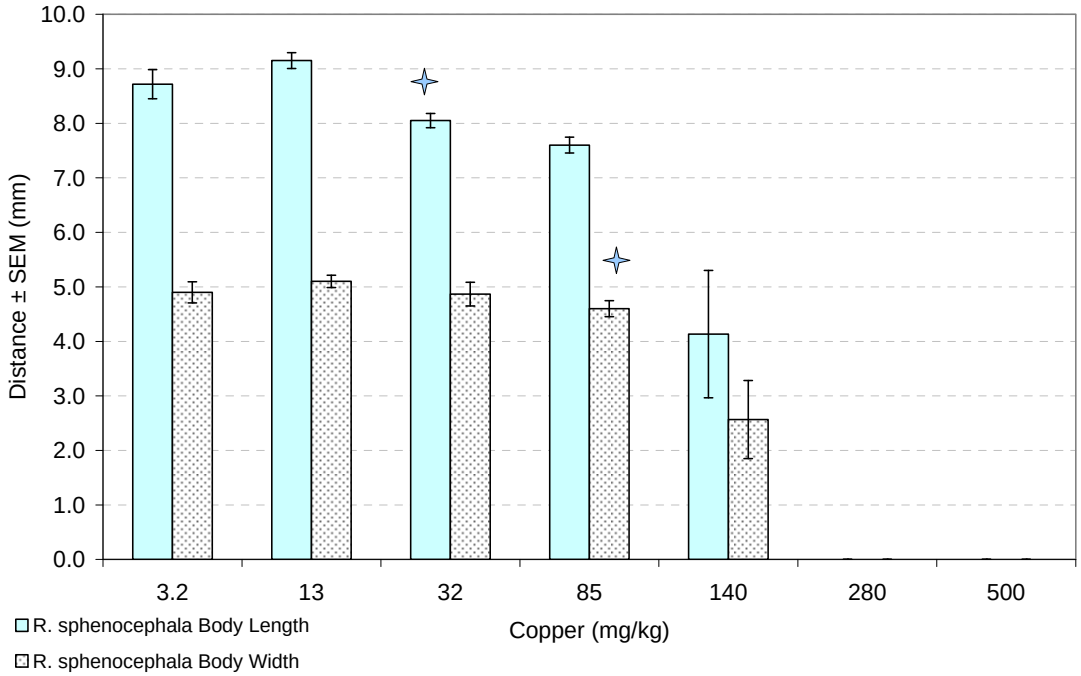


Figure 4-34 Growth of *R. sphenocephala* Following 10-Day Exposure to Copper [Test 120]



Bars represent mean survival and growth measurements at test termination.
Copper and lead concentrations represent measured values
SEM – Standard Error on the Mean
Body width and body length measured as distance (mm)

Error bars represent ±SEM
Indicates NOAEC

Figure 4-35 Survival of *R. sphenocephala* Following 10-Day Exposure to Lead [Test 121]

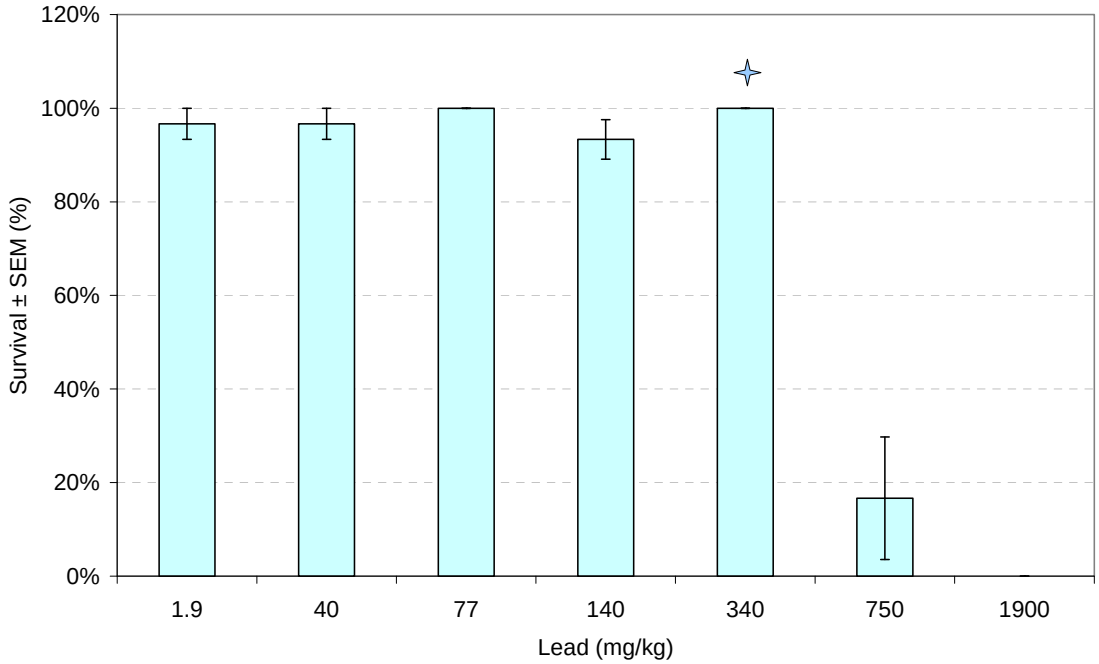
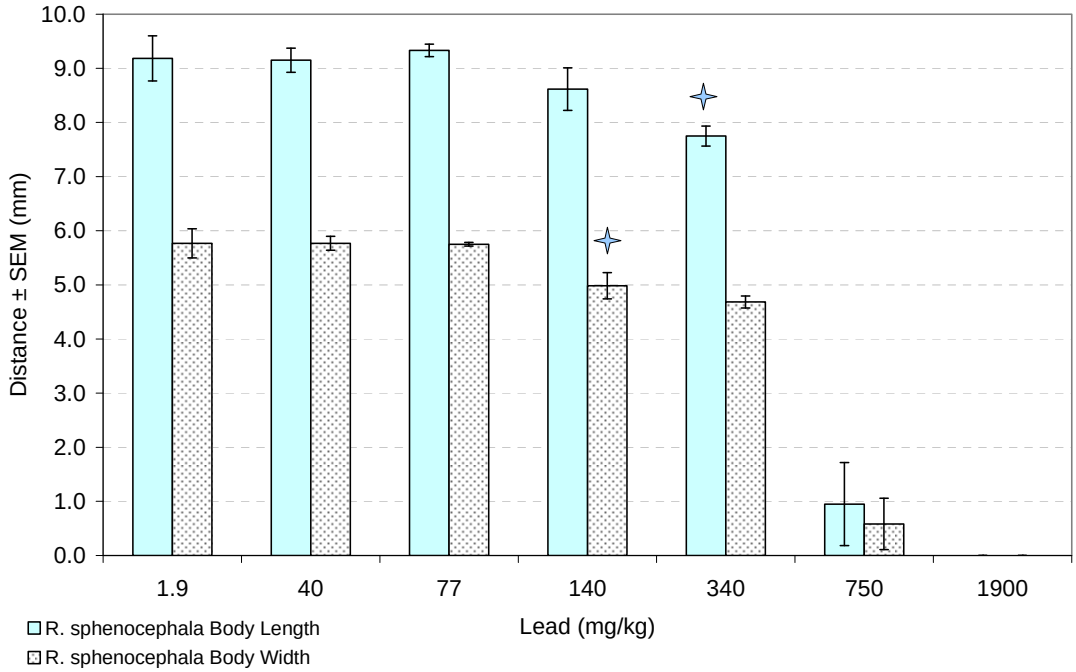


Figure 4-36 Growth of *R. sphenocephala* Following 10-Day Exposure to Lead [Test 121]



Bars represent mean survival and growth measurements at test termination.
Copper and lead concentrations represent measured values
SEM – Standard Error on the Mean
Body width and body length measured as distance (mm)

Error bars represent ±SEM
Indicates NOAEC

Figure 4-37 Survival of *B. americanus* Following 10-Day Exposure to Copper [Test 122]

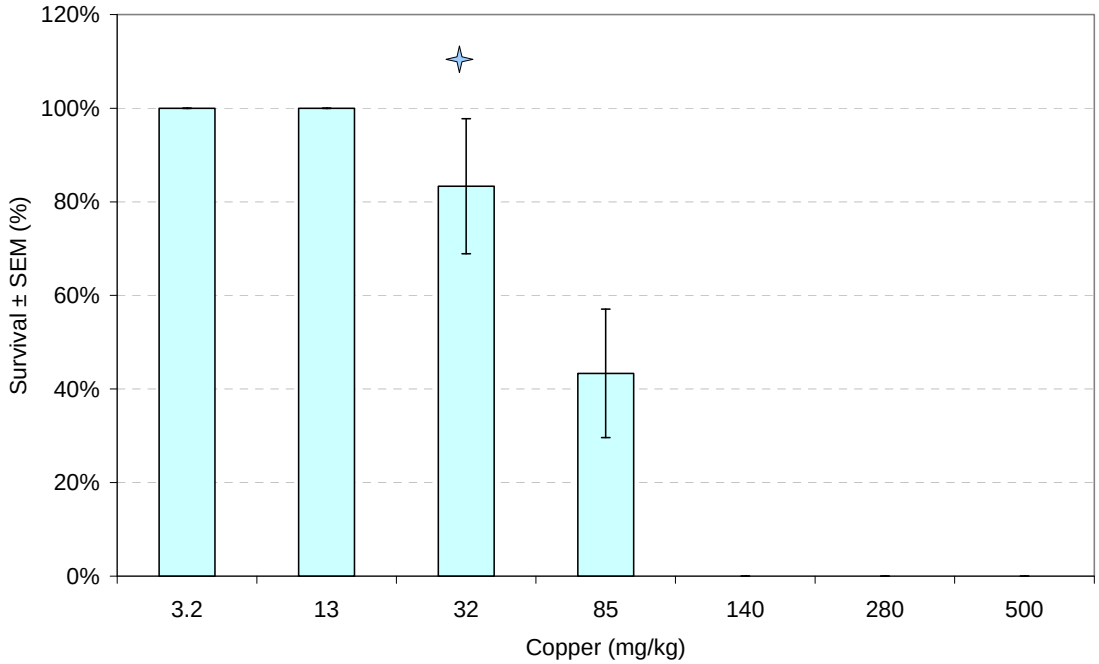
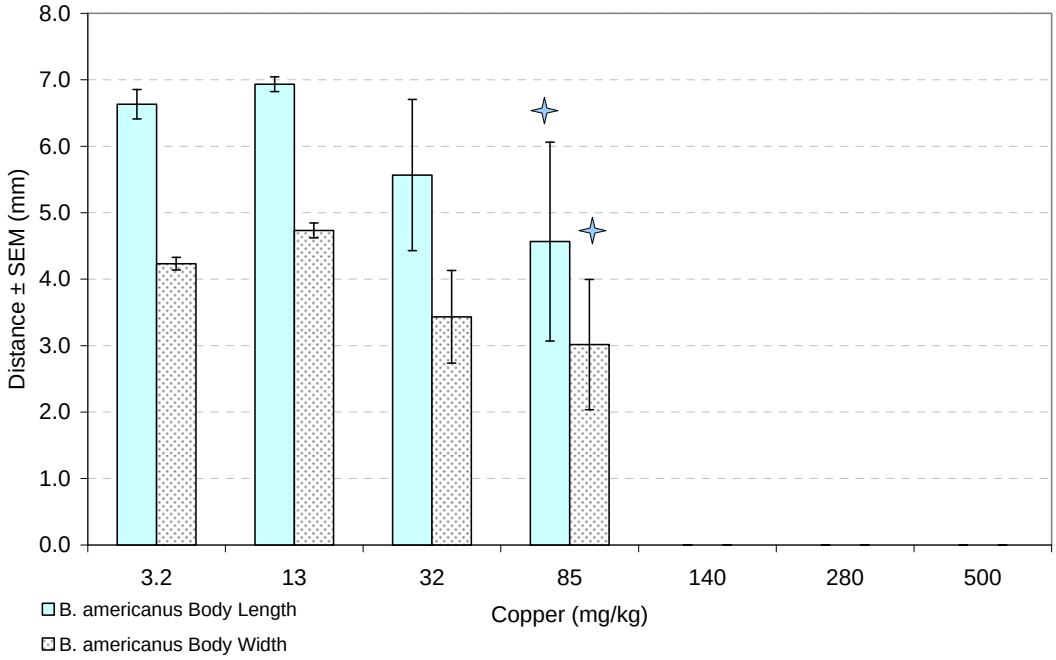


Figure 4-38 Growth of *B. americanus* Following 10-Day Exposure to Copper [Test 122]



Bars represent mean survival and growth measurements at test termination.
Copper and lead concentrations represent measured values
SEM – Standard Error on the Mean
Body width and body length measured as distance (mm)

Error bars represent ±SEM
Indicates NOAEC

Figure 4-39 Survival of *B. americanus* Following 10-Day Exposure to Lead [Test 123]

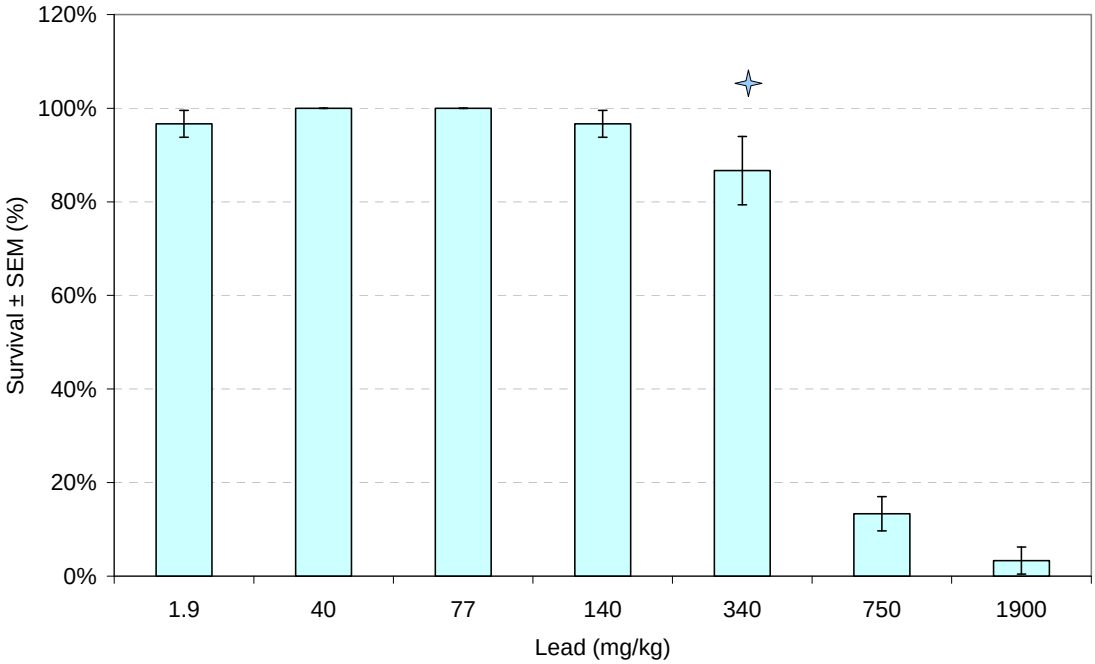
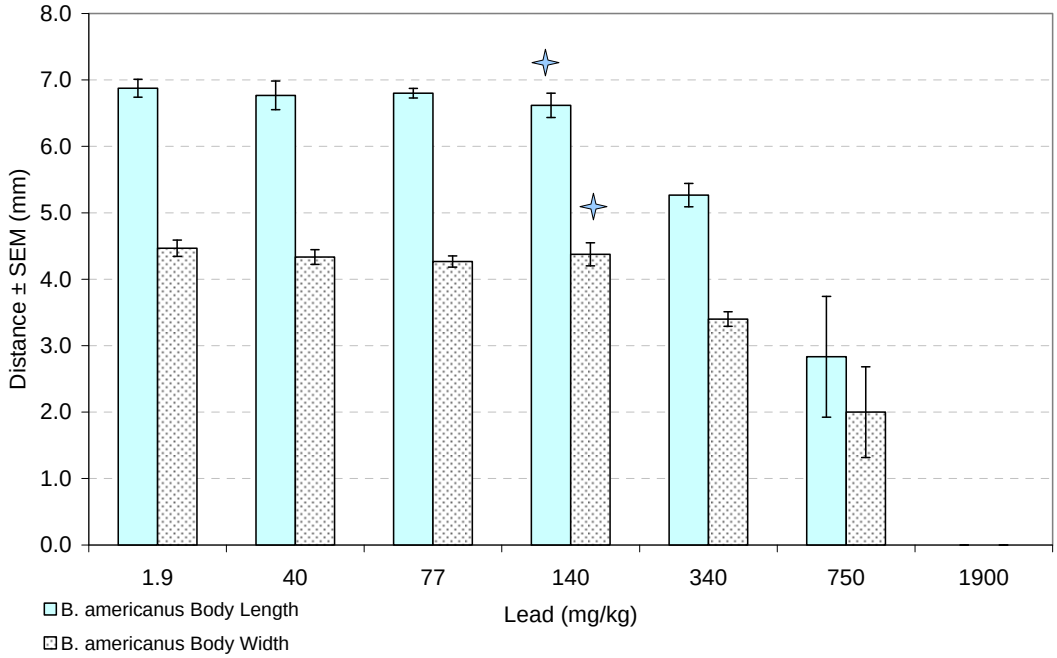


Figure 4-40 Growth of *B. americanus* Following 10-Day Exposure to Lead [Test 123]



Bars represent mean survival and growth measurements at test termination.
Copper and lead concentrations represent measured values
SEM – Standard Error on the Mean
Body width and body length measured as distance (mm)

Error bars represent ±SEM
Indicates NOAEC

Figure 4-41 Survival of *R. catesbeiana* Following 10-Day Exposure to Copper [Test 124]

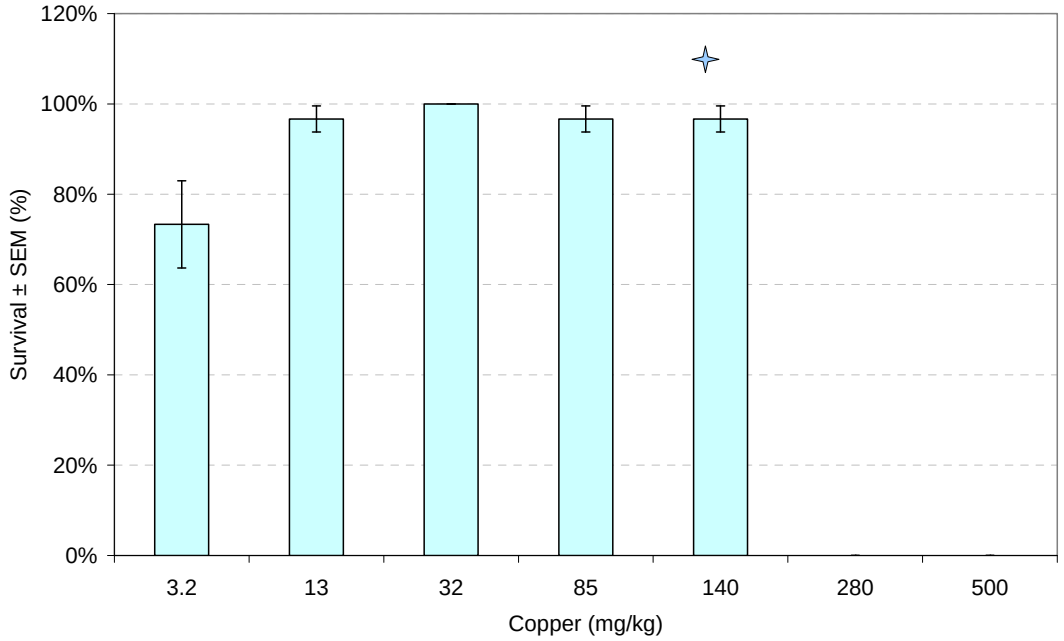
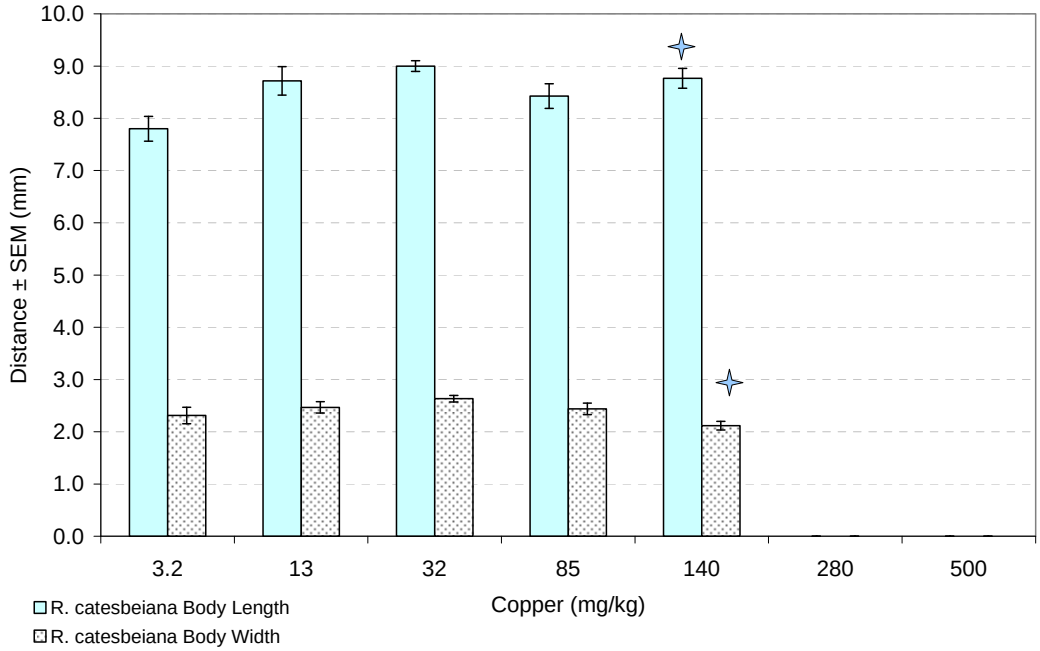


Figure 4-42 Growth of *R. catesbeiana* Following 10-Day Exposure to Copper [Test 124]



Lab control did not meet test acceptability criteria of ≥80% survival. Results should be interpreted with caution.

Bars represent mean survival and growth measurements at test termination.
Copper and lead concentrations represent measured values
SEM – Standard Error on the Mean
Body width and body length measured as distance (mm)

Error bars represent ±SEM
Indicates NOAEC

Figure 4-43 Survival of *R. catesbeiana* Following 10-Day Exposure to Lead [Test 125]

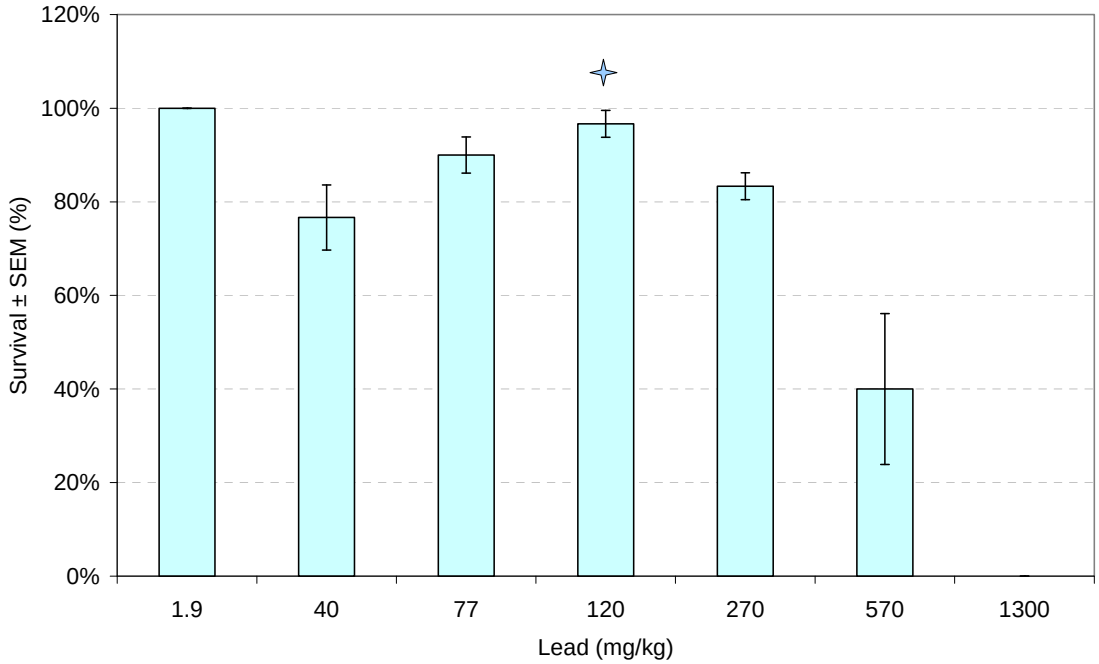
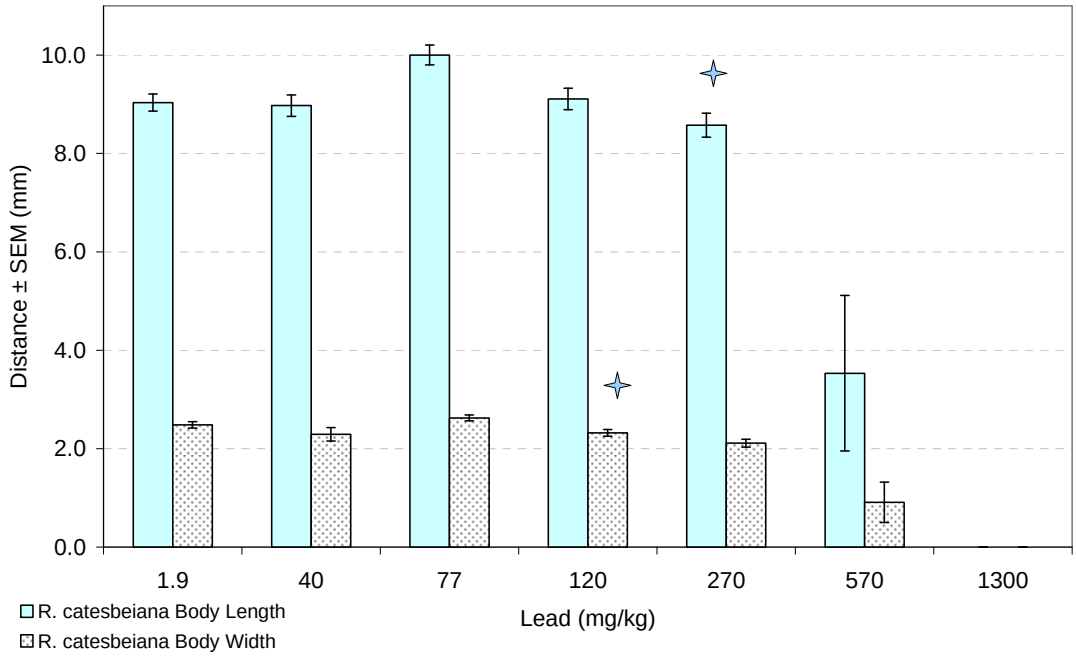


Figure 4-44 Growth of *R. catesbeiana* Following 10-Day Exposure to Lead [Test 125]



Bars represent mean survival and growth measurements at test termination.
Copper and lead concentrations represent measured values
SEM – Standard Error on the Mean
Body width and body length measured as distance (mm)

Error bars represent ±SEM
Indicates NOAEC

5.0 Discussion

Toxicity tests were conducted with 11 different species in order to investigate the differential sensitivities of larval amphibians to copper and lead. Larval amphibians were exposed to copper- and lead-spiked sediments for 10 days with survival evaluated on Days 2, 4, and 10 and body width and body length evaluated at test termination. The results of this testing show that the sediment exposure protocol can be successfully conducted with several different species and that there may be slight differences in the sensitivities of the amphibians to copper and lead.

Previous comparisons of the responses of larval amphibians to inorganic and organic stressors (Birge, et al. 2000; Hoke and Ankley, 2005) have been based on water, not sediment, exposures. Therefore, due to differences in the interaction of the test organisms with the sediment (e.g., resting on the sediment vs. swimming primarily in the water column), the responses of the test organisms in the sediment exposures would not be expected to be the same as in the water only exposures.

Birge, et al. (2000) compiled the largest data set of early life-stage amphibian toxicity data in the literature and classified 25 amphibian species as very sensitive, sensitive, moderately tolerant, or tolerant in comparison to the rainbow trout (*Oncorhynchus mykiss*). In these experiments, embryos were exposed to test chemicals in water from fertilization through 4 days posthatch (test durations ranged from 7 to 12 days due to varying species-specific developmental rates). The classifications were based on studies of 34 inorganic elements and 27 organic compounds and included 447 embryo-larval tests with amphibians and fishes. In this evaluation, the red-spotted toad (*B. punctatus)* was the most tolerant of the 21 amphibian species evaluated for their sensitivity to metals and the African clawed frog (*X. laevis)* was the most tolerant of the eight amphibian species evaluated for their sensitivity to organic chemicals (Table 5-1).

Very sensitive species included several native North American frogs such as the spring peeper (*Pseudacris crucifer*), the northern leopard frog (*R. pipiens*), and the American bullfrog (*R. catesbeiana*). The classification by Birge, et al. (2000) found that, relative to the rainbow trout, several species were highly sensitive to metals exposures in water and somewhat less sensitive to organic compounds. This was the case for the pickerel frog (*R. palustris*) which was classified as 'very sensitive' to metals but 'tolerant' of organic compounds. This evaluation indicates that high sensitivity to one chemical stressor does not necessarily translate to sensitivity to all chemical stressors.

In comparisons made to evaluate the use of Frog Embryo Teratogenesis Assay-Xenopus (FETAX) results in ecological risk assessments (Hoke and Ankley, 2005), native amphibian species such as *Rana* and *Bufo* species, were found to be more sensitive to metals, pesticides, and other organic compounds than the African clawed frog (*X. laevis).* The African clawed frog (*X. laevis)* was also less sensitive to a variety of metals than daphnids, minnows, and trout species in 96 hour water. Overall, FETAX tests using the African clawed frog (*X. laevis)* were found to be the least sensitive test in any comparison based on LC50 values, with the exception of aluminum (Hoke and Ankley, 2005).

While the results evaluated by Birge, et al. (2000) and Hoke and Ankley (2005) were based on water only exposures to metals and organic compounds, it was expected that similar results would be observed for the sediment exposure tests conducted as part of the ER-0514 project. North American species were expected to be more sensitive than the two *Xenopus* species and significant differences were expected between species. Several of the species evaluated by Birge, et al. (2000) were included in the testing conducted as part of the ER-0514 project (see Table 5-1).

As indicated in Figure 5-1, differences in the 10 day LC50 values were observed for tests conducted with each metal. According to these results, *R. clamitans* was the most sensitive species to copper exposure and *X. tropicalis* was the most sensitive species to lead exposure. However, another test conducted with *R. clamitans* resulted in a higher lead LC50 (76.8 mg/kg in Test 076 vs. 37.5 mg/kg in Test 103), suggesting some variability in organism responses from batch to batch.

Based on the results presented by Birge, et al. (2000), *X. tropicalis* and *X. laevis* were expected to be among the more tolerant species. *X. tropicalis* was the most sensitive species in all of the lead tests and *X. laevis* was more sensitive to lead and copper than several of the native North American amphibian species. However, sediment tests using *Xenopus* spp. met with recurring control performance problems and it was difficult to achieve acceptable laboratory control results with both species. Therefore, the results may be biased low due to issues with test organism health. Of the five tests initiated with a *Xenopus* species, only one met the test acceptability criteria with 80% control survival; control survival in the other four tests ranged from 37% to 77%.

It is unclear what stressors may have impacted the health of the test organisms (e.g., shipping, changes in culture water, not suited to direct sediment exposure). Hoke and Ankley (2005) noted that there are no standard approaches for culturing *X. laevis* and that water quality conditions and diet vary widely among laboratories and suppliers. Diet alone can substantially impact growth endpoints with observations that different diets may produce at least a 10-fold difference in larval dry weight (Brown and Rosati, 1997). This lack of uniform dietary requirements was one of the shortcomings that Hoke and Ankley (2005) identified when recommending that FETAX be used for specific applications and not broadly applied as an ecological risk assessment tool.

The range of LC50s displayed in Figure 5-1 is much smaller than the range of LC50s for metals presented by Birge et al (2000) or Hoke and Ankley (2005). The LC50s in Figure 5-1 ranged from 37.5 mg/kg to >230 mg/kg for copper and from 105 mg/kg to >1200 mg/kg for lead. In these tests, all results were within approximately one order of magnitude from each other. In the Birge, et al. (2000) copper data presented in Figure 5-2, the highest LC50 is 1,350 times higher than the lowest LC50. There is some uncertainty in the high end of the LC50 range for the ER-0514 sensitivity testing since both the LC50s for both copper and lead were based on the maximum tested concentration (50% mortality was not observed in those tests so the true LC50 is unknown but higher than the maximum concentration).

A comparison of the four species in common between the two data sets presented in Figure 5-2 shows some differences in the relative sensitivity of the species (from most to least sensitive):

- *R. palustris* > *R. catesbeiana* > *R. pipiens* > *A. maculatum* (Birge, et al., 2000)

- *R. palustris* > *A. maculatum* > *R. catesbeiana* > *R. pipiens* (ER-0514 testing)

With the exception of the *A. maculatum* result, the relative order of the remaining species is similar between the two data sets. Although the overall tolerance classification of amphibians by Birge, et al. (2000) (Table 5-1) based on the responses to 34 different metals may show differences from the responses in the species sensitivity testing, the results for individual metals may be more similar between the two data sets.

A review of the sub-lethal data shows a narrow range of IC25 values for body width and body length (Figures 5-3 and 5-4), relative to the wide LC50 range observed by Birge, et al (2000). For copper, the highest sub-lethal IC25 is only six times the lowest IC25; this is approximately the same difference observed in the copper LC50s. For lead, there is a wider range of growth responses than survival responses. The highest lead IC25 for body width is 36 times the lowest body width IC25, while the highest LC50 is 11 times the lowest LC50. This indicates that, for lead, the growth response is more variable than survival among species. However, the variability is still much less than observed by Birge, et al. (2000).

The data set evaluated as part of the ER-0514 species sensitivity testing was much smaller than that evaluated by Birge, et al. (2000), so additional testing with the sediment exposure protocol could result in a wider variety of responses as different batches of organisms are exposed to copper and lead in sediment. The Birge, et al. (2000) data set also included 32 inorganic compounds other than copper and lead, so other metals contribute to classifications presented in Figure 5-1.

Table 5-1 Tolerance Classification of Amphibians

Tolerance Classification [1]			
Very sensitive	**Sensitive**	**Moderately Tolerant**	**Tolerant**
<u>Metals</u> Spring peeper *(P. crucifer)* **Pickerel frog** ***(R. palustris)*** **Northern leopard frog** ***(R. pipiens)*** Eastern narrow-mouthed toad *(Gastrophryne carolinensis)* **Gray tree frog** ***(H. chrysoscelis)*** Common gray tree frog *(H. versicolor)* **American bullfrog** ***(R. catesbeiana)***	Barking tree frog *(H. gratiosa)* Squirrel tree frog *(H. squirella)* Streamside salamander *barbouri)* Blanchard's cricket frog *(Acris crepitans blanchardi)* Jefferson's salamander *(A. jeffersonianum)* Smallmouth salamander *(A. texanum)*	**Spotted salamander** ***(A. maculatum)*** River frog *(R. heckscheri)* Pig frog *(R. grylio)* Eastern tiger salamander *(A. t. tigrinum)*	Fowler's toad *(B. fowleri)* Marbled salamander *(A. opacum)* Eastern green toad *(B. debilis debilis)* Red-spotted toad *(B. punctatus)*
<u>Organics</u>	Common frog *(R. temporaria)* Northwestern salamander *(A. gracile)*	**American bullfrog** ***(R. catesbeiana)*** **Northern leopard frog** ***(R. pipiens)***	Fowler's toad *(B. fowleri)* **Pickerel frog** ***(R. palustris)*** American toad (*B. americanus)* **African clawed frog** ***(X. laevis)***

1 - Tolerance classifications assigned by Birge et al. (2000) based on geometric mean of amphibian LC50 values relative to rainbow trout LC50 values.
Boldface indicates species evaluated as part of ER-0514 species sensitivity testing.

Figure 5-1 LC50 Values Following 10 Day Exposure to Copper or Lead

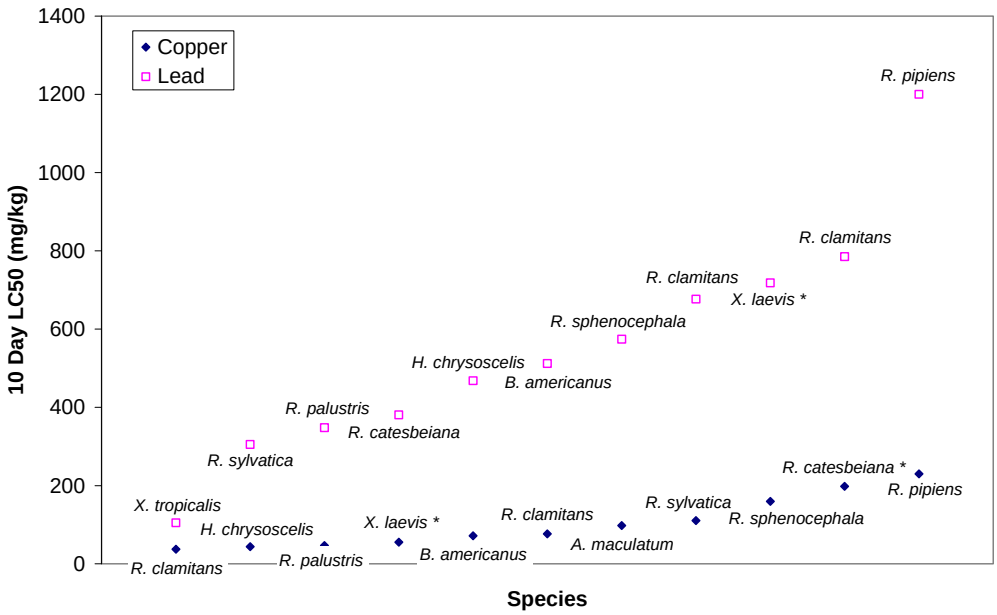
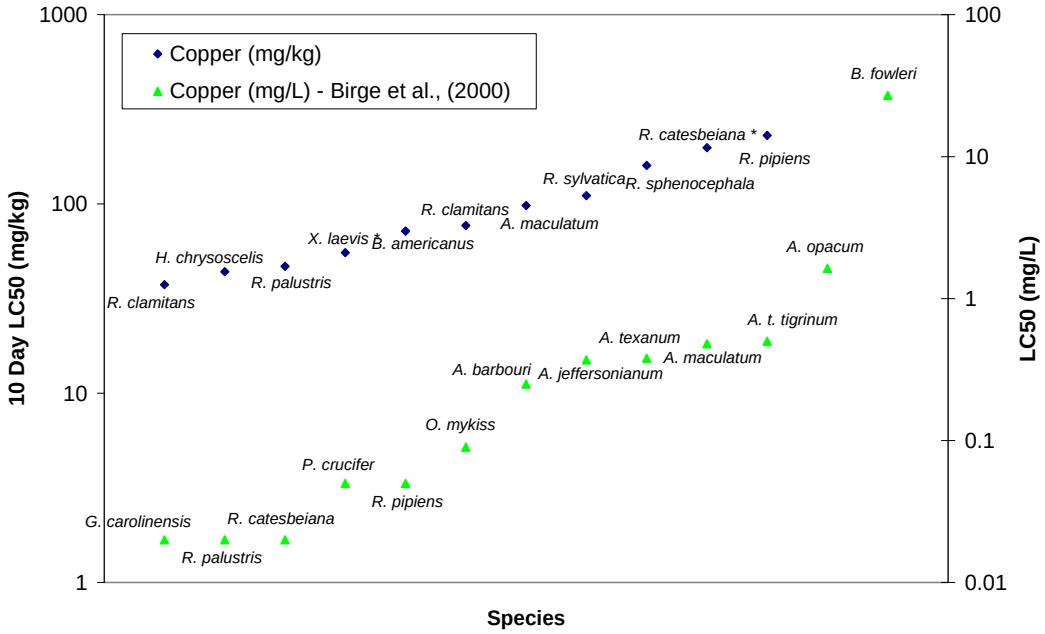


Figure 5-2 Comparison of Copper LC50 Values from Birge, et al. (2000) and ER-0514 Testing



* - Results should be interpreted with caution. Although laboratory control did not meet test acceptability criteria, toxicity statistics were calculated because other treatments met test acceptability criteria and a dose response curve was observed.

Figure 5-3 Body Width IC25 Values Following 10 Day Exposure to Copper or Lead

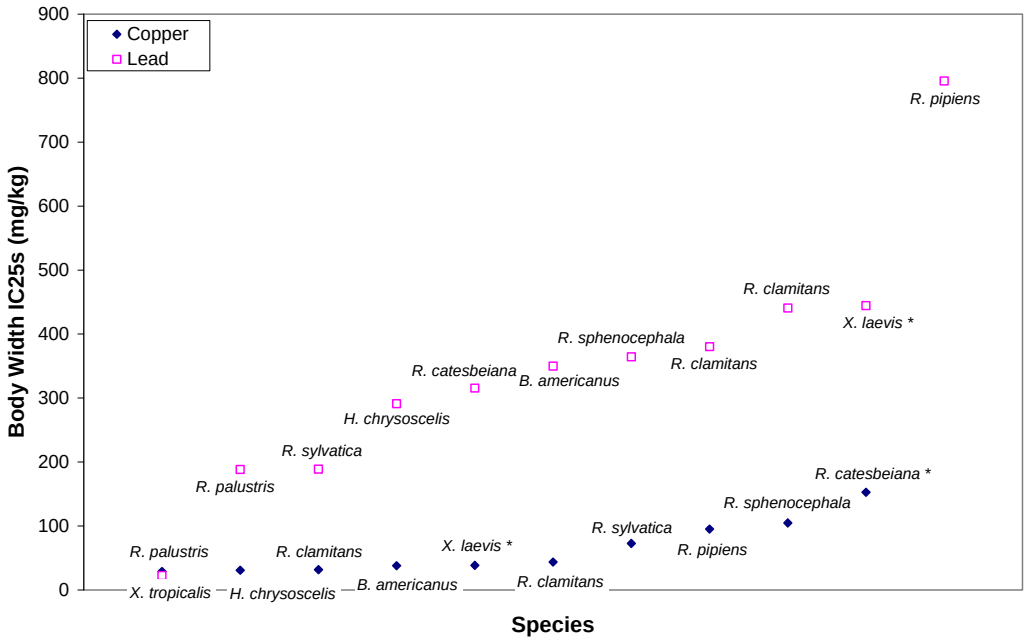
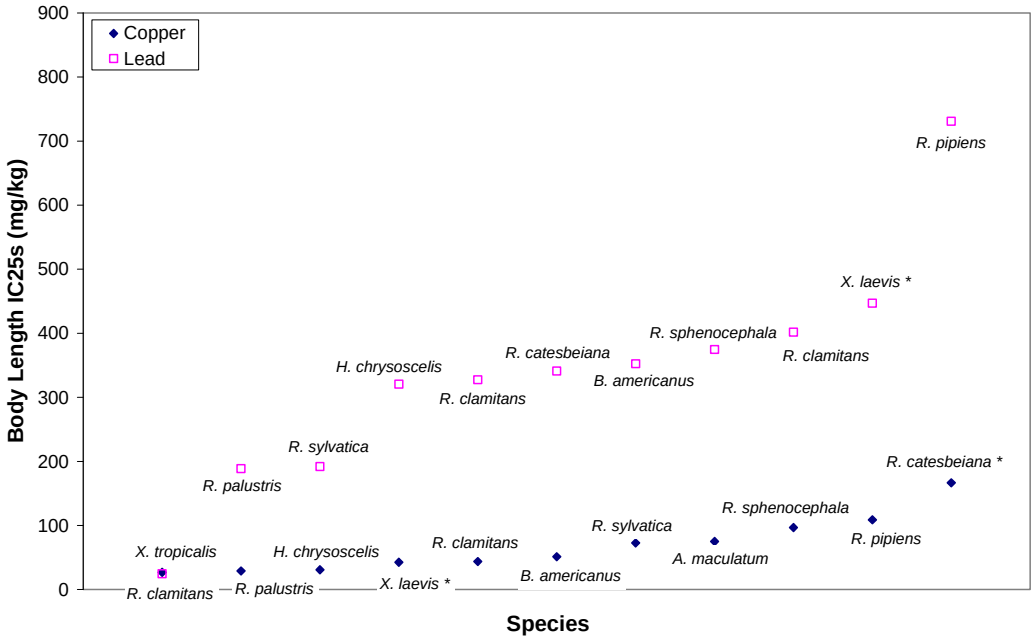


Figure 5-4 Body Length IC25 Values Following 10 Day Exposure to Copper or Lead



* - Results should be interpreted with caution. Although laboratory control did not meet test acceptability criteria, toxicity statistics were calculated because other treatments met test acceptability criteria and a dose response curve was observed.

6.0 Conclusion

The purpose of this phase of the ER-0514 project was to evaluate the responses of different amphibian species exposed to sediments spiked with known concentrations of copper or lead. To accomplish this goal, the 10 day sediment exposure protocol validated as part of the ER-0514 project was used to expose 11 different larval amphibian species to sediment spiked with either copper or lead. Copper and lead concentrations in the sediment were measured, test organism survival was evaluated on Days 2, 4, and 10, and body width and body length were evaluated at test termination.

The results of this testing show there may be slight differences in the sensitivities of the amphibians to copper and lead over the 10 day exposure period. However, the range of responses from species to species is not as great as had been expected based on a review of water only exposures to metals (Birge, et al. 2000; Hoke and Ankley, 2005). For example, the survival results for all species were within about one order of magnitude or less for each metal. Growth differences were somewhat greater for lead with a 30-fold difference between the most and least sensitive species. Sediment tests can often elicit variable response from test organisms due to differences in sediment characteristics that can affect bioavailability of chemicals. However, all tests used the same base sediment (from the Cache La Poudre River) and therefore sediment variability should not have been a major factor. It is likely that additional tests using the same test organisms would yield response data that are different than those already collected, thus illustrating intra-species variability that would affect the overall results. As shown with two rounds of *R. clamitans* testing (Tests 075, 076,103, and 104), some variability in organism response is to be expected when using different batches of organisms. This is clearly illustrated in USEPA water quality criteria documents that often show a substantial response range for species exposed to a chemical stressor under the same conditions but at different times and locations

Due to concerns about test organism health, the apparent sensitivity of the *Xenopus* species should be considered with caution. Although several batches of *Xenopus* were obtained for testing, none of the batches flourished under the laboratory culture conditions used during this investigation, in contrast to the other taxa tested. The results, therefore, may be biased low since the test organism health may have been affected by stressors other than copper or lead. Previous evaluations (Birge, et al. 2000; Hoke and Ankley, 2005) identified *X. laevis* as one of the more tolerant species and did not necessarily recommend the species be included in routine testing conducted for ecological risk assessment purposes.

Neither of the sub-lethal endpoints appears to be consistently more sensitive than the other, but sub-lethal endpoints were nearly always more sensitive than survival. Therefore, it is clear that measurement of sub-lethal endpoints is an important consideration in the tests. Reductions in body width and body length may translate into reduced organism health in the field which may result in less access to food and greater likelihood of loss through predation. In addition, reduced physiological health as a result of one stressor could make organisms more vulnerable to other stressors.

The results of this study further confirm that the sediment exposure protocol, which was approved as an ASTM standard, is applicable at a variety of sites and can be successfully conducted with several different species. As noted during the field demonstration phase of the ER-0514 project, organism availability may be a limiting factor when conducting the sediment exposure test. Drought conditions in 2007 substantially limited the availability of test organisms and delayed the initiation of several tests. The issue of organism availability is one reason that *R. pipiens* was selected as the primary test organism in the ASTM guide (ASTM, 2007). Eggs of this species can generally be obtained from commercial suppliers (eggs induced and fertilized in a laboratory) from approximately November until March; however, there may be some limitations on egg availability even within this window. Therefore, organism availability is likely to be an important factor in the application of the ASTM standard.

When considering the use of the ASTM standard as part of an ecological risk assessment, native North American species are likely to be the most appropriate for use in the United States. The use of *Xenopus* species was problematic in the sediment exposure tests (i.e., low control survival) and they are often among the least sensitive test organisms (Birge, et al. 2000; Hoke and Ankley, 2005). Therefore, these species should only be used when other test organisms are unavailable. The use of *Xenopus* species may be more appropriate comparing the relative toxicity of chemicals, rather than for determining the potential risk to native amphibians.

During the development of the sediment exposure protocol, *R. pipiens* was selected as the recommended test organism due to egg availability and relative sensitivity to chemical stressors (NAVFAC, 2004). Preliminary testing conducted under the YO817 program (Table 2-1) indicated that *R. pipiens* was generally more sensitive to spiked sediments than *B. americanus*. A review of amphibian data presented in U.S. EPA ambient water quality criteria documents for cadmium, copper, and zinc (NAVFAC, 2004) and relative sensitivity data compiled by Birge, et al. (2000) also indicate that *R. pipiens* is considered to be sensitive to metals, relative to other frog, toad, and salamander species. The potential for field-collection of *R. pipiens* eggs with minimal impact to local communities was also a consideration in the selection of this species as the preferred test species.

The species sensitivity testing conducted as part of the ER-0514 project found that the other species may be similarly or slightly more sensitive to copper and lead exposure in sediment than *R. pipiens*. In general the differences between the most and least sensitive species are within an order of magnitude. In addition, there is likely to be intra-species variation that would modify the results presented in Figures 5-1, 5-3, and 5-4 if additional testing was conducted so these results should not be interpreted as definitive classifications of species sensitivities.

In general, *R. palustris* was relatively sensitive to both copper and lead exposures, *R. pipiens* and *R. catesbeiana* were among the more tolerant species, and *B. americanus* was consistently at the approximate mid-point between the most and least sensitive species. These results may be useful to risk managers in selecting the relevant test organism to use when applying the ASTM guide or in interpreting results of a test conducted with a particular species. Test organism availability and the amphibians present at the site under investigation should also be considered when selecting organisms for toxicity testing.

7.0 References

ASTM, 2007. E2591-07 Standard Guide for Conducting Whole Sediment Toxicity Tests with Amphibians. Book of Standards Volume 11.06.

Birge, W.J., A.G. Westerman and J.A. Spromberg. 2000. Chapter 14a. Comparative Toxicology and Risk Assessment of Amphibians. In: Ecotoxicology of Amphibians and Reptiles. D.W. Sparling, G. Linder, and C.A. Bishop eds. SETAC Special Publication. pp 727-791.

Brown LE, Rosati RR. 1997. Effects of three different diets on survival and growth of larvae of the African clawed frog Xenopus laevis. Progressive Fish Culturist 59:54–58.

Goleman, W.L., L.J. Urquidi, T.A. Anderson, E.E. Smith, R.J. Kendall, and J.R. Karr. 2002. Environmentally Relevant Concentrations of Ammonium Perchlorate Inhibit Development and Metamorphosis in *Xenopus laevis*. Environ. Toxicol. Chem. 21: 424-430

Harrahy, E.. and W.H. Clements. 1997. Toxicity and Bioaccumulation of a Mixture of Heavy metals in Chironomus tentans (Diptera: Chironomidae) in Synthetic Sediment. Environ. Toxicol. Chem. 16: 317-327.

Hoke, R.A. and G.T. Ankley. 2005. Application of Frog Embryo Teratogenesis Assay-Xenopus to Ecological Risk Assessment. Environ. Toxicol. Chem. 24: 2677-2690.

Kemble, N.E., F.J. Dwyer, C.G. Ingersoll, T.D. Dawson, and T.J. Norberg-King. 1999. Tolerance of freshwater test organisms to formulated sediments for use as control materials in whole sediment toxicity tests. Environ. Toxicol. Chem. 18: 222-230.

Naval Facilities Engineering Command (NAVFAC). 2004. Development of a Standardized Approach for Assessing Potential Risks to Amphibians Exposed to Sediments and Hydric Soils, March.

Naval Facilities Engineering Command (NAVFAC). 2005. Laboratory Validation Plan for ESTCP Project ER-0514 Demonstration and Certification of Amphibian Ecological Risk Assessment Protocol. December.

Naval Facilities Engineering Command (NAVFAC). 2006. Site Selection Memorandum for ESTCP Project ER-0514 Demonstration and Certification of Amphibian Ecological Risk Assessment Protocol. February.

Naval Facilities Engineering Command (NAVFAC). 2007a. Field Demonstration Plan for ESTCP Project ER-0514 Demonstration and Certification of Amphibian Ecological Risk Assessment Protocol. May.

Naval Facilities Engineering Command (NAVFAC). 2007b. Test Refinement Interim Report for ESTCP Project ER-0514 Demonstration and Certification of Amphibian Ecological Risk Assessment Protocol. June.

Naval Facilities Engineering Command (NAVFAC). 2007c. Species Sensitivity Testing Work Plan for ESTCP Project ER-0514 Demonstration and Certification of Amphibian Ecological Risk Assessment Protocol. August.

Naval Facilities Engineering Command (NAVFAC). 2008. Final Report for ESTCP Project ER-0514 Demonstration and Certification of Amphibian Ecological Risk Assessment Protocol. October 2008.

USEPA. 2000. Guidance for the Data Quality Objectives Process, EPA QA/G-4. August.

Walsh, G.E., D.E. Weber, and T.L. Simon. 1991. Toxicity Tests of Effluents with Marsh Plants in Water and Sediment. Environ. Toxicol. Chem. 10: 517-525.

Attachment A

Scientific Collection Permit from Massachusetts

Division of Fisheries and Wildlife

Commonwealth of Massachusetts

Division of Fisheries & Wildlife

Wayne F. MacCallum, Director

Scientific Collection Permit

REPTILES AND AMPHIBIANS

VALID

2007

ENSR
SCOTT EGAN
2 TECHNOLOGY PARK DRIVE
WESTFORD, MA 01886

DATE: 3/28/2007
PERMIT# : 129.07WRA
NHESP Tracking # : **07-21903**

Subpermitee(s): JOHN BLEILER, JIM GASS, ANDREA DESILETS

is (are) hereby authorized, in accordance with the provisions of Section 4, Chapter 131 and 131A of the Massachusetts General Laws, to remove from the wild within the Commonwealth, subject to conditions set forth below, the following species and numbers:

MAY COLLECT AMPHIBIAN EGG MASSES AS OUTLINED IN THE LETTER DATED MARCH 27, 2007 FROM ENSR TO NHESP. THE PERMIT HOLDER SHALL BE RESPONSIBLE FOR CONDUCTING THE STUDY IN ACCORDANCE WITH ACCEPTED PROFESSIONAL STANDARDS AND PRACTICES. RARE SPECIES OBSERVATION FORMS MUST BE SUBMITTED FOR ALL STATE-LISTED RARE SPECIES ENCOUNTERED. WITHIN 10 DAYS OF THE FIRST OBSERVATION OF A GIVEN STATE-LISTED SPECIES, A RARE SPECIES OBSERVATION FORM MUST BE SUBMITTED TO THE NHESP. ALL OTHER RARE SPECIES OBSERVATION FORMS REPORTING SUBSEQUENT OBSERVATIONS OF A GIVEN SPECIES SHALL BE SUBMITTED BY DECEMBER 31. THIS PERMIT GIVES AUTHORITY TO TRANSPORT EGGS & EMBRYOS OF SPECIES, LISTED IN LETTER, OUT OF STATE, PROVIDED THAT ALL OTHER LEGAL REQUIREMENTS FOR TRANSPORT ARE MET FOR BOTH STATES.

The following method(s) of taking is (are) hereby authorized:

AS INDICATED IN MARCH 27, 2007 LETTER FROM ENSR TO NHESP

Collection activites under this permit shall be restricted to the following locations, subject to the approval of private landowners

STATEWIDE

All specimens secured under this permit shall be donated to the following institutions:

No specimen taken under the authority of this permit may be sold. No specimen may be transferred to another not duly licensed.

This permit of a copy thereof shall be carried at all times by the permittee and subpermittee(s) while engaged in the activities authorized herein.

This permit does not absolve the permittee from compliance in full with any and all other applicable federal, state and local requirements, including the acquisition of a federal endangered species permit if required.

Upon expiration of this permit, a complete report detailing all collection activities shall be filed with this office and must include a listing of all species taken, numbers of specimens, and the disposition of same.

This permit, unless sooner revoked for cause, shall expire on December 31 of the year of issue.

Wayne F. MacCallum

Wayne F. MacCallum, Director

ENSR | AECOM

ENSR
2 Technology Park Drive, Westford, Massachusetts 01886-3140
T 978.589.3000 F 978.589.3100 www.ensr.aecom.com

March 27, 2007

ATTN: Collection Permits
Dr. John Regosin
Division of Fisheries and Wildlife, NHESP
Route 135 North Drive
Westborough, MA 01581

Subject: Fast-Track Application for Commercial Scientific Collection Permit

Dear Dr. Regosin,

ENSR Corporation (ENSR) is interested in obtaining a Scientific Collection Permit that will allow the collection of amphibian egg masses to be used in laboratory experiments that are designed to evaluate the differential sensitivity of larval amphibians to lead and copper.

Background: Because amphibians are a front-line indicator of possible adverse impacts to wetland ecosystems, the Department of Defense has recently funded ENSR to conduct an ongoing laboratory testing program designed to;

- Develop standardized procedures to evaluate the potential toxicity of sediments and hydric soils to amphibians,
- Develop risk-based screening values specific to amphibians for use in screening level risk assessments,
- Develop amphibian risk assessment protocols, and
- Evaluate the potential toxicity of lead and copper impacted sediments and hydric soils to larval amphibians.

This ongoing research has focused on a small number of species that are typically available through biological supply vendors; evaluating the variability in sensitivities between amphibian species was not an *a priori* goal of this program. However, the results of this work yielded several intriguing findings: (1) based on the toxicity testing conducted through this program, there clearly are substantial differences in early life stage sensitivities between the tested frogs and toads, and among the frogs (i.e., within the genus *Rana*); and (2) based on the literature review, certain taxa appear to be more sensitive to metals, whereas others may be more tolerant of contaminant toxicity. In fact, the primary species historically used in amphibian testing the non-native African clawed frog (*Xenopus laevis*), appears to generally be more tolerant of contaminant toxicity than the majority of native species.

Obtaining additional information on amphibian species sensitivity is important from several perspectives:

(1) If amphibians are a species of concern at a particular site, it is important for risk managers to have information regarding the relative and differential sensitivity of various taxa.

(2) Based on our experiences obtaining amphibians for this ongoing program (which has focused on *Rana pipiens*), it is apparent that even experienced project managers may need to use alternative test organisms. Market conditions and climatic factors make it difficult to predict with any certainty which species of amphibians are available at any one time. Understanding the relative sensitivities of differing taxa may be necessary if use of alternative test organisms is required.

(3) There is a universally recognized scarcity of amphibian toxicological literature. These comparative data will be readily publishable and will serve to help fill an important scientific data gap relative to basic amphibian toxicology and ecology. This information can be used by a number of different end users, including conservation professionals and natural resources managers.

(4) The African clawed frog (*X. laevis*) has historically been an important test species for evaluation of potential constituent toxicity and has recently been used by several researchers for evaluation of perchlorate toxicity. These researchers found that *X. laevis* development and metamorphosis was inhibited by environmentally relevant concentrations of perchlorate. In fact, of the various amphibian species reported in the technical ecotoxicology literature, *X. laevis* is perhaps the most common. *X. laevis* eggs and larvae are readily available year-round, this species readily adapts to laboratory conditions, and protocols for evaluation of this species are fairly standardized; it is therefore valuable to include this species in the proposed differential species toxicity testing. However, there are considerable uncertainties with basing environmental cleanups on testing with this species, given that it is very different physiologically from native species (tetraploid genotype), is non-native, is totally aquatic, and perhaps most importantly, appears to be at the relatively insensitive end of species sensitive distribution for amphibians exposed to environmental toxicants.

Methods: As a continuation of this ongoing research, the Department of Defense has funded ENSR to conduct early life stage 10-day toxicity tests with the sediment testing protocol refined under the current project. Up to 10 species of amphibians will be tested using this uniform protocol in order to determine the sensitivity of these organisms to sediment-associated contaminants. Efforts will focus on obtaining amphibian eggs from biological vendors, however, availability is highly variable and some species may need to be field collected under scientific collection permit. Potential test species are based on field collection and commercial availability, and include the following:

Common Name	Scientific Name	Source	
		Field	Vendor
Northern Leopard Frog	*Rana pipiens*		X
Pickerel Frog	*Rana palustris*	X	X
Green Frog	*Rana clamitans*	X	X
Bull Frog	*Rana catesbiana*	X	X
Wood Frog	*Rana sylvatica*	X	
American Toad	*Bufo americanus*	X	X
Fowlers Toad	*Bufo fowleri*	X	
Spotted Salamander	*Ambystoma maculatum*	X	X
African Clawed Frog	*Xenopus laevis*		X
Tiger Salamander	*Ambystoma tigrinum*		X
Southern Leopard Frog	*Rana utricularia*		X
Gray Treefrog	*Hyla chrysoscelis*		X
Spring Peeper	*Pseudacris crucifer*		X
Western Chorus Frog	*Pseudacris triseriata*		X
Cricket Frog	*Acris crepitans*		X
Narrow-mouthed Toad	*Gastrophryne carolinensis*		X

A total of seven species may require field collection of egg masses to perform the proposed study. Following the sediment testing protocol, tests would use 5 organisms in 8 replicates for each treatment, or 40 larvae per concentration or treatment. Assuming 5 concentrations per test, plus a control, that would mean 240 organisms per test, or 480 healthy tadpoles to test two parameters of interest (i.e., lead and copper). In addition, another 120 organisms would be required to run a reference toxicant test. To account for approximately 25% non-viability, we would want to collect 800 eggs/species to complete one round of testing. Additional rounds may be required if we want to rerun some tests because the control fails or the concentration-response is poor.

Prior to removing any egg masses from a particular population, measures will be taken to ensure that only a small proportion of any particular population will be impacted by these collections. For wood frogs and spotted salamanders, the total number of egg masses will be counted, and not more than 10% will be removed for laboratory testing. The average number of embryos per egg mass for wood frogs and spotted salamanders is approximately 1,020 and 125 respectively. Therefore, we would expect to collect not more than 10 wood frog egg masses, and not more than 20 spotted salamander egg masses to reach the embryo numbers outlined above.

For green frogs, bull frogs and toads, the total surface area of the egg sheet will be estimated, and not more than 20% of the surface area will be removed. However, because egg sheets of these species are sometimes difficult to find (particularly green frogs and bull frogs), an estimate of calling intensity will be used to adjust the collection percentage. For areas with high calling intensity (i.e., calling overlaps and number of individuals calling is indistinguishable) as much as 50% of the egg sheet may be taken to reach the embryo numbers outlined above.

Finally, egg masses for pickerel frogs have been documented to contain 2000-3000 embryos per egg mass. Therefore, one egg mass should be sufficient to run the necessary tests. Because egg masses for this species may be difficult to find, as with green and bull frogs, an index of calling intensity will be used to determine whether an encountered egg mass will be collected or not.

Schedule: Because funding for this project was only authorized on March 23, 2007, time is of the essence. We apologize for the extremely short notice relative to this permit application.

Thank you for your time in reviewing this application for a Commercial Scientific Collection Permit. Please call John at (978) 589-3056 or Scott at (978) 589-3179 if you have any questions.

Sincerely yours,

Scott Egan
Wetland and Wildlife Ecologist
segan@ensr.aecom.com

John Bleiler
Natural Resources Department Manager
jbleiler@ensr.aecom.com

Commonwealth of Massachusetts

Division of Fisheries and Wildlife Attn: Collection Permits
Route 135, North Drive, Westborough, MA 01581

****

APPLICATION FOR COMMERCIAL SCIENTIFIC COLLECTION PERMIT:

This Permit Application is for the Scientific Collection of:

☐ Mammals	$100.00
☐ Birds	$100.00
☒ Reptiles & Amphibians	$100.00
☐ Fish	$100.00
☐ Invertebrates	$100.00
☐ Plants	$100.00

If this application is for birds, do you have a Migratory Bird Permit? Y☐ N☐
If yes, give Permit Number: _____

PLEASE SUBMIT SEPARATE APPLICATIONS FOR EACH TAXONOMIC GROUP

PLEASE MAKE CHECKS PAYABLE TO COMMONWEALTH OF MASSACHUSETTS

PROVIDE ATTACHMENTS IF EXTRA SPACE IS NEEDED

NAME: SCOTT EGAN **DATE OF BIRTH:** 3/26/1969

HOME ADDRESS: 29 FOLIAGE WAY, RINDGE, NH 03461

HOME PHONE: 603-547-5651 **CELL PHONE:** —

BUSINESS ADDRESS: 2 TECHNOLOGY PARK DRIVE, WESTFORD, MA 01886

BUSINESS PHONE: 978-589-3179 **EMAIL:** SEGAN@ENSR.AECOM.COM

STATE SPECIFICALLY THE NUMBER AND SPECIES OF ANIMALS OR PLANTS TO BE COLLECTED AND/OR POSSESSED:

SEE ATTACHED LETTER

IS THIS COLLECTION/POSSESION BEING UNDERTAKEN IN RELATION TO A PROPOSED PROJECT THAT HAS BEEN OR WILL BE UNDERGOING AN NHESP RARE SPECIES REGULATORY REVIEW? Y ☐ N ☒

IF YES, NHESP TRACKING NUMBER: ______________________

DID YOU HAVE A NHESP APPROVED SURVEY PROTOCOL? Y ☐ N ☒

Note: A NHESP approved survey protocol may be required for regulatory review.

INSTITUTION TO WHICH COLLECTED SPECIMENS WILL BE DONATED:
NONE

STATE SPECIFICALLY WHERE COLLECTIONS WILL BE MADE:
Attach a copy of a USGS topographic map in the scale of 1:24,000 or 1:25,000 (not copy reduced) with the site location clearly marked and centered on the page
THROUGHOUT THE STATE, WHERE AVAILABLE. A PLAN SHOWING COLLECTION LOCATIONS WILL BE PROVIDED AT THE COMPLETION OF THE STUDY.
→ SOME COLLECTION AT NAS SOUTH WEYMOUTH ANTICIPATED

DESIRED START DATE: APRIL 1, 2007

STATE YOUR QUALIFICATIONS AND ENCLOSE AN OUTLINE OF YOUR PROPOSED STUDY INCLUDING SITE SPECIFIC METHODOLOGY AND SURVEY PROTOCOLS: MS (NATURAL RESOURCES SCIENCE) UNIVERSITY OF RHODE ISLAND
BS (PLANT AND SOIL SCIENCES) UNIVERSITY OF MASSACHUSETTS

NAME(S) OF ANY SUBPERMITTEE(S): JOHN BLEILER, JIM GASS, ANDREA DESILETS

****

Please re-check to assure that all questions have been answered completely.

<u>Failure to follow directions will slow or delay processing of this application.</u>

****

I CERTIFY THAT THE INFORMATION PROVIDED ABOVE IS TRUE AND CORRECT TO THE BEST OF MY KNOWLEDGE AND BELIEF.

Scott Egan — 3/27/07

SIGNATURE OF APPLICANT **DATE**

Note: Permit will expire on December 31 of a given year

Attachment B

Survival and Growth Results for Species Sensitivity Testing

Survival Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Day 0 Specimen Count	Day 1 Specimen Count	Day 2 Specimen Count	Day 3 Specimen Count	Day 4 Specimen Count	Day 5 Specimen Count	Day 6 Specimen Count	Day 7 Specimen Count	Day 8 Specimen Count	Day 9 Specimen Count	Day 10 Specimen Count	Day 10 Survival (%)	Day 10 Average Survival (%)
104	5/12/2007	R. clamitans	Lead	1.9	A	5	5	5	5	5	5	5	4	4	4	4	80%	
104	5/12/2007	R. clamitans	Lead	1.9	B	5	5	5	5	5	5	5	5	5	5	5	100%	
104	5/12/2007	R. clamitans	Lead	1.9	C	5	5	5	5	5	5	5	5	5	5	5	100%	
104	5/12/2007	R. clamitans	Lead	1.9	D	5	5	5	5	5	5	5	5	5	5	5	100%	
104	5/12/2007	R. clamitans	Lead	1.9	E	5	5	5	5	5	5	5	5	5	5	5	100%	
104	5/12/2007	R. clamitans	Lead	1.9	F	5	5	5	5	5	5	5	5	5	5	5	100%	97%
104	5/12/2007	R. clamitans	Lead	27	A	5	5	5	5	5	5	5	5	5	5	5	100%	
104	5/12/2007	R. clamitans	Lead	27	B	5	5	5	5	5	5	5	5	5	5	5	100%	
104	5/12/2007	R. clamitans	Lead	27	C	5	5	5	5	5	5	5	5	5	5	5	100%	
104	5/12/2007	R. clamitans	Lead	27	D	5	5	5	5	5	5	5	5	5	5	5	100%	
104	5/12/2007	R. clamitans	Lead	27	E	5	5	5	5	5	5	5	5	5	5	5	100%	
104	5/12/2007	R. clamitans	Lead	27	F	5	5	5	5	5	5	5	5	5	5	5	100%	100%
104	5/12/2007	R. clamitans	Lead	52	A	5	5	5	5	5	5	5	5	5	5	5	100%	
104	5/12/2007	R. clamitans	Lead	52	B	5	5	5	5	5	5	5	5	5	5	5	100%	
104	5/12/2007	R. clamitans	Lead	52	C	5	5	5	5	5	5	5	5	5	5	5	100%	
104	5/12/2007	R. clamitans	Lead	52	D	5	5	5	5	5	5	5	5	5	5	5	100%	
104	5/12/2007	R. clamitans	Lead	52	E	5	5	5	4	4	4	4	4	4	4	4	80%	
104	5/12/2007	R. clamitans	Lead	52	F	5	5	5	5	5	5	5	5	5	5	5	100%	97%
104	5/12/2007	R. clamitans	Lead	95	A	5	5	5	5	5	5	5	5	5	5	5	100%	
104	5/12/2007	R. clamitans	Lead	95	B	5	5	5	5	5	5	5	5	5	5	5	100%	
104	5/12/2007	R. clamitans	Lead	95	C	5	5	5	5	5	5	5	5	5	5	5	100%	
104	5/12/2007	R. clamitans	Lead	95	D	5	5	5	5	5	5	5	5	5	5	5	100%	
104	5/12/2007	R. clamitans	Lead	95	E	5	5	5	5	5	5	5	5	5	5	5	100%	
104	5/12/2007	R. clamitans	Lead	95	F	5	5	5	5	5	5	5	5	5	5	5	100%	100%
104	5/12/2007	R. clamitans	Lead	250	A	5	5	5	5	5	5	5	5	5	5	5	100%	
104	5/12/2007	R. clamitans	Lead	250	B	5	5	5	5	5	5	5	5	5	5	5	100%	
104	5/12/2007	R. clamitans	Lead	250	C	5	5	5	5	5	5	5	5	5	5	5	100%	
104	5/12/2007	R. clamitans	Lead	250	D	5	5	5	5	5	5	5	5	5	5	5	100%	
104	5/12/2007	R. clamitans	Lead	250	E	5	5	5	5	5	5	5	5	5	5	5	100%	
104	5/12/2007	R. clamitans	Lead	250	F	5	5	5	5	5	5	5	5	5	5	5	100%	100%
104	5/12/2007	R. clamitans	Lead	480	A	5	5	5	5	5	5	5	5	5	5	5	100%	
104	5/12/2007	R. clamitans	Lead	480	B	5	5	5	5	5	5	5	3	3	3	3	60%	
104	5/12/2007	R. clamitans	Lead	480	C	5	5	5	5	5	5	5	5	5	2	2	40%	
104	5/12/2007	R. clamitans	Lead	480	D	5	5	5	5	5	5	5	5	5	3	3	60%	
104	5/12/2007	R. clamitans	Lead	480	E	5	5	5	5	5	5	5	5	5	5	5	100%	
104	5/12/2007	R. clamitans	Lead	480	F	5	5	5	5	5	5	5	5	5	5	5	100%	77%
104	5/12/2007	R. clamitans	Lead	1400	A	5	0	0	0	0	0	0	0	0	0	0	0%	
104	5/12/2007	R. clamitans	Lead	1400	B	5	0	0	0	0	0	0	0	0	0	0	0%	
104	5/12/2007	R. clamitans	Lead	1400	C	5	0	0	0	0	0	0	0	0	0	0	0%	
104	5/12/2007	R. clamitans	Lead	1400	D	5	0	0	0	0	0	0	0	0	0	0	0%	
104	5/12/2007	R. clamitans	Lead	1400	E	5	0	0	0	0	0	0	0	0	0	0	0%	
104	5/12/2007	R. clamitans	Lead	1400	F	5	0	0	0	0	0	0	0	0	0	0	0%	0%
125	6/27/2008	R. catesbeiana	Lead	1.9	A	5	5	5	5	5	5	5	5	5	5	5	100%	
125	6/27/2008	R. catesbeiana	Lead	1.9	B	5	5	5	5	5	5	5	5	5	5	5	100%	
125	6/27/2008	R. catesbeiana	Lead	1.9	C	5	5	5	5	5	5	5	5	5	5	5	100%	
125	6/27/2008	R. catesbeiana	Lead	1.9	D	5	5	5	5	5	5	5	5	5	5	5	100%	
125	6/27/2008	R. catesbeiana	Lead	1.9	E	5	5	5	5	5	5	5	5	5	5	5	100%	
125	6/27/2008	R. catesbeiana	Lead	1.9	F	5	5	5	5	5	5	5	5	5	5	5	100%	100%
125	6/27/2008	R. catesbeiana	Lead	40	A	5	5	5	5	5	5	5	5	5	5	5	100%	
125	6/27/2008	R. catesbeiana	Lead	40	B	5	5	5	5	5	5	5	5	4	4	3	60%	
125	6/27/2008	R. catesbeiana	Lead	40	C	5	5	5	5	5	4	4	4	4	3	3	60%	
125	6/27/2008	R. catesbeiana	Lead	40	D	5	5	5	5	5	5	5	5	3	3	3	60%	
125	6/27/2008	R. catesbeiana	Lead	40	E	5	5	5	5	5	5	5	5	5	5	5	100%	
125	6/27/2008	R. catesbeiana	Lead	40	F	5	5	5	5	5	5	5	5	4	4	4	80%	77%
125	6/27/2008	R. catesbeiana	Lead	77	A	5	5	5	5	5	5	5	5	5	5	5	100%	
125	6/27/2008	R. catesbeiana	Lead	77	B	5	5	5	5	5	5	5	5	5	5	4	80%	
125	6/27/2008	R. catesbeiana	Lead	77	C	5	5	5	5	5	4	4	4	4	4	4	80%	
125	6/27/2008	R. catesbeiana	Lead	77	D	5	5	5	5	5	5	5	5	5	5	5	100%	
125	6/27/2008	R. catesbeiana	Lead	77	E	5	5	5	5	5	5	5	5	5	5	4	80%	
125	6/27/2008	R. catesbeiana	Lead	77	F	5	5	5	5	5	5	5	5	5	5	5	100%	90%
125	6/27/2008	R. catesbeiana	Lead	120	A	5	5	5	5	5	5	5	4	4	4	4	80%	
125	6/27/2008	R. catesbeiana	Lead	120	B	5	5	5	5	5	5	5	5	5	5	5	100%	
125	6/27/2008	R. catesbeiana	Lead	120	C	5	5	5	5	5	5	5	5	5	5	5	100%	
125	6/27/2008	R. catesbeiana	Lead	120	D	5	5	5	5	5	5	5	5	5	5	5	100%	
125	6/27/2008	R. catesbeiana	Lead	120	E	5	5	5	5	5	5	5	5	5	5	5	100%	
125	6/27/2008	R. catesbeiana	Lead	120	F	5	5	5	5	5	5	5	5	5	5	5	100%	97%
125	6/27/2008	R. catesbeiana	Lead	270	A	5	5	5	5	5	5	5	5	5	5	4	80%	
125	6/27/2008	R. catesbeiana	Lead	270	B	5	5	5	5	5	5	5	5	5	4	4	80%	
125	6/27/2008	R. catesbeiana	Lead	270	C	5	5	5	5	5	5	5	5	5	4	4	80%	
125	6/27/2008	R. catesbeiana	Lead	270	D	5	5	5	5	5	5	5	5	5	5	5	100%	
125	6/27/2008	R. catesbeiana	Lead	270	E	5	5	5	5	5	5	5	5	5	5	4	80%	
125	6/27/2008	R. catesbeiana	Lead	270	F	5	5	5	5	5	5	5	5	5	5	4	80%	83%

Survival Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Day 0 Specimen Count	Day 1 Specimen Count	Day 2 Specimen Count	Day 3 Specimen Count	Day 4 Specimen Count	Day 5 Specimen Count	Day 6 Specimen Count	Day 7 Specimen Count	Day 8 Specimen Count	Day 9 Specimen Count	Day 10 Specimen Count	Day 10 Survival (%)	Day 10 Average Survival (%)
125	6/27/2008	R. catesbeiana	Lead	570	A	5	5	5	5	5	0	0	0	0	0	0	0%	
125	6/27/2008	R. catesbeiana	Lead	570	B	5	5	5	5	5	0	0	0	0	0	0	0%	
125	6/27/2008	R. catesbeiana	Lead	570	C	5	5	5	5	5	3	0	0	0	0	0	0%	
125	6/27/2008	R. catesbeiana	Lead	570	D	5	5	5	5	5	5	5	5	5	4	4	80%	
125	6/27/2008	R. catesbeiana	Lead	570	E	5	5	5	5	5	5	4	5	5	3	3	60%	
125	6/27/2008	R. catesbeiana	Lead	570	F	5	5	5	5	5	5	5	5	5	5	5	100%	40%
125	6/27/2008	R. catesbeiana	Lead	1300	A	5	5	5	5	5	0	0	0	0	0	0	0%	
125	6/27/2008	R. catesbeiana	Lead	1300	B	5	5	5	5	5	0	0	0	0	0	0	0%	
125	6/27/2008	R. catesbeiana	Lead	1300	C	5	5	5	5	5	0	0	0	0	0	0	0%	
125	6/27/2008	R. catesbeiana	Lead	1300	D	5	5	5	5	5	0	0	0	0	0	0	0%	
125	6/27/2008	R. catesbeiana	Lead	1300	E	5	5	5	5	5	0	0	0	0	0	0	0%	
125	6/27/2008	R. catesbeiana	Lead	1300	F	5	5	5	5	5	0	0	0	0	0	0	0%	0%
124	6/27/2008	R. catesbeiana	Copper	3.2	A	5	5	5	5	5	1	1	1	1	1	1	20%	
124	6/27/2008	R. catesbeiana	Copper	3.2	B	5	5	5	5	5	5	5	5	4	4	4	80%	
124	6/27/2008	R. catesbeiana	Copper	3.2	C	5	5	5	5	5	5	5	5	5	4	4	80%	
124	6/27/2008	R. catesbeiana	Copper	3.2	D	5	5	5	5	5	5	5	5	4	4	4	80%	
124	6/27/2008	R. catesbeiana	Copper	3.2	E	5	5	5	5	5	5	5	4	4	4	4	80%	
124	6/27/2008	R. catesbeiana	Copper	3.2	F	5	5	5	5	5	5	5	5	5	5	5	100%	73%
124	6/27/2008	R. catesbeiana	Copper	13	A	5	5	5	5	5	5	5	5	5	5	5	100%	
124	6/27/2008	R. catesbeiana	Copper	13	B	5	5	5	5	5	5	5	5	5	5	5	100%	
124	6/27/2008	R. catesbeiana	Copper	13	C	5	5	5	5	5	5	5	5	5	5	5	100%	
124	6/27/2008	R. catesbeiana	Copper	13	D	5	5	5	5	5	5	5	5	5	4	4	80%	
124	6/27/2008	R. catesbeiana	Copper	13	E	5	5	5	5	5	5	5	5	5	5	5	100%	
124	6/27/2008	R. catesbeiana	Copper	13	F	5	5	5	5	5	5	5	5	5	5	5	100%	97%
124	6/27/2008	R. catesbeiana	Copper	32	A	5	5	5	5	5	5	5	5	5	5	5	100%	
124	6/27/2008	R. catesbeiana	Copper	32	B	5	5	5	5	5	5	5	5	5	5	5	100%	
124	6/27/2008	R. catesbeiana	Copper	32	C	5	5	5	5	5	5	5	5	5	5	5	100%	
124	6/27/2008	R. catesbeiana	Copper	32	D	5	5	5	5	5	5	5	5	5	5	5	100%	
124	6/27/2008	R. catesbeiana	Copper	32	E	5	5	5	5	5	5	5	5	5	5	5	100%	
124	6/27/2008	R. catesbeiana	Copper	32	F	5	5	5	5	5	5	5	5	5	5	5	100%	100%
124	6/27/2008	R. catesbeiana	Copper	85	A	5	5	5	5	5	5	5	5	5	5	5	100%	
124	6/27/2008	R. catesbeiana	Copper	85	B	5	5	5	5	5	5	5	5	5	5	5	100%	
124	6/27/2008	R. catesbeiana	Copper	85	C	5	5	5	5	5	5	5	5	5	5	5	100%	
124	6/27/2008	R. catesbeiana	Copper	85	D	5	5	5	5	5	5	5	5	5	5	5	100%	
124	6/27/2008	R. catesbeiana	Copper	85	E	5	5	5	5	5	5	5	5	5	4	4	80%	
124	6/27/2008	R. catesbeiana	Copper	85	F	5	5	5	5	5	5	5	5	5	5	5	100%	97%
124	6/27/2008	R. catesbeiana	Copper	140	A	5	5	5	5	5	5	5	5	5	5	5	100%	
124	6/27/2008	R. catesbeiana	Copper	140	B	5	5	5	5	5	5	5	5	5	5	5	100%	
124	6/27/2008	R. catesbeiana	Copper	140	C	5	5	5	5	5	5	5	5	5	5	5	100%	
124	6/27/2008	R. catesbeiana	Copper	140	D	5	5	5	5	5	5	4	4	4	4	4	80%	
124	6/27/2008	R. catesbeiana	Copper	140	E	5	5	5	5	5	5	5	5	5	5	5	100%	
124	6/27/2008	R. catesbeiana	Copper	140	F	5	5	5	5	5	5	5	5	5	5	5	100%	97%
124	6/27/2008	R. catesbeiana	Copper	280	A	5	5	5	5	5	0	0	0	0	0	0	0%	
124	6/27/2008	R. catesbeiana	Copper	280	B	5	5	5	5	5	0	0	0	0	0	0	0%	
124	6/27/2008	R. catesbeiana	Copper	280	C	5	5	5	5	5	0	0	0	0	0	0	0%	
124	6/27/2008	R. catesbeiana	Copper	280	D	5	5	5	5	5	0	0	0	0	0	0	0%	
124	6/27/2008	R. catesbeiana	Copper	280	E	5	5	5	5	5	0	0	0	0	0	0	0%	
124	6/27/2008	R. catesbeiana	Copper	280	F	5	5	5	5	5	0	0	0	0	0	0	0%	0%
124	6/27/2008	R. catesbeiana	Copper	500	A	5	5	5	5	5	0	0	0	0	0	0	0%	
124	6/27/2008	R. catesbeiana	Copper	500	B	5	5	5	5	5	0	0	0	0	0	0	0%	
124	6/27/2008	R. catesbeiana	Copper	500	C	5	5	5	5	5	0	0	0	0	0	0	0%	
124	6/27/2008	R. catesbeiana	Copper	500	D	5	5	5	5	5	0	0	0	0	0	0	0%	
124	6/27/2008	R. catesbeiana	Copper	500	E	5	5	5	5	5	0	0	0	0	0	0	0%	
124	6/27/2008	R. catesbeiana	Copper	500	F	5	5	5	5	5	0	0	0	0	0	0	0%	0%
121	3/21/2008	R. sphenocephala	Lead	1.9	A	5	5	5	5	5	5	5	5	5	5	5	100%	
121	3/21/2008	R. sphenocephala	Lead	1.9	B	5	5	5	5	5	5	5	5	5	5	5	100%	
121	3/21/2008	R. sphenocephala	Lead	1.9	C	5	5	5	5	5	5	5	5	5	5	5	100%	
121	3/21/2008	R. sphenocephala	Lead	1.9	D	5	5	5	5	5	5	5	5	5	5	5	100%	
121	3/21/2008	R. sphenocephala	Lead	1.9	E	5	5	5	5	5	5	5	4	4	4	4	80%	
121	3/21/2008	R. sphenocephala	Lead	1.9	F	5	5	5	5	5	5	5	5	5	5	5	100%	97%
121	3/21/2008	R. sphenocephala	Lead	40	A	5	5	5	5	5	5	5	5	5	5	5	100%	
121	3/21/2008	R. sphenocephala	Lead	40	B	5	5	5	5	5	5	5	5	5	5	5	100%	
121	3/21/2008	R. sphenocephala	Lead	40	C	5	5	5	5	5	5	5	5	5	5	5	100%	
121	3/21/2008	R. sphenocephala	Lead	40	D	5	5	5	5	5	5	5	5	5	5	5	100%	
121	3/21/2008	R. sphenocephala	Lead	40	E	5	5	5	5	5	5	5	5	5	5	5	100%	
121	3/21/2008	R. sphenocephala	Lead	40	F	5	5	5	5	5	5	4	4	4	4	4	80%	97%
121	3/21/2008	R. sphenocephala	Lead	77	A	5	5	5	5	5	5	5	5	5	5	5	100%	
121	3/21/2008	R. sphenocephala	Lead	77	B	5	5	5	5	5	5	5	5	5	5	5	100%	
121	3/21/2008	R. sphenocephala	Lead	77	C	5	5	5	5	5	5	5	5	5	5	5	100%	
121	3/21/2008	R. sphenocephala	Lead	77	D	5	5	5	5	5	5	5	5	5	5	5	100%	
121	3/21/2008	R. sphenocephala	Lead	77	E	5	5	5	5	5	5	5	5	5	5	5	100%	
121	3/21/2008	R. sphenocephala	Lead	77	F	5	5	5	5	5	5	5	5	5	5	5	100%	100%

Survival Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Day 0 Specimen Count	Day 1 Specimen Count	Day 2 Specimen Count	Day 3 Specimen Count	Day 4 Specimen Count	Day 5 Specimen Count	Day 6 Specimen Count	Day 7 Specimen Count	Day 8 Specimen Count	Day 9 Specimen Count	Day 10 Specimen Count	Day 10 Survival (%)	Day 10 Average Survival (%)
121	3/21/2008	R. sphenocephala	Lead	140	A	5	5	5	5	5	5	5	5	5	5	5	100%	
121	3/21/2008	R. sphenocephala	Lead	140	B	5	5	5	5	5	5	5	5	5	5	5	100%	
121	3/21/2008	R. sphenocephala	Lead	140	C	5	5	5	5	5	5	5	5	5	5	5	100%	
121	3/21/2008	R. sphenocephala	Lead	140	D	5	5	5	5	5	5	4	4	4	4	4	80%	
121	3/21/2008	R. sphenocephala	Lead	140	E	5	5	5	5	5	5	5	4	4	4	4	80%	
121	3/21/2008	R. sphenocephala	Lead	140	F	5	5	5	5	5	5	5	5	5	5	5	100%	93%
121	3/21/2008	R. sphenocephala	Lead	340	A	5	5	5	5	5	5	5	5	5	5	5	100%	
121	3/21/2008	R. sphenocephala	Lead	340	B	5	5	5	5	5	5	5	5	5	5	5	100%	
121	3/21/2008	R. sphenocephala	Lead	340	C	5	5	5	5	5	5	5	5	5	5	5	100%	
121	3/21/2008	R. sphenocephala	Lead	340	D	5	5	5	5	5	5	5	5	5	5	5	100%	
121	3/21/2008	R. sphenocephala	Lead	340	E	5	5	5	5	5	5	5	5	5	5	5	100%	
121	3/21/2008	R. sphenocephala	Lead	340	F	5	5	5	5	5	5	5	5	5	5	5	100%	100%
121	3/21/2008	R. sphenocephala	Lead	750	A	5	5	5	5	0	0	0	0	0	0	0	0%	
121	3/21/2008	R. sphenocephala	Lead	750	B	5	5	5	5	0	0	0	0	0	0	0	0%	
121	3/21/2008	R. sphenocephala	Lead	750	C	5	5	5	5	0	0	0	0	0	0	0	0%	
121	3/21/2008	R. sphenocephala	Lead	750	D	5	5	5	5	4	4	2	2	2	2	1	20%	
121	3/21/2008	R. sphenocephala	Lead	750	E	5	5	5	5	5	5	5	5	4	4	4	80%	
121	3/21/2008	R. sphenocephala	Lead	750	F	5	5	5	5	0	0	0	0	0	0	0	0%	17%
121	3/21/2008	R. sphenocephala	Lead	1900	A	5	0	0	0	0	0	0	0	0	0	0	0%	
121	3/21/2008	R. sphenocephala	Lead	1900	B	5	0	0	0	0	0	0	0	0	0	0	0%	
121	3/21/2008	R. sphenocephala	Lead	1900	C	5	0	0	0	0	0	0	0	0	0	0	0%	
121	3/21/2008	R. sphenocephala	Lead	1900	D	5	0	0	0	0	0	0	0	0	0	0	0%	
121	3/21/2008	R. sphenocephala	Lead	1900	E	5	0	0	0	0	0	0	0	0	0	0	0%	
121	3/21/2008	R. sphenocephala	Lead	1900	F	5	0	0	0	0	0	0	0	0	0	0	0%	0%
120	3/21/2008	R. sphenocephala	Copper	3.2	A	5	5	5	5	5	5	5	5	5	5	5	100%	
120	3/21/2008	R. sphenocephala	Copper	3.2	B	5	5	5	5	5	5	5	5	5	5	5	100%	
120	3/21/2008	R. sphenocephala	Copper	3.2	C	5	5	5	5	5	5	5	5	5	5	5	100%	
120	3/21/2008	R. sphenocephala	Copper	3.2	D	5	5	5	5	5	5	5	5	5	5	5	100%	
120	3/21/2008	R. sphenocephala	Copper	3.2	E	5	5	5	5	5	5	5	5	5	5	5	100%	
120	3/21/2008	R. sphenocephala	Copper	3.2	F	5	5	5	5	5	5	5	4	4	4	4	80%	97%
120	3/21/2008	R. sphenocephala	Copper	13	A	5	5	5	5	5	5	5	5	5	5	5	100%	
120	3/21/2008	R. sphenocephala	Copper	13	B	5	5	5	5	5	5	5	5	5	5	5	100%	
120	3/21/2008	R. sphenocephala	Copper	13	C	5	5	5	5	5	5	5	5	5	5	5	100%	
120	3/21/2008	R. sphenocephala	Copper	13	D	5	5	5	5	5	5	5	5	5	5	5	100%	
120	3/21/2008	R. sphenocephala	Copper	13	E	5	5	5	5	5	5	5	5	5	5	5	100%	
120	3/21/2008	R. sphenocephala	Copper	13	F	5	5	5	5	5	5	5	5	5	5	5	100%	100%
120	3/21/2008	R. sphenocephala	Copper	32	A	5	5	5	5	5	5	5	5	5	5	5	100%	
120	3/21/2008	R. sphenocephala	Copper	32	B	5	5	5	5	5	5	5	5	5	5	5	100%	
120	3/21/2008	R. sphenocephala	Copper	32	C	5	5	5	5	5	5	5	5	5	5	5	100%	
120	3/21/2008	R. sphenocephala	Copper	32	D	5	5	5	5	5	5	5	5	5	5	5	100%	
120	3/21/2008	R. sphenocephala	Copper	32	E	5	5	5	5	5	5	5	5	5	5	5	100%	
120	3/21/2008	R. sphenocephala	Copper	32	F	5	5	5	5	5	5	5	5	5	5	5	100%	100%
120	3/21/2008	R. sphenocephala	Copper	85	A	5	5	5	5	5	5	5	5	5	5	5	100%	
120	3/21/2008	R. sphenocephala	Copper	85	B	5	5	5	5	5	5	5	5	5	5	5	100%	
120	3/21/2008	R. sphenocephala	Copper	85	C	5	5	5	5	5	5	5	5	5	5	5	100%	
120	3/21/2008	R. sphenocephala	Copper	85	D	5	5	5	5	5	5	5	5	5	5	5	100%	
120	3/21/2008	R. sphenocephala	Copper	85	E	5	5	5	5	5	5	5	5	5	5	5	100%	
120	3/21/2008	R. sphenocephala	Copper	85	F	5	5	5	5	5	5	5	5	5	5	5	100%	100%
120	3/21/2008	R. sphenocephala	Copper	140	A	5	5	4	4	2	2	2	2	2	2	2	40%	
120	3/21/2008	R. sphenocephala	Copper	140	B	5	5	5	5	5	5	5	5	5	5	5	100%	
120	3/21/2008	R. sphenocephala	Copper	140	C	5	5	5	5	5	5	5	5	5	5	5	100%	
120	3/21/2008	R. sphenocephala	Copper	140	D	5	4	2	2	0	0	0	0	0	0	0	0%	
120	3/21/2008	R. sphenocephala	Copper	140	E	5	5	4	4	2	2	2	2	2	2	2	40%	
120	3/21/2008	R. sphenocephala	Copper	140	F	5	5	5	5	5	5	5	5	5	5	5	100%	63%
120	3/21/2008	R. sphenocephala	Copper	280	A	5	0	0	0	0	0	0	0	0	0	0	0%	
120	3/21/2008	R. sphenocephala	Copper	280	B	5	0	0	0	0	0	0	0	0	0	0	0%	
120	3/21/2008	R. sphenocephala	Copper	280	C	5	0	0	0	0	0	0	0	0	0	0	0%	
120	3/21/2008	R. sphenocephala	Copper	280	D	5	0	0	0	0	0	0	0	0	0	0	0%	
120	3/21/2008	R. sphenocephala	Copper	280	E	5	0	0	0	0	0	0	0	0	0	0	0%	
120	3/21/2008	R. sphenocephala	Copper	280	F	5	0	0	0	0	0	0	0	0	0	0	0%	0%
120	3/21/2008	R. sphenocephala	Copper	500	A	5	0	0	0	0	0	0	0	0	0	0	0%	
120	3/21/2008	R. sphenocephala	Copper	500	B	5	0	0	0	0	0	0	0	0	0	0	0%	
120	3/21/2008	R. sphenocephala	Copper	500	C	5	0	0	0	0	0	0	0	0	0	0	0%	
120	3/21/2008	R. sphenocephala	Copper	500	D	5	0	0	0	0	0	0	0	0	0	0	0%	
120	3/21/2008	R. sphenocephala	Copper	500	E	5	0	0	0	0	0	0	0	0	0	0	0%	
120	3/21/2008	R. sphenocephala	Copper	500	F	5	0	0	0	0	0	0	0	0	0	0	0%	0%
036	12/9/2005	R.pipiens	Copper	7.2	A	5	5	5	5	5	5	5	5	5	5	5	100%	
036	12/9/2005	R.pipiens	Copper	7.2	B	5	5	5	5	5	5	5	5	5	5	5	100%	
036	12/9/2005	R.pipiens	Copper	7.2	C	5	5	5	5	5	5	5	5	5	5	5	100%	
036	12/9/2005	R.pipiens	Copper	7.2	D	5	5	5	5	5	5	5	5	5	5	5	100%	
036	12/9/2005	R.pipiens	Copper	7.2	E	5	5	5	5	5	5	5	5	5	5	5	100%	
036	12/9/2005	R.pipiens	Copper	7.2	F	5	5	5	5	5	5	5	5	5	5	5	100%	
036	12/9/2005	R.pipiens	Copper	7.2	G	5	5	5	5	5	5	5	5	5	5	5	100%	
036	12/9/2005	R.pipiens	Copper	7.2	H	5	5	5	5	5	5	5	5	5	5	5	100%	100%

Survival Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Day 0 Specimen Count	Day 1 Specimen Count	Day 2 Specimen Count	Day 3 Specimen Count	Day 4 Specimen Count	Day 5 Specimen Count	Day 6 Specimen Count	Day 7 Specimen Count	Day 8 Specimen Count	Day 9 Specimen Count	Day 10 Specimen Count	Day 10 Survival (%)	Day 10 Average Survival (%)
036	12/9/2005	R.pipiens	Copper	15	A	5	5	5	5	5	5	5	5	5	5	5	100%	
036	12/9/2005	R.pipiens	Copper	15	B	5	5	5	5	5	5	5	5	5	5	5	100%	
036	12/9/2005	R.pipiens	Copper	15	C	5	5	5	5	5	5	5	5	5	5	5	100%	
036	12/9/2005	R.pipiens	Copper	15	D	5	5	5	5	5	5	5	5	5	5	5	100%	
036	12/9/2005	R.pipiens	Copper	15	E	5	5	5	5	5	5	5	5	5	5	5	100%	
036	12/9/2005	R.pipiens	Copper	15	F	5	5	5	5	5	5	5	5	5	5	5	100%	
036	12/9/2005	R.pipiens	Copper	15	G	5	5	5	5	5	5	5	5	5	5	5	100%	
036	12/9/2005	R.pipiens	Copper	15	H	5	5	5	5	5	5	5	5	5	5	5	100%	100%
036	12/9/2005	R.pipiens	Copper	21	A	5	5	5	5	5	5	5	5	5	5	5	100%	
036	12/9/2005	R.pipiens	Copper	21	B	5	5	5	5	5	5	5	5	5	5	5	100%	
036	12/9/2005	R.pipiens	Copper	21	C	5	5	5	5	5	5	5	5	5	5	5	100%	
036	12/9/2005	R.pipiens	Copper	21	D	5	5	5	5	5	5	5	5	5	5	4	80%	
036	12/9/2005	R.pipiens	Copper	21	E	5	5	5	5	5	5	5	5	5	5	5	100%	
036	12/9/2005	R.pipiens	Copper	21	F	5	5	5	5	5	5	5	5	5	5	5	100%	
036	12/9/2005	R.pipiens	Copper	21	G	5	5	5	5	5	5	5	5	5	5	5	100%	
036	12/9/2005	R.pipiens	Copper	21	H	5	5	5	5	5	5	5	5	5	5	5	100%	98%
036	12/9/2005	R.pipiens	Copper	87	A	5	5	5	5	5	5	5	5	5	5	5	100%	
036	12/9/2005	R.pipiens	Copper	87	B	5	5	5	5	5	5	5	5	5	5	5	100%	
036	12/9/2005	R.pipiens	Copper	87	C	5	5	5	5	5	5	5	5	5	5	5	100%	
036	12/9/2005	R.pipiens	Copper	87	D	5	5	5	5	5	5	5	5	5	5	5	100%	
036	12/9/2005	R.pipiens	Copper	87	E	5	5	5	5	5	5	5	5	5	5	5	100%	
036	12/9/2005	R.pipiens	Copper	87	F	5	5	5	5	5	5	5	5	5	5	4	80%	
036	12/9/2005	R.pipiens	Copper	87	G	5	5	5	5	5	5	5	5	5	5	5	100%	
036	12/9/2005	R.pipiens	Copper	87	H	5	5	5	5	5	5	5	5	5	5	5	100%	98%
036	12/9/2005	R.pipiens	Copper	140	A	5	5	5	5	5	5	5	5	5	5	5	100%	
036	12/9/2005	R.pipiens	Copper	140	B	5	5	5	5	5	5	4	4	4	4	4	80%	
036	12/9/2005	R.pipiens	Copper	140	C	5	5	5	5	5	5	5	5	5	5	5	100%	
036	12/9/2005	R.pipiens	Copper	140	D	5	5	5	5	5	5	5	5	5	5	5	100%	
036	12/9/2005	R.pipiens	Copper	140	E	5	5	5	5	5	5	5	5	5	5	5	100%	
036	12/9/2005	R.pipiens	Copper	140	F	5	5	5	5	5	5	5	5	5	5	5	100%	
036	12/9/2005	R.pipiens	Copper	140	G	5	5	5	5	5	5	5	5	5	5	5	100%	
036	12/9/2005	R.pipiens	Copper	140	H	5	5	5	5	5	5	5	5	5	5	4	80%	95%
036	12/9/2005	R.pipiens	Copper	230	A	5	5	5	5	5	5	5	5	5	5	5	100%	
036	12/9/2005	R.pipiens	Copper	230	B	5	5	5	5	5	5	4	4	4	4	4	80%	
036	12/9/2005	R.pipiens	Copper	230	C	5	5	5	5	5	5	4	4	4	4	4	80%	
036	12/9/2005	R.pipiens	Copper	230	D	5	5	5	5	4	4	4	3	3	3	3	60%	
036	12/9/2005	R.pipiens	Copper	230	E	5	5	5	5	5	5	5	5	5	5	5	100%	
036	12/9/2005	R.pipiens	Copper	230	F	5	5	5	5	5	5	5	5	5	5	5	100%	
036	12/9/2005	R.pipiens	Copper	230	G	5	3	3	3	3	3	3	3	3	3	3	60%	
036	1/25/2006	R.pipiens	Copper	230	H	5										2	40%	78%
039	12/9/2005	R.pipiens	Lead	4.5	A	5	5	5	5	5	5	5	5	5	5	5	100%	
039	12/9/2005	R.pipiens	Lead	4.5	B	5	5	5	4	4	4	4	4	4	4	4	80%	
039	12/9/2005	R.pipiens	Lead	4.5	C	5	5	5	5	5	5	5	5	5	5	5	100%	
039	12/9/2005	R.pipiens	Lead	4.5	D	5	5	5	5	5	5	5	5	5	5	5	100%	
039	12/9/2005	R.pipiens	Lead	4.5	E	5	5	5	5	5	5	5	5	5	5	5	100%	
039	12/9/2005	R.pipiens	Lead	4.5	F	5	5	5	5	5	5	5	5	5	5	5	100%	
039	12/9/2005	R.pipiens	Lead	4.5	G	5	5	5	5	5	5	5	5	5	5	5	100%	
039	12/9/2005	R.pipiens	Lead	4.5	H	5	5	5	5	5	5	5	5	5	5	5	100%	98%
039	12/9/2005	R.pipiens	Lead	63	A	5	5	5	5	5	5	5	5	5	5	5	100%	
039	12/9/2005	R.pipiens	Lead	63	B	5	5	5	5	5	5	5	5	5	5	5	100%	
039	12/9/2005	R.pipiens	Lead	63	C	5	5	5	5	5	5	5	5	5	5	5	100%	
039	12/9/2005	R.pipiens	Lead	63	D	5	5	5	5	5	5	5	5	5	5	5	100%	
039	12/9/2005	R.pipiens	Lead	63	E	5	5	5	5	5	5	5	5	5	5	5	100%	
039	12/9/2005	R.pipiens	Lead	63	F	5	5	5	5	5	5	5	5	5	5	5	100%	
039	12/9/2005	R.pipiens	Lead	63	G	5	5	5	5	5	5	5	5	5	5	5	100%	
039	12/9/2005	R.pipiens	Lead	63	H	5	5	5	5	5	5	5	5	5	5	5	100%	100%
039	12/9/2005	R.pipiens	Lead	100	A	5	5	5	5	5	5	5	5	5	5	5	100%	
039	12/9/2005	R.pipiens	Lead	100	B	5	5	5	5	5	5	5	5	5	5	5	100%	
039	12/9/2005	R.pipiens	Lead	100	C	5	5	5	5	5	5	5	5	5	5	5	100%	
039	12/9/2005	R.pipiens	Lead	100	D	5	5	5	5	5	5	5	5	5	5	5	100%	
039	12/9/2005	R.pipiens	Lead	100	E	5	5	5	5	5	5	5	5	5	5	5	100%	
039	12/9/2005	R.pipiens	Lead	100	F	5	5	5	5	5	5	5	5	5	5	5	100%	
039	12/9/2005	R.pipiens	Lead	100	G	5	5	5	5	5	5	5	5	5	5	5	100%	
039	12/9/2005	R.pipiens	Lead	100	H	5	5	5	5	5	5	5	5	5	5	5	100%	100%
039	12/9/2005	R.pipiens	Lead	260	A	5	5	5	5	5	5	5	5	5	5	5	100%	
039	12/9/2005	R.pipiens	Lead	260	B	5	5	5	5	5	5	5	5	5	5	5	100%	
039	12/9/2005	R.pipiens	Lead	260	C	5	5	5	5	5	5	5	5	5	5	5	100%	
039	12/9/2005	R.pipiens	Lead	260	D	5	5	5	5	5	5	5	5	5	5	5	100%	
039	12/9/2005	R.pipiens	Lead	260	E	5	5	5	5	5	5	5	5	5	5	5	100%	
039	12/9/2005	R.pipiens	Lead	260	F	5	5	5	5	5	5	5	5	5	5	5	100%	
039	12/9/2005	R.pipiens	Lead	260	G	5	5	5	5	5	5	5	5	5	5	5	100%	
039	12/9/2005	R.pipiens	Lead	260	H	5	5	5	5	5	5	5	5	5	5	5	100%	100%

Survival Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Day 0 Specimen Count	Day 1 Specimen Count	Day 2 Specimen Count	Day 3 Specimen Count	Day 4 Specimen Count	Day 5 Specimen Count	Day 6 Specimen Count	Day 7 Specimen Count	Day 8 Specimen Count	Day 9 Specimen Count	Day 10 Specimen Count	Day 10 Survival (%)	Day 10 Average Survival (%)
039	12/9/2005	R.pipiens	Lead	680	A	5	5	5	5	5	5	5	5	5	5	5	100%	
039	12/9/2005	R.pipiens	Lead	680	B	5	5	5	5	5	5	5	5	5	5	5	100%	
039	12/9/2005	R.pipiens	Lead	680	C	5	5	5	5	5	5	5	5	5	5	5	100%	
039	12/9/2005	R.pipiens	Lead	680	D	5	5	5	5	5	5	5	5	5	5	5	100%	
039	12/9/2005	R.pipiens	Lead	680	E	5	5	5	5	5	5	5	5	5	5	5	100%	
039	12/9/2005	R.pipiens	Lead	680	F	5	5	5	5	5	5	5	5	5	5	5	100%	
039	12/9/2005	R.pipiens	Lead	680	G	5	5	5	5	5	5	5	5	5	5	5	100%	
039	12/9/2005	R.pipiens	Lead	680	H	5	5	5	5	5	5	5	5	5	5	5	100%	100%
039	12/9/2005	R.pipiens	Lead	1200	A	5	5	5	5	5	5	2	2	2	2	2	40%	
039	12/9/2005	R.pipiens	Lead	1200	B	5	5	5	5	5	5	5	5	5	5	5	100%	
039	12/9/2005	R.pipiens	Lead	1200	C	5	5	5	5	5	5	4	4	4	4	4	80%	
039	12/9/2005	R.pipiens	Lead	1200	D	5	5	5	5	4	4	4	4	4	4	4	80%	
039	12/9/2005	R.pipiens	Lead	1200	E	5	5	5	5	5	5	5	5	5	5	5	100%	
039	12/9/2005	R.pipiens	Lead	1200	F	5	5	5	5	5	5	4	4	4	4	4	80%	
039	12/9/2005	R.pipiens	Lead	1200	G	5	5	5	5	5	5	3	3	3	3	2	40%	
039	12/9/2005	R.pipiens	Lead	1200	H	5	5	5	5	5	5	2	2	2	2	2	40%	70%
123	4/21/2008	B. americanus	Lead	1.9	A	5	5	5	5	5	5	5	5	5	5	5	100%	
123	4/21/2008	B. americanus	Lead	1.9	B	5	5	5	5	5	5	5	5	5	5	5	100%	
123	4/21/2008	B. americanus	Lead	1.9	C	5	5	5	5	5	5	5	5	5	5	5	100%	
123	4/21/2008	B. americanus	Lead	1.9	D	5	5	5	5	5	5	5	5	5	5	5	100%	
123	4/21/2008	B. americanus	Lead	1.9	E	5	5	4	4	4	4	4	4	4	4	4	80%	
123	4/21/2008	B. americanus	Lead	1.9	F	5	5	5	5	5	5	5	5	5	5	5	100%	97%
123	4/21/2008	B. americanus	Lead	40	A	5	5	5	5	5	5	5	5	5	5	5	100%	
123	4/21/2008	B. americanus	Lead	40	B	5	5	5	5	5	5	5	5	5	5	5	100%	
123	4/21/2008	B. americanus	Lead	40	C	5	5	5	5	5	5	5	5	5	5	5	100%	
123	4/21/2008	B. americanus	Lead	40	D	5	5	5	5	5	5	5	5	5	5	5	100%	
123	4/21/2008	B. americanus	Lead	40	E	5	5	5	5	5	5	5	5	5	5	5	100%	
123	4/21/2008	B. americanus	Lead	40	F	5	5	5	5	5	5	5	5	5	5	5	100%	100%
123	4/21/2008	B. americanus	Lead	77	A	5	5	5	5	5	5	5	5	5	5	5	100%	
123	4/21/2008	B. americanus	Lead	77	B	5	5	5	5	5	5	5	5	5	5	5	100%	
123	4/21/2008	B. americanus	Lead	77	C	5	5	5	5	5	5	5	5	5	5	5	100%	
123	4/21/2008	B. americanus	Lead	77	D	5	5	5	5	5	5	5	5	5	5	5	100%	
123	4/21/2008	B. americanus	Lead	77	E	5	5	5	5	5	5	5	5	5	5	5	100%	
123	4/21/2008	B. americanus	Lead	77	F	5	5	5	5	5	5	5	5	5	5	5	100%	100%
123	4/21/2008	B. americanus	Lead	140	A	5	5	5	5	5	5	5	5	5	5	5	100%	
123	4/21/2008	B. americanus	Lead	140	B	5	5	5	5	5	5	5	5	5	5	5	100%	
123	4/21/2008	B. americanus	Lead	140	C	5	5	5	5	5	5	5	5	5	5	4	80%	
123	4/21/2008	B. americanus	Lead	140	D	5	5	5	5	5	5	5	5	5	5	5	100%	
123	4/21/2008	B. americanus	Lead	140	E	5	5	5	5	5	5	5	5	5	5	5	100%	
123	4/21/2008	B. americanus	Lead	140	F	5	5	5	5	5	5	5	5	5	5	5	100%	97%
123	4/21/2008	B. americanus	Lead	340	A	5	3	3	3	3	3	3	3	3	3	3	60%	
123	4/21/2008	B. americanus	Lead	340	B	5	5	5	5	4	4	3	3	3	3	3	60%	
123	4/21/2008	B. americanus	Lead	340	C	5	5	5	5	5	5	5	5	5	5	5	100%	
123	4/21/2008	B. americanus	Lead	340	D	5	5	5	5	5	5	5	5	5	5	5	100%	
123	4/21/2008	B. americanus	Lead	340	E	5	5	5	5	5	5	5	5	5	5	5	100%	
123	4/21/2008	B. americanus	Lead	340	F	5	5	5	5	5	5	5	5	5	5	5	100%	87%
123	4/21/2008	B. americanus	Lead	750	A	5	5	5	5	3	1	1	1	1	1	1	20%	
123	4/21/2008	B. americanus	Lead	750	B	5	1	0	0	0	0	0	0	0	0	0	0%	
123	4/21/2008	B. americanus	Lead	750	C	5	0	0	0	0	0	0	0	0	0	0	0%	
123	4/21/2008	B. americanus	Lead	750	D	5	2	2	2	2	2	2	1	1	1	1	20%	
123	4/21/2008	B. americanus	Lead	750	E	5	2	2	2	1	1	1	1	1	1	1	20%	
123	4/21/2008	B. americanus	Lead	750	F	5	1	1	1	1	1	1	1	1	1	1	20%	13%
123	4/21/2008	B. americanus	Lead	1900	A	5	0	0	0	0	0	0	0	0	0	0	0%	
123	4/21/2008	B. americanus	Lead	1900	B	5	0	0	0	0	0	0	0	0	0	0	0%	
123	4/21/2008	B. americanus	Lead	1900	C	5	0	0	0	0	0	0	0	0	0	0	0%	
123	4/21/2008	B. americanus	Lead	1900	D	5	0	0	0	0	0	0	0	0	0	0	0%	
123	4/21/2008	B. americanus	Lead	1900	E	5	0	0	0	0	0	0	0	0	0	0	0%	3%
122	4/21/2008	B. americanus	Copper	3.2	A	5	5	5	5	5	5	5	5	5	5	5	100%	
122	4/21/2008	B. americanus	Copper	3.2	B	5	5	5	5	5	5	5	5	5	5	5	100%	
122	4/21/2008	B. americanus	Copper	3.2	C	5	5	5	5	5	5	5	5	5	5	5	100%	
122	4/21/2008	B. americanus	Copper	3.2	D	5	5	5	5	5	5	5	5	5	5	5	100%	
122	4/21/2008	B. americanus	Copper	3.2	E	5	5	5	5	5	5	5	5	5	5	5	100%	
122	4/21/2008	B. americanus	Copper	3.2	F	5	5	5	5	5	5	5	5	5	5	5	100%	100%
122	4/21/2008	B. americanus	Copper	13	A	5	5	5	5	5	5	5	5	5	5	5	100%	
122	4/21/2008	B. americanus	Copper	13	B	5	5	5	5	5	5	5	5	5	5	5	100%	
122	4/21/2008	B. americanus	Copper	13	C	5	5	5	5	5	5	5	5	5	5	5	100%	
122	4/21/2008	B. americanus	Copper	13	D	5	5	5	5	5	5	5	5	5	5	5	100%	
122	4/21/2008	B. americanus	Copper	13	E	5	5	5	5	5	5	5	5	5	5	5	100%	
122	4/21/2008	B. americanus	Copper	13	F	5	5	5	5	5	5	5	5	5	5	5	100%	100%
122	4/21/2008	B. americanus	Copper	32	A	5	5	5	5	5	5	5	5	5	5	5	100%	
122	4/21/2008	B. americanus	Copper	32	B	5	5	5	5	5	5	5	5	5	5	5	100%	
122	4/21/2008	B. americanus	Copper	32	C	5	5	5	5	5	5	5	5	5	5	5	100%	
122	4/21/2008	B. americanus	Copper	32	D	5	-	-	-	-	-	-	-	-	-	-	0%	
122	4/21/2008	B. americanus	Copper	32	E	5	5	5	5	5	5	5	5	5	5	5	100%	
122	4/21/2008	B. americanus	Copper	32	F	5	5	5	5	5	5	5	5	5	5	5	100%	83%

Survival Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Day 0 Specimen Count	Day 1 Specimen Count	Day 2 Specimen Count	Day 3 Specimen Count	Day 4 Specimen Count	Day 5 Specimen Count	Day 6 Specimen Count	Day 7 Specimen Count	Day 8 Specimen Count	Day 9 Specimen Count	Day 10 Specimen Count	Day 10 Survival (%)	Day 10 Average Survival (%)
122	4/21/2008	B. americanus	Copper	85	A	5	5	5	5	5	5	5	5	5	5	5	100%	
122	4/21/2008	B. americanus	Copper	85	B	5	2	2	2	2	2	2	2	2	2	2	40%	
122	4/21/2008	B. americanus	Copper	85	C	5	4	3	3	3	3	3	3	3	3	3	60%	
122	4/21/2008	B. americanus	Copper	85	D	5	4	4	4	4	4	3	3	3	3	3	60%	
122	4/21/2008	B. americanus	Copper	85	E	5	2	1	1	1	1	0	0	0	0	0	0%	
122	4/21/2008	B. americanus	Copper	85	F	5	0	0	0	0	0	0	0	0	0	0	0%	43%
122	4/21/2008	B. americanus	Copper	140	A	5	0	0	0	0	0	0	0	0	0	0	0%	
122	4/21/2008	B. americanus	Copper	140	B	5	0	0	0	0	0	0	0	0	0	0	0%	
122	4/21/2008	B. americanus	Copper	140	C	5	0	0	0	0	0	0	0	0	0	0	0%	
122	4/21/2008	B. americanus	Copper	140	D	5	0	0	0	0	0	0	0	0	0	0	0%	
122	4/21/2008	B. americanus	Copper	140	E	5	0	0	0	0	0	0	0	0	0	0	0%	
122	4/21/2008	B. americanus	Copper	140	F	5	0	0	0	0	0	0	0	0	0	0	0%	0%
122	4/21/2008	B. americanus	Copper	280	A	5	0	0	0	0	0	0	0	0	0	0	0%	
122	4/21/2008	B. americanus	Copper	280	B	5	0	0	0	0	0	0	0	0	0	0	0%	
122	4/21/2008	B. americanus	Copper	280	C	5	0	0	0	0	0	0	0	0	0	0	0%	
122	4/21/2008	B. americanus	Copper	280	D	5	0	0	0	0	0	0	0	0	0	0	0%	
122	4/21/2008	B. americanus	Copper	280	E	5	0	0	0	0	0	0	0	0	0	0	0%	
122	4/21/2008	B. americanus	Copper	280	F	5	0	0	0	0	0	0	0	0	0	0	0%	0%
122	4/21/2008	B. americanus	Copper	500	A	5	0	0	0	0	0	0	0	0	0	0	0%	
122	4/21/2008	B. americanus	Copper	500	B	5	0	0	0	0	0	0	0	0	0	0	0%	
122	4/21/2008	B. americanus	Copper	500	C	5	0	0	0	0	0	0	0	0	0	0	0%	
122	4/21/2008	B. americanus	Copper	500	D	5	0	0	0	0	0	0	0	0	0	0	0%	
122	4/21/2008	B. americanus	Copper	500	E	5	0	0	0	0	0	0	0	0	0	0	0%	0%
075 [copper]	5/13/2006	R. palustris	Copper	4.2	A	5	5	5	5	5	5	5	5	5	5	5	100%	
075 [copper]	5/13/2006	R. palustris	Copper	4.2	B	5	5	5	5	5	5	5	5	5	5	5	100%	
075 [copper]	5/13/2006	R. palustris	Copper	4.2	C	5	5	5	5	5	5	5	5	5	5	5	100%	
075 [copper]	5/13/2006	R. palustris	Copper	4.2	D	5	5	5	5	5	5	5	5	5	5	5	100%	100%
075 [copper]	5/13/2006	R. palustris	Copper	28.9	A	5	5	5	5	5	5	5	5	5	5	5	100%	
075 [copper]	5/13/2006	R. palustris	Copper	28.9	B	5	5	5	5	5	5	5	5	5	5	5	100%	
075 [copper]	5/13/2006	R. palustris	Copper	28.9	C	5	5	5	5	5	5	5	5	5	5	5	100%	
075 [copper]	5/13/2006	R. palustris	Copper	28.9	D	5	5	4	4	4	4	4	4	4	4	4	80%	95%
075 [copper]	5/13/2006	R. palustris	Copper	79.9	A	5	5	0	0	0	0	0	0	0	0	0	0%	
075 [copper]	5/13/2006	R. palustris	Copper	79.9	B	5	5	0	0	0	0	0	0	0	0	0	0%	
075 [copper]	5/13/2006	R. palustris	Copper	79.9	C	5	5	0	0	0	0	0	0	0	0	0	0%	
075 [copper]	5/13/2006	R. palustris	Copper	79.9	D	5	5	0	0	0	0	0	0	0	0	0	0%	0%
075 [copper]	5/13/2006	R. palustris	Copper	150	A	5	5	0	0	0	0	0	0	0	0	0	0%	
075 [copper]	5/13/2006	R. palustris	Copper	150	B	5	5	0	0	0	0	0	0	0	0	0	0%	
075 [copper]	5/13/2006	R. palustris	Copper	150	C	5	5	0	0	0	0	0	0	0	0	0	0%	
075 [copper]	5/13/2006	R. palustris	Copper	150	D	5	5	0	0	0	0	0	0	0	0	0	0%	0%
075 [copper]	5/13/2006	R. palustris	Copper	265	A	5	5	0	0	0	0	0	0	0	0	0	0%	
075 [copper]	5/13/2006	R. palustris	Copper	265	B	5	5	0	0	0	0	0	0	0	0	0	0%	
075 [copper]	5/13/2006	R. palustris	Copper	265	C	5	5	0	0	0	0	0	0	0	0	0	0%	
075 [copper]	5/13/2006	R. palustris	Copper	265	D	5	5	0	0	0	0	0	0	0	0	0	0%	0%
075 [lead]	5/13/2006	R. palustris	Lead	2.6	A	5	5	5	5	5	5	5	5	5	5	5	100%	
075 [lead]	5/13/2006	R. palustris	Lead	2.6	B	5	5	5	5	5	5	5	5	5	5	5	100%	
075 [lead]	5/13/2006	R. palustris	Lead	2.6	C	5	5	5	5	5	5	5	5	5	5	5	100%	
075 [lead]	5/13/2006	R. palustris	Lead	2.6	D	5	5	5	5	5	5	5	5	5	5	5	100%	100%
075 [lead]	5/13/2006	R. palustris	Lead	116	A	5	5	5	5	5	5	5	5	5	5	5	100%	
075 [lead]	5/13/2006	R. palustris	Lead	116	B	5	5	5	5	5	5	5	5	5	5	5	100%	
075 [lead]	5/13/2006	R. palustris	Lead	116	C	5	5	5	5	5	5	5	5	5	5	5	100%	
075 [lead]	5/13/2006	R. palustris	Lead	116	D	5	5	5	5	5	5	5	5	5	5	5	100%	100%
075 [lead]	5/13/2006	R. palustris	Lead	298	A	5	5	5	5	5	4	4	4	4	4	4	80%	
075 [lead]	5/13/2006	R. palustris	Lead	298	B	5	5	5	5	5	4	4	4	4	4	4	80%	
075 [lead]	5/13/2006	R. palustris	Lead	298	C	5	5	5	5	4	3	3	3	3	3	3	60%	
075 [lead]	5/13/2006	R. palustris	Lead	298	D	5	5	5	5	4	4	3	3	3	3	3	60%	70%
075 [lead]	5/13/2006	R. palustris	Lead	635	A	5	5	5	5	4	4	1	1	0	0	0	0%	
075 [lead]	5/13/2006	R. palustris	Lead	635	B	5	5	5	5	5	3	2	2	1	1	0	0%	
075 [lead]	5/13/2006	R. palustris	Lead	635	C	5	5	4	4	4	1	0	0	0	0	0	0%	
075 [lead]	5/13/2006	R. palustris	Lead	635	D	5	5	4	4	4	0	0	0	0	0	0	0%	0%
075 [lead]	5/13/2006	R. palustris	Lead	1300	A	5	5	0	0	0	0	0	0	0	0	0	0%	
075 [lead]	5/13/2006	R. palustris	Lead	1300	B	5	5	0	0	0	0	0	0	0	0	0	0%	
075 [lead]	5/13/2006	R. palustris	Lead	1300	C	5	5	0	0	0	0	0	0	0	0	0	0%	
075 [lead]	5/13/2006	R. palustris	Lead	1300	D	5	5	1	1	1	0	0	0	0	0	0	0%	0%
076	7/1/2006	R. clamitans	Copper	4.2	A	5	5	5	5	5	5	5	5	5	5	5	100%	
076	7/1/2006	R. clamitans	Copper	4.2	B	5	5	5	5	5	5	3	3	3	3	3	60%	
076	7/1/2006	R. clamitans	Copper	4.2	C	5	5	5	5	3	3	3	3	3	3	3	60%	
076	7/1/2006	R. clamitans	Copper	4.2	D	5	5	5	5	5	5	5	5	5	5	5	100%	
076	7/1/2006	R. clamitans	Copper	4.2	E	5	5	5	5	5	5	5	5	5	5	5	100%	
076	7/1/2006	R. clamitans	Copper	4.2	F	5	5	5	5	5	5	5	5	5	5	5	100%	
076	7/1/2006	R. clamitans	Copper	4.2	G	5	5	5	5	5	5	5	5	5	5	5	100%	
076	7/1/2006	R. clamitans	Copper	4.2	H	5	5	5	5	5	5	5	5	5	5	5	100%	90%
076	7/1/2006	R. clamitans	Copper	28.9	A	5	5	5	5	5	5	5	5	5	5	5	100%	
076	7/1/2006	R. clamitans	Copper	28.9	B	5	5	5	5	5	5	5	5	5	5	5	100%	
076	7/1/2006	R. clamitans	Copper	28.9	C	5	5	5	5	5	5	5	5	5	5	5	100%	
076	7/1/2006	R. clamitans	Copper	28.9	D	5	5	5	5	5	5	5	5	5	5	5	100%	
076	7/1/2006	R. clamitans	Copper	28.9	E	5	5	5	5	5	5	5	5	5	5	5	100%	
076	7/1/2006	R. clamitans	Copper	28.9	F	5	5	5	5	4	4	4	4	4	4	4	80%	
076	7/1/2006	R. clamitans	Copper	28.9	G	5	5	5	5	5	5	5	5	5	5	5	100%	
076	7/1/2006	R. clamitans	Copper	28.9	H	5	5	5	5	5	5	5	5	5	5	5	100%	98%

Survival Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Day 0 Specimen Count	Day 1 Specimen Count	Day 2 Specimen Count	Day 3 Specimen Count	Day 4 Specimen Count	Day 5 Specimen Count	Day 6 Specimen Count	Day 7 Specimen Count	Day 8 Specimen Count	Day 9 Specimen Count	Day 10 Specimen Count	Day 10 Survival (%)	Day 10 Average Survival (%)
076	7/1/2006	R. clamitans	Copper	79.9	A	5	5	0	0	0	0	0	0	0	0	0	0%	
076	7/1/2006	R. clamitans	Copper	79.9	B	5	5	5	5	5	5	5	5	5	5	5	100%	
076	7/1/2006	R. clamitans	Copper	79.9	C	5	5	5	5	5	5	5	5	5	5	5	100%	
076	7/1/2006	R. clamitans	Copper	79.9	D	5	3	3	3	2	2	2	2	2	2	2	40%	
076	7/1/2006	R. clamitans	Copper	79.9	E	5	5	5	5	5	5	5	5	5	5	5	100%	
076	7/1/2006	R. clamitans	Copper	79.9	F	5	0	0	0	0	0	0	0	0	0	0	0%	
076	7/1/2006	R. clamitans	Copper	79.9	G	5	0	0	0	0	0	0	0	0	0	0	0%	
076	7/1/2006	R. clamitans	Copper	79.9	H	5	4	2	2	1	0	0	0	0	0	0	0%	43%
076	7/1/2006	R. clamitans	Copper	150	A	5	0	0	0	0	0	0	0	0	0	0	0%	
076	7/1/2006	R. clamitans	Copper	150	B	5	0	0	0	0	0	0	0	0	0	0	0%	
076	7/1/2006	R. clamitans	Copper	150	C	5	0	0	0	0	0	0	0	0	0	0	0%	
076	7/1/2006	R. clamitans	Copper	150	D	5	0	0	0	0	0	0	0	0	0	0	0%	
076	7/1/2006	R. clamitans	Copper	150	E	5	0	0	0	0	0	0	0	0	0	0	0%	
076	7/1/2006	R. clamitans	Copper	150	F	5	5	4	0	0	0	0	0	0	0	0	0%	
076	7/1/2006	R. clamitans	Copper	150	G	5	0	0	0	0	0	0	0	0	0	0	0%	
076	7/1/2006	R. clamitans	Copper	150	H	5	0	0	0	0	0	0	0	0	0	0	0%	0%
076	7/1/2006	R. clamitans	Copper	265	A	5	0	0	0	0	0	0	0	0	0	0	0%	
076	7/1/2006	R. clamitans	Copper	265	B	5	0	0	0	0	0	0	0	0	0	0	0%	
076	7/1/2006	R. clamitans	Copper	265	C	5	0	0	0	0	0	0	0	0	0	0	0%	
076	7/1/2006	R. clamitans	Copper	265	D	5	0	0	0	0	0	0	0	0	0	0	0%	
076	7/1/2006	R. clamitans	Copper	265	E	5	0	0	0	0	0	0	0	0	0	0	0%	
076	7/1/2006	R. clamitans	Copper	265	F	5	0	0	0	0	0	0	0	0	0	0	0%	
076	7/1/2006	R. clamitans	Copper	265	G	5	0	0	0	0	0	0	0	0	0	0	0%	
076	7/1/2006	R. clamitans	Copper	265	H	5	0	0	0	0	0	0	0	0	0	0	0%	0%
077	7/1/2006	R. clamitans	Lead	2.6	A	5	5	5	5	5	5	5	5	5	5	5	100%	
077	7/1/2006	R. clamitans	Lead	2.6	B	5	5	5	5	5	5	5	5	5	5	5	100%	
077	7/1/2006	R. clamitans	Lead	2.6	C	5	5	5	5	5	5	5	5	5	5	5	100%	
077	7/1/2006	R. clamitans	Lead	2.6	D	5	5	5	5	5	5	5	5	5	5	5	100%	
077	7/1/2006	R. clamitans	Lead	2.6	E	5	5	5	5	5	5	5	5	5	5	5	100%	
077	7/1/2006	R. clamitans	Lead	2.6	F	5	5	5	5	5	5	5	5	5	5	5	100%	
077	7/1/2006	R. clamitans	Lead	2.6	G	5	5	5	5	5	5	5	5	4	4	4	80%	
077	7/1/2006	R. clamitans	Lead	2.6	H	5	5	5	5	5	5	5	5	5	5	5	100%	98%
077	7/1/2006	R. clamitans	Lead	116	A	5	5	5	5	5	5	5	5	5	5	5	100%	
077	7/1/2006	R. clamitans	Lead	116	B	5	5	5	5	5	5	5	5	5	5	5	100%	
077	7/1/2006	R. clamitans	Lead	116	C	5	5	5	5	5	5	5	5	5	5	5	100%	
077	7/1/2006	R. clamitans	Lead	116	D	5	5	5	5	5	5	5	5	5	5	5	100%	
077	7/1/2006	R. clamitans	Lead	116	E	5	5	5	5	5	5	5	5	5	5	5	100%	
077	7/1/2006	R. clamitans	Lead	116	F	5	5	5	5	5	5	5	5	5	5	5	100%	
077	7/1/2006	R. clamitans	Lead	116	G	5	5	5	5	5	5	5	5	5	5	5	100%	
077	7/1/2006	R. clamitans	Lead	116	H	5	5	5	5	5	5	5	5	5	5	5	100%	100%
077	7/1/2006	R. clamitans	Lead	298	A	5	5	5	5	5	5	5	5	5	5	5	100%	
077	7/1/2006	R. clamitans	Lead	298	B	5	5	5	5	5	5	5	5	5	5	5	100%	
077	7/1/2006	R. clamitans	Lead	298	C	5	5	5	5	5	5	5	5	5	5	4	80%	
077	7/1/2006	R. clamitans	Lead	298	D	5	5	5	5	5	5	5	5	5	5	5	100%	
077	7/1/2006	R. clamitans	Lead	298	E	5	5	5	5	5	5	5	5	5	5	5	100%	
077	7/1/2006	R. clamitans	Lead	298	F	5	5	5	5	5	5	5	5	5	5	5	100%	
077	7/1/2006	R. clamitans	Lead	298	G	5	5	5	5	5	5	5	5	5	5	5	100%	
077	7/1/2006	R. clamitans	Lead	298	H	5	5	5	5	5	5	5	5	5	5	5	100%	98%
077	7/1/2006	R. clamitans	Lead	635	A	5	5	5	5	5	5	5	5	4	4	4	80%	
077	7/1/2006	R. clamitans	Lead	635	B	5	5	5	5	5	5	5	5	5	5	4	80%	
077	7/1/2006	R. clamitans	Lead	635	C	5	4	4	4	4	4	4	4	4	4	4	80%	
077	7/1/2006	R. clamitans	Lead	635	D	5	5	5	5	5	5	5	5	5	5	3	60%	
077	7/1/2006	R. clamitans	Lead	635	E	5	5	5	5	5	5	5	5	5	5	5	100%	
077	7/1/2006	R. clamitans	Lead	635	F	5	5	5	5	5	5	5	5	5	5	4	80%	
077	7/1/2006	R. clamitans	Lead	635	G	5	5	5	5	5	5	5	5	5	5	5	100%	
077	7/1/2006	R. clamitans	Lead	635	H	5	5	5	5	5	5	5	5	5	5	3	60%	80%
077	7/1/2006	R. clamitans	Lead	1300	A	5	0	0	0	0	0	0	0	0	0	0	0%	
077	7/1/2006	R. clamitans	Lead	1300	B	5	0	0	0	0	0	0	0	0	0	0	0%	
077	7/1/2006	R. clamitans	Lead	1300	C	5	0	0	0	0	0	0	0	0	0	0	0%	
077	7/1/2006	R. clamitans	Lead	1300	D	5	4	0	0	0	0	0	0	0	0	0	0%	
077	7/1/2006	R. clamitans	Lead	1300	E	5	0	0	0	0	0	0	0	0	0	0	0%	
077	7/1/2006	R. clamitans	Lead	1300	F	5	1	0	0	0	0	0	0	0	0	0	0%	
077	7/1/2006	R. clamitans	Lead	1300	G	5	0	0	0	0	0	0	0	0	0	0	0%	
077	7/1/2006	R. clamitans	Lead	1300	H	5	0	0	0	0	0	0	0	0	0	0	0%	0%
094	4/15/2007	R. sylvatica	Copper	3.2	A	5	5	5	5	5	5	5	5	5	5	5	100%	
094	4/15/2007	R. sylvatica	Copper	3.2	B	5	5	5	5	5	5	5	5	5	5	5	100%	
094	4/15/2007	R. sylvatica	Copper	3.2	C	5	5	5	5	5	5	5	5	5	5	5	100%	
094	4/15/2007	R. sylvatica	Copper	3.2	D	5	5	5	5	5	5	5	5	5	5	5	100%	
094	4/15/2007	R. sylvatica	Copper	3.2	E	5	5	5	5	5	5	5	5	5	5	5	100%	
094	4/15/2007	R. sylvatica	Copper	3.2	F	5	5	5	5	5	5	5	5	5	5	5	100%	100%
094	4/15/2007	R. sylvatica	Copper	13	A	5	5	5	5	5	5	5	5	5	5	5	100%	
094	4/15/2007	R. sylvatica	Copper	13	B	5	5	5	5	5	5	5	5	5	5	5	100%	
094	4/15/2007	R. sylvatica	Copper	13	C	5	5	5	5	5	5	5	5	5	5	4	80%	
094	4/15/2007	R. sylvatica	Copper	13	D	5	5	5	5	5	5	5	5	5	5	5	100%	
094	4/15/2007	R. sylvatica	Copper	13	E	5	5	5	5	5	5	5	5	5	5	5	100%	
094	4/15/2007	R. sylvatica	Copper	13	F	5	5	5	5	5	5	5	5	5	5	5	100%	97%

Survival Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Day 0 Specimen Count	Day 1 Specimen Count	Day 2 Specimen Count	Day 3 Specimen Count	Day 4 Specimen Count	Day 5 Specimen Count	Day 6 Specimen Count	Day 7 Specimen Count	Day 8 Specimen Count	Day 9 Specimen Count	Day 10 Specimen Count	Day 10 Survival (%)	Day 10 Average Survival (%)
094	4/15/2007	R. sylvatica	Copper	32	A	5	5	5	5	5	5	5	5	5	5	5	100%	
094	4/15/2007	R. sylvatica	Copper	32	B	5	5	5	5	5	5	5	5	5	5	5	100%	
094	4/15/2007	R. sylvatica	Copper	32	C	5	5	5	5	5	5	5	5	5	5	5	100%	
094	4/15/2007	R. sylvatica	Copper	32	D	5	5	5	5	5	5	5	5	5	5	5	100%	
094	4/15/2007	R. sylvatica	Copper	32	E	5	5	5	5	5	5	5	5	5	5	5	100%	
094	4/15/2007	R. sylvatica	Copper	32	F	5	5	5	5	5	5	5	5	5	5	5	100%	100%
094	4/15/2007	R. sylvatica	Copper	72.7	A	5	5	5	5	5	5	5	5	5	5	5	100%	
094	4/15/2007	R. sylvatica	Copper	72.7	B	5	5	5	5	5	5	5	5	5	5	5	100%	
094	4/15/2007	R. sylvatica	Copper	72.7	C	5	5	5	5	5	5	5	5	5	5	5	100%	
094	4/15/2007	R. sylvatica	Copper	72.7	D	5	5	5	5	5	5	5	5	5	5	5	100%	
094	4/15/2007	R. sylvatica	Copper	72.7	E	5	5	5	5	5	5	5	5	5	5	5	100%	
094	4/15/2007	R. sylvatica	Copper	72.7	F	5	5	5	5	5	5	5	5	5	5	5	100%	100%
094	4/15/2007	R. sylvatica	Copper	170	A	5	0	0	0	0	0	0	0	0	0	0	0%	
094	4/15/2007	R. sylvatica	Copper	170	B	5	0	0	0	0	0	0	0	0	0	0	0%	
094	4/15/2007	R. sylvatica	Copper	170	C	5	0	0	0	0	0	0	0	0	0	0	0%	
094	4/15/2007	R. sylvatica	Copper	170	D	5	0	0	0	0	0	0	0	0	0	0	0%	
094	4/15/2007	R. sylvatica	Copper	170	E	5	0	0	0	0	0	0	0	0	0	0	0%	
094	4/15/2007	R. sylvatica	Copper	170	F	5	0	0	0	0	0	0	0	0	0	0	0%	0%
094	4/15/2007	R. sylvatica	Copper	280	A	5	0	0	0	0	0	0	0	0	0	0	0%	
094	4/15/2007	R. sylvatica	Copper	280	B	5	0	0	0	0	0	0	0	0	0	0	0%	
094	4/15/2007	R. sylvatica	Copper	280	C	5	0	0	0	0	0	0	0	0	0	0	0%	
094	4/15/2007	R. sylvatica	Copper	280	D	5	0	0	0	0	0	0	0	0	0	0	0%	
094	4/15/2007	R. sylvatica	Copper	280	E	5	0	0	0	0	0	0	0	0	0	0	0%	
094	4/15/2007	R. sylvatica	Copper	280	F	5	0	0	0	0	0	0	0	0	0	0	0%	0%
094	4/15/2007	R. sylvatica	Copper	510	A	5	0	0	0	0	0	0	0	0	0	0	0%	
094	4/15/2007	R. sylvatica	Copper	510	B	5	0	0	0	0	0	0	0	0	0	0	0%	
094	4/15/2007	R. sylvatica	Copper	510	C	5	0	0	0	0	0	0	0	0	0	0	0%	
094	4/15/2007	R. sylvatica	Copper	510	D	5	0	0	0	0	0	0	0	0	0	0	0%	
094	4/15/2007	R. sylvatica	Copper	510	E	5	0	0	0	0	0	0	0	0	0	0	0%	
094	4/15/2007	R. sylvatica	Copper	510	F	5	0	0	0	0	0	0	0	0	0	0	0%	0%
095	4/15/2007	R. sylvatica	Lead	1.9	A	5	5	5	5	5	5	5	5	5	5	5	100%	
095	4/15/2007	R. sylvatica	Lead	1.9	B	5	5	5	5	5	5	5	5	5	5	5	100%	
095	4/15/2007	R. sylvatica	Lead	1.9	C	5	5	5	5	5	5	5	5	5	5	5	100%	
095	4/15/2007	R. sylvatica	Lead	1.9	D	5	5	5	5	5	5	5	5	5	5	5	100%	
095	4/15/2007	R. sylvatica	Lead	1.9	E	5	5	5	5	5	5	5	5	5	5	5	100%	
095	4/15/2007	R. sylvatica	Lead	1.9	F	5	4	4	4	4	4	4	4	4	4	4	80%	97%
095	4/15/2007	R. sylvatica	Lead	67	A	5	5	5	5	5	5	5	5	5	5	5	100%	
095	4/15/2007	R. sylvatica	Lead	67	B	5	5	5	5	5	5	5	5	5	5	5	100%	
095	4/15/2007	R. sylvatica	Lead	67	C	5	5	5	5	5	5	5	5	5	5	4	80%	
095	4/15/2007	R. sylvatica	Lead	67	D	5	5	5	5	5	5	5	5	5	5	5	100%	
095	4/15/2007	R. sylvatica	Lead	67	E	5	5	5	5	5	5	5	5	5	5	5	100%	
095	4/15/2007	R. sylvatica	Lead	67	F	5	5	5	5	5	5	5	5	5	5	5	100%	97%
095	4/15/2007	R. sylvatica	Lead	130	A	5	5	5	5	5	5	5	5	5	5	5	100%	
095	4/15/2007	R. sylvatica	Lead	130	B	5	5	5	5	5	5	5	5	5	5	5	100%	
095	4/15/2007	R. sylvatica	Lead	130	C	5	5	5	5	5	5	5	5	5	5	5	100%	
095	4/15/2007	R. sylvatica	Lead	130	D	5	5	5	5	5	5	5	5	5	5	5	100%	
095	4/15/2007	R. sylvatica	Lead	130	E	5	5	5	5	5	5	5	5	5	5	5	100%	
095	4/15/2007	R. sylvatica	Lead	130	F	5	5	5	5	5	5	5	5	3	3	3	60%	93%
095	4/15/2007	R. sylvatica	Lead	340	A	5	5	5	5	5	5	5	4	4	4	4	80%	
095	4/15/2007	R. sylvatica	Lead	340	B	5	5	4	2	2	2	2	2	2	2	2	40%	
095	4/15/2007	R. sylvatica	Lead	340	C	5	5	5	5	5	5	5	5	5	5	4	80%	
095	4/15/2007	R. sylvatica	Lead	340	D	5	5	5	5	5	5	3	1	1	1	1	20%	
095	4/15/2007	R. sylvatica	Lead	340	E	5	5	5	5	5	5	3	2	0	0	0	0%	
095	4/15/2007	R. sylvatica	Lead	340	F	5	5	4	3	3	3	1	0	0	0	0	0%	37%
095	4/15/2007	R. sylvatica	Lead	630	A	5	0	0	0	0	0	0	0	0	0	0	0%	
095	4/15/2007	R. sylvatica	Lead	630	B	5	0	0	0	0	0	0	0	0	0	0	0%	
095	4/15/2007	R. sylvatica	Lead	630	C	5	0	0	0	0	0	0	0	0	0	0	0%	
095	4/15/2007	R. sylvatica	Lead	630	D	5	5	3	3	0	0	0	0	0	0	0	0%	
095	4/15/2007	R. sylvatica	Lead	630	E	5	3	3	3	3	0	0	0	0	0	0	0%	
095	4/15/2007	R. sylvatica	Lead	630	F	5	0	0	0	0	0	0	0	0	0	0	0%	0%
095	4/15/2007	R. sylvatica	Lead	1300	A	5	0	0	0	0	0	0	0	0	0	0	0%	
095	4/15/2007	R. sylvatica	Lead	1300	B	5	0	0	0	0	0	0	0	0	0	0	0%	
095	4/15/2007	R. sylvatica	Lead	1300	C	5	0	0	0	0	0	0	0	0	0	0	0%	
095	4/15/2007	R. sylvatica	Lead	1300	D	5	0	0	0	0	0	0	0	0	0	0	0%	
095	4/15/2007	R. sylvatica	Lead	1300	E	5	0	0	0	0	0	0	0	0	0	0	0%	
095	4/15/2007	R. sylvatica	Lead	1300	F	5	0	0	0	0	0	0	0	0	0	0	0%	0%
095	4/15/2007	R. sylvatica	Lead	1600	A	5	0	0	0	0	0	0	0	0	0	0	0%	
095	4/15/2007	R. sylvatica	Lead	1600	B	5	0	0	0	0	0	0	0	0	0	0	0%	
095	4/15/2007	R. sylvatica	Lead	1600	C	5	0	0	0	0	0	0	0	0	0	0	0%	
095	4/15/2007	R. sylvatica	Lead	1600	D	5	0	0	0	0	0	0	0	0	0	0	0%	
095	4/15/2007	R. sylvatica	Lead	1600	E	5	0	0	0	0	0	0	0	0	0	0	0%	
095	4/15/2007	R. sylvatica	Lead	1600	F	5	0	0	0	0	0	0	0	0	0	0	0%	0%
100	5/2/2007	A. maculatum	Copper	3.2	A	5	5	5	5	5	5	5	5	5	5	5	100%	
100	5/2/2007	A. maculatum	Copper	3.2	B	5	5	5	5	5	5	5	5	5	5	5	100%	100%
100	5/2/2007	A. maculatum	Copper	30.7	A	5	5	5	5	5	5	5	5	5	5	5	100%	
100	5/2/2007	A. maculatum	Copper	30.7	B	5	5	5	5	5	5	5	5	5	5	5	100%	100%
100	5/2/2007	A. maculatum	Copper	75	A	5	5	5	5	5	5	5	5	5	5	5	100%	
100	5/2/2007	A. maculatum	Copper	75	B	5	5	5	5	5	5	5	5	5	5	4	80%	90%
100	5/2/2007	A. maculatum	Copper	150	A	5	0	0	0	0	0	0	0	0	0	0	0%	
100	5/2/2007	A. maculatum	Copper	150	B	5	0	0	0	0	0	0	0	0	0	0	0%	0%
100	5/2/2007	A. maculatum	Copper	270	A	5	0	0	0	0	0	0	0	0	0	0	0%	
100	5/2/2007	A. maculatum	Copper	270	B	5	0	0	0	0	0	0	0	0	0	0	0%	0%
100	5/2/2007	A. maculatum	Copper	470	A	5	0	0	0	0	0	0	0	0	0	0	0%	
100	5/2/2007	A. maculatum	Copper	470	B	5	0	0	0	0	0	0	0	0	0	0	0%	0%
101	5/10/2007	H. chrysoscelis	Copper	3.2	A	5	5	5	5	5	5	5	5	5	5	5	100%	
101	5/10/2007	H. chrysoscelis	Copper	3.2	B	5	5	5	5	5	5	5	5	5	3	3	60%	

Survival Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Day 0 Specimen Count	Day 1 Specimen Count	Day 2 Specimen Count	Day 3 Specimen Count	Day 4 Specimen Count	Day 5 Specimen Count	Day 6 Specimen Count	Day 7 Specimen Count	Day 8 Specimen Count	Day 9 Specimen Count	Day 10 Specimen Count	Day 10 Survival (%)	Day 10 Average Survival (%)
101	5/10/2007	H. chrysoscelis	Copper	3.2	C	5	5	5	5	5	5	5	5	5	5	5	100%	
101	5/10/2007	H. chrysoscelis	Copper	3.2	D	5	5	5	5	5	5	5	5	5	5	5	100%	
101	5/10/2007	H. chrysoscelis	Copper	3.2	E	5	5	5	5	5	5	5	5	5	5	5	100%	
101	5/10/2007	H. chrysoscelis	Copper	3.2	F	5	5	5	5	5	5	5	5	5	5	5	100%	93%
101	5/10/2007	H. chrysoscelis	Copper	13	A	5	5	5	5	5	5	5	5	5	5	5	100%	
101	5/10/2007	H. chrysoscelis	Copper	13	B	5	5	5	5	5	5	5	5	5	5	5	100%	
101	5/10/2007	H. chrysoscelis	Copper	13	C	5	5	5	5	5	5	5	5	5	5	5	100%	
101	5/10/2007	H. chrysoscelis	Copper	13	D	5	5	5	5	5	5	5	5	5	5	5	100%	
101	5/10/2007	H. chrysoscelis	Copper	13	E	5	5	5	5	5	5	5	5	5	5	5	100%	
101	5/10/2007	H. chrysoscelis	Copper	13	F	5	5	5	5	5	5	5	5	5	5	5	100%	100%
101	5/10/2007	H. chrysoscelis	Copper	30.7	A	5	5	5	5	5	4	4	4	4	4	4	80%	
101	5/10/2007	H. chrysoscelis	Copper	30.7	B	5	5	5	5	5	5	5	5	5	5	5	100%	
101	5/10/2007	H. chrysoscelis	Copper	30.7	C	5	5	5	5	5	5	5	5	5	5	4	80%	
101	5/10/2007	H. chrysoscelis	Copper	30.7	D	5	5	5	5	5	5	5	5	5	5	5	100%	
101	5/10/2007	H. chrysoscelis	Copper	30.7	E	5	5	5	5	5	5	5	5	5	5	5	100%	
101	5/10/2007	H. chrysoscelis	Copper	30.7	F	5	5	5	3	3	3	3	3	3	3	3	60%	87%
101	5/10/2007	H. chrysoscelis	Copper	75	A	5	0	0	0	0	0	0	0	0	0	0	0%	
101	5/10/2007	H. chrysoscelis	Copper	75	B	5	0	0	0	0	0	0	0	0	0	0	0%	
101	5/10/2007	H. chrysoscelis	Copper	75	C	5	0	0	0	0	0	0	0	0	0	0	0%	
101	5/10/2007	H. chrysoscelis	Copper	75	D	5	0	0	0	0	0	0	0	0	0	0	0%	
101	5/10/2007	H. chrysoscelis	Copper	75	E	5	0	0	0	0	0	0	0	0	0	0	0%	
101	5/10/2007	H. chrysoscelis	Copper	75	F	5	0	0	0	0	0	0	0	0	0	0	0%	0%
101	5/10/2007	H. chrysoscelis	Copper	150	A	5	0	0	0	0	0	0	0	0	0	0	0%	
101	5/10/2007	H. chrysoscelis	Copper	150	B	5	0	0	0	0	0	0	0	0	0	0	0%	
101	5/10/2007	H. chrysoscelis	Copper	150	C	5	0	0	0	0	0	0	0	0	0	0	0%	
101	5/10/2007	H. chrysoscelis	Copper	150	D	5	0	0	0	0	0	0	0	0	0	0	0%	
101	5/10/2007	H. chrysoscelis	Copper	150	E	5	0	0	0	0	0	0	0	0	0	0	0%	
101	5/10/2007	H. chrysoscelis	Copper	150	F	5	0	0	0	0	0	0	0	0	0	0	0%	0%
101	5/10/2007	H. chrysoscelis	Copper	270	A	5	0	0	0	0	0	0	0	0	0	0	0%	
101	5/10/2007	H. chrysoscelis	Copper	270	B	5	0	0	0	0	0	0	0	0	0	0	0%	
101	5/10/2007	H. chrysoscelis	Copper	270	C	5	0	0	0	0	0	0	0	0	0	0	0%	
101	5/10/2007	H. chrysoscelis	Copper	270	D	5	0	0	0	0	0	0	0	0	0	0	0%	
101	5/10/2007	H. chrysoscelis	Copper	270	E	5	0	0	0	0	0	0	0	0	0	0	0%	
101	5/10/2007	H. chrysoscelis	Copper	270	F	5	0	0	0	0	0	0	0	0	0	0	0%	0%
101	5/10/2007	H. chrysoscelis	Copper	470	A	5	0	0	0	0	0	0	0	0	0	0	0%	
101	5/10/2007	H. chrysoscelis	Copper	470	B	5	0	0	0	0	0	0	0	0	0	0	0%	
101	5/10/2007	H. chrysoscelis	Copper	470	C	5	0	0	0	0	0	0	0	0	0	0	0%	
101	5/10/2007	H. chrysoscelis	Copper	470	D	5	0	0	0	0	0	0	0	0	0	0	0%	
101	5/10/2007	H. chrysoscelis	Copper	470	E	5	0	0	0	0	0	0	0	0	0	0	0%	
101	5/10/2007	H. chrysoscelis	Copper	470	F	5	0	0	0	0	0	0	0	0	0	0	0%	0%
102	5/10/2007	H. chrysoscelis	Lead	1.9	A	5	5	5	5	5	5	5	5	5	5	5	100%	
102	5/10/2007	H. chrysoscelis	Lead	1.9	B	5	5	5	5	5	5	5	5	5	5	5	100%	
102	5/10/2007	H. chrysoscelis	Lead	1.9	C	5	5	5	5	5	5	5	5	5	5	5	100%	
102	5/10/2007	H. chrysoscelis	Lead	1.9	D	5	5	5	5	5	5	5	5	5	5	5	100%	
102	5/10/2007	H. chrysoscelis	Lead	1.9	E	5	5	5	5	5	5	5	5	5	5	5	100%	
102	5/10/2007	H. chrysoscelis	Lead	1.9	F	5	5	5	5	5	5	5	5	5	5	5	100%	100%
102	5/10/2007	H. chrysoscelis	Lead	27	A	5	5	5	5	5	5	5	5	5	5	5	100%	
102	5/10/2007	H. chrysoscelis	Lead	27	B	5	5	5	5	5	5	5	5	5	5	5	100%	
102	5/10/2007	H. chrysoscelis	Lead	27	C	5	5	5	5	5	5	5	5	5	5	5	100%	
102	5/10/2007	H. chrysoscelis	Lead	27	D	5	5	5	5	5	5	5	5	5	5	5	100%	
102	5/10/2007	H. chrysoscelis	Lead	27	E	5	5	5	5	5	5	5	5	5	5	5	100%	
102	5/10/2007	H. chrysoscelis	Lead	27	F	5	5	5	5	5	5	5	5	5	5	5	100%	100%
102	5/10/2007	H. chrysoscelis	Lead	52	A	5	5	5	5	5	5	5	5	5	5	5	100%	
102	5/10/2007	H. chrysoscelis	Lead	52	B	5	5	5	5	5	5	5	5	5	5	5	100%	
102	5/10/2007	H. chrysoscelis	Lead	52	C	5	5	5	5	5	5	5	5	5	5	5	100%	
102	5/10/2007	H. chrysoscelis	Lead	52	D	5	5	5	5	5	5	5	5	5	5	5	100%	
102	5/10/2007	H. chrysoscelis	Lead	52	E	5	5	5	5	5	5	5	5	5	5	5	100%	
102	5/10/2007	H. chrysoscelis	Lead	52	F	5	5	5	5	5	5	5	5	5	5	5	100%	100%
102	5/10/2007	H. chrysoscelis	Lead	95	A	5	5	5	5	5	5	5	5	5	5	5	100%	
102	5/10/2007	H. chrysoscelis	Lead	95	B	5	5	5	5	5	5	5	5	5	5	5	100%	
102	5/10/2007	H. chrysoscelis	Lead	95	C	5	5	5	5	5	5	5	5	5	5	5	100%	
102	5/10/2007	H. chrysoscelis	Lead	95	D	5	5	5	5	5	5	5	5	5	5	5	100%	
102	5/10/2007	H. chrysoscelis	Lead	95	E	5	5	5	5	4	4	4	4	4	4	3	60%	
102	5/10/2007	H. chrysoscelis	Lead	95	F	5	5	5	5	5	5	5	5	5	5	5	100%	93%
102	5/10/2007	H. chrysoscelis	Lead	250	A	5	5	5	5	5	5	5	5	5	5	5	100%	
102	5/10/2007	H. chrysoscelis	Lead	250	B	5	5	5	5	5	5	5	5	5	5	5	100%	
102	5/10/2007	H. chrysoscelis	Lead	250	C	5	5	5	5	5	5	5	5	5	5	5	100%	
102	5/10/2007	H. chrysoscelis	Lead	250	D	5	5	5	5	5	5	5	5	5	5	5	100%	
102	5/10/2007	H. chrysoscelis	Lead	250	E	5	5	5	5	5	5	5	5	5	5	4	80%	
102	5/10/2007	H. chrysoscelis	Lead	250	F	5	5	5	5	5	5	5	5	5	5	5	100%	97%

Survival Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Day 0 Specimen Count	Day 1 Specimen Count	Day 2 Specimen Count	Day 3 Specimen Count	Day 4 Specimen Count	Day 5 Specimen Count	Day 6 Specimen Count	Day 7 Specimen Count	Day 8 Specimen Count	Day 9 Specimen Count	Day 10 Specimen Count	Day 10 Survival (%)	Day 10 Average Survival (%)
102	5/10/2007	H. chrysoscelis	Lead	480	A	5	5	5	5	5	5	5	3	3	3	2	40%	
102	5/10/2007	H. chrysoscelis	Lead	480	B	5	5	5	4	4	4	4	4	4	4	4	80%	
102	5/10/2007	H. chrysoscelis	Lead	480	C	5	5	5	5	5	4	4	4	4	3	3	60%	
102	5/10/2007	H. chrysoscelis	Lead	480	D	5	5	5	0	0	0	0	0	0	0	0	0%	
102	5/10/2007	H. chrysoscelis	Lead	480	E	5	5	5	4	4	3	3	3	3	3	2	40%	
102	5/10/2007	H. chrysoscelis	Lead	480	F	5	5	5	5	5	4	4	4	4	4	4	80%	50%
102	5/10/2007	H. chrysoscelis	Lead	1400	A	5	0	0	0	0	0	0	0	0	0	0	0%	
102	5/10/2007	H. chrysoscelis	Lead	1400	B	5	0	0	0	0	0	0	0	0	0	0	0%	
102	5/10/2007	H. chrysoscelis	Lead	1400	C	5	0	0	0	0	0	0	0	0	0	0	0%	
102	5/10/2007	H. chrysoscelis	Lead	1400	D	5	0	0	0	0	0	0	0	0	0	0	0%	
102	5/10/2007	H. chrysoscelis	Lead	1400	E	5	0	0	0	0	0	0	0	0	0	0	0%	
102	5/10/2007	H. chrysoscelis	Lead	1400	F	5	0	0	0	0	0	0	0	0	0	0	0%	0%
103	5/12/2007	R. clamitans	Copper	3.2	A	5	5	5	5	5	5	5	5	5	5	5	100%	
103	5/12/2007	R. clamitans	Copper	3.2	B	5	5	5	5	5	5	5	5	5	5	5	100%	
103	5/12/2007	R. clamitans	Copper	3.2	C	5	5	5	5	5	5	5	5	5	5	5	100%	
103	5/12/2007	R. clamitans	Copper	3.2	D	5	5	5	5	5	5	5	5	5	5	5	100%	
103	5/12/2007	R. clamitans	Copper	3.2	E	5	5	5	5	5	5	5	5	5	5	5	100%	
103	5/12/2007	R. clamitans	Copper	3.2	F	5	5	5	5	5	5	5	5	5	5	5	100%	100%
103	5/12/2007	R. clamitans	Copper	13	A	5	5	5	5	5	5	5	5	5	5	5	100%	
103	5/12/2007	R. clamitans	Copper	13	B	5	5	5	5	5	5	5	3	3	3	3	60%	
103	5/12/2007	R. clamitans	Copper	13	C	5	5	5	5	5	5	5	5	5	5	5	100%	
103	5/12/2007	R. clamitans	Copper	13	D	5	5	5	5	5	5	5	5	5	4	4	80%	
103	5/12/2007	R. clamitans	Copper	13	E	5	5	5	5	5	5	5	5	5	5	5	100%	
103	5/12/2007	R. clamitans	Copper	13	F	5	5	5	5	5	5	5	5	5	5	5	100%	90%
103	5/12/2007	R. clamitans	Copper	30.7	A	5	5	5	5	5	5	5	5	5	5	5	100%	
103	5/12/2007	R. clamitans	Copper	30.7	B	5	5	5	5	5	5	5	5	5	5	5	100%	
103	5/12/2007	R. clamitans	Copper	30.7	C	5	5	5	5	5	5	5	5	5	5	5	100%	
103	5/12/2007	R. clamitans	Copper	30.7	D	5	5	5	5	5	3	3	2	2	2	2	40%	
103	5/12/2007	R. clamitans	Copper	30.7	E	5	5	5	5	5	3	3	3	2	2	2	40%	
103	5/12/2007	R. clamitans	Copper	30.7	F	5	5	5	5	5	5	5	5	5	5	5	100%	80%
103	5/12/2007	R. clamitans	Copper	75	A	5	0	0	0	0	0	0	0	0	0	0	0%	
103	5/12/2007	R. clamitans	Copper	75	B	5	0	0	0	0	0	0	0	0	0	0	0%	
103	5/12/2007	R. clamitans	Copper	75	C	5	5	5	3	2	2	2	2	2	2	2	40%	
103	5/12/2007	R. clamitans	Copper	75	D	5	0	0	0	0	0	0	0	0	0	0	0%	
103	5/12/2007	R. clamitans	Copper	75	E	5	0	0	0	0	0	0	0	0	0	0	0%	
103	5/12/2007	R. clamitans	Copper	75	F	5	0	0	0	0	0	0	0	0	0	0	0%	7%
103	5/12/2007	R. clamitans	Copper	150	A	5	0	0	0	0	0	0	0	0	0	0	0%	
103	5/12/2007	R. clamitans	Copper	150	B	5	0	0	0	0	0	0	0	0	0	0	0%	
103	5/12/2007	R. clamitans	Copper	150	C	5	0	0	0	0	0	0	0	0	0	0	0%	
103	5/12/2007	R. clamitans	Copper	150	D	5	0	0	0	0	0	0	0	0	0	0	0%	
103	5/12/2007	R. clamitans	Copper	150	E	5	0	0	0	0	0	0	0	0	0	0	0%	
103	5/12/2007	R. clamitans	Copper	150	F	5	0	0	0	0	0	0	0	0	0	0	0%	0%
103	5/12/2007	R. clamitans	Copper	270	A	5	0	0	0	0	0	0	0	0	0	0	0%	
103	5/12/2007	R. clamitans	Copper	270	B	5	0	0	0	0	0	0	0	0	0	0	0%	
103	5/12/2007	R. clamitans	Copper	270	C	5	0	0	0	0	0	0	0	0	0	0	0%	
103	5/12/2007	R. clamitans	Copper	270	D	5	0	0	0	0	0	0	0	0	0	0	0%	
103	5/12/2007	R. clamitans	Copper	270	E	5	0	0	0	0	0	0	0	0	0	0	0%	
103	5/12/2007	R. clamitans	Copper	270	F	5	0	0	0	0	0	0	0	0	0	0	0%	0%
103	5/12/2007	R. clamitans	Copper	470	A	5	0	0	0	0	0	0	0	0	0	0	0%	
103	5/12/2007	R. clamitans	Copper	470	B	5	0	0	0	0	0	0	0	0	0	0	0%	
103	5/12/2007	R. clamitans	Copper	470	C	5	0	0	0	0	0	0	0	0	0	0	0%	
103	5/12/2007	R. clamitans	Copper	470	D	5	0	0	0	0	0	0	0	0	0	0	0%	
103	5/12/2007	R. clamitans	Copper	470	E	5	0	0	0	0	0	0	0	0	0	0	0%	
103	5/12/2007	R. clamitans	Copper	470	F	5	0	0	0	0	0	0	0	0	0	0	0%	0%
107	7/6/2007	X. laevis	Copper	3.2	A	5	--	--	4	4	4	4	4	4	4	4	80%	
107	7/6/2007	X. laevis	Copper	3.2	B	5	--	--	5	5	5	4	4	4	4	4	80%	
107	7/6/2007	X. laevis	Copper	3.2	C	5	--	--	5	5	5	5	5	5	5	5	100%	
107	7/6/2007	X. laevis	Copper	3.2	D	5	--	--	5	5	5	4	3	3	3	2	40%	
107	7/6/2007	X. laevis	Copper	3.2	E	5	--	--	4	4	4	3	3	3	3	3	60%	
107	7/6/2007	X. laevis	Copper	3.2	F	5	--	--	5	5	5	5	5	5	5	5	100%	77%
107	7/6/2007	X. laevis	Copper	13	A	5	--	--	5	5	5	5	5	5	5	5	100%	
107	7/6/2007	X. laevis	Copper	13	B	5	--	--	5	5	5	5	5	5	5	5	100%	
107	7/6/2007	X. laevis	Copper	13	C	5	--	--	5	5	5	5	5	5	5	5	100%	
107	7/6/2007	X. laevis	Copper	13	D	5	--	--	5	5	5	5	5	5	5	5	100%	
107	7/6/2007	X. laevis	Copper	13	E	5	--	--	5	5	5	5	5	5	5	5	100%	
107	7/6/2007	X. laevis	Copper	13	F	5	--	--	5	5	5	5	5	5	5	5	100%	100%
107	7/6/2007	X. laevis	Copper	32	A	5	--	--	5	5	5	5	5	5	5	4	80%	
107	7/6/2007	X. laevis	Copper	32	B	5	--	--	4	4	4	4	4	4	3	2	40%	
107	7/6/2007	X. laevis	Copper	32	C	5	--	--	5	5	5	4	4	4	4	4	80%	
107	7/6/2007	X. laevis	Copper	32	D	5	--	--	5	5	5	5	5	5	5	5	100%	
107	7/6/2007	X. laevis	Copper	32	E	5	--	--	5	5	5	5	5	5	5	5	100%	
107	7/6/2007	X. laevis	Copper	32	F	5	--	--	5	5	5	5	5	5	5	5	100%	83%
107	7/6/2007	X. laevis	Copper	85	A	5	--	--	4	4	4	4	4	4	4	4	80%	
107	7/6/2007	X. laevis	Copper	85	B	5	--	--	0	0	0	0	0	0	0	0	0%	
107	7/6/2007	X. laevis	Copper	85	C	5	--	--	0	0	0	0	0	0	0	0	0%	
107	7/6/2007	X. laevis	Copper	85	D	5	--	--	0	0	0	0	0	0	0	0	0%	
107	7/6/2007	X. laevis	Copper	85	E	5	--	--	0	0	0	0	0	0	0	0	0%	
107	7/6/2007	X. laevis	Copper	85	F	5	--	--	0	0	0	0	0	0	0	0	0%	13%

Survival Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Day 0 Specimen Count	Day 1 Specimen Count	Day 2 Specimen Count	Day 3 Specimen Count	Day 4 Specimen Count	Day 5 Specimen Count	Day 6 Specimen Count	Day 7 Specimen Count	Day 8 Specimen Count	Day 9 Specimen Count	Day 10 Specimen Count	Day 10 Survival (%)	Day 10 Average Survival (%)
107	7/6/2007	X. laevis	Copper	140	A	5	--	--	0	0	0	0	0	0	0	0	0%	
107	7/6/2007	X. laevis	Copper	140	B	5	--	--	0	0	0	0	0	0	0	0	0%	
107	7/6/2007	X. laevis	Copper	140	C	5	--	--	0	0	0	0	0	0	0	0	0%	
107	7/6/2007	X. laevis	Copper	140	D	5	--	--	0	0	0	0	0	0	0	0	0%	
107	7/6/2007	X. laevis	Copper	140	E	5	--	--	0	0	0	0	0	0	0	0	0%	
107	7/6/2007	X. laevis	Copper	140	F	5	--	--	0	0	0	0	0	0	0	0	0%	0%
107	7/6/2007	X. laevis	Copper	280	A	5	--	--	0	0	0	0	0	0	0	0	0%	
107	7/6/2007	X. laevis	Copper	280	B	5	--	--	0	0	0	0	0	0	0	0	0%	
107	7/6/2007	X. laevis	Copper	280	C	5	--	--	0	0	0	0	0	0	0	0	0%	
107	7/6/2007	X. laevis	Copper	280	D	5	--	--	0	0	0	0	0	0	0	0	0%	
107	7/6/2007	X. laevis	Copper	280	E	5	--	--	0	0	0	0	0	0	0	0	0%	
107	7/6/2007	X. laevis	Copper	280	F	5	--	--	0	0	0	0	0	0	0	0	0%	0%
107	7/6/2007	X. laevis	Copper	500	A	5	--	--	0	0	0	0	0	0	0	0	0%	
107	7/6/2007	X. laevis	Copper	500	B	5	--	--	0	0	0	0	0	0	0	0	0%	
107	7/6/2007	X. laevis	Copper	500	C	5	--	--	0	0	0	0	0	0	0	0	0%	
107	7/6/2007	X. laevis	Copper	500	D	5	--	--	0	0	0	0	0	0	0	0	0%	
107	7/6/2007	X. laevis	Copper	500	E	5	--	--	0	0	0	0	0	0	0	0	0%	
107	7/6/2007	X. laevis	Copper	500	F	5	--	--	0	0	0	0	0	0	0	0	0%	0%
108	7/6/2007	X. laevis	Lead	1.9	A	5	--	--	5	5	5	5	5	5	5	5	100%	
108	7/6/2007	X. laevis	Lead	1.9	B	5	--	--	4	4	4	2	2	4	4	1	20%	
108	7/6/2007	X. laevis	Lead	1.9	C	5	--	--	5	5	5	5	5	5	5	5	100%	
108	7/6/2007	X. laevis	Lead	1.9	D	5	--	--	5	5	5	5	3	3	3	0	0%	
108	7/6/2007	X. laevis	Lead	1.9	E	5	--	--	5	5	3	3	3	5	3	1	20%	
108	7/6/2007	X. laevis	Lead	1.9	F	5	--	--	5	5	5	5	5	5	3	3	60%	50%
108	7/6/2007	X. laevis	Lead	40	A	5	--	--	5	5	5	3	4	4	4	4	80%	
108	7/6/2007	X. laevis	Lead	40	B	5	--	--	5	5	5	5	5	5	5	4	80%	
108	7/6/2007	X. laevis	Lead	40	C	5	--	--	5	5	5	5	5	5	4	4	80%	
108	7/6/2007	X. laevis	Lead	40	D	5	--	--	5	5	5	5	5	5	5	5	100%	
108	7/6/2007	X. laevis	Lead	40	E	5	--	--	5	5	5	5	5	5	5	4	80%	
108	7/6/2007	X. laevis	Lead	40	F	5	--	--	5	5	5	5	5	5	5	5	100%	87%
108	7/6/2007	X. laevis	Lead	77	A	5	--	--	5	5	5	5	5	5	5	5	100%	
108	7/6/2007	X. laevis	Lead	77	B	5	--	--	4	4	4	4	4	4	4	3	60%	
108	7/6/2007	X. laevis	Lead	77	C	5	--	--	5	5	5	5	5	5	5	4	80%	
108	7/6/2007	X. laevis	Lead	77	D	5	--	--	5	5	5	5	5	5	5	5	100%	
108	7/6/2007	X. laevis	Lead	77	E	5	--	--	5	5	5	4	4	4	4	4	80%	
108	7/6/2007	X. laevis	Lead	77	F	5	--	--	5	5	5	5	5	5	5	5	100%	87%
108	7/6/2007	X. laevis	Lead	140	A	5	--	--	5	5	5	5	5	5	5	5	100%	
108	7/6/2007	X. laevis	Lead	140	B	5	--	--	4	4	4	4	4	4	4	4	80%	
108	7/6/2007	X. laevis	Lead	140	C	5	--	--	5	5	5	5	5	5	5	5	100%	
108	7/6/2007	X. laevis	Lead	140	D	5	--	--	5	5	5	5	5	5	5	5	100%	
108	7/6/2007	X. laevis	Lead	140	E	5	--	--	4	4	4	4	4	4	4	3	60%	
108	7/6/2007	X. laevis	Lead	140	F	6	--	--	6	6	6	6	6	6	6	6	100%	90%
108	7/6/2007	X. laevis	Lead	340	A	5	--	--	5	5	5	5	5	5	5	5	100%	
108	7/6/2007	X. laevis	Lead	340	B	5	--	--	4	4	4	4	4	4	4	4	80%	
108	7/6/2007	X. laevis	Lead	340	C	5	--	--	5	5	5	5	5	5	5	5	100%	
108	7/6/2007	X. laevis	Lead	340	D	5	--	--	5	5	5	5	5	5	5	5	100%	
108	7/6/2007	X. laevis	Lead	340	E	5	--	--	5	5	5	5	5	5	5	5	100%	
108	7/6/2007	X. laevis	Lead	340	F	5	--	--	5	4	4	4	4	4	4	4	80%	93%
108	7/6/2007	X. laevis	Lead	750	A	5	--	--	0	0	0	0	0	0	0	0	0%	
108	7/6/2007	X. laevis	Lead	750	B	5	--	--	2	2	2	2	2	2	2	2	40%	
108	7/6/2007	X. laevis	Lead	750	C	5	--	--	3	3	3	3	3	3	3	3	60%	
108	7/6/2007	X. laevis	Lead	750	D	5	--	--	1	1	1	1	1	1	1	0	0%	
108	7/6/2007	X. laevis	Lead	750	E	5	--	--	2	2	5	5	5	5	5	5	100%	
108	7/6/2007	X. laevis	Lead	750	F	5	--	--	0	0	0	0	0	0	0	0	0%	33%
108	7/6/2007	X. laevis	Lead	1900	A	5	--	--	0	0	0	0	0	0	0	0	0%	
108	7/6/2007	X. laevis	Lead	1900	B	5	--	--	0	0	0	0	0	0	0	0	0%	
108	7/6/2007	X. laevis	Lead	1900	C	5	--	--	0	0	0	0	0	0	0	0	0%	
108	7/6/2007	X. laevis	Lead	1900	D	5	--	--	0	0	0	0	0	0	0	0	0%	
108	7/6/2007	X. laevis	Lead	1900	E	5	--	--	0	0	0	0	0	0	0	0	0%	
108	7/6/2007	X. laevis	Lead	1900	F	5	--	--	0	0	0	0	0	0	0	0	0%	0%
114	9/28/2007	X. tropicalis	Lead	1.9	A	5	5	5	5	5	5	5	5	5	5	5	100%	
114	9/28/2007	X. tropicalis	Lead	1.9	B	5	5	5	5	5	5	4	4	4	4	4	80%	
114	9/28/2007	X. tropicalis	Lead	1.9	C	5	5	5	4	3	3	3	3	3	2	2	40%	
114	9/28/2007	X. tropicalis	Lead	1.9	D	5	5	4	3	3	3	3	3	3	3	3	60%	
114	9/28/2007	X. tropicalis	Lead	1.9	E	5	5	5	5	5	5	5	5	5	5	5	100%	
114	9/28/2007	X. tropicalis	Lead	1.9	F	5	5	5	5	5	5	5	5	5	5	5	100%	80%
114	9/28/2007	X. tropicalis	Lead	40	A	5	5	5	5	5	5	5	5	5	5	5	100%	
114	9/28/2007	X. tropicalis	Lead	40	B	5	5	5	5	5	5	5	5	5	5	5	100%	
114	9/28/2007	X. tropicalis	Lead	40	C	5	5	5	5	5	4	3	2	1	1	1	20%	
114	9/28/2007	X. tropicalis	Lead	40	D	5	5	5	5	5	3	1	1	1	1	1	20%	
114	9/28/2007	X. tropicalis	Lead	40	E	5	5	5	5	5	5	4	4	4	4	4	80%	
114	9/28/2007	X. tropicalis	Lead	40	F	5	5	5	5	4	4	3	3	3	3	3	60%	63%
114	9/28/2007	X. tropicalis	Lead	77	A	5	5	2	2	2	2	2	2	2	2	2	40%	
114	9/28/2007	X. tropicalis	Lead	77	B	5	5	5	5	5	5	4	4	4	4	4	80%	
114	9/28/2007	X. tropicalis	Lead	77	C	5	5	5	5	5	4	3	2	1	1	1	20%	
114	9/28/2007	X. tropicalis	Lead	77	D	5	5	1	0	0	0	0	0	0	0	0	0%	
114	9/28/2007	X. tropicalis	Lead	77	E	5	5	4	4	4	4	4	4	4	4	4	80%	
114	9/28/2007	X. tropicalis	Lead	77	F	5	5	4	4	4	4	4	4	4	4	4	80%	50%

Survival Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Day 0 Specimen Count	Day 1 Specimen Count	Day 2 Specimen Count	Day 3 Specimen Count	Day 4 Specimen Count	Day 5 Specimen Count	Day 6 Specimen Count	Day 7 Specimen Count	Day 8 Specimen Count	Day 9 Specimen Count	Day 10 Specimen Count	Day 10 Survival (%)	Day 10 Average Survival (%)
114	9/28/2007	X. tropicalis	Lead	140	A	5	5	4	4	4	4	3	3	3	3	3	60%	
114	9/28/2007	X. tropicalis	Lead	140	B	5	5	4	4	3	3	3	3	2	2	2	40%	
114	9/28/2007	X. tropicalis	Lead	140	C	5	5	5	5	5	5	3	3	3	3	0	0%	
114	9/28/2007	X. tropicalis	Lead	140	D	5	5	2	2	2	2	2	2	2	2	0	0%	
114	9/28/2007	X. tropicalis	Lead	140	E	5	5	5	5	5	5	5	3	2	2	2	40%	
114	9/28/2007	X. tropicalis	Lead	140	F	5	5	3	3	3	3	3	3	3	3	3	60%	33%
114	9/28/2007	X. tropicalis	Lead	340	A	5	5	0	0	0	0	0	0	0	0	0	0%	
114	9/28/2007	X. tropicalis	Lead	340	B	5	5	1	1	1	1	1	1	1	1	1	20%	
114	9/28/2007	X. tropicalis	Lead	340	C	5	5	2	2	2	1	1	1	1	0	0	0%	
114	9/28/2007	X. tropicalis	Lead	340	D	5	5	1	1	1	1	0	0	0	0	0	0%	
114	9/28/2007	X. tropicalis	Lead	340	E	5	5	0	0	0	0	0	0	0	0	0	0%	
114	9/28/2007	X. tropicalis	Lead	340	F	5	5	1	1	1	1	0	0	0	0	0	0%	3%
114	9/28/2007	X. tropicalis	Lead	750	A	5	5	2	2	2	2	1	1	1	0	0	0%	
114	9/28/2007	X. tropicalis	Lead	750	B	5	5	1	1	1	1	1	1	1	0	0	0%	
114	9/28/2007	X. tropicalis	Lead	750	C	5	4	1	0	0	0	0	0	0	0	0	0%	
114	9/28/2007	X. tropicalis	Lead	750	D	5	5	0	0	0	0	0	0	0	0	0	0%	
114	9/28/2007	X. tropicalis	Lead	750	E	5	5	1	0	0	0	0	0	0	0	0	0%	
114	9/28/2007	X. tropicalis	Lead	750	F	5	5	3	3	3	2	0	0	0	0	0	0%	0%
114	9/28/2007	X. tropicalis	Lead	1900	A	5	0	0	0	0	0	0	0	0	0	0	0%	
114	9/28/2007	X. tropicalis	Lead	1900	B	5	0	0	0	0	0	0	0	0	0	0	0%	
114	9/28/2007	X. tropicalis	Lead	1900	C	5	0	0	0	0	0	0	0	0	0	0	0%	
114	9/28/2007	X. tropicalis	Lead	1900	D	5	0	0	0	0	0	0	0	0	0	0	0%	
114	9/28/2007	X. tropicalis	Lead	1900	E	5	0	0	0	0	0	0	0	0	0	0	0%	
114	9/28/2007	X. tropicalis	Lead	1900	F	5	0	0	0	0	0	0	0	0	0	0	0%	0%
113	9/28/2007	X. tropicalis	Copper	3.2	A	5	5	1	1	1	1	1	1	1	1	1	20%	
113	9/28/2007	X. tropicalis	Copper	3.2	B	5	5	3	3	3	2	1	0	0	0	0	0%	
113	9/28/2007	X. tropicalis	Copper	3.2	C	5	5	5	5	5	5	5	5	5	5	4	80%	
113	9/28/2007	X. tropicalis	Copper	3.2	D	5	5	4	4	4	4	3	3	3	3	3	60%	
113	9/28/2007	X. tropicalis	Copper	3.2	E	5	5	5	5	5	5	5	4	4	3	3	60%	
113	9/28/2007	X. tropicalis	Copper	3.2	F	5	5	5	4	3	3	3	3	2	2	2	40%	43%
113	9/28/2007	X. tropicalis	Copper	13	A	5	5	4	4	4	4	4	3	3	3	3	60%	
113	9/28/2007	X. tropicalis	Copper	13	B	5	5	5	5	5	5	4	4	4	4	4	80%	
113	9/28/2007	X. tropicalis	Copper	13	C	5	5	4	4	4	4	4	2	1	1	1	20%	
113	9/28/2007	X. tropicalis	Copper	13	D	5	5	3	3	3	1	1	1	1	1	1	20%	
113	9/28/2007	X. tropicalis	Copper	13	E	5	5	5	4	4	3	2	2	2	2	2	40%	
113	9/28/2007	X. tropicalis	Copper	13	F	5	5	2	0	0	0	0	0	0	0	0	0%	37%
113	9/28/2007	X. tropicalis	Copper	32	A	5	5	2	2	2	2	1	1	1	1	1	20%	
113	9/28/2007	X. tropicalis	Copper	32	B	5	5	2	1	1	1	1	1	1	1	1	20%	
113	9/28/2007	X. tropicalis	Copper	32	C	5	5	4	4	4	2	2	2	2	2	2	40%	
113	9/28/2007	X. tropicalis	Copper	32	D	5	5	5	5	4	2	1	1	1	1	1	20%	
113	9/28/2007	X. tropicalis	Copper	32	E	5	5	4	3	2	2	0	0	0	0	0	0%	
113	9/28/2007	X. tropicalis	Copper	32	F	5	5	4	3	3	3	3	3	2	2	2	40%	23%
113	9/28/2007	X. tropicalis	Copper	85	A	5	0	0	0	0	0	0	0	0	0	0	0%	
113	9/28/2007	X. tropicalis	Copper	85	B	5	0	0	0	0	0	0	0	0	0	0	0%	
113	9/28/2007	X. tropicalis	Copper	85	C	5	0	0	0	0	0	0	0	0	0	0	0%	
113	9/28/2007	X. tropicalis	Copper	85	D	5	0	0	0	0	0	0	0	0	0	0	0%	
113	9/28/2007	X. tropicalis	Copper	85	E	5	0	0	0	0	0	0	0	0	0	0	0%	
113	9/28/2007	X. tropicalis	Copper	85	F	5	0	0	0	0	0	0	0	0	0	0	0%	0%
113	9/28/2007	X. tropicalis	Copper	140	A	5	0	0	0	0	0	0	0	0	0	0	0%	
113	9/28/2007	X. tropicalis	Copper	140	B	5	0	0	0	0	0	0	0	0	0	0	0%	
113	9/28/2007	X. tropicalis	Copper	140	C	5	0	0	0	0	0	0	0	0	0	0	0%	
113	9/28/2007	X. tropicalis	Copper	140	D	5	0	0	0	0	0	0	0	0	0	0	0%	
113	9/28/2007	X. tropicalis	Copper	140	E	5	0	0	0	0	0	0	0	0	0	0	0%	
113	9/28/2007	X. tropicalis	Copper	140	F	5	0	0	0	0	0	0	0	0	0	0	0%	0%
113	9/28/2007	X. tropicalis	Copper	280	A	5	0	0	0	0	0	0	0	0	0	0	0%	
113	9/28/2007	X. tropicalis	Copper	280	B	5	0	0	0	0	0	0	0	0	0	0	0%	
113	9/28/2007	X. tropicalis	Copper	280	C	5	0	0	0	0	0	0	0	0	0	0	0%	
113	9/28/2007	X. tropicalis	Copper	280	D	5	0	0	0	0	0	0	0	0	0	0	0%	
113	9/28/2007	X. tropicalis	Copper	280	E	5	0	0	0	0	0	0	0	0	0	0	0%	
113	9/28/2007	X. tropicalis	Copper	280	F	5	0	0	0	0	0	0	0	0	0	0	0%	0%
113	9/28/2007	X. tropicalis	Copper	500	A	5	0	0	0	0	0	0	0	0	0	0	0%	
113	9/28/2007	X. tropicalis	Copper	500	B	5	0	0	0	0	0	0	0	0	0	0	0%	
113	9/28/2007	X. tropicalis	Copper	500	C	5	0	0	0	0	0	0	0	0	0	0	0%	
113	9/28/2007	X. tropicalis	Copper	500	D	5	0	0	0	0	0	0	0	0	0	0	0%	
113	9/28/2007	X. tropicalis	Copper	500	E	5	0	0	0	0	0	0	0	0	0	0	0%	
113	9/28/2007	X. tropicalis	Copper	500	F	5	0	0	0	0	0	0	0	0	0	0	0%	0%
119	2/19/2008	X. laevis	Lead	1.9	A	5	5	5	5	5	5	5	5	5	5	5	100%	
119	2/19/2008	X. laevis	Lead	1.9	B	5	5	5	5	5	0	0	0	0	0	0	0%	
119	2/19/2008	X. laevis	Lead	1.9	C	5	5	5	5	5	5	5	5	5	5	5	100%	
119	2/19/2008	X. laevis	Lead	1.9	D	5	5	5	5	5	0	0	0	0	0	0	0%	
119	2/19/2008	X. laevis	Lead	1.9	E	5	5	5	5	5	5	0	0	0	0	0	0%	
119	2/19/2008	X. laevis	Lead	1.9	F	5	5	5	5	5	5	3	2	1	1	1	20%	37%
119	2/19/2008	X. laevis	Lead	140	A	5	5	5	4	4	4	1	1	1	1	1	20%	
119	2/19/2008	X. laevis	Lead	140	B	5	5	5	5	5	5	1	1	1	1	1	20%	
119	2/19/2008	X. laevis	Lead	140	C	5	5	5	5	5	5	5	5	5	5	5	100%	
119	2/19/2008	X. laevis	Lead	140	D	5	5	5	5	5	5	2	2	2	2	2	40%	
119	2/19/2008	X. laevis	Lead	140	E	5	5	5	5	5	5	2	1	1	1	1	20%	
119	2/19/2008	X. laevis	Lead	140	F	5	5	5	5	5	5	5	5	1	1	1	20%	37%
119	2/19/2008	X. laevis	Lead	340	A	5	5	5	5	5	5	5	5	5	5	5	100%	
119	2/19/2008	X. laevis	Lead	340	B	5	5	5	4	4	4	3	3	3	3	3	60%	
119	2/19/2008	X. laevis	Lead	340	C	5	5	5	4	4	4	4	4	4	4	4	80%	
119	2/19/2008	X. laevis	Lead	340	D	5	5	5	5	5	5	5	5	5	5	5	100%	
119	2/19/2008	X. laevis	Lead	340	E	5	5	5	4	4	4	4	4	4	4	4	80%	
119	2/19/2008	X. laevis	Lead	340	F	5	5	5	5	5	5	5	5	5	5	5	100%	87%
119	2/19/2008	X. laevis	Lead	750	A	5	5	1	1	1	1	1	1	1	1	1	20%	
119	2/19/2008	X. laevis	Lead	750	B	5	5	2	2	2	2	2	2	2	2	2	40%	

Survival Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Day 0 Specimen Count	Day 1 Specimen Count	Day 2 Specimen Count	Day 3 Specimen Count	Day 4 Specimen Count	Day 5 Specimen Count	Day 6 Specimen Count	Day 7 Specimen Count	Day 8 Specimen Count	Day 9 Specimen Count	Day 10 Specimen Count	Day 10 Survival (%)	Day 10 Average Survival (%)
119	2/19/2008	X. laevis	Lead	750	C	5	5	3	3	3	3	2	2	2	2	2	40%	
119	2/19/2008	X. laevis	Lead	750	D	5	5	1	0	0	0	0	0	0	0	0	0%	
119	2/19/2008	X. laevis	Lead	750	E	5	4	1	1	1	1	1	1	1	1	1	20%	
119	2/19/2008	X. laevis	Lead	750	F	5	5	0	0	0	0	0	0	0	0	0	0%	20%
119	2/19/2008	X. laevis	Lead	1900	A	5	0	0	0	0	0	0	0	0	0	0	0%	
119	2/19/2008	X. laevis	Lead	1900	B	5	0	0	0	0	0	0	0	0	0	0	0%	
119	2/19/2008	X. laevis	Lead	1900	C	5	0	0	0	0	0	0	0	0	0	0	0%	
119	2/19/2008	X. laevis	Lead	1900	D	5	0	0	0	0	0	0	0	0	0	0	0%	
119	2/19/2008	X. laevis	Lead	1900	E	5	0	0	0	0	0	0	0	0	0	0	0%	
119	2/19/2008	X. laevis	Lead	1900	F	5	0	0	0	0	0	0	0	0	0	0	0%	0%

-- Survival not measured

SubLethal Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Organism	Body Width (mm)	Body Length (mm)	Day 10 Average Body Width (mm)	Day 10 Average Body Length (mm)	Day 10 Average Treatment Body Width (mm)	Day 10 Average Treatment Body Length (mm)
104	5/12/2007	R. clamitans	Lead	1.9	A	1	4	5.5				
104	5/12/2007	R. clamitans	Lead	1.9	A	2	4	5.5				
104	5/12/2007	R. clamitans	Lead	1.9	A	3	3.5	5.5				
104	5/12/2007	R. clamitans	Lead	1.9	A	4	4	6				
104	5/12/2007	R. clamitans	Lead	1.9	A	5	-	-	3.1	4.5		
104	5/12/2007	R. clamitans	Lead	1.9	B	1	4	5				
104	5/12/2007	R. clamitans	Lead	1.9	B	2	4	6				
104	5/12/2007	R. clamitans	Lead	1.9	B	3	4	6				
104	5/12/2007	R. clamitans	Lead	1.9	B	4	3	5.5				
104	5/12/2007	R. clamitans	Lead	1.9	B	5	3	5	3.6	5.5		
104	5/12/2007	R. clamitans	Lead	1.9	C	1	3.5	5.5				
104	5/12/2007	R. clamitans	Lead	1.9	C	2	3	5				
104	5/12/2007	R. clamitans	Lead	1.9	C	3	4	6				
104	5/12/2007	R. clamitans	Lead	1.9	C	4	3.5	5.5				
104	5/12/2007	R. clamitans	Lead	1.9	C	5	4	6	3.6	5.6		
104	5/12/2007	R. clamitans	Lead	1.9	D	1	3	5.5				
104	5/12/2007	R. clamitans	Lead	1.9	D	2	3	5				
104	5/12/2007	R. clamitans	Lead	1.9	D	3	4	6				
104	5/12/2007	R. clamitans	Lead	1.9	D	4	4	5.5				
104	5/12/2007	R. clamitans	Lead	1.9	D	5	3	6	3.4	5.6		
104	5/12/2007	R. clamitans	Lead	1.9	E	1	3.5	6				
104	5/12/2007	R. clamitans	Lead	1.9	E	2	3.5	5.5				
104	5/12/2007	R. clamitans	Lead	1.9	E	3	4	6				
104	5/12/2007	R. clamitans	Lead	1.9	E	4	4	6				
104	5/12/2007	R. clamitans	Lead	1.9	E	5	4	6.5	3.8	6.0		
104	5/12/2007	R. clamitans	Lead	1.9	F	1	4	5.5				
104	5/12/2007	R. clamitans	Lead	1.9	F	2	3	6				
104	5/12/2007	R. clamitans	Lead	1.9	F	3	4	5.5				
104	5/12/2007	R. clamitans	Lead	1.9	F	4	4	6				
104	5/12/2007	R. clamitans	Lead	1.9	F	5	3.5	6	3.7	5.8	3.5	5.5
104	5/12/2007	R. clamitans	Lead	27	A	1	4	5.5				
104	5/12/2007	R. clamitans	Lead	27	A	2	4	5.5				
104	5/12/2007	R. clamitans	Lead	27	A	3	3.5	6				
104	5/12/2007	R. clamitans	Lead	27	A	4	4	5				
104	5/12/2007	R. clamitans	Lead	27	A	5	4	5.5	3.9	5.5		
104	5/12/2007	R. clamitans	Lead	27	B	1	3.5	4.5				
104	5/12/2007	R. clamitans	Lead	27	B	2	5	6				
104	5/12/2007	R. clamitans	Lead	27	B	3	4	5				
104	5/12/2007	R. clamitans	Lead	27	B	4	4	5				
104	5/12/2007	R. clamitans	Lead	27	B	5	4	5.5	4.1	5.2		
104	5/12/2007	R. clamitans	Lead	27	C	1	4	5.5				
104	5/12/2007	R. clamitans	Lead	27	C	2	3.5	5				
104	5/12/2007	R. clamitans	Lead	27	C	3	3	4.5				
104	5/12/2007	R. clamitans	Lead	27	C	4	4	5				
104	5/12/2007	R. clamitans	Lead	27	C	5	4	5.5	3.7	5.1		
104	5/12/2007	R. clamitans	Lead	27	D	1	3.5	4.5				
104	5/12/2007	R. clamitans	Lead	27	D	2	3	4				
104	5/12/2007	R. clamitans	Lead	27	D	3	3.5	5				
104	5/12/2007	R. clamitans	Lead	27	D	4	4	5				
104	5/12/2007	R. clamitans	Lead	27	D	5	3	4.5	3.4	4.6		
104	5/12/2007	R. clamitans	Lead	27	E	1	3.5	4.5				
104	5/12/2007	R. clamitans	Lead	27	E	2	3.5	4.5				
104	5/12/2007	R. clamitans	Lead	27	E	3	3	4.5				
104	5/12/2007	R. clamitans	Lead	27	E	4	3.5	4				
104	5/12/2007	R. clamitans	Lead	27	E	5	3	4.5	3.3	4.4		
104	5/12/2007	R. clamitans	Lead	27	F	1	3.5	4.5				
104	5/12/2007	R. clamitans	Lead	27	F	2	4	5.5				
104	5/12/2007	R. clamitans	Lead	27	F	3	4.5	6				
104	5/12/2007	R. clamitans	Lead	27	F	4	3.5	5.5				
104	5/12/2007	R. clamitans	Lead	27	F	5	4	6	3.9	5.5	3.7	5.1
104	5/12/2007	R. clamitans	Lead	52	A	1	3.5	5.5				
104	5/12/2007	R. clamitans	Lead	52	A	2	3	5				
104	5/12/2007	R. clamitans	Lead	52	A	3	3	5				
104	5/12/2007	R. clamitans	Lead	52	A	4	4	6				
104	5/12/2007	R. clamitans	Lead	52	A	5	3	5	3.3	5.3		
104	5/12/2007	R. clamitans	Lead	52	B	1	4	6				
104	5/12/2007	R. clamitans	Lead	52	B	2	4	6				
104	5/12/2007	R. clamitans	Lead	52	B	3	3.5	6				
104	5/12/2007	R. clamitans	Lead	52	B	4	3	5				
104	5/12/2007	R. clamitans	Lead	52	B	5	3.5	5.5	3.6	5.7		
104	5/12/2007	R. clamitans	Lead	52	C	1	3	5				
104	5/12/2007	R. clamitans	Lead	52	C	2	3.5	5.5				
104	5/12/2007	R. clamitans	Lead	52	C	3	4	6				
104	5/12/2007	R. clamitans	Lead	52	C	4	4	6				
104	5/12/2007	R. clamitans	Lead	52	C	5	3	6	3.5	5.7		
104	5/12/2007	R. clamitans	Lead	52	D	1	3	4.5				
104	5/12/2007	R. clamitans	Lead	52	D	2	4	6				
104	5/12/2007	R. clamitans	Lead	52	D	3	3	5.5				
104	5/12/2007	R. clamitans	Lead	52	D	4	3	5				
104	5/12/2007	R. clamitans	Lead	52	D	5	3.5	5.5	3.3	5.3		
104	5/12/2007	R. clamitans	Lead	52	E	1	4	6				
104	5/12/2007	R. clamitans	Lead	52	E	2	3	6				
104	5/12/2007	R. clamitans	Lead	52	E	3	4	6				
104	5/12/2007	R. clamitans	Lead	52	E	4	4	6				
104	5/12/2007	R. clamitans	Lead	52	E	5	-	-	3.0	4.8		
104	5/12/2007	R. clamitans	Lead	52	F	1	4	6				
104	5/12/2007	R. clamitans	Lead	52	F	2	3.5	5				
104	5/12/2007	R. clamitans	Lead	52	F	3	4	6				
104	5/12/2007	R. clamitans	Lead	52	F	4	3.5	5.5				
104	5/12/2007	R. clamitans	Lead	52	F	5	3	5	3.6	5.5	3.4	5.4

SubLethal Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Organism	Body Width (mm)	Body Length (mm)	Day 10 Average Body Width (mm)	Day 10 Average Body Length (mm)	Day 10 Average Treatment Body Width (mm)	Day 10 Average Treatment Body Length (mm)
104	5/12/2007	R. clamitans	Lead	95	A	1	4	5.5				
104	5/12/2007	R. clamitans	Lead	95	A	2	3.5	5				
104	5/12/2007	R. clamitans	Lead	95	A	3	3.5	5				
104	5/12/2007	R. clamitans	Lead	95	A	4	3.5	5				
104	5/12/2007	R. clamitans	Lead	95	A	5	4	5.5	3.7	5.2		
104	5/12/2007	R. clamitans	Lead	95	B	1	4	5.5				
104	5/12/2007	R. clamitans	Lead	95	B	2	3.5	5				
104	5/12/2007	R. clamitans	Lead	95	B	3	4	5.5				
104	5/12/2007	R. clamitans	Lead	95	B	4	4	5				
104	5/12/2007	R. clamitans	Lead	95	B	5	4	5	3.9	5.2		
104	5/12/2007	R. clamitans	Lead	95	C	1	3.5	5				
104	5/12/2007	R. clamitans	Lead	95	C	2	4	5.5				
104	5/12/2007	R. clamitans	Lead	95	C	3	4	5.5				
104	5/12/2007	R. clamitans	Lead	95	C	4	4	5				
104	5/12/2007	R. clamitans	Lead	95	C	5	3	4.5	3.7	5.1		
104	5/12/2007	R. clamitans	Lead	95	D	1	3.5	5				
104	5/12/2007	R. clamitans	Lead	95	D	2	4	6				
104	5/12/2007	R. clamitans	Lead	95	D	3	4	5.5				
104	5/12/2007	R. clamitans	Lead	95	D	4	3.5	5				
104	5/12/2007	R. clamitans	Lead	95	D	5	3.5	5	3.7	5.3		
104	5/12/2007	R. clamitans	Lead	95	E	1	4	5				
104	5/12/2007	R. clamitans	Lead	95	E	2	4	5				
104	5/12/2007	R. clamitans	Lead	95	E	3	3.5	4.5				
104	5/12/2007	R. clamitans	Lead	95	E	4	3.5	4.5				
104	5/12/2007	R. clamitans	Lead	95	E	5	3.5	4.5	3.7	4.7		
104	5/12/2007	R. clamitans	Lead	95	F	1	4	5				
104	5/12/2007	R. clamitans	Lead	95	F	2	5	6				
104	5/12/2007	R. clamitans	Lead	95	F	3	4	5				
104	5/12/2007	R. clamitans	Lead	95	F	4	4	5				
104	5/12/2007	R. clamitans	Lead	95	F	5	4	5	4.2	5.2	3.8	5.1
104	5/12/2007	R. clamitans	Lead	250	A	1	4	5				
104	5/12/2007	R. clamitans	Lead	250	A	2	3.5	5				
104	5/12/2007	R. clamitans	Lead	250	A	3	4	5.5				
104	5/12/2007	R. clamitans	Lead	250	A	4	2.5	4				
104	5/12/2007	R. clamitans	Lead	250	A	5	3.5	4	3.5	4.7		
104	5/12/2007	R. clamitans	Lead	250	B	1	3.5	5				
104	5/12/2007	R. clamitans	Lead	250	B	2	3.5	5				
104	5/12/2007	R. clamitans	Lead	250	B	3	3.5	5				
104	5/12/2007	R. clamitans	Lead	250	B	4	3	4				
104	5/12/2007	R. clamitans	Lead	250	B	5	4	5	3.5	4.8		
104	5/12/2007	R. clamitans	Lead	250	C	1	4	5				
104	5/12/2007	R. clamitans	Lead	250	C	2	4	5.5				
104	5/12/2007	R. clamitans	Lead	250	C	3	4	5				
104	5/12/2007	R. clamitans	Lead	250	C	4	3.5	5				
104	5/12/2007	R. clamitans	Lead	250	C	5	4	5	3.9	5.1		
104	5/12/2007	R. clamitans	Lead	250	D	1	4	4				
104	5/12/2007	R. clamitans	Lead	250	D	2	4	5				
104	5/12/2007	R. clamitans	Lead	250	D	3	3.5	5				
104	5/12/2007	R. clamitans	Lead	250	D	4	3	4				
104	5/12/2007	R. clamitans	Lead	250	D	5	4	5	3.7	4.6		
104	5/12/2007	R. clamitans	Lead	250	E	1	3	4.5				
104	5/12/2007	R. clamitans	Lead	250	E	2	3.5	4.5				
104	5/12/2007	R. clamitans	Lead	250	E	3	4	4.5				
104	5/12/2007	R. clamitans	Lead	250	E	4	4	5				
104	5/12/2007	R. clamitans	Lead	250	E	5	4	5	3.7	4.7		
104	5/12/2007	R. clamitans	Lead	250	F	1	4	5				
104	5/12/2007	R. clamitans	Lead	250	F	2	3.5	4				
104	5/12/2007	R. clamitans	Lead	250	F	3	4	5				
104	5/12/2007	R. clamitans	Lead	250	F	4	3.5	5				
104	5/12/2007	R. clamitans	Lead	250	F	5	4	5.5	3.8	4.9	3.7	4.8
104	5/12/2007	R. clamitans	Lead	480	A	1	3	4				
104	5/12/2007	R. clamitans	Lead	480	A	2	3	4.5				
104	5/12/2007	R. clamitans	Lead	480	A	3	3	4				
104	5/12/2007	R. clamitans	Lead	480	A	4	3	4.5				
104	5/12/2007	R. clamitans	Lead	480	A	5	2.5	4	2.9	4.2		
104	5/12/2007	R. clamitans	Lead	480	B	1	3	5				
104	5/12/2007	R. clamitans	Lead	480	B	2	3	4				
104	5/12/2007	R. clamitans	Lead	480	B	3	2.5	4				
104	5/12/2007	R. clamitans	Lead	480	B	4	-	-				
104	5/12/2007	R. clamitans	Lead	480	B	5	-	-	1.7	2.6		
104	5/12/2007	R. clamitans	Lead	480	C	1	3	4				
104	5/12/2007	R. clamitans	Lead	480	C	2	2.5	3				
104	5/12/2007	R. clamitans	Lead	480	C	3	-	-				
104	5/12/2007	R. clamitans	Lead	480	C	4	-	-				
104	5/12/2007	R. clamitans	Lead	480	C	5	-	-	1.1	1.4		
104	5/12/2007	R. clamitans	Lead	480	D	1	3	4				
104	5/12/2007	R. clamitans	Lead	480	D	2	3	4				
104	5/12/2007	R. clamitans	Lead	480	D	3	2.5	3.5				
104	5/12/2007	R. clamitans	Lead	480	D	4	-	-				
104	5/12/2007	R. clamitans	Lead	480	D	5	-	-	1.7	2.3		
104	5/12/2007	R. clamitans	Lead	480	E	1	3.5	4.5				
104	5/12/2007	R. clamitans	Lead	480	E	2	3.5	5				
104	5/12/2007	R. clamitans	Lead	480	E	3	3	4.5				
104	5/12/2007	R. clamitans	Lead	480	E	4	3	5				
104	5/12/2007	R. clamitans	Lead	480	E	5	3	5	3.2	4.8		
104	5/12/2007	R. clamitans	Lead	480	F	1	3.5	5				
104	5/12/2007	R. clamitans	Lead	480	F	2	3.5	4.5				
104	5/12/2007	R. clamitans	Lead	480	F	3	3	4.5				
104	5/12/2007	R. clamitans	Lead	480	F	4	3.5	4.5				
104	5/12/2007	R. clamitans	Lead	480	F	5	-	-	2.7	3.7	2.2	3.2

SubLethal Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Organism	Body Width (mm)	Body Length (mm)	Day 10 Average Body Width (mm)	Day 10 Average Body Length (mm)	Day 10 Average Treatment Body Width (mm)	Day 10 Average Treatment Body Length (mm)
125	6/27/2008	R. catesbeiana	Lead	1.9	A	1	2.5	9				
125	6/27/2008	R. catesbeiana	Lead	1.9	A	2	2.5	9				
125	6/27/2008	R. catesbeiana	Lead	1.9	A	3	2.5	9				
125	6/27/2008	R. catesbeiana	Lead	1.9	A	4	2	9				
125	6/27/2008	R. catesbeiana	Lead	1.9	A	5	2	9	2.3	9.0		
125	6/27/2008	R. catesbeiana	Lead	1.9	B	1	3	10				
125	6/27/2008	R. catesbeiana	Lead	1.9	B	2	1.5	7				
125	6/27/2008	R. catesbeiana	Lead	1.9	B	3	2.5	10				
125	6/27/2008	R. catesbeiana	Lead	1.9	B	4	2	9				
125	6/27/2008	R. catesbeiana	Lead	1.9	B	5	2.5	10	2.3	9.2		
125	6/27/2008	R. catesbeiana	Lead	1.9	C	1	2.5	10				
125	6/27/2008	R. catesbeiana	Lead	1.9	C	2	2.5	9				
125	6/27/2008	R. catesbeiana	Lead	1.9	C	3	2	9				
125	6/27/2008	R. catesbeiana	Lead	1.9	C	4	3	10				
125	6/27/2008	R. catesbeiana	Lead	1.9	C	5	2.5	9	2.5	9.4		
125	6/27/2008	R. catesbeiana	Lead	1.9	D	1	2	8				
125	6/27/2008	R. catesbeiana	Lead	1.9	D	2	3	10				
125	6/27/2008	R. catesbeiana	Lead	1.9	D	3	3	9				
125	6/27/2008	R. catesbeiana	Lead	1.9	D	4	2	7				
125	6/27/2008	R. catesbeiana	Lead	1.9	D	5	2.5	7	2.5	8.2		
125	6/27/2008	R. catesbeiana	Lead	1.9	E	1	2.5	9				
125	6/27/2008	R. catesbeiana	Lead	1.9	E	2	3	10				
125	6/27/2008	R. catesbeiana	Lead	1.9	E	3	2.5	9				
125	6/27/2008	R. catesbeiana	Lead	1.9	E	4	3	9				
125	6/27/2008	R. catesbeiana	Lead	1.9	E	5	2.5	9	2.7	9.2		
125	6/27/2008	R. catesbeiana	Lead	1.9	F	1	3	11				
125	6/27/2008	R. catesbeiana	Lead	1.9	F	2	2	8				
125	6/27/2008	R. catesbeiana	Lead	1.9	F	3	3	10				
125	6/27/2008	R. catesbeiana	Lead	1.9	F	4	3	9				
125	6/27/2008	R. catesbeiana	Lead	1.9	F	5	2	8	2.6	9.2	2.5	9.0
125	6/27/2008	R. catesbeiana	Lead	40	A	1	3	10				
125	6/27/2008	R. catesbeiana	Lead	40	A	2	3	11				
125	6/27/2008	R. catesbeiana	Lead	40	A	3	3	10				
125	6/27/2008	R. catesbeiana	Lead	40	A	4	3	10				
125	6/27/2008	R. catesbeiana	Lead	40	A	5	2	7	2.8	9.6		
125	6/27/2008	R. catesbeiana	Lead	40	B	1	2	9				
125	6/27/2008	R. catesbeiana	Lead	40	B	2	1.5	7				
125	6/27/2008	R. catesbeiana	Lead	40	B	3	2	9				
125	6/27/2008	R. catesbeiana	Lead	40	B	4	-	-				
125	6/27/2008	R. catesbeiana	Lead	40	B	5	-	-	1.8	8.3		
125	6/27/2008	R. catesbeiana	Lead	40	C	1	2	7				
125	6/27/2008	R. catesbeiana	Lead	40	C	2	3	10				
125	6/27/2008	R. catesbeiana	Lead	40	C	3	2	9				
125	6/27/2008	R. catesbeiana	Lead	40	C	4	-	-				
125	6/27/2008	R. catesbeiana	Lead	40	C	5	-	-	2.3	8.7		
125	6/27/2008	R. catesbeiana	Lead	40	D	1	2	9				
125	6/27/2008	R. catesbeiana	Lead	40	D	2	2.5	10				
125	6/27/2008	R. catesbeiana	Lead	40	D	3	2	9				
125	6/27/2008	R. catesbeiana	Lead	40	D	4	-	-				
125	6/27/2008	R. catesbeiana	Lead	40	D	5	-	-	2.2	9.3		
125	6/27/2008	R. catesbeiana	Lead	40	E	1	2.5	9				
125	6/27/2008	R. catesbeiana	Lead	40	E	2	2.5	9				
125	6/27/2008	R. catesbeiana	Lead	40	E	3	3	11				
125	6/27/2008	R. catesbeiana	Lead	40	E	4	2	8				
125	6/27/2008	R. catesbeiana	Lead	40	E	5	2.5	10	2.5	9.4		
125	6/27/2008	R. catesbeiana	Lead	40	F	1	2.5	10				
125	6/27/2008	R. catesbeiana	Lead	40	F	2	2	8				
125	6/27/2008	R. catesbeiana	Lead	40	F	3	2	9				
125	6/27/2008	R. catesbeiana	Lead	40	F	4	2	7				
125	6/27/2008	R. catesbeiana	Lead	40	F	5	-	-	2.1	8.5	2.3	9.0
125	6/27/2008	R. catesbeiana	Lead	77	A	1	2.5	9				
125	6/27/2008	R. catesbeiana	Lead	77	A	2	3	10				
125	6/27/2008	R. catesbeiana	Lead	77	A	3	2	8				
125	6/27/2008	R. catesbeiana	Lead	77	A	4	3	11				
125	6/27/2008	R. catesbeiana	Lead	77	A	5	2.5	9	2.6	9.4		
125	6/27/2008	R. catesbeiana	Lead	77	B	1	3	11				
125	6/27/2008	R. catesbeiana	Lead	77	B	2	2.5	9				
125	6/27/2008	R. catesbeiana	Lead	77	B	3	2.5	10				
125	6/27/2008	R. catesbeiana	Lead	77	B	4	2.5	10				
125	6/27/2008	R. catesbeiana	Lead	77	B	5	-	-	2.6	10.0		
125	6/27/2008	R. catesbeiana	Lead	77	C	1	2.5	10				
125	6/27/2008	R. catesbeiana	Lead	77	C	2	3	10				
125	6/27/2008	R. catesbeiana	Lead	77	C	3	2	9				
125	6/27/2008	R. catesbeiana	Lead	77	C	4	2	9				
125	6/27/2008	R. catesbeiana	Lead	77	C	5	-	-	2.4	9.5		
125	6/27/2008	R. catesbeiana	Lead	77	D	1	3	10				
125	6/27/2008	R. catesbeiana	Lead	77	D	2	3	10				
125	6/27/2008	R. catesbeiana	Lead	77	D	3	3	11				
125	6/27/2008	R. catesbeiana	Lead	77	D	4	3	10				
125	6/27/2008	R. catesbeiana	Lead	77	D	5	2	9	2.8	10.0		
125	6/27/2008	R. catesbeiana	Lead	77	E	1	3	11				
125	6/27/2008	R. catesbeiana	Lead	77	E	2	2	9				
125	6/27/2008	R. catesbeiana	Lead	77	E	3	3	11				
125	6/27/2008	R. catesbeiana	Lead	77	E	4	3	11				
125	6/27/2008	R. catesbeiana	Lead	77	E	5	-	-	2.8	10.5		
125	6/27/2008	R. catesbeiana	Lead	77	F	1	3	11				
125	6/27/2008	R. catesbeiana	Lead	77	F	2	2	10				
125	6/27/2008	R. catesbeiana	Lead	77	F	3	2.5	10				
125	6/27/2008	R. catesbeiana	Lead	77	F	4	3	11				
125	6/27/2008	R. catesbeiana	Lead	77	F	5	2.5	11	2.6	10.6	2.6	10.0

SubLethal Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Organism	Body Width (mm)	Body Length (mm)	Day 10 Average Body Width (mm)	Day 10 Average Body Length (mm)	Day 10 Average Treatment Body Width (mm)	Day 10 Average Treatment Body Length (mm)
125	6/27/2008	R. catesbeiana	Lead	120	A	1	2	9				
125	6/27/2008	R. catesbeiana	Lead	120	A	2	2	9				
125	6/27/2008	R. catesbeiana	Lead	120	A	3	2.5	10				
125	6/27/2008	R. catesbeiana	Lead	120	A	4	2	9				
125	6/27/2008	R. catesbeiana	Lead	120	A	5	-	-	2.1	9.3		
125	6/27/2008	R. catesbeiana	Lead	120	B	1	2.5	10				
125	6/27/2008	R. catesbeiana	Lead	120	B	2	2.5	9				
125	6/27/2008	R. catesbeiana	Lead	120	B	3	2.5	10				
125	6/27/2008	R. catesbeiana	Lead	120	B	4	2	10				
125	6/27/2008	R. catesbeiana	Lead	120	B	5	2.5	10	2.4	9.8		
125	6/27/2008	R. catesbeiana	Lead	120	C	1	2.5	9				
125	6/27/2008	R. catesbeiana	Lead	120	C	2	2	9				
125	6/27/2008	R. catesbeiana	Lead	120	C	3	2	9				
125	6/27/2008	R. catesbeiana	Lead	120	C	4	2.5	9				
125	6/27/2008	R. catesbeiana	Lead	120	C	5	2	9	2.2	9.0		
125	6/27/2008	R. catesbeiana	Lead	120	D	1	2	8				
125	6/27/2008	R. catesbeiana	Lead	120	D	2	2.5	9				
125	6/27/2008	R. catesbeiana	Lead	120	D	3	2.5	9				
125	6/27/2008	R. catesbeiana	Lead	120	D	4	2.5	9				
125	6/27/2008	R. catesbeiana	Lead	120	D	5	3	10	2.5	9.0		
125	6/27/2008	R. catesbeiana	Lead	120	E	1	2.5	10				
125	6/27/2008	R. catesbeiana	Lead	120	E	2	2.5	9				
125	6/27/2008	R. catesbeiana	Lead	120	E	3	3	10				
125	6/27/2008	R. catesbeiana	Lead	120	E	4	2.5	10				
125	6/27/2008	R. catesbeiana	Lead	120	E	5	2	8	2.5	9.4		
125	6/27/2008	R. catesbeiana	Lead	120	F	1	2	8				
125	6/27/2008	R. catesbeiana	Lead	120	F	2	2	8				
125	6/27/2008	R. catesbeiana	Lead	120	F	3	2.5	9				
125	6/27/2008	R. catesbeiana	Lead	120	F	4	2.5	8				
125	6/27/2008	R. catesbeiana	Lead	120	F	5	2	8	2.2	8.2	2.3	9.1
125	6/27/2008	R. catesbeiana	Lead	270	A	1	2	8				
125	6/27/2008	R. catesbeiana	Lead	270	A	2	2	8				
125	6/27/2008	R. catesbeiana	Lead	270	A	3	2	8				
125	6/27/2008	R. catesbeiana	Lead	270	A	4	2	7				
125	6/27/2008	R. catesbeiana	Lead	270	A	5	-	-	2.0	7.8		
125	6/27/2008	R. catesbeiana	Lead	270	B	1	2	9				
125	6/27/2008	R. catesbeiana	Lead	270	B	2	2.5	10				
125	6/27/2008	R. catesbeiana	Lead	270	B	3	2	7				
125	6/27/2008	R. catesbeiana	Lead	270	B	4	2	9				
125	6/27/2008	R. catesbeiana	Lead	270	B	5	-	-	2.1	8.6		
125	6/27/2008	R. catesbeiana	Lead	270	C	1	2.5	10				
125	6/27/2008	R. catesbeiana	Lead	270	C	2	2.5	9				
125	6/27/2008	R. catesbeiana	Lead	270	C	3	2	8				
125	6/27/2008	R. catesbeiana	Lead	270	C	4	2.5	10				
125	6/27/2008	R. catesbeiana	Lead	270	C	5	-	-	2.4	9.3		
125	6/27/2008	R. catesbeiana	Lead	270	D	1	2	9				
125	6/27/2008	R. catesbeiana	Lead	270	D	2	2.5	9				
125	6/27/2008	R. catesbeiana	Lead	270	D	3	2.5	9				
125	6/27/2008	R. catesbeiana	Lead	270	D	4	2.5	10				
125	6/27/2008	R. catesbeiana	Lead	270	D	5	2	9	2.3	9.2		
125	6/27/2008	R. catesbeiana	Lead	270	E	1	2	9				
125	6/27/2008	R. catesbeiana	Lead	270	E	2	2	9				
125	6/27/2008	R. catesbeiana	Lead	270	E	3	1.5	7				
125	6/27/2008	R. catesbeiana	Lead	270	E	4	2	8				
125	6/27/2008	R. catesbeiana	Lead	270	E	5	-	-	1.9	8.3		
125	6/27/2008	R. catesbeiana	Lead	270	F	1	2	9				
125	6/27/2008	R. catesbeiana	Lead	270	F	2	2	7				
125	6/27/2008	R. catesbeiana	Lead	270	F	3	2	9				
125	6/27/2008	R. catesbeiana	Lead	270	F	4	2	8				
125	6/27/2008	R. catesbeiana	Lead	270	F	5	-	-	2.0	8.3	2.1	8.6
125	6/27/2008	R. catesbeiana	Lead	570	A	1	-	-				
125	6/27/2008	R. catesbeiana	Lead	570	A	2	-	-				
125	6/27/2008	R. catesbeiana	Lead	570	A	3	-	-				
125	6/27/2008	R. catesbeiana	Lead	570	A	4	-	-				
125	6/27/2008	R. catesbeiana	Lead	570	A	5	-	-	0.0	0.0		
125	6/27/2008	R. catesbeiana	Lead	570	B	1	-	-				
125	6/27/2008	R. catesbeiana	Lead	570	B	2	-	-				
125	6/27/2008	R. catesbeiana	Lead	570	B	3	-	-				
125	6/27/2008	R. catesbeiana	Lead	570	B	4	-	-				
125	6/27/2008	R. catesbeiana	Lead	570	B	5	-	-	0.0	0.0		
125	6/27/2008	R. catesbeiana	Lead	570	C	1	-	-				
125	6/27/2008	R. catesbeiana	Lead	570	C	2	-	-				
125	6/27/2008	R. catesbeiana	Lead	570	C	3	-	-				
125	6/27/2008	R. catesbeiana	Lead	570	C	4	-	-				
125	6/27/2008	R. catesbeiana	Lead	570	C	5	-	-	0.0	0.0		
125	6/27/2008	R. catesbeiana	Lead	570	D	1	2	7				
125	6/27/2008	R. catesbeiana	Lead	570	D	2	2	7				
125	6/27/2008	R. catesbeiana	Lead	570	D	3	2	7				
125	6/27/2008	R. catesbeiana	Lead	570	D	4	2	7				
125	6/27/2008	R. catesbeiana	Lead	570	D	5	-	-	2.0	7.0		
125	6/27/2008	R. catesbeiana	Lead	570	E	1	1.5	7				
125	6/27/2008	R. catesbeiana	Lead	570	E	2	1.5	7				
125	6/27/2008	R. catesbeiana	Lead	570	E	3	2	7				
125	6/27/2008	R. catesbeiana	Lead	570	E	4	-	-				
125	6/27/2008	R. catesbeiana	Lead	570	E	5	-	-	1.7	7.0		
125	6/27/2008	R. catesbeiana	Lead	570	F	1	2	7				
125	6/27/2008	R. catesbeiana	Lead	570	F	2	2	8				
125	6/27/2008	R. catesbeiana	Lead	570	F	3	2	7				
125	6/27/2008	R. catesbeiana	Lead	570	F	4	1.5	7				
125	6/27/2008	R. catesbeiana	Lead	570	F	5	1.5	7	1.8	7.2	0.9	3.5

SubLethal Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Organism	Body Width (mm)	Body Length (mm)	Day 10 Average Body Width (mm)	Day 10 Average Body Length (mm)	Day 10 Average Treatment Body Width (mm)	Day 10 Average Treatment Body Length (mm)
125	6/27/2008	R. catesbeiana	Lead	1300	A	1	-	-				
125	6/27/2008	R. catesbeiana	Lead	1300	A	2	-	-				
125	6/27/2008	R. catesbeiana	Lead	1300	A	3	-	-				
125	6/27/2008	R. catesbeiana	Lead	1300	A	4	-	-				
125	6/27/2008	R. catesbeiana	Lead	1300	A	5	-	-	0.0	0.0		
125	6/27/2008	R. catesbeiana	Lead	1300	B	1	-	-				
125	6/27/2008	R. catesbeiana	Lead	1300	B	2	-	-				
125	6/27/2008	R. catesbeiana	Lead	1300	B	3	-	-				
125	6/27/2008	R. catesbeiana	Lead	1300	B	4	-	-				
125	6/27/2008	R. catesbeiana	Lead	1300	B	5	-	-	0.0	0.0		
125	6/27/2008	R. catesbeiana	Lead	1300	C	1	-	-				
125	6/27/2008	R. catesbeiana	Lead	1300	C	2	-	-				
125	6/27/2008	R. catesbeiana	Lead	1300	C	3	-	-				
125	6/27/2008	R. catesbeiana	Lead	1300	C	4	-	-				
125	6/27/2008	R. catesbeiana	Lead	1300	C	5	-	-	0.0	0.0		
125	6/27/2008	R. catesbeiana	Lead	1300	D	1	-	-				
125	6/27/2008	R. catesbeiana	Lead	1300	D	2	-	-				
125	6/27/2008	R. catesbeiana	Lead	1300	D	3	-	-				
125	6/27/2008	R. catesbeiana	Lead	1300	D	4	-	-				
125	6/27/2008	R. catesbeiana	Lead	1300	D	5	-	-	0.0	0.0		
125	6/27/2008	R. catesbeiana	Lead	1300	E	1	-	-				
125	6/27/2008	R. catesbeiana	Lead	1300	E	2	-	-				
125	6/27/2008	R. catesbeiana	Lead	1300	E	3	-	-				
125	6/27/2008	R. catesbeiana	Lead	1300	E	4	-	-				
125	6/27/2008	R. catesbeiana	Lead	1300	E	5	-	-	0.0	0.0		
125	6/27/2008	R. catesbeiana	Lead	1300	F	1	-	-				
125	6/27/2008	R. catesbeiana	Lead	1300	F	2	-	-				
125	6/27/2008	R. catesbeiana	Lead	1300	F	3	-	-				
125	6/27/2008	R. catesbeiana	Lead	1300	F	4	-	-				
125	6/27/2008	R. catesbeiana	Lead	1300	F	5	-	-	0.0	0.0	0.0	0.0
124	6/27/2008	R. catesbeiana	Copper	3.2	A	1	3	8				
124	6/27/2008	R. catesbeiana	Copper	3.2	A	2	-	-				
124	6/27/2008	R. catesbeiana	Copper	3.2	A	3	-	-				
124	6/27/2008	R. catesbeiana	Copper	3.2	A	4	-	-				
124	6/27/2008	R. catesbeiana	Copper	3.2	A	5	-	-	3.0	8.0		
124	6/27/2008	R. catesbeiana	Copper	3.2	B	1	3	10				
124	6/27/2008	R. catesbeiana	Copper	3.2	B	2	2	8				
124	6/27/2008	R. catesbeiana	Copper	3.2	B	3	2	10				
124	6/27/2008	R. catesbeiana	Copper	3.2	B	4	3	6				
124	6/27/2008	R. catesbeiana	Copper	3.2	B	5	-	-	2.5	8.5		
124	6/27/2008	R. catesbeiana	Copper	3.2	C	1	2	8				
124	6/27/2008	R. catesbeiana	Copper	3.2	C	2	2	7				
124	6/27/2008	R. catesbeiana	Copper	3.2	C	3	2	9				
124	6/27/2008	R. catesbeiana	Copper	3.2	C	4	2	9				
124	6/27/2008	R. catesbeiana	Copper	3.2	C	5	-	-	2.0	8.3		
124	6/27/2008	R. catesbeiana	Copper	3.2	D	1	2	6				
124	6/27/2008	R. catesbeiana	Copper	3.2	D	2	2	7				
124	6/27/2008	R. catesbeiana	Copper	3.2	D	3	2	8				
124	6/27/2008	R. catesbeiana	Copper	3.2	D	4	2	7				
124	6/27/2008	R. catesbeiana	Copper	3.2	D	5	-	-	2.0	7.0		
124	6/27/2008	R. catesbeiana	Copper	3.2	E	1	2	8				
124	6/27/2008	R. catesbeiana	Copper	3.2	E	2	1.5	6				
124	6/27/2008	R. catesbeiana	Copper	3.2	E	3	3	8				
124	6/27/2008	R. catesbeiana	Copper	3.2	E	4	2.5	7				
124	6/27/2008	R. catesbeiana	Copper	3.2	E	5	-	-	2.3	7.3		
124	6/27/2008	R. catesbeiana	Copper	3.2	F	1	2.5	9				
124	6/27/2008	R. catesbeiana	Copper	3.2	F	2	2	7				
124	6/27/2008	R. catesbeiana	Copper	3.2	F	3	2	10				
124	6/27/2008	R. catesbeiana	Copper	3.2	F	4	2.6	7				
124	6/27/2008	R. catesbeiana	Copper	3.2	F	5	1.5	6	2.1	7.8	2.3	7.8
124	6/27/2008	R. catesbeiana	Copper	13	A	1	3	9				
124	6/27/2008	R. catesbeiana	Copper	13	A	2	2.5	10				
124	6/27/2008	R. catesbeiana	Copper	13	A	3	2.5	8				
124	6/27/2008	R. catesbeiana	Copper	13	A	4	2.5	9				
124	6/27/2008	R. catesbeiana	Copper	13	A	5	2.5	9	2.6	9.0		
124	6/27/2008	R. catesbeiana	Copper	13	B	1	2.5	8				
124	6/27/2008	R. catesbeiana	Copper	13	B	2	3	10				
124	6/27/2008	R. catesbeiana	Copper	13	B	3	2.5	10				
124	6/27/2008	R. catesbeiana	Copper	13	B	4	2.5	8				
124	6/27/2008	R. catesbeiana	Copper	13	B	5	2.5	9	2.6	9.0		
124	6/27/2008	R. catesbeiana	Copper	13	C	1	3	11				
124	6/27/2008	R. catesbeiana	Copper	13	C	2	3	9				
124	6/27/2008	R. catesbeiana	Copper	13	C	3	2.5	9				
124	6/27/2008	R. catesbeiana	Copper	13	C	4	2.5	10				
124	6/27/2008	R. catesbeiana	Copper	13	C	5	2.5	7	2.7	9.2		
124	6/27/2008	R. catesbeiana	Copper	13	D	1	2	7				
124	6/27/2008	R. catesbeiana	Copper	13	D	2	2	8				
124	6/27/2008	R. catesbeiana	Copper	13	D	3	2.5	9				
124	6/27/2008	R. catesbeiana	Copper	13	D	4	1.5	6				
124	6/27/2008	R. catesbeiana	Copper	13	D	5	-	-	2.0	7.5		
124	6/27/2008	R. catesbeiana	Copper	13	E	1	2	8				
124	6/27/2008	R. catesbeiana	Copper	13	E	2	2.5	7				
124	6/27/2008	R. catesbeiana	Copper	13	E	3	2	8				
124	6/27/2008	R. catesbeiana	Copper	13	E	4	2.5	9				
124	6/27/2008	R. catesbeiana	Copper	13	E	5	2.5	10	2.3	8.4		
124	6/27/2008	R. catesbeiana	Copper	13	F	1	2.5	9				
124	6/27/2008	R. catesbeiana	Copper	13	F	2	2.5	9				
124	6/27/2008	R. catesbeiana	Copper	13	F	3	3	10				
124	6/27/2008	R. catesbeiana	Copper	13	F	4	2.5	8				
124	6/27/2008	R. catesbeiana	Copper	13	F	5	2.5	10	2.6	9.2	2.5	8.7

SubLethal Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Organism	Body Width (mm)	Body Length (mm)	Day 10 Average Body Width (mm)	Day 10 Average Body Length (mm)	Day 10 Average Treatment Body Width (mm)	Day 10 Average Treatment Body Length (mm)
124	6/27/2008	R. catesbeiana	Copper	32	A	1	2.5	9				
124	6/27/2008	R. catesbeiana	Copper	32	A	2	2.5	10				
124	6/27/2008	R. catesbeiana	Copper	32	A	3	2.5	8				
124	6/27/2008	R. catesbeiana	Copper	32	A	4	3	9				
124	6/27/2008	R. catesbeiana	Copper	32	A	5	2.5	8	2.6	8.8		
124	6/27/2008	R. catesbeiana	Copper	32	B	1	3	9				
124	6/27/2008	R. catesbeiana	Copper	32	B	2	2.5	9				
124	6/27/2008	R. catesbeiana	Copper	32	B	3	2	7				
124	6/27/2008	R. catesbeiana	Copper	32	B	4	2.5	9				
124	6/27/2008	R. catesbeiana	Copper	32	B	5	3	10	2.6	8.8		
124	6/27/2008	R. catesbeiana	Copper	32	C	1	2	9				
124	6/27/2008	R. catesbeiana	Copper	32	C	2	2.5	9				
124	6/27/2008	R. catesbeiana	Copper	32	C	3	2.5	9				
124	6/27/2008	R. catesbeiana	Copper	32	C	4	2	8				
124	6/27/2008	R. catesbeiana	Copper	32	C	5	3	9	2.4	8.8		
124	6/27/2008	R. catesbeiana	Copper	32	D	1	2.5	9				
124	6/27/2008	R. catesbeiana	Copper	32	D	2	3	10				
124	6/27/2008	R. catesbeiana	Copper	32	D	3	3	9				
124	6/27/2008	R. catesbeiana	Copper	32	D	4	3	9				
124	6/27/2008	R. catesbeiana	Copper	32	D	5	2.5	9	2.8	9.2		
124	6/27/2008	R. catesbeiana	Copper	32	E	1	3	10				
124	6/27/2008	R. catesbeiana	Copper	32	E	2	1.5	7				
124	6/27/2008	R. catesbeiana	Copper	32	E	3	3	10				
124	6/27/2008	R. catesbeiana	Copper	32	E	4	2.5	8				
124	6/27/2008	R. catesbeiana	Copper	32	E	5	3	10	2.6	9.0		
124	6/27/2008	R. catesbeiana	Copper	32	F	1	2.5	9				
124	6/27/2008	R. catesbeiana	Copper	32	F	2	3	10				
124	6/27/2008	R. catesbeiana	Copper	32	F	3	3	10				
124	6/27/2008	R. catesbeiana	Copper	32	F	4	2.5	9				
124	6/27/2008	R. catesbeiana	Copper	32	F	5	3	9	2.8	9.4	2.6	9.0
124	6/27/2008	R. catesbeiana	Copper	85	A	1	2	6				
124	6/27/2008	R. catesbeiana	Copper	85	A	2	3	10				
124	6/27/2008	R. catesbeiana	Copper	85	A	3	2.5	9				
124	6/27/2008	R. catesbeiana	Copper	85	A	4	3	9				
124	6/27/2008	R. catesbeiana	Copper	85	A	5	2.5	8	2.6	8.4		
124	6/27/2008	R. catesbeiana	Copper	85	B	1	2.5	9				
124	6/27/2008	R. catesbeiana	Copper	85	B	2	2.5	9				
124	6/27/2008	R. catesbeiana	Copper	85	B	3	2.5	10				
124	6/27/2008	R. catesbeiana	Copper	85	B	4	2.5	9				
124	6/27/2008	R. catesbeiana	Copper	85	B	5	3	8	2.6	9.0		
124	6/27/2008	R. catesbeiana	Copper	85	C	1	2	8				
124	6/27/2008	R. catesbeiana	Copper	85	C	2	2	8				
124	6/27/2008	R. catesbeiana	Copper	85	C	3	2.5	8				
124	6/27/2008	R. catesbeiana	Copper	85	C	4	2	6				
124	6/27/2008	R. catesbeiana	Copper	85	C	5	2.5	7	2.2	7.4		
124	6/27/2008	R. catesbeiana	Copper	85	D	1	2.5	8				
124	6/27/2008	R. catesbeiana	Copper	85	D	2	2.5	8				
124	6/27/2008	R. catesbeiana	Copper	85	D	3	2.5	8				
124	6/27/2008	R. catesbeiana	Copper	85	D	4	2.5	9				
124	6/27/2008	R. catesbeiana	Copper	85	D	5	3	8	2.6	8.2		
124	6/27/2008	R. catesbeiana	Copper	85	E	1	2	6				
124	6/27/2008	R. catesbeiana	Copper	85	E	2	2.5	10				
124	6/27/2008	R. catesbeiana	Copper	85	E	3	3	10				
124	6/27/2008	R. catesbeiana	Copper	85	E	4	3	9				
124	6/27/2008	R. catesbeiana	Copper	85	E	5	-	-	2.6	8.8		
124	6/27/2008	R. catesbeiana	Copper	85	F	1	2	9				
124	6/27/2008	R. catesbeiana	Copper	85	F	2	2	9				
124	6/27/2008	R. catesbeiana	Copper	85	F	3	2	9				
124	6/27/2008	R. catesbeiana	Copper	85	F	4	2	8				
124	6/27/2008	R. catesbeiana	Copper	85	F	5	2	9	2.0	8.8	2.4	8.4
124	6/27/2008	R. catesbeiana	Copper	140	A	1	2.5	9				
124	6/27/2008	R. catesbeiana	Copper	140	A	2	2	10				
124	6/27/2008	R. catesbeiana	Copper	140	A	3	2	9				
124	6/27/2008	R. catesbeiana	Copper	140	A	4	2	9				
124	6/27/2008	R. catesbeiana	Copper	140	A	5	2	9	2.1	9.2		
124	6/27/2008	R. catesbeiana	Copper	140	B	1	2	9				
124	6/27/2008	R. catesbeiana	Copper	140	B	2	2	9				
124	6/27/2008	R. catesbeiana	Copper	140	B	3	2	9				
124	6/27/2008	R. catesbeiana	Copper	140	B	4	2	9				
124	6/27/2008	R. catesbeiana	Copper	140	B	5	2.5	9	2.1	9.0		
124	6/27/2008	R. catesbeiana	Copper	140	C	1	1.5	8				
124	6/27/2008	R. catesbeiana	Copper	140	C	2	2	8				
124	6/27/2008	R. catesbeiana	Copper	140	C	3	2	8				
124	6/27/2008	R. catesbeiana	Copper	140	C	4	2	8				
124	6/27/2008	R. catesbeiana	Copper	140	C	5	2	8	1.9	8.0		
124	6/27/2008	R. catesbeiana	Copper	140	D	1	2	9				
124	6/27/2008	R. catesbeiana	Copper	140	D	2	3	9				
124	6/27/2008	R. catesbeiana	Copper	140	D	3	2.5	9				
124	6/27/2008	R. catesbeiana	Copper	140	D	4	2.5	9				
124	6/27/2008	R. catesbeiana	Copper	140	D	5	-	-	2.5	9.0		
124	6/27/2008	R. catesbeiana	Copper	140	E	1	2	9				
124	6/27/2008	R. catesbeiana	Copper	140	E	2	2	8				
124	6/27/2008	R. catesbeiana	Copper	140	E	3	2	8				
124	6/27/2008	R. catesbeiana	Copper	140	E	4	2	9				
124	6/27/2008	R. catesbeiana	Copper	140	E	5	2	8	2.0	8.4		
124	6/27/2008	R. catesbeiana	Copper	140	F	1	2.5	9				
124	6/27/2008	R. catesbeiana	Copper	140	F	2	2	9				
124	6/27/2008	R. catesbeiana	Copper	140	F	3	2	9				
124	6/27/2008	R. catesbeiana	Copper	140	F	4	2	9				
124	6/27/2008	R. catesbeiana	Copper	140	F	5	2	9	2.1	9.0	2.1	8.8

SubLethal Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Organism	Body Width (mm)	Body Length (mm)	Day 10 Average Body Width (mm)	Day 10 Average Body Length (mm)	Day 10 Average Treatment Body Width (mm)	Day 10 Average Treatment Body Length (mm)
124	6/27/2008	R. catesbeiana	Copper	230	A	1	-	-				
124	6/27/2008	R. catesbeiana	Copper	230	A	2	-	-				
124	6/27/2008	R. catesbeiana	Copper	230	A	3	-	-				
124	6/27/2008	R. catesbeiana	Copper	230	A	4	-	-				
124	6/27/2008	R. catesbeiana	Copper	230	A	5	-	-	0.0	0.0		
124	6/27/2008	R. catesbeiana	Copper	230	B	1	-	-				
124	6/27/2008	R. catesbeiana	Copper	230	B	2	-	-				
124	6/27/2008	R. catesbeiana	Copper	230	B	3	-	-				
124	6/27/2008	R. catesbeiana	Copper	230	B	4	-	-				
124	6/27/2008	R. catesbeiana	Copper	230	B	5	-	-	0.0	0.0		
124	6/27/2008	R. catesbeiana	Copper	230	C	1	-	-				
124	6/27/2008	R. catesbeiana	Copper	230	C	2	-	-				
124	6/27/2008	R. catesbeiana	Copper	230	C	3	-	-				
124	6/27/2008	R. catesbeiana	Copper	230	C	4	-	-				
124	6/27/2008	R. catesbeiana	Copper	230	C	5	-	-	0.0	0.0		
124	6/27/2008	R. catesbeiana	Copper	230	D	1	-	-				
124	6/27/2008	R. catesbeiana	Copper	230	D	2	-	-				
124	6/27/2008	R. catesbeiana	Copper	230	D	3	-	-				
124	6/27/2008	R. catesbeiana	Copper	230	D	4	-	-				
124	6/27/2008	R. catesbeiana	Copper	230	D	5	-	-	0.0	0.0		
124	6/27/2008	R. catesbeiana	Copper	230	E	1	-	-				
124	6/27/2008	R. catesbeiana	Copper	230	E	2	-	-				
124	6/27/2008	R. catesbeiana	Copper	230	E	3	-	-				
124	6/27/2008	R. catesbeiana	Copper	230	E	4	-	-				
124	6/27/2008	R. catesbeiana	Copper	230	E	5	-	-	0.0	0.0		
124	6/27/2008	R. catesbeiana	Copper	230	F	1	-	-				
124	6/27/2008	R. catesbeiana	Copper	230	F	2	-	-				
124	6/27/2008	R. catesbeiana	Copper	230	F	3	-	-				
124	6/27/2008	R. catesbeiana	Copper	230	F	4	-	-				
124	6/27/2008	R. catesbeiana	Copper	230	F	5	-	-	0.0	0.0	0.0	0.0
124	6/27/2008	R. catesbeiana	Copper	500	A	1	-	-				
124	6/27/2008	R. catesbeiana	Copper	500	A	2	-	-				
124	6/27/2008	R. catesbeiana	Copper	500	A	3	-	-				
124	6/27/2008	R. catesbeiana	Copper	500	A	4	-	-				
124	6/27/2008	R. catesbeiana	Copper	500	A	5	-	-	0.0	0.0		
124	6/27/2008	R. catesbeiana	Copper	500	B	1	-	-				
124	6/27/2008	R. catesbeiana	Copper	500	B	2	-	-				
124	6/27/2008	R. catesbeiana	Copper	500	B	3	-	-				
124	6/27/2008	R. catesbeiana	Copper	500	B	4	-	-				
124	6/27/2008	R. catesbeiana	Copper	500	B	5	-	-	0.0	0.0		
124	6/27/2008	R. catesbeiana	Copper	500	C	1	-	-				
124	6/27/2008	R. catesbeiana	Copper	500	C	2	-	-				
124	6/27/2008	R. catesbeiana	Copper	500	C	3	-	-				
124	6/27/2008	R. catesbeiana	Copper	500	C	4	-	-				
124	6/27/2008	R. catesbeiana	Copper	500	C	5	-	-	0.0	0.0		
124	6/27/2008	R. catesbeiana	Copper	500	D	1	-	-				
124	6/27/2008	R. catesbeiana	Copper	500	D	2	-	-				
124	6/27/2008	R. catesbeiana	Copper	500	D	3	-	-				
124	6/27/2008	R. catesbeiana	Copper	500	D	4	-	-				
124	6/27/2008	R. catesbeiana	Copper	500	D	5	-	-	0.0	0.0		
124	6/27/2008	R. catesbeiana	Copper	500	E	1	-	-				
124	6/27/2008	R. catesbeiana	Copper	500	E	2	-	-				
124	6/27/2008	R. catesbeiana	Copper	500	E	3	-	-				
124	6/27/2008	R. catesbeiana	Copper	500	E	4	-	-				
124	6/27/2008	R. catesbeiana	Copper	500	E	5	-	-	0.0	0.0		
124	6/27/2008	R. catesbeiana	Copper	500	F	1	-	-				
124	6/27/2008	R. catesbeiana	Copper	500	F	2	-	-				
124	6/27/2008	R. catesbeiana	Copper	500	F	3	-	-				
124	6/27/2008	R. catesbeiana	Copper	500	F	4	-	-				
124	6/27/2008	R. catesbeiana	Copper	500	F	5	-	-	0.0	0.0	0.0	0.0
120	3/21/2008	R. sphenocephala	Copper	3.2	A	1	5	9.5				
120	3/21/2008	R. sphenocephala	Copper	3.2	A	2	5	8.5				
120	3/21/2008	R. sphenocephala	Copper	3.2	A	3	5	8.5				
120	3/21/2008	R. sphenocephala	Copper	3.2	A	4	5	9				
120	3/21/2008	R. sphenocephala	Copper	3.2	A	5	5	9	5.0	8.9		
120	3/21/2008	R. sphenocephala	Copper	3.2	B	1	6	9.5				
120	3/21/2008	R. sphenocephala	Copper	3.2	B	2	5.5	8.5				
120	3/21/2008	R. sphenocephala	Copper	3.2	B	3	5.5	9.5				
120	3/21/2008	R. sphenocephala	Copper	3.2	B	4	5.5	9.5				
120	3/21/2008	R. sphenocephala	Copper	3.2	B	5	3	8	5.1	9.0		
120	3/21/2008	R. sphenocephala	Copper	3.2	C	1	4.5	8				
120	3/21/2008	R. sphenocephala	Copper	3.2	C	2	5	8				
120	3/21/2008	R. sphenocephala	Copper	3.2	C	3	5	8.5				
120	3/21/2008	R. sphenocephala	Copper	3.2	C	4	5	8.5				
120	3/21/2008	R. sphenocephala	Copper	3.2	C	5	5	9.5	4.9	8.5		
120	3/21/2008	R. sphenocephala	Copper	3.2	D	1	5	8				
120	3/21/2008	R. sphenocephala	Copper	3.2	D	2	5.5	9				
120	3/21/2008	R. sphenocephala	Copper	3.2	D	3	5.5	9.5				
120	3/21/2008	R. sphenocephala	Copper	3.2	D	4	5.5	9.5				
120	3/21/2008	R. sphenocephala	Copper	3.2	D	5	5.5	9.5	5.4	9.1		
120	3/21/2008	R. sphenocephala	Copper	3.2	E	1	5	9.5				
120	3/21/2008	R. sphenocephala	Copper	3.2	E	2	5	9.5				
120	3/21/2008	R. sphenocephala	Copper	3.2	E	3	5	9				
120	3/21/2008	R. sphenocephala	Copper	3.2	E	4	5	9				
120	3/21/2008	R. sphenocephala	Copper	3.2	E	5	5	9.5	5.0	9.3		
120	3/21/2008	R. sphenocephala	Copper	3.2	F	1	5	9				
120	3/21/2008	R. sphenocephala	Copper	3.2	F	2	5	9.5				
120	3/21/2008	R. sphenocephala	Copper	3.2	F	3	5	9.5				
120	3/21/2008	R. sphenocephala	Copper	3.2	F	4	5	9.5				
120	3/21/2008	R. sphenocephala	Copper	3.2	F	5	-	-	4.0	7.5	4.9	8.7

SubLethal Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Organism	Body Width (mm)	Body Length (mm)	Day 10 Average Body Width (mm)	Day 10 Average Body Length (mm)	Day 10 Average Treatment Body Width (mm)	Day 10 Average Treatment Body Length (mm)
120	3/21/2008	R. sphenocephala	Copper	13	A	1	5.5	10				
120	3/21/2008	R. sphenocephala	Copper	13	A	2	5	9.5				
120	3/21/2008	R. sphenocephala	Copper	13	A	3	5	9.5				
120	3/21/2008	R. sphenocephala	Copper	13	A	4	5	9				
120	3/21/2008	R. sphenocephala	Copper	13	A	5	5.5	9.5	5.2	9.5		
120	3/21/2008	R. sphenocephala	Copper	13	B	1	5.5	9.5				
120	3/21/2008	R. sphenocephala	Copper	13	B	2	5	9				
120	3/21/2008	R. sphenocephala	Copper	13	B	3	5	9				
120	3/21/2008	R. sphenocephala	Copper	13	B	4	5.5	9.5				
120	3/21/2008	R. sphenocephala	Copper	13	B	5	5	9.5	5.2	9.3		
120	3/21/2008	R. sphenocephala	Copper	13	C	1	5	8.5				
120	3/21/2008	R. sphenocephala	Copper	13	C	2	5	9				
120	3/21/2008	R. sphenocephala	Copper	13	C	3	5	8.5				
120	3/21/2008	R. sphenocephala	Copper	13	C	4	4.5	8.5				
120	3/21/2008	R. sphenocephala	Copper	13	C	5	5	8.5	4.9	8.6		
120	3/21/2008	R. sphenocephala	Copper	13	D	1	5.5	9.5				
120	3/21/2008	R. sphenocephala	Copper	13	D	2	5.5	9.5				
120	3/21/2008	R. sphenocephala	Copper	13	D	3	5.5	9.5				
120	3/21/2008	R. sphenocephala	Copper	13	D	4	5.5	9.5				
120	3/21/2008	R. sphenocephala	Copper	13	D	5	5.5	9.5	5.5	9.5		
120	3/21/2008	R. sphenocephala	Copper	13	E	1	5	9				
120	3/21/2008	R. sphenocephala	Copper	13	E	2	5.5	9.5				
120	3/21/2008	R. sphenocephala	Copper	13	E	3	5	9				
120	3/21/2008	R. sphenocephala	Copper	13	E	4	5	9				
120	3/21/2008	R. sphenocephala	Copper	13	E	5	5	9	5.1	9.1		
120	3/21/2008	R. sphenocephala	Copper	13	F	1	3	7				
120	3/21/2008	R. sphenocephala	Copper	13	F	2	5.5	10				
120	3/21/2008	R. sphenocephala	Copper	13	F	3	5	9.5				
120	3/21/2008	R. sphenocephala	Copper	13	F	4	5	9				
120	3/21/2008	R. sphenocephala	Copper	13	F	5	5	9	4.7	8.9	5.1	9.2
120	3/21/2008	R. sphenocephala	Copper	32	A	1	5	8				
120	3/21/2008	R. sphenocephala	Copper	32	A	2	5	8				
120	3/21/2008	R. sphenocephala	Copper	32	A	3	4	6				
120	3/21/2008	R. sphenocephala	Copper	32	A	4	6	8				
120	3/21/2008	R. sphenocephala	Copper	32	A	5	5	8	5.0	7.6		
120	3/21/2008	R. sphenocephala	Copper	32	B	1	6	8				
120	3/21/2008	R. sphenocephala	Copper	32	B	2	5	8				
120	3/21/2008	R. sphenocephala	Copper	32	B	3	5	8				
120	3/21/2008	R. sphenocephala	Copper	32	B	4	5	8				
120	3/21/2008	R. sphenocephala	Copper	32	B	5	5	8	5.2	8.0		
120	3/21/2008	R. sphenocephala	Copper	32	C	1	5	9				
120	3/21/2008	R. sphenocephala	Copper	32	C	2	6	9				
120	3/21/2008	R. sphenocephala	Copper	32	C	3	4	6				
120	3/21/2008	R. sphenocephala	Copper	32	C	4	6	9				
120	3/21/2008	R. sphenocephala	Copper	32	C	5	5	8	5.2	8.2		
120	3/21/2008	R. sphenocephala	Copper	32	D	1	5	9				
120	3/21/2008	R. sphenocephala	Copper	32	D	2	4	7				
120	3/21/2008	R. sphenocephala	Copper	32	D	3	5	8				
120	3/21/2008	R. sphenocephala	Copper	32	D	4	6	9				
120	3/21/2008	R. sphenocephala	Copper	32	D	5	5	8	5.0	8.2		
120	3/21/2008	R. sphenocephala	Copper	32	E	1	5	8.5				
120	3/21/2008	R. sphenocephala	Copper	32	E	2	5	8.5				
120	3/21/2008	R. sphenocephala	Copper	32	E	3	5	8.5				
120	3/21/2008	R. sphenocephala	Copper	32	E	4	5	8.5				
120	3/21/2008	R. sphenocephala	Copper	32	E	5	5	8.5	5.0	8.5		
120	3/21/2008	R. sphenocephala	Copper	32	F	1	3	7.5				
120	3/21/2008	R. sphenocephala	Copper	32	F	2	3	7.5				
120	3/21/2008	R. sphenocephala	Copper	32	F	3	4	7.5				
120	3/21/2008	R. sphenocephala	Copper	32	F	4	5	8.5				
120	3/21/2008	R. sphenocephala	Copper	32	F	5	4	8	3.8	7.8	4.9	8.1
120	3/21/2008	R. sphenocephala	Copper	85	A	1	6	8				
120	3/21/2008	R. sphenocephala	Copper	85	A	2	5	9				
120	3/21/2008	R. sphenocephala	Copper	85	A	3	4	9				
120	3/21/2008	R. sphenocephala	Copper	85	A	4	6	7				
120	3/21/2008	R. sphenocephala	Copper	85	A	5	5	8	5.2	8.2		
120	3/21/2008	R. sphenocephala	Copper	85	B	1	4	8				
120	3/21/2008	R. sphenocephala	Copper	85	B	2	4	7				
120	3/21/2008	R. sphenocephala	Copper	85	B	3	4	7				
120	3/21/2008	R. sphenocephala	Copper	85	B	4	5	8				
120	3/21/2008	R. sphenocephala	Copper	85	B	5	5	8	4.4	7.6		
120	3/21/2008	R. sphenocephala	Copper	85	C	1	5	9				
120	3/21/2008	R. sphenocephala	Copper	85	C	2	5	7				
120	3/21/2008	R. sphenocephala	Copper	85	C	3	5	8				
120	3/21/2008	R. sphenocephala	Copper	85	C	4	4	8				
120	3/21/2008	R. sphenocephala	Copper	85	C	5	5	7	4.8	7.8		
120	3/21/2008	R. sphenocephala	Copper	85	D	1	4	7				
120	3/21/2008	R. sphenocephala	Copper	85	D	2	5	7				
120	3/21/2008	R. sphenocephala	Copper	85	D	3	5	7				
120	3/21/2008	R. sphenocephala	Copper	85	D	4	4	8				
120	3/21/2008	R. sphenocephala	Copper	85	D	5	4	7	4.4	7.2		
120	3/21/2008	R. sphenocephala	Copper	85	E	1	4	7				
120	3/21/2008	R. sphenocephala	Copper	85	E	2	5	8				
120	3/21/2008	R. sphenocephala	Copper	85	E	3	4	8				
120	3/21/2008	R. sphenocephala	Copper	85	E	4	4	7				
120	3/21/2008	R. sphenocephala	Copper	85	E	5	4	7	4.2	7.4		
120	3/21/2008	R. sphenocephala	Copper	85	F	1	4	7				
120	3/21/2008	R. sphenocephala	Copper	85	F	2	4	7				
120	3/21/2008	R. sphenocephala	Copper	85	F	3	5	8				
120	3/21/2008	R. sphenocephala	Copper	85	F	4	5	7				
120	3/21/2008	R. sphenocephala	Copper	85	F	5	5	8	4.6	7.4	4.6	7.6

SubLethal Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Organism	Body Width (mm)	Body Length (mm)	Day 10 Average Body Width (mm)	Day 10 Average Body Length (mm)	Day 10 Average Treatment Body Width (mm)	Day 10 Average Treatment Body Length (mm)
120	3/21/2008	R. sphenocephala	Copper	140	A	1	4	6				
120	3/21/2008	R. sphenocephala	Copper	140	A	2	4	7				
120	3/21/2008	R. sphenocephala	Copper	140	A	3	-	-				
120	3/21/2008	R. sphenocephala	Copper	140	A	4	-	-				
120	3/21/2008	R. sphenocephala	Copper	140	A	5	-	-	1.6	2.6		
120	3/21/2008	R. sphenocephala	Copper	140	B	1	3	6				
120	3/21/2008	R. sphenocephala	Copper	140	B	2	4	6				
120	3/21/2008	R. sphenocephala	Copper	140	B	3	4	7				
120	3/21/2008	R. sphenocephala	Copper	140	B	4	4	6				
120	3/21/2008	R. sphenocephala	Copper	140	B	5	4	6	3.8	6.2		
120	3/21/2008	R. sphenocephala	Copper	140	C	1	4	6				
120	3/21/2008	R. sphenocephala	Copper	140	C	2	4	7				
120	3/21/2008	R. sphenocephala	Copper	140	C	3	5	7				
120	3/21/2008	R. sphenocephala	Copper	140	C	4	4	7				
120	3/21/2008	R. sphenocephala	Copper	140	C	5	5	8	4.4	7.0		
120	3/21/2008	R. sphenocephala	Copper	140	D	1	-	-				
120	3/21/2008	R. sphenocephala	Copper	140	D	2	-	-				
120	3/21/2008	R. sphenocephala	Copper	140	D	3	-	-				
120	3/21/2008	R. sphenocephala	Copper	140	D	4	-	-				
120	3/21/2008	R. sphenocephala	Copper	140	D	5	-	-	0.0	0.0		
120	3/21/2008	R. sphenocephala	Copper	140	E	1	4	6				
120	3/21/2008	R. sphenocephala	Copper	140	E	2	4	6				
120	3/21/2008	R. sphenocephala	Copper	140	E	3	-	-				
120	3/21/2008	R. sphenocephala	Copper	140	E	4	-	-				
120	3/21/2008	R. sphenocephala	Copper	140	E	5	-	-	1.6	2.4		
120	3/21/2008	R. sphenocephala	Copper	140	F	1	4	6				
120	3/21/2008	R. sphenocephala	Copper	140	F	2	4	6				
120	3/21/2008	R. sphenocephala	Copper	140	F	3	4	7				
120	3/21/2008	R. sphenocephala	Copper	140	F	4	4	7				
120	3/21/2008	R. sphenocephala	Copper	140	F	5	4	7	4.0	6.6	2.6	4.1
120	3/21/2008	R. sphenocephala	Copper	280	A	1	-	-				
120	3/21/2008	R. sphenocephala	Copper	280	A	2	-	-				
120	3/21/2008	R. sphenocephala	Copper	280	A	3	-	-				
120	3/21/2008	R. sphenocephala	Copper	280	A	4	-	-				
120	3/21/2008	R. sphenocephala	Copper	280	A	5	-	-	0.0	0.0		
120	3/21/2008	R. sphenocephala	Copper	280	B	1	-	-				
120	3/21/2008	R. sphenocephala	Copper	280	B	2	-	-				
120	3/21/2008	R. sphenocephala	Copper	280	B	3	-	-				
120	3/21/2008	R. sphenocephala	Copper	280	B	4	-	-				
120	3/21/2008	R. sphenocephala	Copper	280	B	5	-	-	0.0	0.0		
120	3/21/2008	R. sphenocephala	Copper	280	C	1	-	-				
120	3/21/2008	R. sphenocephala	Copper	280	C	2	-	-				
120	3/21/2008	R. sphenocephala	Copper	280	C	3	-	-				
120	3/21/2008	R. sphenocephala	Copper	280	C	4	-	-				
120	3/21/2008	R. sphenocephala	Copper	280	C	5	-	-	0.0	0.0		
120	3/21/2008	R. sphenocephala	Copper	280	D	1	-	-				
120	3/21/2008	R. sphenocephala	Copper	280	D	2	-	-				
120	3/21/2008	R. sphenocephala	Copper	280	D	3	-	-				
120	3/21/2008	R. sphenocephala	Copper	280	D	4	-	-				
120	3/21/2008	R. sphenocephala	Copper	280	D	5	-	-	0.0	0.0		
120	3/21/2008	R. sphenocephala	Copper	280	E	1	-	-				
120	3/21/2008	R. sphenocephala	Copper	280	E	2	-	-				
120	3/21/2008	R. sphenocephala	Copper	280	E	3	-	-				
120	3/21/2008	R. sphenocephala	Copper	280	E	4	-	-				
120	3/21/2008	R. sphenocephala	Copper	280	E	5	-	-	0.0	0.0		
120	3/21/2008	R. sphenocephala	Copper	280	F	1	-	-				
120	3/21/2008	R. sphenocephala	Copper	280	F	2	-	-				
120	3/21/2008	R. sphenocephala	Copper	280	F	3	-	-				
120	3/21/2008	R. sphenocephala	Copper	280	F	4	-	-				
120	3/21/2008	R. sphenocephala	Copper	280	F	5	-	-	0.0	0.0	0.0	0.0
120	3/21/2008	R. sphenocephala	Copper	500	A	1	-	-				
120	3/21/2008	R. sphenocephala	Copper	500	A	2	-	-				
120	3/21/2008	R. sphenocephala	Copper	500	A	3	-	-				
120	3/21/2008	R. sphenocephala	Copper	500	A	4	-	-				
120	3/21/2008	R. sphenocephala	Copper	500	A	5	-	-	0.0	0.0		
120	3/21/2008	R. sphenocephala	Copper	500	B	1	-	-				
120	3/21/2008	R. sphenocephala	Copper	500	B	2	-	-				
120	3/21/2008	R. sphenocephala	Copper	500	B	3	-	-				
120	3/21/2008	R. sphenocephala	Copper	500	B	4	-	-				
120	3/21/2008	R. sphenocephala	Copper	500	B	5	-	-	0.0	0.0		
120	3/21/2008	R. sphenocephala	Copper	500	C	1	-	-				
120	3/21/2008	R. sphenocephala	Copper	500	C	2	-	-				
120	3/21/2008	R. sphenocephala	Copper	500	C	3	-	-				
120	3/21/2008	R. sphenocephala	Copper	500	C	4	-	-				
120	3/21/2008	R. sphenocephala	Copper	500	C	5	-	-	0.0	0.0		
120	3/21/2008	R. sphenocephala	Copper	500	D	1	-	-				
120	3/21/2008	R. sphenocephala	Copper	500	D	2	-	-				
120	3/21/2008	R. sphenocephala	Copper	500	D	3	-	-				
120	3/21/2008	R. sphenocephala	Copper	500	D	4	-	-				
120	3/21/2008	R. sphenocephala	Copper	500	D	5	-	-	0.0	0.0		
120	3/21/2008	R. sphenocephala	Copper	500	E	1	-	-				
120	3/21/2008	R. sphenocephala	Copper	500	E	2	-	-				
120	3/21/2008	R. sphenocephala	Copper	500	E	3	-	-				
120	3/21/2008	R. sphenocephala	Copper	500	E	4	-	-				
120	3/21/2008	R. sphenocephala	Copper	500	E	5	-	-	0.0	0.0		
120	3/21/2008	R. sphenocephala	Copper	500	F	1	-	-				
120	3/21/2008	R. sphenocephala	Copper	500	F	2	-	-				
120	3/21/2008	R. sphenocephala	Copper	500	F	3	-	-				
120	3/21/2008	R. sphenocephala	Copper	500	F	4	-	-				
120	3/21/2008	R. sphenocephala	Copper	500	F	5	-	-	0.0	0.0	0.0	0.0

SubLethal Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Organism	Body Width (mm)	Body Length (mm)	Day 10 Average Body Width (mm)	Day 10 Average Body Length (mm)	Day 10 Average Treatment Body Width (mm)	Day 10 Average Treatment Body Length (mm)
121	3/21/2008	R. sphenocephala	Lead	1.9	A	1	5	8.5				
121	3/21/2008	R. sphenocephala	Lead	1.9	A	2	5	8.5				
121	3/21/2008	R. sphenocephala	Lead	1.9	A	3	6	9				
121	3/21/2008	R. sphenocephala	Lead	1.9	A	4	5	9				
121	3/21/2008	R. sphenocephala	Lead	1.9	A	5	7	10	5.6	9.0		
121	3/21/2008	R. sphenocephala	Lead	1.9	B	1	7.5	11				
121	3/21/2008	R. sphenocephala	Lead	1.9	B	2	7	10				
121	3/21/2008	R. sphenocephala	Lead	1.9	B	3	6.5	10				
121	3/21/2008	R. sphenocephala	Lead	1.9	B	4	6.5	10				
121	3/21/2008	R. sphenocephala	Lead	1.9	B	5	7	11	6.9	10.4		
121	3/21/2008	R. sphenocephala	Lead	1.9	C	1	6	10				
121	3/21/2008	R. sphenocephala	Lead	1.9	C	2	6	10				
121	3/21/2008	R. sphenocephala	Lead	1.9	C	3	6	9.5				
121	3/21/2008	R. sphenocephala	Lead	1.9	C	4	6	10				
121	3/21/2008	R. sphenocephala	Lead	1.9	C	5	6	10	6.0	9.9		
121	3/21/2008	R. sphenocephala	Lead	1.9	D	1	5	9				
121	3/21/2008	R. sphenocephala	Lead	1.9	D	2	5	9				
121	3/21/2008	R. sphenocephala	Lead	1.9	D	3	6	9				
121	3/21/2008	R. sphenocephala	Lead	1.9	D	4	6	9				
121	3/21/2008	R. sphenocephala	Lead	1.9	D	5	6	10	5.6	9.2		
121	3/21/2008	R. sphenocephala	Lead	1.9	E	1	6.5	10				
121	3/21/2008	R. sphenocephala	Lead	1.9	E	2	6.5	9				
121	3/21/2008	R. sphenocephala	Lead	1.9	E	3	6.5	10				
121	3/21/2008	R. sphenocephala	Lead	1.9	E	4	5	8				
121	3/21/2008	R. sphenocephala	Lead	1.9	E	5	-	-	4.9	7.4		
121	3/21/2008	R. sphenocephala	Lead	1.9	F	1	6.5	10				
121	3/21/2008	R. sphenocephala	Lead	1.9	F	2	5.5	9.5				
121	3/21/2008	R. sphenocephala	Lead	1.9	F	3	6	9				
121	3/21/2008	R. sphenocephala	Lead	1.9	F	4	5	8.5				
121	3/21/2008	R. sphenocephala	Lead	1.9	F	5	5	9	5.6	9.2	5.8	9.2
121	3/21/2008	R. sphenocephala	Lead	40	A	1	6	10				
121	3/21/2008	R. sphenocephala	Lead	40	A	2	6	10				
121	3/21/2008	R. sphenocephala	Lead	40	A	3	5	9				
121	3/21/2008	R. sphenocephala	Lead	40	A	4	6	9.5				
121	3/21/2008	R. sphenocephala	Lead	40	A	5	6	9	5.8	9.5		
121	3/21/2008	R. sphenocephala	Lead	40	B	1	6	9.5				
121	3/21/2008	R. sphenocephala	Lead	40	B	2	6	9				
121	3/21/2008	R. sphenocephala	Lead	40	B	3	6	8.5				
121	3/21/2008	R. sphenocephala	Lead	40	B	4	5	9				
121	3/21/2008	R. sphenocephala	Lead	40	B	5	6	9	5.8	9.0		
121	3/21/2008	R. sphenocephala	Lead	40	C	1	6	9				
121	3/21/2008	R. sphenocephala	Lead	40	C	2	6	9				
121	3/21/2008	R. sphenocephala	Lead	40	C	3	5	8.5				
121	3/21/2008	R. sphenocephala	Lead	40	C	4	6	8.5				
121	3/21/2008	R. sphenocephala	Lead	40	C	5	5.5	9	5.7	8.8		
121	3/21/2008	R. sphenocephala	Lead	40	D	1	6	9.5				
121	3/21/2008	R. sphenocephala	Lead	40	D	2	6	10				
121	3/21/2008	R. sphenocephala	Lead	40	D	3	6	9				
121	3/21/2008	R. sphenocephala	Lead	40	D	4	6.5	10				
121	3/21/2008	R. sphenocephala	Lead	40	D	5	6	10	6.1	9.7		
121	3/21/2008	R. sphenocephala	Lead	40	E	1	6	9				
121	3/21/2008	R. sphenocephala	Lead	40	E	2	6	9.5				
121	3/21/2008	R. sphenocephala	Lead	40	E	3	6	10				
121	3/21/2008	R. sphenocephala	Lead	40	E	4	6	9.5				
121	3/21/2008	R. sphenocephala	Lead	40	E	5	6	10	6.0	9.6		
121	3/21/2008	R. sphenocephala	Lead	40	F	1	7	10				
121	3/21/2008	R. sphenocephala	Lead	40	F	2	6	11				
121	3/21/2008	R. sphenocephala	Lead	40	F	3	6	10.5				
121	3/21/2008	R. sphenocephala	Lead	40	F	4	7	10				
121	3/21/2008	R. sphenocephala	Lead	40	F	5	-	-	5.2	8.3	5.8	9.2
121	3/21/2008	R. sphenocephala	Lead	77	A	1	6	10				
121	3/21/2008	R. sphenocephala	Lead	77	A	2	6	9				
121	3/21/2008	R. sphenocephala	Lead	77	A	3	6	10				
121	3/21/2008	R. sphenocephala	Lead	77	A	4	5	9				
121	3/21/2008	R. sphenocephala	Lead	77	A	5	6	10	5.8	9.8		
121	3/21/2008	R. sphenocephala	Lead	77	B	1	6	9				
121	3/21/2008	R. sphenocephala	Lead	77	B	2	5	9				
121	3/21/2008	R. sphenocephala	Lead	77	B	3	6	10				
121	3/21/2008	R. sphenocephala	Lead	77	B	4	6	9.5				
121	3/21/2008	R. sphenocephala	Lead	77	B	5	6	9.5	5.8	9.4		
121	3/21/2008	R. sphenocephala	Lead	77	C	1	6	10				
121	3/21/2008	R. sphenocephala	Lead	77	C	2	5	9				
121	3/21/2008	R. sphenocephala	Lead	77	C	3	6	9				
121	3/21/2008	R. sphenocephala	Lead	77	C	4	5.5	9				
121	3/21/2008	R. sphenocephala	Lead	77	C	5	6	9	5.7	9.2		
121	3/21/2008	R. sphenocephala	Lead	77	D	1	6	9.5				
121	3/21/2008	R. sphenocephala	Lead	77	D	2	5	9.5				
121	3/21/2008	R. sphenocephala	Lead	77	D	3	6	10				
121	3/21/2008	R. sphenocephala	Lead	77	D	4	6	10				
121	3/21/2008	R. sphenocephala	Lead	77	D	5	6	9.5	5.8	9.7		
121	3/21/2008	R. sphenocephala	Lead	77	E	1	5.5	9				
121	3/21/2008	R. sphenocephala	Lead	77	E	2	5.5	9.5				
121	3/21/2008	R. sphenocephala	Lead	77	E	3	5	8.5				
121	3/21/2008	R. sphenocephala	Lead	77	E	4	6	9				
121	3/21/2008	R. sphenocephala	Lead	77	E	5	6	9	5.6	9.0		
121	3/21/2008	R. sphenocephala	Lead	77	F	1	6	9				
121	3/21/2008	R. sphenocephala	Lead	77	F	2	6	9				
121	3/21/2008	R. sphenocephala	Lead	77	F	3	6	8.5				
121	3/21/2008	R. sphenocephala	Lead	77	F	4	6	10				
121	3/21/2008	R. sphenocephala	Lead	77	F	5	5	9	5.8	9.1	5.8	9.3

SubLethal Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Organism	Body Width (mm)	Body Length (mm)	Day 10 Average Body Width (mm)	Day 10 Average Body Length (mm)	Day 10 Average Treatment Body Width (mm)	Day 10 Average Treatment Body Length (mm)
121	3/21/2008	R. sphenocephala	Lead	140	A	1	5	9				
121	3/21/2008	R. sphenocephala	Lead	140	A	2	6	9				
121	3/21/2008	R. sphenocephala	Lead	140	A	3	5.5	9				
121	3/21/2008	R. sphenocephala	Lead	140	A	4	5.5	10				
121	3/21/2008	R. sphenocephala	Lead	140	A	5	6.5	9.5	5.7	9.3		
121	3/21/2008	R. sphenocephala	Lead	140	B	1	4.5	9				
121	3/21/2008	R. sphenocephala	Lead	140	B	2	5.5	9.5				
121	3/21/2008	R. sphenocephala	Lead	140	B	3	5.5	9.5				
121	3/21/2008	R. sphenocephala	Lead	140	B	4	5.5	9.5				
121	3/21/2008	R. sphenocephala	Lead	140	B	5	5.5	9	5.3	9.3		
121	3/21/2008	R. sphenocephala	Lead	140	C	1	5.5	9.5				
121	3/21/2008	R. sphenocephala	Lead	140	C	2	5.5	9.5				
121	3/21/2008	R. sphenocephala	Lead	140	C	3	5.5	9.5				
121	3/21/2008	R. sphenocephala	Lead	140	C	4	5	9				
121	3/21/2008	R. sphenocephala	Lead	140	C	5	5.5	9.5	5.4	9.4		
121	3/21/2008	R. sphenocephala	Lead	140	D	1	5.5	9				
121	3/21/2008	R. sphenocephala	Lead	140	D	2	5.5	9				
121	3/21/2008	R. sphenocephala	Lead	140	D	3	5	8.5				
121	3/21/2008	R. sphenocephala	Lead	140	D	4	5	9.5				
121	3/21/2008	R. sphenocephala	Lead	140	D	5	-	-	4.2	7.2		
121	3/21/2008	R. sphenocephala	Lead	140	E	1	5.5	9.5				
121	3/21/2008	R. sphenocephala	Lead	140	E	2	5.5	9.5				
121	3/21/2008	R. sphenocephala	Lead	140	E	3	5.5	9.5				
121	3/21/2008	R. sphenocephala	Lead	140	E	4	5.5	9.5				
121	3/21/2008	R. sphenocephala	Lead	140	E	5	-	-	4.4	7.6		
121	3/21/2008	R. sphenocephala	Lead	140	F	1	5	9				
121	3/21/2008	R. sphenocephala	Lead	140	F	2	5	9				
121	3/21/2008	R. sphenocephala	Lead	140	F	3	5	9				
121	3/21/2008	R. sphenocephala	Lead	140	F	4	5	9				
121	3/21/2008	R. sphenocephala	Lead	140	F	5	4.5	8.5	4.9	8.9	5.0	8.6
121	3/21/2008	R. sphenocephala	Lead	340	A	1	5	9				
121	3/21/2008	R. sphenocephala	Lead	340	A	2	5	9				
121	3/21/2008	R. sphenocephala	Lead	340	A	3	4.5	7				
121	3/21/2008	R. sphenocephala	Lead	340	A	4	4.5	7				
121	3/21/2008	R. sphenocephala	Lead	340	A	5	4.5	8	4.7	8.0		
121	3/21/2008	R. sphenocephala	Lead	340	B	1	4.5	7				
121	3/21/2008	R. sphenocephala	Lead	340	B	2	4.5	8				
121	3/21/2008	R. sphenocephala	Lead	340	B	3	5	7.5				
121	3/21/2008	R. sphenocephala	Lead	340	B	4	5	8				
121	3/21/2008	R. sphenocephala	Lead	340	B	5	5	7.5	4.8	7.6		
121	3/21/2008	R. sphenocephala	Lead	340	C	1	5.5	9.5				
121	3/21/2008	R. sphenocephala	Lead	340	C	2	4.5	8				
121	3/21/2008	R. sphenocephala	Lead	340	C	3	5	8				
121	3/21/2008	R. sphenocephala	Lead	340	C	4	5	8				
121	3/21/2008	R. sphenocephala	Lead	340	C	5	3	7	4.6	8.1		
121	3/21/2008	R. sphenocephala	Lead	340	D	1	5	8.5				
121	3/21/2008	R. sphenocephala	Lead	340	D	2	5	7.5				
121	3/21/2008	R. sphenocephala	Lead	340	D	3	5	7.5				
121	3/21/2008	R. sphenocephala	Lead	340	D	4	5	8.5				
121	3/21/2008	R. sphenocephala	Lead	340	D	5	5	7.5	5.0	7.9		
121	3/21/2008	R. sphenocephala	Lead	340	E	1	5	8.5				
121	3/21/2008	R. sphenocephala	Lead	340	E	2	5	8.5				
121	3/21/2008	R. sphenocephala	Lead	340	E	3	4.5	7.5				
121	3/21/2008	R. sphenocephala	Lead	340	E	4	5	8				
121	3/21/2008	R. sphenocephala	Lead	340	E	5	4.5	7.5	4.8	8.0		
121	3/21/2008	R. sphenocephala	Lead	340	F	1	5	8				
121	3/21/2008	R. sphenocephala	Lead	340	F	2	4.5	7				
121	3/21/2008	R. sphenocephala	Lead	340	F	3	4.5	7				
121	3/21/2008	R. sphenocephala	Lead	340	F	4	4	6.5				
121	3/21/2008	R. sphenocephala	Lead	340	F	5	3	6	4.2	6.9	4.7	7.8
121	3/21/2008	R. sphenocephala	Lead	750	A	1	-	-				
121	3/21/2008	R. sphenocephala	Lead	750	A	2	-	-				
121	3/21/2008	R. sphenocephala	Lead	750	A	3	-	-				
121	3/21/2008	R. sphenocephala	Lead	750	A	4	-	-				
121	3/21/2008	R. sphenocephala	Lead	750	A	5	-	-	0.0	0.0		
121	3/21/2008	R. sphenocephala	Lead	750	B	1	-	-				
121	3/21/2008	R. sphenocephala	Lead	750	B	2	-	-				
121	3/21/2008	R. sphenocephala	Lead	750	B	3	-	-				
121	3/21/2008	R. sphenocephala	Lead	750	B	4	-	-				
121	3/21/2008	R. sphenocephala	Lead	750	B	5	-	-	0.0	0.0		
121	3/21/2008	R. sphenocephala	Lead	750	C	1	-	-				
121	3/21/2008	R. sphenocephala	Lead	750	C	2	-	-				
121	3/21/2008	R. sphenocephala	Lead	750	C	3	-	-				
121	3/21/2008	R. sphenocephala	Lead	750	C	4	-	-				
121	3/21/2008	R. sphenocephala	Lead	750	C	5	-	-	0.0	0.0		
121	3/21/2008	R. sphenocephala	Lead	750	D	1	3	5				
121	3/21/2008	R. sphenocephala	Lead	750	D	2	-	-				
121	3/21/2008	R. sphenocephala	Lead	750	D	3	-	-				
121	3/21/2008	R. sphenocephala	Lead	750	D	4	-	-				
121	3/21/2008	R. sphenocephala	Lead	750	D	5	-	-	0.6	1.0		
121	3/21/2008	R. sphenocephala	Lead	750	E	1	3	4.5				
121	3/21/2008	R. sphenocephala	Lead	750	E	2	3.5	5.5				
121	3/21/2008	R. sphenocephala	Lead	750	E	3	3.5	6				
121	3/21/2008	R. sphenocephala	Lead	750	E	4	4.5	7.5				
121	3/21/2008	R. sphenocephala	Lead	750	E	5	-	-	2.5	4.7		
121	3/21/2008	R. sphenocephala	Lead	750	F	1	-	-				
121	3/21/2008	R. sphenocephala	Lead	750	F	2	-	-				
121	3/21/2008	R. sphenocephala	Lead	750	F	3	-	-				
121	3/21/2008	R. sphenocephala	Lead	750	F	4	-	-				
121	3/21/2008	R. sphenocephala	Lead	750	F	5	-	-	0.0	0.0	0.6	1.0

SubLethal Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Organism	Body Width (mm)	Body Length (mm)	Day 10 Average Body Width (mm)	Day 10 Average Body Length (mm)	Day 10 Average Treatment Body Width (mm)	Day 10 Average Treatment Body Length (mm)
121	3/21/2008	R. sphenocephala	Lead	1900	A	1	-	-				
121	3/21/2008	R. sphenocephala	Lead	1900	A	2	-	-				
121	3/21/2008	R. sphenocephala	Lead	1900	A	3	-	-				
121	3/21/2008	R. sphenocephala	Lead	1900	A	4	-	-				
121	3/21/2008	R. sphenocephala	Lead	1900	A	5	-	-	0.0	0.0		
121	3/21/2008	R. sphenocephala	Lead	1900	B	1	-	-				
121	3/21/2008	R. sphenocephala	Lead	1900	B	2	-	-				
121	3/21/2008	R. sphenocephala	Lead	1900	B	3	-	-				
121	3/21/2008	R. sphenocephala	Lead	1900	B	4	-	-				
121	3/21/2008	R. sphenocephala	Lead	1900	B	5	-	-	0.0	0.0		
121	3/21/2008	R. sphenocephala	Lead	1900	C	1	-	-				
121	3/21/2008	R. sphenocephala	Lead	1900	C	2	-	-				
121	3/21/2008	R. sphenocephala	Lead	1900	C	3	-	-				
121	3/21/2008	R. sphenocephala	Lead	1900	C	4	-	-				
121	3/21/2008	R. sphenocephala	Lead	1900	C	5	-	-	0.0	0.0		
121	3/21/2008	R. sphenocephala	Lead	1900	D	1	-	-				
121	3/21/2008	R. sphenocephala	Lead	1900	D	2	-	-				
121	3/21/2008	R. sphenocephala	Lead	1900	D	3	-	-				
121	3/21/2008	R. sphenocephala	Lead	1900	D	4	-	-				
121	3/21/2008	R. sphenocephala	Lead	1900	D	5	-	-	0.0	0.0		
121	3/21/2008	R. sphenocephala	Lead	1900	E	1	-	-				
121	3/21/2008	R. sphenocephala	Lead	1900	E	2	-	-				
121	3/21/2008	R. sphenocephala	Lead	1900	E	3	-	-				
121	3/21/2008	R. sphenocephala	Lead	1900	E	4	-	-				
121	3/21/2008	R. sphenocephala	Lead	1900	E	5	-	-	0.0	0.0		
121	3/21/2008	R. sphenocephala	Lead	1900	F	1	-	-				
121	3/21/2008	R. sphenocephala	Lead	1900	F	2	-	-				
121	3/21/2008	R. sphenocephala	Lead	1900	F	3	-	-				
121	3/21/2008	R. sphenocephala	Lead	1900	F	4	-	-				
121	3/21/2008	R. sphenocephala	Lead	1900	F	5	-	-	0.0	0.0	0.0	0.0
036	12/9/2005	R.pipiens	Copper	7.2	A	1	4	6				
036	12/9/2005	R.pipiens	Copper	7.2	A	2	4	5				
036	12/9/2005	R.pipiens	Copper	7.2	A	3	4	6				
036	12/9/2005	R.pipiens	Copper	7.2	A	4	4	5				
036	12/9/2005	R.pipiens	Copper	7.2	A	5	4	5	4.0	5.4		
036	12/9/2005	R.pipiens	Copper	7.2	B	1	5	7				
036	12/9/2005	R.pipiens	Copper	7.2	B	2	4	6				
036	12/9/2005	R.pipiens	Copper	7.2	B	3	4	6				
036	12/9/2005	R.pipiens	Copper	7.2	B	4	5	6				
036	12/9/2005	R.pipiens	Copper	7.2	B	5	5	6	4.6	6.2		
036	12/9/2005	R.pipiens	Copper	7.2	C	1	4	5				
036	12/9/2005	R.pipiens	Copper	7.2	C	2	5	7				
036	12/9/2005	R.pipiens	Copper	7.2	C	3	4	6				
036	12/9/2005	R.pipiens	Copper	7.2	C	4	4	6				
036	12/9/2005	R.pipiens	Copper	7.2	C	5	4	6	4.2	6.0		
036	12/9/2005	R.pipiens	Copper	7.2	D	1	5	7				
036	12/9/2005	R.pipiens	Copper	7.2	D	2	5	7				
036	12/9/2005	R.pipiens	Copper	7.2	D	3	6	6				
036	12/9/2005	R.pipiens	Copper	7.2	D	4	4	6				
036	12/9/2005	R.pipiens	Copper	7.2	D	5	5	6	5.0	6.4		
036	12/9/2005	R.pipiens	Copper	7.2	E	1	4	6				
036	12/9/2005	R.pipiens	Copper	7.2	E	2	4	6				
036	12/9/2005	R.pipiens	Copper	7.2	E	3	5	7				
036	12/9/2005	R.pipiens	Copper	7.2	E	4	5	7				
036	12/9/2005	R.pipiens	Copper	7.2	E	5	5	6	4.6	6.4		
036	12/9/2005	R.pipiens	Copper	7.2	F	1	4	6				
036	12/9/2005	R.pipiens	Copper	7.2	F	2	5	7				
036	12/9/2005	R.pipiens	Copper	7.2	F	3	5	7				
036	12/9/2005	R.pipiens	Copper	7.2	F	4	5	7				
036	12/9/2005	R.pipiens	Copper	7.2	F	5	4	7	4.6	6.8		
036	12/9/2005	R.pipiens	Copper	7.2	G	1	4	6				
036	12/9/2005	R.pipiens	Copper	7.2	G	2	4	6				
036	12/9/2005	R.pipiens	Copper	7.2	G	3	4	5				
036	12/9/2005	R.pipiens	Copper	7.2	G	4	4	6				
036	12/9/2005	R.pipiens	Copper	7.2	G	5	4	6	4.0	5.8		
036	12/9/2005	R.pipiens	Copper	7.2	H	1	5	6				
036	12/9/2005	R.pipiens	Copper	7.2	H	2	5	6				
036	12/9/2005	R.pipiens	Copper	7.2	H	3	5	7				
036	12/9/2005	R.pipiens	Copper	7.2	H	4	4	6				
036	12/9/2005	R.pipiens	Copper	7.2	H	5	4	7	4.6	6.4	4.5	6.2
036	12/9/2005	R.pipiens	Copper	15	A	1	4	6				
036	12/9/2005	R.pipiens	Copper	15	A	2	4	6				
036	12/9/2005	R.pipiens	Copper	15	A	3	4	6				
036	12/9/2005	R.pipiens	Copper	15	A	4	5	7				
036	12/9/2005	R.pipiens	Copper	15	A	5	5	7	4.4	6.4		
036	12/9/2005	R.pipiens	Copper	15	B	1	5	7				
036	12/9/2005	R.pipiens	Copper	15	B	2	5	6				
036	12/9/2005	R.pipiens	Copper	15	B	3	4	6				
036	12/9/2005	R.pipiens	Copper	15	B	4	4	6				
036	12/9/2005	R.pipiens	Copper	15	B	5	4	6	4.4	6.2		
036	12/9/2005	R.pipiens	Copper	15	C	1	5	6				
036	12/9/2005	R.pipiens	Copper	15	C	2	5	6				
036	12/9/2005	R.pipiens	Copper	15	C	3	4	6				
036	12/9/2005	R.pipiens	Copper	15	C	4	5	7				
036	12/9/2005	R.pipiens	Copper	15	C	5	4	7	4.6	6.4		
036	12/9/2005	R.pipiens	Copper	15	D	1	4	6				
036	12/9/2005	R.pipiens	Copper	15	D	2	4	6				
036	12/9/2005	R.pipiens	Copper	15	D	3	4	6				
036	12/9/2005	R.pipiens	Copper	15	D	4	4	6				
036	12/9/2005	R.pipiens	Copper	15	D	5	4	7	4.0	6.2		
036	12/9/2005	R.pipiens	Copper	15	E	1	4	6				
036	12/9/2005	R.pipiens	Copper	15	E	2	4	6				
036	12/9/2005	R.pipiens	Copper	15	E	3	5	7				
036	12/9/2005	R.pipiens	Copper	15	E	4	4	6				
036	12/9/2005	R.pipiens	Copper	15	E	5	4	6	4.2	6.2		
036	12/9/2005	R.pipiens	Copper	15	F	1	4	6				
036	12/9/2005	R.pipiens	Copper	15	F	2	4	6				
036	12/9/2005	R.pipiens	Copper	15	F	3	4	6				
036	12/9/2005	R.pipiens	Copper	15	F	4	4	6				
036	12/9/2005	R.pipiens	Copper	15	F	5	5	7	4.2	6.2		
036	12/9/2005	R.pipiens	Copper	15	G	1	4	6				
036	12/9/2005	R.pipiens	Copper	15	G	2	4	5				
036	12/9/2005	R.pipiens	Copper	15	G	3	4	6				
036	12/9/2005	R.pipiens	Copper	15	G	4	5	6				
036	12/9/2005	R.pipiens	Copper	15	G	5	4	5	4.2	5.8		
036	12/9/2005	R.pipiens	Copper	15	H	1	4	6				
036	12/9/2005	R.pipiens	Copper	15	H	2	4	6				
036	12/9/2005	R.pipiens	Copper	15	H	3	4	6				
036	12/9/2005	R.pipiens	Copper	15	H	4	5	7				
036	12/9/2005	R.pipiens	Copper	15	H	5	4	6	4.2	6.2	4.3	6.2

SubLethal Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Organism	Body Width (mm)	Body Length (mm)	Day 10 Average Body Width (mm)	Day 10 Average Body Length (mm)	Day 10 Average Treatment Body Width (mm)	Day 10 Average Treatment Body Length (mm)
036	12/9/2005	R.pipiens	Copper	21	A	1	4	7				
036	12/9/2005	R.pipiens	Copper	21	A	2	4	6				
036	12/9/2005	R.pipiens	Copper	21	A	3	4	6				
036	12/9/2005	R.pipiens	Copper	21	A	4	3	5				
036	12/9/2005	R.pipiens	Copper	21	A	5	4	5	3.8	5.8		
036	12/9/2005	R.pipiens	Copper	21	B	1	4	7				
036	12/9/2005	R.pipiens	Copper	21	B	2	4	6				
036	12/9/2005	R.pipiens	Copper	21	B	3	4	6				
036	12/9/2005	R.pipiens	Copper	21	B	4	4	6				
036	12/9/2005	R.pipiens	Copper	21	B	5	4	6	4.0	6.2		
036	12/9/2005	R.pipiens	Copper	21	C	1	4	6				
036	12/9/2005	R.pipiens	Copper	21	C	2	4	6				
036	12/9/2005	R.pipiens	Copper	21	C	3	4	6				
036	12/9/2005	R.pipiens	Copper	21	C	4	4	6				
036	12/9/2005	R.pipiens	Copper	21	C	5	4	6	4.0	6.0		
036	12/9/2005	R.pipiens	Copper	21	D	1	5	6				
036	12/9/2005	R.pipiens	Copper	21	D	2	5	6				
036	12/9/2005	R.pipiens	Copper	21	D	3	5	7				
036	12/9/2005	R.pipiens	Copper	21	D	4	4	6				
036	12/9/2005	R.pipiens	Copper	21	D	5	-	-	3.8	5.0		
036	12/9/2005	R.pipiens	Copper	21	E	1	5	7				
036	12/9/2005	R.pipiens	Copper	21	E	2	4	6				
036	12/9/2005	R.pipiens	Copper	21	E	3	4	7				
036	12/9/2005	R.pipiens	Copper	21	E	4	5	7				
036	12/9/2005	R.pipiens	Copper	21	E	5	4	7	4.4	6.8		
036	12/9/2005	R.pipiens	Copper	21	F	1	4	6				
036	12/9/2005	R.pipiens	Copper	21	F	2	4	6				
036	12/9/2005	R.pipiens	Copper	21	F	3	5	6				
036	12/9/2005	R.pipiens	Copper	21	F	4	7	6				
036	12/9/2005	R.pipiens	Copper	21	F	5	4	6	4.8	6.0		
036	12/9/2005	R.pipiens	Copper	21	G	1	4	6				
036	12/9/2005	R.pipiens	Copper	21	G	2	4	6				
036	12/9/2005	R.pipiens	Copper	21	G	3	4	5				
036	12/9/2005	R.pipiens	Copper	21	G	4	3	5				
036	12/9/2005	R.pipiens	Copper	21	G	5	3	5	3.6	5.4		
036	12/9/2005	R.pipiens	Copper	21	H	1	4	6				
036	12/9/2005	R.pipiens	Copper	21	H	2	4	6				
036	12/9/2005	R.pipiens	Copper	21	H	3	5	7				
036	12/9/2005	R.pipiens	Copper	21	H	4	4	6				
036	12/9/2005	R.pipiens	Copper	21	H	5	4	6	4.2	6.2	4.1	5.9
036	38695	R.pipiens	Copper	87	A	1	4	6				
036	38695	R.pipiens	Copper	87	A	2	4	6				
036	38695	R.pipiens	Copper	87	A	3	4	6				
036	38695	R.pipiens	Copper	87	A	4	3	6				
036	38695	R.pipiens	Copper	87	A	5	3	6	3.6	6.0		
036	38695	R.pipiens	Copper	87	B	1	3	5				
036	38695	R.pipiens	Copper	87	B	2	4	5				
036	38695	R.pipiens	Copper	87	B	3	3	5				
036	38695	R.pipiens	Copper	87	B	4	4	6				
036	38695	R.pipiens	Copper	87	B	5	4	5	3.6	5.2		
036	38695	R.pipiens	Copper	87	C	1	3	4				
036	38695	R.pipiens	Copper	87	C	2	3	5				
036	38695	R.pipiens	Copper	87	C	3	3	5				
036	38695	R.pipiens	Copper	87	C	4	3	5				
036	38695	R.pipiens	Copper	87	C	5	3	5	3.0	4.8		
036	38695	R.pipiens	Copper	87	D	1	4	5				
036	38695	R.pipiens	Copper	87	D	2	4	5				
036	38695	R.pipiens	Copper	87	D	3	4	5				
036	38695	R.pipiens	Copper	87	D	4	4	5				
036	38695	R.pipiens	Copper	87	D	5	3	5	3.8	5.0		
036	38695	R.pipiens	Copper	87	E	1	4	5				
036	38695	R.pipiens	Copper	87	E	2	4	5				
036	38695	R.pipiens	Copper	87	E	3	3	5				
036	38695	R.pipiens	Copper	87	E	4	3	5				
036	38695	R.pipiens	Copper	87	E	5	3	5	3.4	5.0		
036	38695	R.pipiens	Copper	87	F	1	4	6				
036	38695	R.pipiens	Copper	87	F	2	4	6				
036	38695	R.pipiens	Copper	87	F	3	4	6				
036	38695	R.pipiens	Copper	87	F	4	4	6				
036	38695	R.pipiens	Copper	87	F	5	-	-	3.2	4.8		
036	38695	R.pipiens	Copper	87	G	1	4	5				
036	38695	R.pipiens	Copper	87	G	2	4	5				
036	38695	R.pipiens	Copper	87	G	3	4	5				
036	38695	R.pipiens	Copper	87	G	4	4	5				
036	38695	R.pipiens	Copper	87	G	5	4	5	4.0	5.0		
036	38695	R.pipiens	Copper	87	H	1	3	5				
036	38695	R.pipiens	Copper	87	H	2	3	5				
036	38695	R.pipiens	Copper	87	H	3	3	5				
036	38695	R.pipiens	Copper	87	H	4	3	5				
036	38695	R.pipiens	Copper	87	H	5	3	5	3.0	5.0	3.5	5.1

SubLethal Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Organism	Body Width (mm)	Body Length (mm)	Day 10 Average Body Width (mm)	Day 10 Average Body Length (mm)	Day 10 Average Treatment Body Width (mm)	Day 10 Average Treatment Body Length (mm)
036	12/9/2005	R.pipiens	Copper	140	A	1	3	5				
036	12/9/2005	R.pipiens	Copper	140	A	2	3	4				
036	12/9/2005	R.pipiens	Copper	140	A	3	3	5				
036	12/9/2005	R.pipiens	Copper	140	A	4	3	5				
036	12/9/2005	R.pipiens	Copper	140	A	5	3	4	3.0	4.6		
036	12/9/2005	R.pipiens	Copper	140	B	1	3	4				
036	12/9/2005	R.pipiens	Copper	140	B	2	3	4				
036	12/9/2005	R.pipiens	Copper	140	B	3	3	4				
036	12/9/2005	R.pipiens	Copper	140	B	4	3	4				
036	12/9/2005	R.pipiens	Copper	140	B	5	-	-	2.4	3.2		
036	12/9/2005	R.pipiens	Copper	140	C	1	3	4				
036	12/9/2005	R.pipiens	Copper	140	C	2	3	4				
036	12/9/2005	R.pipiens	Copper	140	C	3	3	5				
036	12/9/2005	R.pipiens	Copper	140	C	4	3	4				
036	12/9/2005	R.pipiens	Copper	140	C	5	3	4	3.0	4.2		
036	12/9/2005	R.pipiens	Copper	140	D	1	3	5				
036	12/9/2005	R.pipiens	Copper	140	D	2	3	4				
036	12/9/2005	R.pipiens	Copper	140	D	3	3	4				
036	12/9/2005	R.pipiens	Copper	140	D	4	3	4				
036	12/9/2005	R.pipiens	Copper	140	D	5	3	4	3.0	4.2		
036	12/9/2005	R.pipiens	Copper	140	E	1	3	4				
036	12/9/2005	R.pipiens	Copper	140	E	2	3	4				
036	12/9/2005	R.pipiens	Copper	140	E	3	3	4				
036	12/9/2005	R.pipiens	Copper	140	E	4	3	5				
036	12/9/2005	R.pipiens	Copper	140	E	5	3	5	3.0	4.4		
036	12/9/2005	R.pipiens	Copper	140	F	1	3	5				
036	12/9/2005	R.pipiens	Copper	140	F	2	3	5				
036	12/9/2005	R.pipiens	Copper	140	F	3	3	4				
036	12/9/2005	R.pipiens	Copper	140	F	4	3	4				
036	12/9/2005	R.pipiens	Copper	140	F	5	3	5	3.0	4.6		
036	12/9/2005	R.pipiens	Copper	140	G	1	3	4				
036	12/9/2005	R.pipiens	Copper	140	G	2	3	4				
036	12/9/2005	R.pipiens	Copper	140	G	3	3	4				
036	12/9/2005	R.pipiens	Copper	140	G	4	3	4				
036	12/9/2005	R.pipiens	Copper	140	G	5	3	5	3.0	4.2		
036	12/9/2005	R.pipiens	Copper	140	H	1	3	5				
036	12/9/2005	R.pipiens	Copper	140	H	2	3	4				
036	12/9/2005	R.pipiens	Copper	140	H	3	3	4				
036	12/9/2005	R.pipiens	Copper	140	H	4	3	4				
036	12/9/2005	R.pipiens	Copper	140	H	5	-	-	2.4	3.4	2.9	4.1
036	12/9/2005	R.pipiens	Copper	230	A	1	3	4				
036	12/9/2005	R.pipiens	Copper	230	A	2	3	4				
036	12/9/2005	R.pipiens	Copper	230	A	3	3	4				
036	12/9/2005	R.pipiens	Copper	230	A	4	3	4				
036	12/9/2005	R.pipiens	Copper	230	A	5	3	4	3.0	4.0		
036	12/9/2005	R.pipiens	Copper	230	B	1	3	4				
036	12/9/2005	R.pipiens	Copper	230	B	2	3	4				
036	12/9/2005	R.pipiens	Copper	230	B	3	3	4				
036	12/9/2005	R.pipiens	Copper	230	B	4	3	4				
036	12/9/2005	R.pipiens	Copper	230	B	5	-	-	2.4	3.2		
036	12/9/2005	R.pipiens	Copper	230	C	1	2	4				
036	12/9/2005	R.pipiens	Copper	230	C	2	2	4				
036	12/9/2005	R.pipiens	Copper	230	C	3	2	4				
036	12/9/2005	R.pipiens	Copper	230	C	4	3	4				
036	12/9/2005	R.pipiens	Copper	230	C	5	-	-	1.8	3.2		
036	12/9/2005	R.pipiens	Copper	230	D	1	3	4				
036	12/9/2005	R.pipiens	Copper	230	D	2	4	5				
036	12/9/2005	R.pipiens	Copper	230	D	3	5	5				
036	12/9/2005	R.pipiens	Copper	230	D	4	-	-				
036	12/9/2005	R.pipiens	Copper	230	D	5	-	-	2.4	2.8		
036	12/9/2005	R.pipiens	Copper	230	E	1	2	4				
036	12/9/2005	R.pipiens	Copper	230	E	2	2	4				
036	12/9/2005	R.pipiens	Copper	230	E	3	2	4				
036	12/9/2005	R.pipiens	Copper	230	E	4	3	4				
036	12/9/2005	R.pipiens	Copper	230	E	5	3	4	2.4	4.0		
036	12/9/2005	R.pipiens	Copper	230	F	1	2	4				
036	12/9/2005	R.pipiens	Copper	230	F	2	3	4				
036	12/9/2005	R.pipiens	Copper	230	F	3	2	4				
036	12/9/2005	R.pipiens	Copper	230	F	4	2	3				
036	12/9/2005	R.pipiens	Copper	230	F	5	3	4	2.4	3.8		
036	12/9/2005	R.pipiens	Copper	230	G	1	2	3				
036	12/9/2005	R.pipiens	Copper	230	G	2	3	4				
036	12/9/2005	R.pipiens	Copper	230	G	3	2	3				
036	12/9/2005	R.pipiens	Copper	230	G	4	-	-				
036	12/9/2005	R.pipiens	Copper	230	G	5	-	-	1.4	2.0		
036	12/9/2005	R.pipiens	Copper	230	H	1	3	4				
036	12/9/2005	R.pipiens	Copper	230	H	2	3	4				
036	12/9/2005	R.pipiens	Copper	230	H	3	-	-				
036	12/9/2005	R.pipiens	Copper	230	H	4	-	-				
036	12/9/2005	R.pipiens	Copper	230	H	5	-	-	1.2	1.6	2.1	3.1

SubLethal Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Organism	Body Width (mm)	Body Length (mm)	Day 10 Average Body Width (mm)	Day 10 Average Body Length (mm)	Day 10 Average Treatment Body Width (mm)	Day 10 Average Treatment Body Length (mm)
039	12/9/2005	R.pipiens	Lead	4.5	A	1	4	6				
039	12/9/2005	R.pipiens	Lead	4.5	A	2	4	6				
039	12/9/2005	R.pipiens	Lead	4.5	A	3	5	7				
039	12/9/2005	R.pipiens	Lead	4.5	A	4	4	7				
039	12/9/2005	R.pipiens	Lead	4.5	A	5	4	7	4.2	6.6		
039	12/9/2005	R.pipiens	Lead	4.5	B	1	5	8				
039	12/9/2005	R.pipiens	Lead	4.5	B	2	5	9				
039	12/9/2005	R.pipiens	Lead	4.5	B	3	5	8				
039	12/9/2005	R.pipiens	Lead	4.5	B	4	5	8				
039	12/9/2005	R.pipiens	Lead	4.5	B	5	-	-	4.0	6.6		
039	12/9/2005	R.pipiens	Lead	4.5	C	1	5	7				
039	12/9/2005	R.pipiens	Lead	4.5	C	2	4	6				
039	12/9/2005	R.pipiens	Lead	4.5	C	3	4	7				
039	12/9/2005	R.pipiens	Lead	4.5	C	4	5	8				
039	12/9/2005	R.pipiens	Lead	4.5	C	5	5	7	4.6	7.0		
039	12/9/2005	R.pipiens	Lead	4.5	D	1	5	8				
039	12/9/2005	R.pipiens	Lead	4.5	D	2	4	7				
039	12/9/2005	R.pipiens	Lead	4.5	D	3	5	8				
039	12/9/2005	R.pipiens	Lead	4.5	D	4	5	7				
039	12/9/2005	R.pipiens	Lead	4.5	D	5	4	8	4.6	7.6		
039	12/9/2005	R.pipiens	Lead	4.5	E	1	4	7				
039	12/9/2005	R.pipiens	Lead	4.5	E	2	4	5				
039	12/9/2005	R.pipiens	Lead	4.5	E	3	5	7				
039	12/9/2005	R.pipiens	Lead	4.5	E	4	4	6				
039	12/9/2005	R.pipiens	Lead	4.5	E	5	5	6	4.4	6.2		
039	12/9/2005	R.pipiens	Lead	4.5	F	1	4	6				
039	12/9/2005	R.pipiens	Lead	4.5	F	2	4	7				
039	12/9/2005	R.pipiens	Lead	4.5	F	3	4	7				
039	12/9/2005	R.pipiens	Lead	4.5	F	4	5	6				
039	12/9/2005	R.pipiens	Lead	4.5	F	5	4	7	4.2	6.6		
039	12/9/2005	R.pipiens	Lead	4.5	G	1	5	7				
039	12/9/2005	R.pipiens	Lead	4.5	G	2	4	7				
039	12/9/2005	R.pipiens	Lead	4.5	G	3	5	9				
039	12/9/2005	R.pipiens	Lead	4.5	G	4	5	8				
039	12/9/2005	R.pipiens	Lead	4.5	G	5	3	6	4.4	7.4		
039	12/9/2005	R.pipiens	Lead	4.5	H	1	4	7				
039	12/9/2005	R.pipiens	Lead	4.5	H	2	4	7				
039	12/9/2005	R.pipiens	Lead	4.5	H	3	5	7				
039	12/9/2005	R.pipiens	Lead	4.5	H	4	4	8				
039	12/9/2005	R.pipiens	Lead	4.5	H	5	4	7	4.2	7.2	4.3	6.9
039	12/9/2005	R.pipiens	Lead	63	A	1	5	7				
039	12/9/2005	R.pipiens	Lead	63	A	2	4	6				
039	12/9/2005	R.pipiens	Lead	63	A	3	5	7				
039	12/9/2005	R.pipiens	Lead	63	A	4	4	8				
039	12/9/2005	R.pipiens	Lead	63	A	5	5	7	4.6	7.0		
039	12/9/2005	R.pipiens	Lead	63	B	1	4	7				
039	12/9/2005	R.pipiens	Lead	63	B	2	5	7				
039	12/9/2005	R.pipiens	Lead	63	B	3	5	8				
039	12/9/2005	R.pipiens	Lead	63	B	4	5	7				
039	12/9/2005	R.pipiens	Lead	63	B	5	4	7	4.6	7.2		
039	12/9/2005	R.pipiens	Lead	63	C	1	5	9				
039	12/9/2005	R.pipiens	Lead	63	C	2	5	9				
039	12/9/2005	R.pipiens	Lead	63	C	3	5	9				
039	12/9/2005	R.pipiens	Lead	63	C	4	5	8				
039	12/9/2005	R.pipiens	Lead	63	C	5	5	8	5.0	8.6		
039	12/9/2005	R.pipiens	Lead	63	D	1	5	7				
039	12/9/2005	R.pipiens	Lead	63	D	2	5	7				
039	12/9/2005	R.pipiens	Lead	63	D	3	5	7				
039	12/9/2005	R.pipiens	Lead	63	D	4	5	8				
039	12/9/2005	R.pipiens	Lead	63	D	5	5	7	5.0	7.2		
039	12/9/2005	R.pipiens	Lead	63	E	1	5	8				
039	12/9/2005	R.pipiens	Lead	63	E	2	5	8				
039	12/9/2005	R.pipiens	Lead	63	E	3	4	7				
039	12/9/2005	R.pipiens	Lead	63	E	4	4	7				
039	12/9/2005	R.pipiens	Lead	63	E	5	5	7	4.6	7.4		
039	12/9/2005	R.pipiens	Lead	63	F	1	4	7				
039	12/9/2005	R.pipiens	Lead	63	F	2	5	6				
039	12/9/2005	R.pipiens	Lead	63	F	3	4	6				
039	12/9/2005	R.pipiens	Lead	63	F	4	5	6				
039	12/9/2005	R.pipiens	Lead	63	F	5	5	7	4.6	6.4		
039	12/9/2005	R.pipiens	Lead	63	G	1	5	8				
039	12/9/2005	R.pipiens	Lead	63	G	2	5	8				
039	12/9/2005	R.pipiens	Lead	63	G	3	4	8				
039	12/9/2005	R.pipiens	Lead	63	G	4	4	7				
039	12/9/2005	R.pipiens	Lead	63	G	5	4	7	4.4	7.6		
039	12/9/2005	R.pipiens	Lead	63	H	1	6	9				
039	12/9/2005	R.pipiens	Lead	63	H	2	5	7				
039	12/9/2005	R.pipiens	Lead	63	H	3	5	7				
039	12/9/2005	R.pipiens	Lead	63	H	4	5	7				
039	12/9/2005	R.pipiens	Lead	63	H	5	5	8	5.2	7.6	4.8	7.4

SubLethal Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Organism	Body Width (mm)	Body Length (mm)	Day 10 Average Body Width (mm)	Day 10 Average Body Length (mm)	Day 10 Average Treatment Body Width (mm)	Day 10 Average Treatment Body Length (mm)
039	12/9/2005	R.pipiens	Lead	100	A	1	4	6				
039	12/9/2005	R.pipiens	Lead	100	A	2	5	7				
039	12/9/2005	R.pipiens	Lead	100	A	3	5	7				
039	12/9/2005	R.pipiens	Lead	100	A	4	4	6				
039	12/9/2005	R.pipiens	Lead	100	A	5	5	7	4.6	6.6		
039	12/9/2005	R.pipiens	Lead	100	B	1	5	7				
039	12/9/2005	R.pipiens	Lead	100	B	2	5	7				
039	12/9/2005	R.pipiens	Lead	100	B	3	5	6				
039	12/9/2005	R.pipiens	Lead	100	B	4	5	7				
039	12/9/2005	R.pipiens	Lead	100	B	5	5	6	5.0	6.6		
039	12/9/2005	R.pipiens	Lead	100	C	1	6	8				
039	12/9/2005	R.pipiens	Lead	100	C	2	6	7				
039	12/9/2005	R.pipiens	Lead	100	C	3	5	7				
039	12/9/2005	R.pipiens	Lead	100	C	4	6	8				
039	12/9/2005	R.pipiens	Lead	100	C	5	5	7	5.6	7.4		
039	12/9/2005	R.pipiens	Lead	100	D	1	4	6				
039	12/9/2005	R.pipiens	Lead	100	D	2	5	7				
039	12/9/2005	R.pipiens	Lead	100	D	3	5	7				
039	12/9/2005	R.pipiens	Lead	100	D	4	5	7				
039	12/9/2005	R.pipiens	Lead	100	D	5	4	6	4.6	6.6		
039	12/9/2005	R.pipiens	Lead	100	E	1	4	7				
039	12/9/2005	R.pipiens	Lead	100	E	2	4	7				
039	12/9/2005	R.pipiens	Lead	100	E	3	4	7				
039	12/9/2005	R.pipiens	Lead	100	E	4	4	7				
039	12/9/2005	R.pipiens	Lead	100	E	5	5	7	4.2	7.0		
039	12/9/2005	R.pipiens	Lead	100	F	1	4	6				
039	12/9/2005	R.pipiens	Lead	100	F	2	5	7				
039	12/9/2005	R.pipiens	Lead	100	F	3	4	6				
039	12/9/2005	R.pipiens	Lead	100	F	4	4	5				
039	12/9/2005	R.pipiens	Lead	100	F	5	4	6	4.2	6.0		
039	12/9/2005	R.pipiens	Lead	100	G	1	5	7				
039	12/9/2005	R.pipiens	Lead	100	G	2	5	7				
039	12/9/2005	R.pipiens	Lead	100	G	3	5	8				
039	12/9/2005	R.pipiens	Lead	100	G	4	4	7				
039	12/9/2005	R.pipiens	Lead	100	G	5	5	7	4.8	7.2		
039	12/9/2005	R.pipiens	Lead	100	H	1	4	6				
039	12/9/2005	R.pipiens	Lead	100	H	2	4	6				
039	12/9/2005	R.pipiens	Lead	100	H	3	4	6				
039	12/9/2005	R.pipiens	Lead	100	H	4	4	7				
039	12/9/2005	R.pipiens	Lead	100	H	5	4	6	4.0	6.2	4.6	6.7
039	12/9/2005	R.pipiens	Lead	260	A	1	4	7				
039	12/9/2005	R.pipiens	Lead	260	A	2	5	6				
039	12/9/2005	R.pipiens	Lead	260	A	3	4	6				
039	12/9/2005	R.pipiens	Lead	260	A	4	4	6				
039	12/9/2005	R.pipiens	Lead	260	A	5	4	7	4.2	6.4		
039	12/9/2005	R.pipiens	Lead	260	B	1	5	6				
039	12/9/2005	R.pipiens	Lead	260	B	2	4	6				
039	12/9/2005	R.pipiens	Lead	260	B	3	4	7				
039	12/9/2005	R.pipiens	Lead	260	B	4	4	7				
039	12/9/2005	R.pipiens	Lead	260	B	5	4	6	4.2	6.4		
039	12/9/2005	R.pipiens	Lead	260	C	1	4	7				
039	12/9/2005	R.pipiens	Lead	260	C	2	4	7				
039	12/9/2005	R.pipiens	Lead	260	C	3	4	7				
039	12/9/2005	R.pipiens	Lead	260	C	4	4	7				
039	12/9/2005	R.pipiens	Lead	260	C	5	4	6	4.0	6.8		
039	12/9/2005	R.pipiens	Lead	260	D	1	4	7				
039	12/9/2005	R.pipiens	Lead	260	D	2	4	7				
039	12/9/2005	R.pipiens	Lead	260	D	3	4	6				
039	12/9/2005	R.pipiens	Lead	260	D	4	4	7				
039	12/9/2005	R.pipiens	Lead	260	D	5	4	6	4.0	6.6		
039	12/9/2005	R.pipiens	Lead	260	E	1	4	6				
039	12/9/2005	R.pipiens	Lead	260	E	2	4	6				
039	12/9/2005	R.pipiens	Lead	260	E	3	4	6				
039	12/9/2005	R.pipiens	Lead	260	E	4	3	6				
039	12/9/2005	R.pipiens	Lead	260	E	5	4	6	3.8	6.0		
039	12/9/2005	R.pipiens	Lead	260	F	1	4	7				
039	12/9/2005	R.pipiens	Lead	260	F	2	4	7				
039	12/9/2005	R.pipiens	Lead	260	F	3	4	7				
039	12/9/2005	R.pipiens	Lead	260	F	4	5	6				
039	12/9/2005	R.pipiens	Lead	260	F	5	3	5	4.0	6.4		
039	12/9/2005	R.pipiens	Lead	260	G	1	5	6				
039	12/9/2005	R.pipiens	Lead	260	G	2	5	8				
039	12/9/2005	R.pipiens	Lead	260	G	3	5	8				
039	12/9/2005	R.pipiens	Lead	260	G	4	4	7				
039	12/9/2005	R.pipiens	Lead	260	G	5	4	7	4.6	7.2		
039	12/9/2005	R.pipiens	Lead	260	H	1	4	5				
039	12/9/2005	R.pipiens	Lead	260	H	2	4	6				
039	12/9/2005	R.pipiens	Lead	260	H	3	4	6				
039	12/9/2005	R.pipiens	Lead	260	H	4	4	7				
039	12/9/2005	R.pipiens	Lead	260	H	5	4	6	4.0	6.0	4.1	6.5

SubLethal Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Organism	Body Width (mm)	Body Length (mm)	Day 10 Average Body Width (mm)	Day 10 Average Body Length (mm)	Day 10 Average Treatment Body Width (mm)	Day 10 Average Treatment Body Length (mm)
039	12/9/2005	R.pipiens	Lead	680	A	1	4	6				
039	12/9/2005	R.pipiens	Lead	680	A	2	4	5				
039	12/9/2005	R.pipiens	Lead	680	A	3	4	6				
039	12/9/2005	R.pipiens	Lead	680	A	4	4	6				
039	12/9/2005	R.pipiens	Lead	680	A	5	5	6	4.2	5.8		
039	12/9/2005	R.pipiens	Lead	680	B	1	5	7				
039	12/9/2005	R.pipiens	Lead	680	B	2	5	7				
039	12/9/2005	R.pipiens	Lead	680	B	3	4	6				
039	12/9/2005	R.pipiens	Lead	680	B	4	4	7				
039	12/9/2005	R.pipiens	Lead	680	B	5	4	6	4.4	6.6		
039	12/9/2005	R.pipiens	Lead	680	C	1	4	6				
039	12/9/2005	R.pipiens	Lead	680	C	2	4	6				
039	12/9/2005	R.pipiens	Lead	680	C	3	3	5				
039	12/9/2005	R.pipiens	Lead	680	C	4	4	6				
039	12/9/2005	R.pipiens	Lead	680	C	5	3	5	3.6	5.6		
039	12/9/2005	R.pipiens	Lead	680	D	1	4	6				
039	12/9/2005	R.pipiens	Lead	680	D	2	4	5				
039	12/9/2005	R.pipiens	Lead	680	D	3	4	5				
039	12/9/2005	R.pipiens	Lead	680	D	4	4	5				
039	12/9/2005	R.pipiens	Lead	680	D	5	4	6	4.0	5.4		
039	12/9/2005	R.pipiens	Lead	680	E	1	4	6				
039	12/9/2005	R.pipiens	Lead	680	E	2	3	4				
039	12/9/2005	R.pipiens	Lead	680	E	3	3	5				
039	12/9/2005	R.pipiens	Lead	680	E	4	3	4				
039	12/9/2005	R.pipiens	Lead	680	E	5	4	6	3.4	5.0		
039	12/9/2005	R.pipiens	Lead	680	F	1	4	6				
039	12/9/2005	R.pipiens	Lead	680	F	2	4	5				
039	12/9/2005	R.pipiens	Lead	680	F	3	4	6				
039	12/9/2005	R.pipiens	Lead	680	F	4	4	6				
039	12/9/2005	R.pipiens	Lead	680	F	5	4	6	4.0	5.8		
039	12/9/2005	R.pipiens	Lead	680	G	1	4	6				
039	12/9/2005	R.pipiens	Lead	680	G	2	4	6				
039	12/9/2005	R.pipiens	Lead	680	G	3	4	5				
039	12/9/2005	R.pipiens	Lead	680	G	4	4	7				
039	12/9/2005	R.pipiens	Lead	680	G	5	4	6	4.0	6.0		
039	12/9/2005	R.pipiens	Lead	680	H	1	4	5				
039	12/9/2005	R.pipiens	Lead	680	H	2	4	5				
039	12/9/2005	R.pipiens	Lead	680	H	3	4	5				
039	12/9/2005	R.pipiens	Lead	680	H	4	4	6				
039	12/9/2005	R.pipiens	Lead	680	H	5	4	6	4.0	5.4	4.0	5.7
039	12/9/2005	R.pipiens	Lead	1200	A	1	2	4				
039	12/9/2005	R.pipiens	Lead	1200	A	2	2	3				
039	12/9/2005	R.pipiens	Lead	1200	A	3	-	-				
039	12/9/2005	R.pipiens	Lead	1200	A	4	-	-				
039	12/9/2005	R.pipiens	Lead	1200	A	5	-	-	0.8	1.4		
039	12/9/2005	R.pipiens	Lead	1200	B	1	3	5				
039	12/9/2005	R.pipiens	Lead	1200	B	2	3	4				
039	12/9/2005	R.pipiens	Lead	1200	B	3	4	6				
039	12/9/2005	R.pipiens	Lead	1200	B	4	3	5				
039	12/9/2005	R.pipiens	Lead	1200	B	5	3	5	3.2	5.0		
039	12/9/2005	R.pipiens	Lead	1200	C	1	3	4				
039	12/9/2005	R.pipiens	Lead	1200	C	2	3	5				
039	12/9/2005	R.pipiens	Lead	1200	C	3	3	4				
039	12/9/2005	R.pipiens	Lead	1200	C	4	2	4				
039	12/9/2005	R.pipiens	Lead	1200	C	5	-	-	2.2	3.4		
039	12/9/2005	R.pipiens	Lead	1200	D	1	3	4				
039	12/9/2005	R.pipiens	Lead	1200	D	2	4	5				
039	12/9/2005	R.pipiens	Lead	1200	D	3	3	4				
039	12/9/2005	R.pipiens	Lead	1200	D	4	3	5				
039	12/9/2005	R.pipiens	Lead	1200	D	5	-	-	2.6	3.6		
039	12/9/2005	R.pipiens	Lead	1200	E	1	1	2				
039	12/9/2005	R.pipiens	Lead	1200	E	2	3	4				
039	12/9/2005	R.pipiens	Lead	1200	E	3	4	5				
039	12/9/2005	R.pipiens	Lead	1200	E	4	3	5				
039	12/9/2005	R.pipiens	Lead	1200	E	5	3	5	2.8	4.2		
039	12/9/2005	R.pipiens	Lead	1200	F	1	2	3				
039	12/9/2005	R.pipiens	Lead	1200	F	2	4	5				
039	12/9/2005	R.pipiens	Lead	1200	F	3	4	5				
039	12/9/2005	R.pipiens	Lead	1200	F	4	1	2				
039	12/9/2005	R.pipiens	Lead	1200	F	5	-	-	2.2	3.0		
039	12/9/2005	R.pipiens	Lead	1200	G	1	3	3				
039	12/9/2005	R.pipiens	Lead	1200	G	2	3	5				
039	12/9/2005	R.pipiens	Lead	1200	G	3	-	-				
039	12/9/2005	R.pipiens	Lead	1200	G	4	-	-				
039	12/9/2005	R.pipiens	Lead	1200	G	5	-	-	1.2	1.6		
039	12/9/2005	R.pipiens	Lead	1200	H	1	3	4				
039	12/9/2005	R.pipiens	Lead	1200	H	2	4	4				
039	12/9/2005	R.pipiens	Lead	1200	H	3	-	-				
039	12/9/2005	R.pipiens	Lead	1200	H	4	-	-				
039	12/9/2005	R.pipiens	Lead	1200	H	5	-	-	1.4	1.6	2.1	3.0
123	4/21/2008	B. americanus	Lead	1.9	A	1	5	7				
123	4/21/2008	B. americanus	Lead	1.9	A	2	5	7				
123	4/21/2008	B. americanus	Lead	1.9	A	3	4	7				
123	4/21/2008	B. americanus	Lead	1.9	A	4	4	7				
123	4/21/2008	B. americanus	Lead	1.9	A	5	4	7	4.4	7.0		
123	4/21/2008	B. americanus	Lead	1.9	B	1	4	6				
123	4/21/2008	B. americanus	Lead	1.9	B	2	5	7				
123	4/21/2008	B. americanus	Lead	1.9	B	3	4	7				
123	4/21/2008	B. americanus	Lead	1.9	B	4	5	7				
123	4/21/2008	B. americanus	Lead	1.9	B	5	4	7	4.4	6.8		
123	4/21/2008	B. americanus	Lead	1.9	C	1	5	8				
123	4/21/2008	B. americanus	Lead	1.9	C	2	5	7				
123	4/21/2008	B. americanus	Lead	1.9	C	3	4	6				
123	4/21/2008	B. americanus	Lead	1.9	C	4	5	7				
123	4/21/2008	B. americanus	Lead	1.9	C	5	5	7	4.8	7.0		
123	4/21/2008	B. americanus	Lead	1.9	D	1	5	7				
123	4/21/2008	B. americanus	Lead	1.9	D	2	5	8				
123	4/21/2008	B. americanus	Lead	1.9	D	3	5	7				
123	4/21/2008	B. americanus	Lead	1.9	D	4	5	7				
123	4/21/2008	B. americanus	Lead	1.9	D	5	4	7	4.8	7.2		
123	4/21/2008	B. americanus	Lead	1.9	E	1	4	6				
123	4/21/2008	B. americanus	Lead	1.9	E	2	4	7				
123	4/21/2008	B. americanus	Lead	1.9	E	3	4	6				
123	4/21/2008	B. americanus	Lead	1.9	E	4	4	6				
123	4/21/2008	B. americanus	Lead	1.9	E	5	-	-	4.0	6.3		
123	4/21/2008	B. americanus	Lead	1.9	F	1	5	7				
123	4/21/2008	B. americanus	Lead	1.9	F	2	4	7				
123	4/21/2008	B. americanus	Lead	1.9	F	3	4	7				
123	4/21/2008	B. americanus	Lead	1.9	F	4	5	7				
123	4/21/2008	B. americanus	Lead	1.9	F	5	4	7	4.4	7.0	4.5	6.9

SubLethal Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Organism	Body Width (mm)	Body Length (mm)	Day 10 Average Body Width (mm)	Day 10 Average Body Length (mm)	Day 10 Average Treatment Body Width (mm)	Day 10 Average Treatment Body Length (mm)
123	4/21/2008	B. americanus	Lead	40	A	1	4	6				
123	4/21/2008	B. americanus	Lead	40	A	2	4	6				
123	4/21/2008	B. americanus	Lead	40	A	3	5	7				
123	4/21/2008	B. americanus	Lead	40	A	4	3	5				
123	4/21/2008	B. americanus	Lead	40	A	5	5	7	4.2	6.2		
123	4/21/2008	B. americanus	Lead	40	B	1	5	8				
123	4/21/2008	B. americanus	Lead	40	B	2	5	8				
123	4/21/2008	B. americanus	Lead	40	B	3	5	7				
123	4/21/2008	B. americanus	Lead	40	B	4	5	8				
123	4/21/2008	B. americanus	Lead	40	B	5	4	7	4.8	7.6		
123	4/21/2008	B. americanus	Lead	40	C	1	4	7				
123	4/21/2008	B. americanus	Lead	40	C	2	4	7				
123	4/21/2008	B. americanus	Lead	40	C	3	4	7				
123	4/21/2008	B. americanus	Lead	40	C	4	5	8				
123	4/21/2008	B. americanus	Lead	40	C	5	4	6	4.2	7.0		
123	4/21/2008	B. americanus	Lead	40	D	1	5	7				
123	4/21/2008	B. americanus	Lead	40	D	2	4	7				
123	4/21/2008	B. americanus	Lead	40	D	3	5	7				
123	4/21/2008	B. americanus	Lead	40	D	4	4	7				
123	4/21/2008	B. americanus	Lead	40	D	5	4	6	4.4	6.8		
123	4/21/2008	B. americanus	Lead	40	E	1	4	6				
123	4/21/2008	B. americanus	Lead	40	E	2	4	6				
123	4/21/2008	B. americanus	Lead	40	E	3	4	7				
123	4/21/2008	B. americanus	Lead	40	E	4	4	6				
123	4/21/2008	B. americanus	Lead	40	E	5	4	6	4.0	6.2		
123	4/21/2008	B. americanus	Lead	40	F	1	4	7				
123	4/21/2008	B. americanus	Lead	40	F	2	4	6				
123	4/21/2008	B. americanus	Lead	40	F	3	5	7				
123	4/21/2008	B. americanus	Lead	40	F	4	5	7				
123	4/21/2008	B. americanus	Lead	40	F	5	4	7	4.4	6.8	4.3	6.8
123	4/21/2008	B. americanus	Lead	77	A	1	5	6				
123	4/21/2008	B. americanus	Lead	77	A	2	4	7				
123	4/21/2008	B. americanus	Lead	77	A	3	5	7				
123	4/21/2008	B. americanus	Lead	77	A	4	4	6				
123	4/21/2008	B. americanus	Lead	77	A	5	5	7	4.6	6.6		
123	4/21/2008	B. americanus	Lead	77	B	1	4	7				
123	4/21/2008	B. americanus	Lead	77	B	2	4	7				
123	4/21/2008	B. americanus	Lead	77	B	3	4	7				
123	4/21/2008	B. americanus	Lead	77	B	4	5	7				
123	4/21/2008	B. americanus	Lead	77	B	5	4	7	4.2	7.0		
123	4/21/2008	B. americanus	Lead	77	C	1	5	7				
123	4/21/2008	B. americanus	Lead	77	C	2	4	6				
123	4/21/2008	B. americanus	Lead	77	C	3	5	7				
123	4/21/2008	B. americanus	Lead	77	C	4	4	7				
123	4/21/2008	B. americanus	Lead	77	C	5	4	7	4.4	6.8		
123	4/21/2008	B. americanus	Lead	77	D	1	4	7				
123	4/21/2008	B. americanus	Lead	77	D	2	5	7				
123	4/21/2008	B. americanus	Lead	77	D	3	4	7				
123	4/21/2008	B. americanus	Lead	77	D	4	4	7				
123	4/21/2008	B. americanus	Lead	77	D	5	4	7	4.2	7.0		
123	4/21/2008	B. americanus	Lead	77	E	1	4	7				
123	4/21/2008	B. americanus	Lead	77	E	2	4	7				
123	4/21/2008	B. americanus	Lead	77	E	3	4	7				
123	4/21/2008	B. americanus	Lead	77	E	4	4	6				
123	4/21/2008	B. americanus	Lead	77	E	5	5	7	4.2	6.8		
123	4/21/2008	B. americanus	Lead	77	F	1	4	6				
123	4/21/2008	B. americanus	Lead	77	F	2	4	7				
123	4/21/2008	B. americanus	Lead	77	F	3	4	7				
123	4/21/2008	B. americanus	Lead	77	F	4	4	6				
123	4/21/2008	B. americanus	Lead	77	F	5	4	7	4.0	6.6	4.3	6.8
123	4/21/2008	B. americanus	Lead	140	A	1	5	7				
123	4/21/2008	B. americanus	Lead	140	A	2	5	7				
123	4/21/2008	B. americanus	Lead	140	A	3	5	6				
123	4/21/2008	B. americanus	Lead	140	A	4	4	7				
123	4/21/2008	B. americanus	Lead	140	A	5	5	7	4.8	6.8		
123	4/21/2008	B. americanus	Lead	140	B	1	3	6				
123	4/21/2008	B. americanus	Lead	140	B	2	4	6				
123	4/21/2008	B. americanus	Lead	140	B	3	4	6				
123	4/21/2008	B. americanus	Lead	140	B	4	4	6				
123	4/21/2008	B. americanus	Lead	140	B	5	3	5	3.6	5.8		
123	4/21/2008	B. americanus	Lead	140	C	1	4	6				
123	4/21/2008	B. americanus	Lead	140	C	2	5	7				
123	4/21/2008	B. americanus	Lead	140	C	3	4	7				
123	4/21/2008	B. americanus	Lead	140	C	4	4	6				
123	4/21/2008	B. americanus	Lead	140	C	5	-	-	4.3	6.5		
123	4/21/2008	B. americanus	Lead	140	D	1	4	7				
123	4/21/2008	B. americanus	Lead	140	D	2	5	7				
123	4/21/2008	B. americanus	Lead	140	D	3	4	7				
123	4/21/2008	B. americanus	Lead	140	D	4	5	7				
123	4/21/2008	B. americanus	Lead	140	D	5	5	7	4.6	7.0		
123	4/21/2008	B. americanus	Lead	140	E	1	5	7				
123	4/21/2008	B. americanus	Lead	140	E	2	5	7				
123	4/21/2008	B. americanus	Lead	140	E	3	4	6				
123	4/21/2008	B. americanus	Lead	140	E	4	4	7				
123	4/21/2008	B. americanus	Lead	140	E	5	5	6	4.6	6.8		
123	4/21/2008	B. americanus	Lead	140	F	1	4	7				
123	4/21/2008	B. americanus	Lead	140	F	2	5	7				
123	4/21/2008	B. americanus	Lead	140	F	3	4	7				
123	4/21/2008	B. americanus	Lead	140	F	4	4	7				
123	4/21/2008	B. americanus	Lead	140	F	5	5	7	4.4	7.0	4.4	6.6

SubLethal Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Organism	Body Width (mm)	Body Length (mm)	Day 10 Average Body Width (mm)	Day 10 Average Body Length (mm)	Day 10 Average Treatment Body Width (mm)	Day 10 Average Treatment Body Length (mm)
123	4/21/2008	B. americanus	Lead	340	A	1	4	6				
123	4/21/2008	B. americanus	Lead	340	A	2	4	6				
123	4/21/2008	B. americanus	Lead	340	A	3	3	6				
123	4/21/2008	B. americanus	Lead	340	A	4	-	-				
123	4/21/2008	B. americanus	Lead	340	A	5	-	-	3.7	6.0		
123	4/21/2008	B. americanus	Lead	340	B	1	3	5				
123	4/21/2008	B. americanus	Lead	340	B	2	4	6				
123	4/21/2008	B. americanus	Lead	340	B	3	3	4				
123	4/21/2008	B. americanus	Lead	340	B	4	-	-				
123	4/21/2008	B. americanus	Lead	340	B	5	-	-	3.3	5.0		
123	4/21/2008	B. americanus	Lead	340	C	1	4	5				
123	4/21/2008	B. americanus	Lead	340	C	2	4	5				
123	4/21/2008	B. americanus	Lead	340	C	3	3	5				
123	4/21/2008	B. americanus	Lead	340	C	4	4	5				
123	4/21/2008	B. americanus	Lead	340	C	5	3	5	3.6	5.0		
123	4/21/2008	B. americanus	Lead	340	D	1	4	6				
123	4/21/2008	B. americanus	Lead	340	D	2	3	5				
123	4/21/2008	B. americanus	Lead	340	D	3	3	5				
123	4/21/2008	B. americanus	Lead	340	D	4	3	4				
123	4/21/2008	B. americanus	Lead	340	D	5	3	5	3.2	5.0		
123	4/21/2008	B. americanus	Lead	340	E	1	4	6				
123	4/21/2008	B. americanus	Lead	340	E	2	3	5				
123	4/21/2008	B. americanus	Lead	340	E	3	4	6				
123	4/21/2008	B. americanus	Lead	340	E	4	4	5				
123	4/21/2008	B. americanus	Lead	340	E	5	3	6	3.6	5.6		
123	4/21/2008	B. americanus	Lead	340	F	1	2	4				
123	4/21/2008	B. americanus	Lead	340	F	2	3	4				
123	4/21/2008	B. americanus	Lead	340	F	3	3	6				
123	4/21/2008	B. americanus	Lead	340	F	4	4	6				
123	4/21/2008	B. americanus	Lead	340	F	5	3	5	3.0	5.0	3.4	5.3
123	4/21/2008	B. americanus	Lead	750	A	1	3	4				
123	4/21/2008	B. americanus	Lead	750	A	2	-	-				
123	4/21/2008	B. americanus	Lead	750	A	3	-	-				
123	4/21/2008	B. americanus	Lead	750	A	4	-	-				
123	4/21/2008	B. americanus	Lead	750	A	5	-	-	3.0	4.0		
123	4/21/2008	B. americanus	Lead	750	B	1	-	-				
123	4/21/2008	B. americanus	Lead	750	B	2	-	-				
123	4/21/2008	B. americanus	Lead	750	B	3	-	-				
123	4/21/2008	B. americanus	Lead	750	B	4	-	-				
123	4/21/2008	B. americanus	Lead	750	B	5	-	-	0.0	0.0		
123	4/21/2008	B. americanus	Lead	750	C	1	-	-				
123	4/21/2008	B. americanus	Lead	750	C	2	-	-				
123	4/21/2008	B. americanus	Lead	750	C	3	-	-				
123	4/21/2008	B. americanus	Lead	750	C	4	-	-				
123	4/21/2008	B. americanus	Lead	750	C	5	-	-	0.0	0.0		
123	4/21/2008	B. americanus	Lead	750	D	1	4	4				
123	4/21/2008	B. americanus	Lead	750	D	2	-	-				
123	4/21/2008	B. americanus	Lead	750	D	3	-	-				
123	4/21/2008	B. americanus	Lead	750	D	4	-	-				
123	4/21/2008	B. americanus	Lead	750	D	5	-	-	4.0	4.0		
123	4/21/2008	B. americanus	Lead	750	E	1	3	5				
123	4/21/2008	B. americanus	Lead	750	E	2	-	-				
123	4/21/2008	B. americanus	Lead	750	E	3	-	-				
123	4/21/2008	B. americanus	Lead	750	E	4	-	-				
123	4/21/2008	B. americanus	Lead	750	E	5	-	-	3.0	5.0		
123	4/21/2008	B. americanus	Lead	750	F	1	2	4				
123	4/21/2008	B. americanus	Lead	750	F	2	-	-				
123	4/21/2008	B. americanus	Lead	750	F	3	-	-				
123	4/21/2008	B. americanus	Lead	750	F	4	-	-				
123	4/21/2008	B. americanus	Lead	750	F	5	-	-	2.0	4.0	2.0	2.8
122	4/21/2008	B. americanus	Copper	3.2	A	1	5	8				
122	4/21/2008	B. americanus	Copper	3.2	A	2	4	8				
122	4/21/2008	B. americanus	Copper	3.2	A	3	4	7				
122	4/21/2008	B. americanus	Copper	3.2	A	4	5	8				
122	4/21/2008	B. americanus	Copper	3.2	A	5	4	7	4.4	7.6		
122	4/21/2008	B. americanus	Copper	3.2	B	1	4	7				
122	4/21/2008	B. americanus	Copper	3.2	B	2	4	7				
122	4/21/2008	B. americanus	Copper	3.2	B	3	4	7				
122	4/21/2008	B. americanus	Copper	3.2	B	4	3	6				
122	4/21/2008	B. americanus	Copper	3.2	B	5	4	7	3.8	6.8		
122	4/21/2008	B. americanus	Copper	3.2	C	1	5	6				
122	4/21/2008	B. americanus	Copper	3.2	C	2	4	6				
122	4/21/2008	B. americanus	Copper	3.2	C	3	4	6				
122	4/21/2008	B. americanus	Copper	3.2	C	4	4	6				
122	4/21/2008	B. americanus	Copper	3.2	C	5	4	6	4.2	6.0		
122	4/21/2008	B. americanus	Copper	3.2	D	1	4	7				
122	4/21/2008	B. americanus	Copper	3.2	D	2	4	6				
122	4/21/2008	B. americanus	Copper	3.2	D	3	4	6				
122	4/21/2008	B. americanus	Copper	3.2	D	4	4	6				
122	4/21/2008	B. americanus	Copper	3.2	D	5	5	7	4.2	6.4		
122	4/21/2008	B. americanus	Copper	3.2	E	1	4	7				
122	4/21/2008	B. americanus	Copper	3.2	E	2	5	7				
122	4/21/2008	B. americanus	Copper	3.2	E	3	4	7				
122	4/21/2008	B. americanus	Copper	3.2	E	4	5	6				
122	4/21/2008	B. americanus	Copper	3.2	E	5	4	6	4.4	6.8		
122	4/21/2008	B. americanus	Copper	3.2	F	1	5	7				
122	4/21/2008	B. americanus	Copper	3.2	F	2	4	6				
122	4/21/2008	B. americanus	Copper	3.2	F	3	4	6				
122	4/21/2008	B. americanus	Copper	3.2	F	4	5	7				
122	4/21/2008	B. americanus	Copper	3.2	F	5	4	6	4.4	6.4	4.2	6.6

SubLethal Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Organism	Body Width (mm)	Body Length (mm)	Day 10 Average Body Width (mm)	Day 10 Average Body Length (mm)	Day 10 Average Treatment Body Width (mm)	Day 10 Average Treatment Body Length (mm)
122	4/21/2008	B. americanus	Copper	13	A	1	5	7				
122	4/21/2008	B. americanus	Copper	13	A	2	5	7				
122	4/21/2008	B. americanus	Copper	13	A	3	4	7				
122	4/21/2008	B. americanus	Copper	13	A	4	5	7				
122	4/21/2008	B. americanus	Copper	13	A	5	5	7	4.8	7.0		
122	4/21/2008	B. americanus	Copper	13	B	1	4	7				
122	4/21/2008	B. americanus	Copper	13	B	2	4	7				
122	4/21/2008	B. americanus	Copper	13	B	3	5	7				
122	4/21/2008	B. americanus	Copper	13	B	4	4	6				
122	4/21/2008	B. americanus	Copper	13	B	5	5	7	4.4	6.8		
122	4/21/2008	B. americanus	Copper	13	C	1	5	7				
122	4/21/2008	B. americanus	Copper	13	C	2	5	7				
122	4/21/2008	B. americanus	Copper	13	C	3	5	7				
122	4/21/2008	B. americanus	Copper	13	C	4	5	7				
122	4/21/2008	B. americanus	Copper	13	C	5	4	6	4.8	6.8		
122	4/21/2008	B. americanus	Copper	13	D	1	5	7				
122	4/21/2008	B. americanus	Copper	13	D	2	5	7				
122	4/21/2008	B. americanus	Copper	13	D	3	5	7				
122	4/21/2008	B. americanus	Copper	13	D	4	5	8				
122	4/21/2008	B. americanus	Copper	13	D	5	5	8	5.0	7.4		
122	4/21/2008	B. americanus	Copper	13	E	1	4	6				
122	4/21/2008	B. americanus	Copper	13	E	2	4	6				
122	4/21/2008	B. americanus	Copper	13	E	3	5	7				
122	4/21/2008	B. americanus	Copper	13	E	4	4	7				
122	4/21/2008	B. americanus	Copper	13	E	5	5	7	4.4	6.6		
122	4/21/2008	B. americanus	Copper	13	F	1	5	7				
122	4/21/2008	B. americanus	Copper	13	F	2	5	7				
122	4/21/2008	B. americanus	Copper	13	F	3	5	7				
122	4/21/2008	B. americanus	Copper	13	F	4	5	7				
122	4/21/2008	B. americanus	Copper	13	F	5	5	7	5.0	7.0	4.7	6.9
122	4/21/2008	B. americanus	Copper	32	A	1	4	7				
122	4/21/2008	B. americanus	Copper	32	A	2	4	7				
122	4/21/2008	B. americanus	Copper	32	A	3	5	7				
122	4/21/2008	B. americanus	Copper	32	A	4	5	7				
122	4/21/2008	B. americanus	Copper	32	A	5	4	7	4.4	7.0		
122	4/21/2008	B. americanus	Copper	32	B	1	4	6				
122	4/21/2008	B. americanus	Copper	32	B	2	3	5				
122	4/21/2008	B. americanus	Copper	32	B	3	4	6				
122	4/21/2008	B. americanus	Copper	32	B	4	4	6				
122	4/21/2008	B. americanus	Copper	32	B	5	3	5	3.6	5.6		
122	4/21/2008	B. americanus	Copper	32	C	1	4	7				
122	4/21/2008	B. americanus	Copper	32	C	2	4	7				
122	4/21/2008	B. americanus	Copper	32	C	3	5	7				
122	4/21/2008	B. americanus	Copper	32	C	4	4	7				
122	4/21/2008	B. americanus	Copper	32	C	5	4	7	4.2	7.0		
122	4/21/2008	B. americanus	Copper	32	D	1	-	-				
122	4/21/2008	B. americanus	Copper	32	D	2	-	-				
122	4/21/2008	B. americanus	Copper	32	D	3	-	-				
122	4/21/2008	B. americanus	Copper	32	D	4	-	-				
122	4/21/2008	B. americanus	Copper	32	D	5	-	-	0.0	0.0		
122	4/21/2008	B. americanus	Copper	32	E	1	3	6				
122	4/21/2008	B. americanus	Copper	32	E	2	5	7				
122	4/21/2008	B. americanus	Copper	32	E	3	4	7				
122	4/21/2008	B. americanus	Copper	32	E	4	4	6				
122	4/21/2008	B. americanus	Copper	32	E	5	4	7	4.0	6.6		
122	4/21/2008	B. americanus	Copper	32	F	1	4	7				
122	4/21/2008	B. americanus	Copper	32	F	2	5	8				
122	4/21/2008	B. americanus	Copper	32	F	3	4	7				
122	4/21/2008	B. americanus	Copper	32	F	4	5	7				
122	4/21/2008	B. americanus	Copper	32	F	5	4	7	4.4	7.2	3.4	5.6
122	4/21/2008	B. americanus	Copper	85	A	1	5	7				
122	4/21/2008	B. americanus	Copper	85	A	2	4	8				
122	4/21/2008	B. americanus	Copper	85	A	3	4	7				
122	4/21/2008	B. americanus	Copper	85	A	4	5	7				
122	4/21/2008	B. americanus	Copper	85	A	5	5	8	4.6	7.4		
122	4/21/2008	B. americanus	Copper	85	B	1	2	3				
122	4/21/2008	B. americanus	Copper	85	B	2	5	7				
122	4/21/2008	B. americanus	Copper	85	B	3	-	-				
122	4/21/2008	B. americanus	Copper	85	B	4	-	-				
122	4/21/2008	B. americanus	Copper	85	B	5	-	-	3.5	5.0		
122	4/21/2008	B. americanus	Copper	85	C	1	5	8				
122	4/21/2008	B. americanus	Copper	85	C	2	5	7				
122	4/21/2008	B. americanus	Copper	85	C	3	5	8				
122	4/21/2008	B. americanus	Copper	85	C	4	-	-				
122	4/21/2008	B. americanus	Copper	85	C	5	-	-	5.0	7.7		
122	4/21/2008	B. americanus	Copper	85	D	1	5	7				
122	4/21/2008	B. americanus	Copper	85	D	2	5	7				
122	4/21/2008	B. americanus	Copper	85	D	3	5	8				
122	4/21/2008	B. americanus	Copper	85	D	4	-	-				
122	4/21/2008	B. americanus	Copper	85	D	5	-	-	5.0	7.3		
122	4/21/2008	B. americanus	Copper	85	E	1	-	-				
122	4/21/2008	B. americanus	Copper	85	E	2	-	-				
122	4/21/2008	B. americanus	Copper	85	E	3	-	-				
122	4/21/2008	B. americanus	Copper	85	E	4	-	-				
122	4/21/2008	B. americanus	Copper	85	E	5	-	-	0.0	0.0		
122	4/21/2008	B. americanus	Copper	85	F	1	-	-				
122	4/21/2008	B. americanus	Copper	85	F	2	-	-				
122	4/21/2008	B. americanus	Copper	85	F	3	-	-				
122	4/21/2008	B. americanus	Copper	85	F	4	-	-				
122	4/21/2008	B. americanus	Copper	85	F	5	-	-	0.0	0.0	3.0	4.6

SubLethal Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Organism	Body Width (mm)	Body Length (mm)	Day 10 Average Body Width (mm)	Day 10 Average Body Length (mm)	Day 10 Average Treatment Body Width (mm)	Day 10 Average Treatment Body Length (mm)
122	4/21/2008	B. americanus	Copper	140	A	1	-					
122	4/21/2008	B. americanus	Copper	140	A	2	-					
122	4/21/2008	B. americanus	Copper	140	A	3	-					
122	4/21/2008	B. americanus	Copper	140	A	4	-					
122	4/21/2008	B. americanus	Copper	140	A	5	-		0.0	0.0		
122	4/21/2008	B. americanus	Copper	140	B	1	-					
122	4/21/2008	B. americanus	Copper	140	B	2	-					
122	4/21/2008	B. americanus	Copper	140	B	3	-					
122	4/21/2008	B. americanus	Copper	140	B	4	-					
122	4/21/2008	B. americanus	Copper	140	B	5	-		0.0	0.0		
122	4/21/2008	B. americanus	Copper	140	C	1	-					
122	4/21/2008	B. americanus	Copper	140	C	2	-					
122	4/21/2008	B. americanus	Copper	140	C	3	-					
122	4/21/2008	B. americanus	Copper	140	C	4	-					
122	4/21/2008	B. americanus	Copper	140	C	5	-		0.0	0.0		
122	4/21/2008	B. americanus	Copper	140	D	1	-					
122	4/21/2008	B. americanus	Copper	140	D	2	-					
122	4/21/2008	B. americanus	Copper	140	D	3	-					
122	4/21/2008	B. americanus	Copper	140	D	4	-					
122	4/21/2008	B. americanus	Copper	140	D	5	-		0.0	0.0		
122	4/21/2008	B. americanus	Copper	140	E	1	-					
122	4/21/2008	B. americanus	Copper	140	E	2	-					
122	4/21/2008	B. americanus	Copper	140	E	3	-					
122	4/21/2008	B. americanus	Copper	140	E	4	-					
122	4/21/2008	B. americanus	Copper	140	E	5	-		0.0	0.0	0.0	0.0
102	5/10/2007	H. chrysoscelis	Lead	1.9	A	1	3	5				
102	5/10/2007	H. chrysoscelis	Lead	1.9	A	2	3	4				
102	5/10/2007	H. chrysoscelis	Lead	1.9	A	3	3	4				
102	5/10/2007	H. chrysoscelis	Lead	1.9	A	4	3	4				
102	5/10/2007	H. chrysoscelis	Lead	1.9	A	5	3	4	3.0	4.2		
102	5/10/2007	H. chrysoscelis	Lead	1.9	B	1	3	4				
102	5/10/2007	H. chrysoscelis	Lead	1.9	B	2	2	3				
102	5/10/2007	H. chrysoscelis	Lead	1.9	B	3	3	5				
102	5/10/2007	H. chrysoscelis	Lead	1.9	B	4	3	4				
102	5/10/2007	H. chrysoscelis	Lead	1.9	B	5	3	4	2.8	4.0		
102	5/10/2007	H. chrysoscelis	Lead	1.9	C	1	3	4				
102	5/10/2007	H. chrysoscelis	Lead	1.9	C	2	3	4				
102	5/10/2007	H. chrysoscelis	Lead	1.9	C	3	2	3				
102	5/10/2007	H. chrysoscelis	Lead	1.9	C	4	4	5				
102	5/10/2007	H. chrysoscelis	Lead	1.9	C	5	3	4	3.0	4.0		
102	5/10/2007	H. chrysoscelis	Lead	1.9	D	1	3	4				
102	5/10/2007	H. chrysoscelis	Lead	1.9	D	2	3	5				
102	5/10/2007	H. chrysoscelis	Lead	1.9	D	3	3	4				
102	5/10/2007	H. chrysoscelis	Lead	1.9	D	4	3	4				
102	5/10/2007	H. chrysoscelis	Lead	1.9	D	5	4	5	3.2	4.4		
102	5/10/2007	H. chrysoscelis	Lead	1.9	E	1	3	4				
102	5/10/2007	H. chrysoscelis	Lead	1.9	E	2	3	4				
102	5/10/2007	H. chrysoscelis	Lead	1.9	E	3	3	4				
102	5/10/2007	H. chrysoscelis	Lead	1.9	E	4	3	4				
102	5/10/2007	H. chrysoscelis	Lead	1.9	E	5	3	4	3.0	4.0		
102	5/10/2007	H. chrysoscelis	Lead	1.9	F	1	4	4				
102	5/10/2007	H. chrysoscelis	Lead	1.9	F	2	3	3				
102	5/10/2007	H. chrysoscelis	Lead	1.9	F	3	4	4				
102	5/10/2007	H. chrysoscelis	Lead	1.9	F	4	3	4				
102	5/10/2007	H. chrysoscelis	Lead	1.9	F	5	2	3	3.2	3.6	3.0	4.0
102	5/10/2007	H. chrysoscelis	Lead	27	A	1	3	4				
102	5/10/2007	H. chrysoscelis	Lead	27	A	2	3	4				
102	5/10/2007	H. chrysoscelis	Lead	27	A	3	2	3				
102	5/10/2007	H. chrysoscelis	Lead	27	A	4	2	3				
102	5/10/2007	H. chrysoscelis	Lead	27	A	5	2	3	2.4	3.4		
102	5/10/2007	H. chrysoscelis	Lead	27	B	1	3	5				
102	5/10/2007	H. chrysoscelis	Lead	27	B	2	3	4				
102	5/10/2007	H. chrysoscelis	Lead	27	B	3	3	4				
102	5/10/2007	H. chrysoscelis	Lead	27	B	4	3	4				
102	5/10/2007	H. chrysoscelis	Lead	27	B	5	2	4	2.8	4.2		
102	5/10/2007	H. chrysoscelis	Lead	27	C	1	3	4				
102	5/10/2007	H. chrysoscelis	Lead	27	C	2	3	4				
102	5/10/2007	H. chrysoscelis	Lead	27	C	3	3	4				
102	5/10/2007	H. chrysoscelis	Lead	27	C	4	2	3				
102	5/10/2007	H. chrysoscelis	Lead	27	C	5	2	3	2.6	3.6		
102	5/10/2007	H. chrysoscelis	Lead	27	D	1	3	4				
102	5/10/2007	H. chrysoscelis	Lead	27	D	2	2	4				
102	5/10/2007	H. chrysoscelis	Lead	27	D	3	2	4				
102	5/10/2007	H. chrysoscelis	Lead	27	D	4	2	4				
102	5/10/2007	H. chrysoscelis	Lead	27	D	5	2	3	2.2	3.8		
102	5/10/2007	H. chrysoscelis	Lead	27	E	1	3	5				
102	5/10/2007	H. chrysoscelis	Lead	27	E	2	3	5				
102	5/10/2007	H. chrysoscelis	Lead	27	E	3	3	4				
102	5/10/2007	H. chrysoscelis	Lead	27	E	4	3	4				
102	5/10/2007	H. chrysoscelis	Lead	27	E	5	2	3	2.8	4.2		
102	5/10/2007	H. chrysoscelis	Lead	27	F	1	3	5				
102	5/10/2007	H. chrysoscelis	Lead	27	F	2	3	5				
102	5/10/2007	H. chrysoscelis	Lead	27	F	3	3	5				
102	5/10/2007	H. chrysoscelis	Lead	27	F	4	3	5				
102	5/10/2007	H. chrysoscelis	Lead	27	F	5	2	4	2.8	4.8	2.6	4.0

SubLethal Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Organism	Body Width (mm)	Body Length (mm)	Day 10 Average Body Width (mm)	Day 10 Average Body Length (mm)	Day 10 Average Treatment Body Width (mm)	Day 10 Average Treatment Body Length (mm)
102	5/10/2007	H. chrysoscelis	Lead	52	A	1	3	4.5				
102	5/10/2007	H. chrysoscelis	Lead	52	A	2	2	3				
102	5/10/2007	H. chrysoscelis	Lead	52	A	3	2.5	3.5				
102	5/10/2007	H. chrysoscelis	Lead	52	A	4	3	4				
102	5/10/2007	H. chrysoscelis	Lead	52	A	5	2	4	2.5	3.8		
102	5/10/2007	H. chrysoscelis	Lead	52	B	1	2	3.5				
102	5/10/2007	H. chrysoscelis	Lead	52	B	2	2	4				
102	5/10/2007	H. chrysoscelis	Lead	52	B	3	2.5	5				
102	5/10/2007	H. chrysoscelis	Lead	52	B	4	2	4				
102	5/10/2007	H. chrysoscelis	Lead	52	B	5	2.5	4	2.2	4.1		
102	5/10/2007	H. chrysoscelis	Lead	52	C	1	3	6				
102	5/10/2007	H. chrysoscelis	Lead	52	C	2	3	5				
102	5/10/2007	H. chrysoscelis	Lead	52	C	3	3	5				
102	5/10/2007	H. chrysoscelis	Lead	52	C	4	2.5	4				
102	5/10/2007	H. chrysoscelis	Lead	52	C	5	2.5	4	2.8	4.8		
102	5/10/2007	H. chrysoscelis	Lead	52	D	1	2.5	4				
102	5/10/2007	H. chrysoscelis	Lead	52	D	2	3	4				
102	5/10/2007	H. chrysoscelis	Lead	52	D	3	2.5	4				
102	5/10/2007	H. chrysoscelis	Lead	52	D	4	2	3				
102	5/10/2007	H. chrysoscelis	Lead	52	D	5	3	3	2.6	3.6		
102	5/10/2007	H. chrysoscelis	Lead	52	E	1	3	5				
102	5/10/2007	H. chrysoscelis	Lead	52	E	2	2.5	4				
102	5/10/2007	H. chrysoscelis	Lead	52	E	3	2.5	4				
102	5/10/2007	H. chrysoscelis	Lead	52	E	4	3	4				
102	5/10/2007	H. chrysoscelis	Lead	52	E	5	3	4.5	2.8	4.9		
102	5/10/2007	H. chrysoscelis	Lead	52	F	1	3	4				
102	5/10/2007	H. chrysoscelis	Lead	52	F	2	2.5	3.5				
102	5/10/2007	H. chrysoscelis	Lead	52	F	3	2	3				
102	5/10/2007	H. chrysoscelis	Lead	52	F	4	2.5	4				
102	5/10/2007	H. chrysoscelis	Lead	52	F	5	3	4	2.6	3.7	2.6	4.1
102	5/10/2007	H. chrysoscelis	Lead	95	A	1	3	4				
102	5/10/2007	H. chrysoscelis	Lead	95	A	2	3	4				
102	5/10/2007	H. chrysoscelis	Lead	95	A	3	2	4				
102	5/10/2007	H. chrysoscelis	Lead	95	A	4	2	4				
102	5/10/2007	H. chrysoscelis	Lead	95	A	5	2	4	2.4	4.0		
102	5/10/2007	H. chrysoscelis	Lead	95	B	1	3	4				
102	5/10/2007	H. chrysoscelis	Lead	95	B	2	3	4				
102	5/10/2007	H. chrysoscelis	Lead	95	B	3	2	4				
102	5/10/2007	H. chrysoscelis	Lead	95	B	4	2	4				
102	5/10/2007	H. chrysoscelis	Lead	95	B	5	2	4	2.4	4.0		
102	5/10/2007	H. chrysoscelis	Lead	95	C	1	3	5				
102	5/10/2007	H. chrysoscelis	Lead	95	C	2	2.5	4				
102	5/10/2007	H. chrysoscelis	Lead	95	C	3	2	4				
102	5/10/2007	H. chrysoscelis	Lead	95	C	4	2.5	4				
102	5/10/2007	H. chrysoscelis	Lead	95	C	5	3	5	2.6	4.4		
102	5/10/2007	H. chrysoscelis	Lead	95	D	1	3	4.5				
102	5/10/2007	H. chrysoscelis	Lead	95	D	2	2.5	4				
102	5/10/2007	H. chrysoscelis	Lead	95	D	3	2.5	4				
102	5/10/2007	H. chrysoscelis	Lead	95	D	4	2.5	4				
102	5/10/2007	H. chrysoscelis	Lead	95	D	5	2.5	4	2.6	4.1		
102	5/10/2007	H. chrysoscelis	Lead	95	E	1	3	4.5				
102	5/10/2007	H. chrysoscelis	Lead	95	E	2	3	4.5				
102	5/10/2007	H. chrysoscelis	Lead	95	E	3	2.5	4				
102	5/10/2007	H. chrysoscelis	Lead	95	E	4	-	-				
102	5/10/2007	H. chrysoscelis	Lead	95	E	5	-	-	1.7	2.6		
102	5/10/2007	H. chrysoscelis	Lead	95	F	1	2	3.5				
102	5/10/2007	H. chrysoscelis	Lead	95	F	2	2.5	4				
102	5/10/2007	H. chrysoscelis	Lead	95	F	3	2.5	4				
102	5/10/2007	H. chrysoscelis	Lead	95	F	4	2.5	4				
102	5/10/2007	H. chrysoscelis	Lead	95	F	5	3	4.5	2.5	4.0	2.4	3.9
102	5/10/2007	H. chrysoscelis	Lead	250	A	1	3	4.5				
102	5/10/2007	H. chrysoscelis	Lead	250	A	2	3	4				
102	5/10/2007	H. chrysoscelis	Lead	250	A	3	3	3				
102	5/10/2007	H. chrysoscelis	Lead	250	A	4	3	4				
102	5/10/2007	H. chrysoscelis	Lead	250	A	5	3	4	3.0	3.9		
102	5/10/2007	H. chrysoscelis	Lead	250	B	1	3	4				
102	5/10/2007	H. chrysoscelis	Lead	250	B	2	3.5	4				
102	5/10/2007	H. chrysoscelis	Lead	250	B	3	3.5	4.5				
102	5/10/2007	H. chrysoscelis	Lead	250	B	4	3	4				
102	5/10/2007	H. chrysoscelis	Lead	250	B	5	2.5	3	3.1	3.9		
102	5/10/2007	H. chrysoscelis	Lead	250	C	1	3	4				
102	5/10/2007	H. chrysoscelis	Lead	250	C	2	3	3.5				
102	5/10/2007	H. chrysoscelis	Lead	250	C	3	2.5	4				
102	5/10/2007	H. chrysoscelis	Lead	250	C	4	3	4.5				
102	5/10/2007	H. chrysoscelis	Lead	250	C	5	3	4	2.9	4.0		
102	5/10/2007	H. chrysoscelis	Lead	250	D	1	3	4.5				
102	5/10/2007	H. chrysoscelis	Lead	250	D	2	3	4				
102	5/10/2007	H. chrysoscelis	Lead	250	D	3	3	3.5				
102	5/10/2007	H. chrysoscelis	Lead	250	D	4	3	3.5				
102	5/10/2007	H. chrysoscelis	Lead	250	D	5	3	3	3.0	3.7		
102	5/10/2007	H. chrysoscelis	Lead	250	E	1	2.5	3				
102	5/10/2007	H. chrysoscelis	Lead	250	E	2	3	3.5				
102	5/10/2007	H. chrysoscelis	Lead	250	E	3	3.5	4				
102	5/10/2007	H. chrysoscelis	Lead	250	E	4	3	4				
102	5/10/2007	H. chrysoscelis	Lead	250	E	5	3	4	3.0	3.7		
102	5/10/2007	H. chrysoscelis	Lead	250	F	1	3	4				
102	5/10/2007	H. chrysoscelis	Lead	250	F	2	3	3.5				
102	5/10/2007	H. chrysoscelis	Lead	250	F	3	2.5	4				
102	5/10/2007	H. chrysoscelis	Lead	250	F	4	2.5	3.5				
102	5/10/2007	H. chrysoscelis	Lead	250	F	5	4	4.5	3.0	3.9	3.0	3.9

SubLethal Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Organism	Body Width (mm)	Body Length (mm)	Day 10 Average Body Width (mm)	Day 10 Average Body Length (mm)	Day 10 Average Treatment Body Width (mm)	Day 10 Average Treatment Body Length (mm)
102	5/10/2007	H. chrysoscelis	Lead	480	A	1	1.5	3				
102	5/10/2007	H. chrysoscelis	Lead	480	A	2	2	3				
102	5/10/2007	H. chrysoscelis	Lead	480	A	3	-	-				
102	5/10/2007	H. chrysoscelis	Lead	480	A	4	-	-				
102	5/10/2007	H. chrysoscelis	Lead	480	A	5	-	-	0.7	1.2		
102	5/10/2007	H. chrysoscelis	Lead	480	B	1	2.5	3.5				
102	5/10/2007	H. chrysoscelis	Lead	480	B	2	2	3				
102	5/10/2007	H. chrysoscelis	Lead	480	B	3	2	3				
102	5/10/2007	H. chrysoscelis	Lead	480	B	4	2.5	5				
102	5/10/2007	H. chrysoscelis	Lead	480	B	5	-	-	1.8	2.9		
102	5/10/2007	H. chrysoscelis	Lead	480	C	1	2	3				
102	5/10/2007	H. chrysoscelis	Lead	480	C	2	2.5	3.5				
102	5/10/2007	H. chrysoscelis	Lead	480	C	3	2	3				
102	5/10/2007	H. chrysoscelis	Lead	480	C	4	-	-				
102	5/10/2007	H. chrysoscelis	Lead	480	C	5	-	-	1.3	1.9		
102	5/10/2007	H. chrysoscelis	Lead	480	D	1	-	-				
102	5/10/2007	H. chrysoscelis	Lead	480	D	2	-	-				
102	5/10/2007	H. chrysoscelis	Lead	480	D	3	-	-				
102	5/10/2007	H. chrysoscelis	Lead	480	D	4	-	-				
102	5/10/2007	H. chrysoscelis	Lead	480	D	5	-	-	0.0	0.0		
102	5/10/2007	H. chrysoscelis	Lead	480	E	1	2	3				
102	5/10/2007	H. chrysoscelis	Lead	480	E	2	2.5	3				
102	5/10/2007	H. chrysoscelis	Lead	480	E	3	-	-				
102	5/10/2007	H. chrysoscelis	Lead	480	E	4	-	-				
102	5/10/2007	H. chrysoscelis	Lead	480	E	5	-	-	0.9	1.2		
102	5/10/2007	H. chrysoscelis	Lead	480	F	1	2.5	4				
102	5/10/2007	H. chrysoscelis	Lead	480	F	2	2.5	3.5				
102	5/10/2007	H. chrysoscelis	Lead	480	F	3	2	3.5				
102	5/10/2007	H. chrysoscelis	Lead	480	F	4	2	3.5				
102	5/10/2007	H. chrysoscelis	Lead	480	F	5	-	-	1.8	2.9	1.1	1.7
095	4/15/2007	R. sylvatica	Lead	1.9	A	1	5	7.5				
095	4/15/2007	R. sylvatica	Lead	1.9	A	2	4	7				
095	4/15/2007	R. sylvatica	Lead	1.9	A	3	5	7.5				
095	4/15/2007	R. sylvatica	Lead	1.9	A	4	4.5	7				
095	4/15/2007	R. sylvatica	Lead	1.9	A	5	5	7	4.7	7.2		
095	4/15/2007	R. sylvatica	Lead	1.9	B	1	4	6				
095	4/15/2007	R. sylvatica	Lead	1.9	B	2	4.5	7.5				
095	4/15/2007	R. sylvatica	Lead	1.9	B	3	4.5	7				
095	4/15/2007	R. sylvatica	Lead	1.9	B	4	4.5	6.5				
095	4/15/2007	R. sylvatica	Lead	1.9	B	5	5	7	4.5	6.6		
095	4/15/2007	R. sylvatica	Lead	1.9	C	1	5	8				
095	4/15/2007	R. sylvatica	Lead	1.9	C	2	5	8				
095	4/15/2007	R. sylvatica	Lead	1.9	C	3	5	7.5				
095	4/15/2007	R. sylvatica	Lead	1.9	C	4	4.5	8				
095	4/15/2007	R. sylvatica	Lead	1.9	C	5	4.5	7.5	4.8	7.8		
095	4/15/2007	R. sylvatica	Lead	1.9	D	1	5	7.5				
095	4/15/2007	R. sylvatica	Lead	1.9	D	2	5	8				
095	4/15/2007	R. sylvatica	Lead	1.9	D	3	5	7.5				
095	4/15/2007	R. sylvatica	Lead	1.9	D	4	5	8				
095	4/15/2007	R. sylvatica	Lead	1.9	D	5	5	8	5.0	7.8		
095	4/15/2007	R. sylvatica	Lead	1.9	E	1	5	8				
095	4/15/2007	R. sylvatica	Lead	1.9	E	2	4.5	7.5				
095	4/15/2007	R. sylvatica	Lead	1.9	E	3	5	8.5				
095	4/15/2007	R. sylvatica	Lead	1.9	E	4	5	8				
095	4/15/2007	R. sylvatica	Lead	1.9	E	5	5	8	4.9	8.0		
095	4/15/2007	R. sylvatica	Lead	1.9	F	1	5	7.5				
095	4/15/2007	R. sylvatica	Lead	1.9	F	2	5	7.5				
095	4/15/2007	R. sylvatica	Lead	1.9	F	3	4.5	7				
095	4/15/2007	R. sylvatica	Lead	1.9	F	4	5	8				
095	4/15/2007	R. sylvatica	Lead	1.9	F	5	-	-	3.9	6.0	4.6	7.3
095	4/15/2007	R. sylvatica	Lead	67	A	1	4	7				
095	4/15/2007	R. sylvatica	Lead	67	A	2	4.5	8				
095	4/15/2007	R. sylvatica	Lead	67	A	3	4	7				
095	4/15/2007	R. sylvatica	Lead	67	A	4	4.5	7				
095	4/15/2007	R. sylvatica	Lead	67	A	5	4	7	4.2	7.2		
095	4/15/2007	R. sylvatica	Lead	67	B	1	6	8.5				
095	4/15/2007	R. sylvatica	Lead	67	B	2	6	8.5				
095	4/15/2007	R. sylvatica	Lead	67	B	3	6	8.5				
095	4/15/2007	R. sylvatica	Lead	67	B	4	5.5	8				
095	4/15/2007	R. sylvatica	Lead	67	B	5	6	8.5	5.9	8.4		
095	4/15/2007	R. sylvatica	Lead	67	C	1	4.5	7				
095	4/15/2007	R. sylvatica	Lead	67	C	2	4	6.5				
095	4/15/2007	R. sylvatica	Lead	67	C	3	4	6				
095	4/15/2007	R. sylvatica	Lead	67	C	4	4.5	7				
095	4/15/2007	R. sylvatica	Lead	67	C	5	-	-	3.4	5.3		
095	4/15/2007	R. sylvatica	Lead	67	D	1	5	8				
095	4/15/2007	R. sylvatica	Lead	67	D	2	5	8.5				
095	4/15/2007	R. sylvatica	Lead	67	D	3	6	9				
095	4/15/2007	R. sylvatica	Lead	67	D	4	4.5	7				
095	4/15/2007	R. sylvatica	Lead	67	D	5	5	8.5	5.1	8.2		
095	4/15/2007	R. sylvatica	Lead	67	E	1	5	8				
095	4/15/2007	R. sylvatica	Lead	67	E	2	4.5	8				
095	4/15/2007	R. sylvatica	Lead	67	E	3	5	8				
095	4/15/2007	R. sylvatica	Lead	67	E	4	5	8				
095	4/15/2007	R. sylvatica	Lead	67	E	5	5	8	4.9	8.0		
095	4/15/2007	R. sylvatica	Lead	67	F	1	5	8				
095	4/15/2007	R. sylvatica	Lead	67	F	2	5	8				
095	4/15/2007	R. sylvatica	Lead	67	F	3	5	8				
095	4/15/2007	R. sylvatica	Lead	67	F	4	5	8				
095	4/15/2007	R. sylvatica	Lead	67	F	5	5	7.5	5.0	7.9	4.8	7.5

SubLethal Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Organism	Body Width (mm)	Body Length (mm)	Day 10 Average Body Width (mm)	Day 10 Average Body Length (mm)	Day 10 Average Treatment Body Width (mm)	Day 10 Average Treatment Body Length (mm)
095	4/15/2007	R. sylvatica	Lead	130	A	1	5	8				
095	4/15/2007	R. sylvatica	Lead	130	A	2	4.5	8				
095	4/15/2007	R. sylvatica	Lead	130	A	3	5	8.5				
095	4/15/2007	R. sylvatica	Lead	130	A	4	5	8.5				
095	4/15/2007	R. sylvatica	Lead	130	A	5	5	8.5	4.9	8.3		
095	4/15/2007	R. sylvatica	Lead	130	B	1	5	8				
095	4/15/2007	R. sylvatica	Lead	130	B	2	5	8				
095	4/15/2007	R. sylvatica	Lead	130	B	3	5	8				
095	4/15/2007	R. sylvatica	Lead	130	B	4	5.5	8.5				
095	4/15/2007	R. sylvatica	Lead	130	B	5	5	8	5.1	8.1		
095	4/15/2007	R. sylvatica	Lead	130	C	1	4	6				
095	4/15/2007	R. sylvatica	Lead	130	C	2	4	6				
095	4/15/2007	R. sylvatica	Lead	130	C	3	4	6				
095	4/15/2007	R. sylvatica	Lead	130	C	4	4.5	7				
095	4/15/2007	R. sylvatica	Lead	130	C	5	4	6	4.1	6.2		
095	4/15/2007	R. sylvatica	Lead	130	D	1	5	8.5				
095	4/15/2007	R. sylvatica	Lead	130	D	2	5	8				
095	4/15/2007	R. sylvatica	Lead	130	D	3	5	8.5				
095	4/15/2007	R. sylvatica	Lead	130	D	4	5.5	8.5				
095	4/15/2007	R. sylvatica	Lead	130	D	5	5	8.5	5.1	8.4		
095	4/15/2007	R. sylvatica	Lead	130	E	1	5	8.5				
095	4/15/2007	R. sylvatica	Lead	130	E	2	5	8				
095	4/15/2007	R. sylvatica	Lead	130	E	3	5	8				
095	4/15/2007	R. sylvatica	Lead	130	E	4	5.5	9				
095	4/15/2007	R. sylvatica	Lead	130	E	5	5	8	5.1	8.3		
095	4/15/2007	R. sylvatica	Lead	130	F	1	5.5	8				
095	4/15/2007	R. sylvatica	Lead	130	F	2	5	8				
095	4/15/2007	R. sylvatica	Lead	130	F	3	5	8				
095	4/15/2007	R. sylvatica	Lead	130	F	4	-	-				
095	4/15/2007	R. sylvatica	Lead	130	F	5	-	-	3.1	4.8	4.6	7.4
095	4/15/2007	R. sylvatica	Lead	340	A	1	5	8				
095	4/15/2007	R. sylvatica	Lead	340	A	2	5	8				
095	4/15/2007	R. sylvatica	Lead	340	A	3	5	8				
095	4/15/2007	R. sylvatica	Lead	340	A	4	5	8				
095	4/15/2007	R. sylvatica	Lead	340	A	5	-	-	4.0	6.4		
095	4/15/2007	R. sylvatica	Lead	340	B	1	4	7				
095	4/15/2007	R. sylvatica	Lead	340	B	2	4	7				
095	4/15/2007	R. sylvatica	Lead	340	B	3	-	-				
095	4/15/2007	R. sylvatica	Lead	340	B	4	-	-				
095	4/15/2007	R. sylvatica	Lead	340	B	5	-	-	1.6	2.6		
095	4/15/2007	R. sylvatica	Lead	340	C	1	4	6				
095	4/15/2007	R. sylvatica	Lead	340	C	2	5	7				
095	4/15/2007	R. sylvatica	Lead	340	C	3	5	7				
095	4/15/2007	R. sylvatica	Lead	340	C	4	5	7				
095	4/15/2007	R. sylvatica	Lead	340	C	5	5	7	4.8	6.8		
095	4/15/2007	R. sylvatica	Lead	340	D	1	4	6				
095	4/15/2007	R. sylvatica	Lead	340	D	2	-	-				
095	4/15/2007	R. sylvatica	Lead	340	D	3	-	-				
095	4/15/2007	R. sylvatica	Lead	340	D	4	-	-				
095	4/15/2007	R. sylvatica	Lead	340	D	5	-	-	0.8	1.2		
095	4/15/2007	R. sylvatica	Lead	340	E	1	-	-				
095	4/15/2007	R. sylvatica	Lead	340	E	2	-	-				
095	4/15/2007	R. sylvatica	Lead	340	E	3	-	-				
095	4/15/2007	R. sylvatica	Lead	340	E	4	-	-				
095	4/15/2007	R. sylvatica	Lead	340	E	5	-	-	0.0	0.0		
095	4/15/2007	R. sylvatica	Lead	340	F	1	-	-				
095	4/15/2007	R. sylvatica	Lead	340	F	2	-	-				
095	4/15/2007	R. sylvatica	Lead	340	F	3	-	-				
095	4/15/2007	R. sylvatica	Lead	340	F	4	-	-				
095	4/15/2007	R. sylvatica	Lead	340	F	5	-	-	0.0	0.0	1.9	2.9
094	4/15/2007	R. sylvatica	Copper	3.2	A	1	5	8				
094	4/15/2007	R. sylvatica	Copper	3.2	A	2	5	8				
094	4/15/2007	R. sylvatica	Copper	3.2	A	3	5	7				
094	4/15/2007	R. sylvatica	Copper	3.2	A	4	5	8				
094	4/15/2007	R. sylvatica	Copper	3.2	A	5	5	8	5.0	7.8		
094	4/15/2007	R. sylvatica	Copper	3.2	B	1	5	8				
094	4/15/2007	R. sylvatica	Copper	3.2	B	2	6	8				
094	4/15/2007	R. sylvatica	Copper	3.2	B	3	5	8				
094	4/15/2007	R. sylvatica	Copper	3.2	B	4	4	8				
094	4/15/2007	R. sylvatica	Copper	3.2	B	5	5	8	5.0	8.0		
094	4/15/2007	R. sylvatica	Copper	3.2	C	1	5	8				
094	4/15/2007	R. sylvatica	Copper	3.2	C	2	5	8				
094	4/15/2007	R. sylvatica	Copper	3.2	C	3	5	8				
094	4/15/2007	R. sylvatica	Copper	3.2	C	4	5	8				
094	4/15/2007	R. sylvatica	Copper	3.2	C	5	5	8	5.0	8.0		
094	4/15/2007	R. sylvatica	Copper	3.2	D	1	5	8				
094	4/15/2007	R. sylvatica	Copper	3.2	D	2	5	8				
094	4/15/2007	R. sylvatica	Copper	3.2	D	3	5	8				
094	4/15/2007	R. sylvatica	Copper	3.2	D	4	5	8				
094	4/15/2007	R. sylvatica	Copper	3.2	D	5	5	8	5.0	8.0		
094	4/15/2007	R. sylvatica	Copper	3.2	E	1	5	8				
094	4/15/2007	R. sylvatica	Copper	3.2	E	2	5	8				
094	4/15/2007	R. sylvatica	Copper	3.2	E	3	5	8				
094	4/15/2007	R. sylvatica	Copper	3.2	E	4	5	8				
094	4/15/2007	R. sylvatica	Copper	3.2	E	5	5	8	5.0	8.0		
094	4/15/2007	R. sylvatica	Copper	3.2	F	1	5	8				
094	4/15/2007	R. sylvatica	Copper	3.2	F	2	5	8				
094	4/15/2007	R. sylvatica	Copper	3.2	F	3	5	8				
094	4/15/2007	R. sylvatica	Copper	3.2	F	4	5	8				
094	4/15/2007	R. sylvatica	Copper	3.2	F	5	5	8	5.0	8.0	5.0	8.0

SubLethal Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Organism	Body Width (mm)	Body Length (mm)	Day 10 Average Body Width (mm)	Day 10 Average Body Length (mm)	Day 10 Average Treatment Body Width (mm)	Day 10 Average Treatment Body Length (mm)
094	4/15/2007	R. sylvatica	Copper	13	A	1	5	8				
094	4/15/2007	R. sylvatica	Copper	13	A	2	5	8				
094	4/15/2007	R. sylvatica	Copper	13	A	3	5	8				
094	4/15/2007	R. sylvatica	Copper	13	A	4	5	9				
094	4/15/2007	R. sylvatica	Copper	13	A	5	5	8	5.0	8.2		
094	4/15/2007	R. sylvatica	Copper	13	B	1	5	8				
094	4/15/2007	R. sylvatica	Copper	13	B	2	5	8				
094	4/15/2007	R. sylvatica	Copper	13	B	3	5	8				
094	4/15/2007	R. sylvatica	Copper	13	B	4	5	8				
094	4/15/2007	R. sylvatica	Copper	13	B	5	5	8	5.0	8.0		
094	4/15/2007	R. sylvatica	Copper	13	C	1	4	7				
094	4/15/2007	R. sylvatica	Copper	13	C	2	4	8				
094	4/15/2007	R. sylvatica	Copper	13	C	3	5	7				
094	4/15/2007	R. sylvatica	Copper	13	C	4	5	8				
094	4/15/2007	R. sylvatica	Copper	13	C	5	-	-	3.6	6.0		
094	4/15/2007	R. sylvatica	Copper	13	D	1	5	8				
094	4/15/2007	R. sylvatica	Copper	13	D	2	5	8				
094	4/15/2007	R. sylvatica	Copper	13	D	3	5	8				
094	4/15/2007	R. sylvatica	Copper	13	D	4	5	8				
094	4/15/2007	R. sylvatica	Copper	13	D	5	5	8	5.0	8.0		
094	4/15/2007	R. sylvatica	Copper	13	E	1	5	8				
094	4/15/2007	R. sylvatica	Copper	13	E	2	5	8				
094	4/15/2007	R. sylvatica	Copper	13	E	3	5	8				
094	4/15/2007	R. sylvatica	Copper	13	E	4	5	9				
094	4/15/2007	R. sylvatica	Copper	13	E	5	5	8	5.0	8.2		
094	4/15/2007	R. sylvatica	Copper	13	F	1	4	7				
094	4/15/2007	R. sylvatica	Copper	13	F	2	5	7				
094	4/15/2007	R. sylvatica	Copper	13	F	3	5	8				
094	4/15/2007	R. sylvatica	Copper	13	F	4	5	7				
094	4/15/2007	R. sylvatica	Copper	13	F	5	5	8	4.8	7.4	4.7	7.6
094	4/15/2007	R. sylvatica	Copper	32	A	1	4	7				
094	4/15/2007	R. sylvatica	Copper	32	A	2	5	8				
094	4/15/2007	R. sylvatica	Copper	32	A	3	4	7				
094	4/15/2007	R. sylvatica	Copper	32	A	4	4	7				
094	4/15/2007	R. sylvatica	Copper	32	A	5	4	7	4.2	7.2		
094	4/15/2007	R. sylvatica	Copper	32	B	1	5	8				
094	4/15/2007	R. sylvatica	Copper	32	B	2	5	7				
094	4/15/2007	R. sylvatica	Copper	32	B	3	4	7				
094	4/15/2007	R. sylvatica	Copper	32	B	4	4	7				
094	4/15/2007	R. sylvatica	Copper	32	B	5	4.5	7	4.5	7.2		
094	4/15/2007	R. sylvatica	Copper	32	C	1	5	8				
094	4/15/2007	R. sylvatica	Copper	32	C	2	5	8				
094	4/15/2007	R. sylvatica	Copper	32	C	3	5	8				
094	4/15/2007	R. sylvatica	Copper	32	C	4	5	8				
094	4/15/2007	R. sylvatica	Copper	32	C	5	5	8	5.0	8.0		
094	4/15/2007	R. sylvatica	Copper	32	D	1	5	8				
094	4/15/2007	R. sylvatica	Copper	32	D	2	5	7				
094	4/15/2007	R. sylvatica	Copper	32	D	3	5	7				
094	4/15/2007	R. sylvatica	Copper	32	D	4	5	8				
094	4/15/2007	R. sylvatica	Copper	32	D	5	5	8	5.0	7.6		
094	4/15/2007	R. sylvatica	Copper	32	E	1	5	8				
094	4/15/2007	R. sylvatica	Copper	32	E	2	5	8				
094	4/15/2007	R. sylvatica	Copper	32	E	3	5	8				
094	4/15/2007	R. sylvatica	Copper	32	E	4	5	8				
094	4/15/2007	R. sylvatica	Copper	32	E	5	5	8	5.0	8.0		
094	4/15/2007	R. sylvatica	Copper	32	F	1	4	6				
094	4/15/2007	R. sylvatica	Copper	32	F	2	4	7				
094	4/15/2007	R. sylvatica	Copper	32	F	3	4	6				
094	4/15/2007	R. sylvatica	Copper	32	F	4	4	7				
094	4/15/2007	R. sylvatica	Copper	32	F	5	4.5	7	4.1	6.6	4.6	7.4
094	4/15/2007	R. sylvatica	Copper	72.7	A	1	5	7				
094	4/15/2007	R. sylvatica	Copper	72.7	A	2	4	6				
094	4/15/2007	R. sylvatica	Copper	72.7	A	3	4	6				
094	4/15/2007	R. sylvatica	Copper	72.7	A	4	5	7				
094	4/15/2007	R. sylvatica	Copper	72.7	A	5	5	6	4.6	6.4		
094	4/15/2007	R. sylvatica	Copper	72.7	B	1	4	7				
094	4/15/2007	R. sylvatica	Copper	72.7	B	2	4	6				
094	4/15/2007	R. sylvatica	Copper	72.7	B	3	4	6				
094	4/15/2007	R. sylvatica	Copper	72.7	B	4	4	7				
094	4/15/2007	R. sylvatica	Copper	72.7	B	5	4	6	4.0	6.4		
094	4/15/2007	R. sylvatica	Copper	72.7	C	1	4	6				
094	4/15/2007	R. sylvatica	Copper	72.7	C	2	4	5				
094	4/15/2007	R. sylvatica	Copper	72.7	C	3	4	6				
094	4/15/2007	R. sylvatica	Copper	72.7	C	4	4	6				
094	4/15/2007	R. sylvatica	Copper	72.7	C	5	4	6	4.0	5.8		
094	4/15/2007	R. sylvatica	Copper	72.7	D	1	4	6				
094	4/15/2007	R. sylvatica	Copper	72.7	D	2	4	7				
094	4/15/2007	R. sylvatica	Copper	72.7	D	3	4	6				
094	4/15/2007	R. sylvatica	Copper	72.7	D	4	4	6				
094	4/15/2007	R. sylvatica	Copper	72.7	D	5	4	6	4.0	6.2		
094	4/15/2007	R. sylvatica	Copper	72.7	E	1	4	6				
094	4/15/2007	R. sylvatica	Copper	72.7	E	2	4	6				
094	4/15/2007	R. sylvatica	Copper	72.7	E	3	5	7				
094	4/15/2007	R. sylvatica	Copper	72.7	E	4	4	6				
094	4/15/2007	R. sylvatica	Copper	72.7	E	5	5	7	4.4	6.4		
094	4/15/2007	R. sylvatica	Copper	72.7	F	1	4	6				
094	4/15/2007	R. sylvatica	Copper	72.7	F	2	4	7				
094	4/15/2007	R. sylvatica	Copper	72.7	F	3	4	6				
094	4/15/2007	R. sylvatica	Copper	72.7	F	4	4	6				
094	4/15/2007	R. sylvatica	Copper	72.7	F	5	5	7	4.2	6.4	4.2	6.3
100	5/2/2007	A. maculatum	Copper	3.2	A	1	NA	16				
100	5/2/2007	A. maculatum	Copper	3.2	A	2	NA	15				
100	5/2/2007	A. maculatum	Copper	3.2	A	3	NA	16				
100	5/2/2007	A. maculatum	Copper	3.2	A	4	NA	16				
100	5/2/2007	A. maculatum	Copper	3.2	A	5	NA	15	0.0	15.6		
100	5/2/2007	A. maculatum	Copper	3.2	B	1	NA	15				
100	5/2/2007	A. maculatum	Copper	3.2	B	2	NA	14				
100	5/2/2007	A. maculatum	Copper	3.2	B	3	NA	15				
100	5/2/2007	A. maculatum	Copper	3.2	B	4	NA	15				
100	5/2/2007	A. maculatum	Copper	3.2	B	5	NA	15	0.0	14.8	NA	15.2
100	5/2/2007	A. maculatum	Copper	30.7	A	1	NA	15				
100	5/2/2007	A. maculatum	Copper	30.7	A	2	NA	15				
100	5/2/2007	A. maculatum	Copper	30.7	A	3	NA	13				
100	5/2/2007	A. maculatum	Copper	30.7	A	4	NA	14				
100	5/2/2007	A. maculatum	Copper	30.7	A	5	NA	14	0.0	14.2		
100	5/2/2007	A. maculatum	Copper	30.7	B	1	NA	14				
100	5/2/2007	A. maculatum	Copper	30.7	B	2	NA	12				
100	5/2/2007	A. maculatum	Copper	30.7	B	3	NA	14				
100	5/2/2007	A. maculatum	Copper	30.7	B	4	NA	13				
100	5/2/2007	A. maculatum	Copper	30.7	B	5	NA	13	0.0	13.2	NA	13.7

SubLethal Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Organism	Body Width (mm)	Body Length (mm)	Day 10 Average Body Width (mm)	Day 10 Average Body Length (mm)	Day 10 Average Treatment Body Width (mm)	Day 10 Average Treatment Body Length (mm)
100	5/2/2007	A. maculatum	Copper	75	A	1	NA	15				
100	5/2/2007	A. maculatum	Copper	75	A	2	NA	15				
100	5/2/2007	A. maculatum	Copper	75	A	3	NA	15				
100	5/2/2007	A. maculatum	Copper	75	A	4	NA	14				
100	5/2/2007	A. maculatum	Copper	75	A	5	NA	14	0.0	14.6		
100	5/2/2007	A. maculatum	Copper	75	B	1	NA	11				
100	5/2/2007	A. maculatum	Copper	75	B	2	NA	13				
100	5/2/2007	A. maculatum	Copper	75	B	3	NA	14				
100	5/2/2007	A. maculatum	Copper	75	B	4	NA	12				
100	5/2/2007	A. maculatum	Copper	75	B	5	NA	-	0.0	10.0	NA	12.3
101	5/10/2007	H. chrysoscelis	Copper	3.2	A	1	3	4.5				
101	5/10/2007	H. chrysoscelis	Copper	3.2	A	2	3	4.5				
101	5/10/2007	H. chrysoscelis	Copper	3.2	A	3	4	6				
101	5/10/2007	H. chrysoscelis	Copper	3.2	A	4	3	4				
101	5/10/2007	H. chrysoscelis	Copper	3.2	A	5	3	4	3.2	4.6		
101	5/10/2007	H. chrysoscelis	Copper	3.2	B	1	3	4				
101	5/10/2007	H. chrysoscelis	Copper	3.2	B	2	3	4.5				
101	5/10/2007	H. chrysoscelis	Copper	3.2	B	3	3	4				
101	5/10/2007	H. chrysoscelis	Copper	3.2	B	4	-	-				
101	5/10/2007	H. chrysoscelis	Copper	3.2	B	5	-	-	1.8	2.5		
101	5/10/2007	H. chrysoscelis	Copper	3.2	C	1	3.5	5				
101	5/10/2007	H. chrysoscelis	Copper	3.2	C	2	3.5	5				
101	5/10/2007	H. chrysoscelis	Copper	3.2	C	3	4	6				
101	5/10/2007	H. chrysoscelis	Copper	3.2	C	4	3.5	5				
101	5/10/2007	H. chrysoscelis	Copper	3.2	C	5	3	4	3.5	5.0		
101	5/10/2007	H. chrysoscelis	Copper	3.2	D	1	3.5	5				
101	5/10/2007	H. chrysoscelis	Copper	3.2	D	2	3.5	4.5				
101	5/10/2007	H. chrysoscelis	Copper	3.2	D	3	4	5				
101	5/10/2007	H. chrysoscelis	Copper	3.2	D	4	3	4				
101	5/10/2007	H. chrysoscelis	Copper	3.2	D	5	4.5	6	3.7	4.9		
101	5/10/2007	H. chrysoscelis	Copper	3.2	E	1	3.5	5				
101	5/10/2007	H. chrysoscelis	Copper	3.2	E	2	3.5	5				
101	5/10/2007	H. chrysoscelis	Copper	3.2	E	3	4	5.5				
101	5/10/2007	H. chrysoscelis	Copper	3.2	E	4	4	5.5				
101	5/10/2007	H. chrysoscelis	Copper	3.2	E	5	3	4.5	3.6	5.1		
101	5/10/2007	H. chrysoscelis	Copper	3.2	F	1	3.5	5				
101	5/10/2007	H. chrysoscelis	Copper	3.2	F	2	4	5				
101	5/10/2007	H. chrysoscelis	Copper	3.2	F	3	3.5	5.5				
101	5/10/2007	H. chrysoscelis	Copper	3.2	F	4	3.5	5				
101	5/10/2007	H. chrysoscelis	Copper	3.2	F	5	3.5	5	3.6	5.1	3.2	4.5
101	5/10/2007	H. chrysoscelis	Copper	13	A	1	3	4.5				
101	5/10/2007	H. chrysoscelis	Copper	13	A	2	3.5	5				
101	5/10/2007	H. chrysoscelis	Copper	13	A	3	3.5	5				
101	5/10/2007	H. chrysoscelis	Copper	13	A	4	3.5	5				
101	5/10/2007	H. chrysoscelis	Copper	13	A	5	3.5	5.5	3.4	5.0		
101	5/10/2007	H. chrysoscelis	Copper	13	B	1	4	5				
101	5/10/2007	H. chrysoscelis	Copper	13	B	2	4	6				
101	5/10/2007	H. chrysoscelis	Copper	13	B	3	3.5	5				
101	5/10/2007	H. chrysoscelis	Copper	13	B	4	4	6				
101	5/10/2007	H. chrysoscelis	Copper	13	B	5	3.5	5	3.8	5.4		
101	5/10/2007	H. chrysoscelis	Copper	13	C	1	3	4				
101	5/10/2007	H. chrysoscelis	Copper	13	C	2	3.5	5				
101	5/10/2007	H. chrysoscelis	Copper	13	C	3	3.5	4.5				
101	5/10/2007	H. chrysoscelis	Copper	13	C	4	3.5	5				
101	5/10/2007	H. chrysoscelis	Copper	13	C	5	3	4.5	3.3	4.6		
101	5/10/2007	H. chrysoscelis	Copper	13	D	1	5	6				
101	5/10/2007	H. chrysoscelis	Copper	13	D	2	4	5.5				
101	5/10/2007	H. chrysoscelis	Copper	13	D	3	4	6				
101	5/10/2007	H. chrysoscelis	Copper	13	D	4	4	5.5				
101	5/10/2007	H. chrysoscelis	Copper	13	D	5	4	7	4.2	6.0		
101	5/10/2007	H. chrysoscelis	Copper	13	E	1	4	6				
101	5/10/2007	H. chrysoscelis	Copper	13	E	2	5	6				
101	5/10/2007	H. chrysoscelis	Copper	13	E	3	4.5	6				
101	5/10/2007	H. chrysoscelis	Copper	13	E	4	4.5	5				
101	5/10/2007	H. chrysoscelis	Copper	13	E	5	5	6	4.6	5.8		
101	5/10/2007	H. chrysoscelis	Copper	13	F	1	5	6				
101	5/10/2007	H. chrysoscelis	Copper	13	F	2	4.5	6				
101	5/10/2007	H. chrysoscelis	Copper	13	F	3	4	5.5				
101	5/10/2007	H. chrysoscelis	Copper	13	F	4	3.5	5				
101	5/10/2007	H. chrysoscelis	Copper	13	F	5	4	6	4.2	5.7	3.9	5.4
101	5/10/2007	H. chrysoscelis	Copper	30.7	A	1	4	5				
101	5/10/2007	H. chrysoscelis	Copper	30.7	A	2	4	6				
101	5/10/2007	H. chrysoscelis	Copper	30.7	A	3	4	6.5				
101	5/10/2007	H. chrysoscelis	Copper	30.7	A	4	4	5.5				
101	5/10/2007	H. chrysoscelis	Copper	30.7	A	5	-	-	3.2	4.6		
101	5/10/2007	H. chrysoscelis	Copper	30.7	B	1	3.5	5				
101	5/10/2007	H. chrysoscelis	Copper	30.7	B	2	3.5	6				
101	5/10/2007	H. chrysoscelis	Copper	30.7	B	3	3	4.5				
101	5/10/2007	H. chrysoscelis	Copper	30.7	B	4	4	5				
101	5/10/2007	H. chrysoscelis	Copper	30.7	B	5	4.5	6	3.7	5.3		
101	5/10/2007	H. chrysoscelis	Copper	30.7	C	1	4	6				
101	5/10/2007	H. chrysoscelis	Copper	30.7	C	2	3	4.5				
101	5/10/2007	H. chrysoscelis	Copper	30.7	C	3	4	5				
101	5/10/2007	H. chrysoscelis	Copper	30.7	C	4	3.5	5				
101	5/10/2007	H. chrysoscelis	Copper	30.7	C	5	-	-	2.9	4.1		
101	5/10/2007	H. chrysoscelis	Copper	30.7	D	1	4	5				
101	5/10/2007	H. chrysoscelis	Copper	30.7	D	2	3.5	5				
101	5/10/2007	H. chrysoscelis	Copper	30.7	D	3	3.5	5				
101	5/10/2007	H. chrysoscelis	Copper	30.7	D	4	4	5				
101	5/10/2007	H. chrysoscelis	Copper	30.7	D	5	3.5	4.5	3.7	4.9		
101	5/10/2007	H. chrysoscelis	Copper	30.7	E	1	4	6				
101	5/10/2007	H. chrysoscelis	Copper	30.7	E	2	3.5	5				
101	5/10/2007	H. chrysoscelis	Copper	30.7	E	3	4	5.5				
101	5/10/2007	H. chrysoscelis	Copper	30.7	E	4	4	6				
101	5/10/2007	H. chrysoscelis	Copper	30.7	E	5	4	4.5	3.9	5.4		
101	5/10/2007	H. chrysoscelis	Copper	30.7	F	1	4	5				
101	5/10/2007	H. chrysoscelis	Copper	30.7	F	2	4	6				
101	5/10/2007	H. chrysoscelis	Copper	30.7	F	3	2	3				
101	5/10/2007	H. chrysoscelis	Copper	30.7	F	4	-	-				
101	5/10/2007	H. chrysoscelis	Copper	30.7	F	5	-	-	2.0	2.8	3.2	4.5

SubLethal Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Organism	Body Width (mm)	Body Length (mm)	Day 10 Average Body Width (mm)	Day 10 Average Body Length (mm)	Day 10 Average Treatment Body Width (mm)	Day 10 Average Treatment Body Length (mm)
075 [copper]	5/13/2006	R. palustris	Copper	4.2	A	1	5	8				
075 [copper]	5/13/2006	R. palustris	Copper	4.2	A	2	5	8				
075 [copper]	5/13/2006	R. palustris	Copper	4.2	A	3	6	8				
075 [copper]	5/13/2006	R. palustris	Copper	4.2	A	4	5	8				
075 [copper]	5/13/2006	R. palustris	Copper	4.2	A	5	6	8	5.4	8.0		
075 [copper]	5/13/2006	R. palustris	Copper	4.2	B	1	5	7				
075 [copper]	5/13/2006	R. palustris	Copper	4.2	B	2	6	8				
075 [copper]	5/13/2006	R. palustris	Copper	4.2	B	3	6	8				
075 [copper]	5/13/2006	R. palustris	Copper	4.2	B	4	6	8				
075 [copper]	5/13/2006	R. palustris	Copper	4.2	B	5	6	8	5.8	7.8		
075 [copper]	5/13/2006	R. palustris	Copper	4.2	C	1	5	8				
075 [copper]	5/13/2006	R. palustris	Copper	4.2	C	2	5	7				
075 [copper]	5/13/2006	R. palustris	Copper	4.2	C	3	6	8				
075 [copper]	5/13/2006	R. palustris	Copper	4.2	C	4	5	7				
075 [copper]	5/13/2006	R. palustris	Copper	4.2	C	5	5	7	5.2	7.4		
075 [copper]	5/13/2006	R. palustris	Copper	4.2	D	1	5	8				
075 [copper]	5/13/2006	R. palustris	Copper	4.2	D	2	5	8				
075 [copper]	5/13/2006	R. palustris	Copper	4.2	D	3	5	8				
075 [copper]	5/13/2006	R. palustris	Copper	4.2	D	4	3	6				
075 [copper]	5/13/2006	R. palustris	Copper	4.2	D	5	6	8	4.8	7.6	5.3	7.7
075 [copper]	5/13/2006	R. palustris	Copper	28.9	A	1	5	7				
075 [copper]	5/13/2006	R. palustris	Copper	28.9	A	2	4	6				
075 [copper]	5/13/2006	R. palustris	Copper	28.9	A	3	4	7				
075 [copper]	5/13/2006	R. palustris	Copper	28.9	A	4	4	7				
075 [copper]	5/13/2006	R. palustris	Copper	28.9	A	5	4	6	4.2	6.6		
075 [copper]	5/13/2006	R. palustris	Copper	28.9	B	1	4	7				
075 [copper]	5/13/2006	R. palustris	Copper	28.9	B	2	4	6				
075 [copper]	5/13/2006	R. palustris	Copper	28.9	B	3	5	7				
075 [copper]	5/13/2006	R. palustris	Copper	28.9	B	4	4	7				
075 [copper]	5/13/2006	R. palustris	Copper	28.9	B	5	4	6	4.2	6.6		
075 [copper]	5/13/2006	R. palustris	Copper	28.9	C	1	3	5				
075 [copper]	5/13/2006	R. palustris	Copper	28.9	C	2	5	7				
075 [copper]	5/13/2006	R. palustris	Copper	28.9	C	3	4	7				
075 [copper]	5/13/2006	R. palustris	Copper	28.9	C	4	4	7				
075 [copper]	5/13/2006	R. palustris	Copper	28.9	C	5	5	7	4.2	6.6		
075 [copper]	5/13/2006	R. palustris	Copper	28.9	D	1	5	7				
075 [copper]	5/13/2006	R. palustris	Copper	28.9	D	2	4	6				
075 [copper]	5/13/2006	R. palustris	Copper	28.9	D	3	4	5				
075 [copper]	5/13/2006	R. palustris	Copper	28.9	D	4	5	7				
075 [copper]	5/13/2006	R. palustris	Copper	28.9	D	5	-	-	3.6	5.0	4.1	6.2
075 [lead]	5/13/2006	R. palustris	Lead	4.2	A	1	5	7				
075 [lead]	5/13/2006	R. palustris	Lead	4.2	A	2	5	7				
075 [lead]	5/13/2006	R. palustris	Lead	4.2	A	3	5	7				
075 [lead]	5/13/2006	R. palustris	Lead	4.2	A	4	5	7				
075 [lead]	5/13/2006	R. palustris	Lead	4.2	A	5	4	7	4.8	7.0		
075 [lead]	5/13/2006	R. palustris	Lead	4.2	B	1	5	7				
075 [lead]	5/13/2006	R. palustris	Lead	4.2	B	2	5	8				
075 [lead]	5/13/2006	R. palustris	Lead	4.2	B	3	5	7				
075 [lead]	5/13/2006	R. palustris	Lead	4.2	B	4	5	7				
075 [lead]	5/13/2006	R. palustris	Lead	4.2	B	5	5	7	5.0	7.2		
075 [lead]	5/13/2006	R. palustris	Lead	4.2	C	1	5	7				
075 [lead]	5/13/2006	R. palustris	Lead	4.2	C	2	4	7				
075 [lead]	5/13/2006	R. palustris	Lead	4.2	C	3	5	7				
075 [lead]	5/13/2006	R. palustris	Lead	4.2	C	4	5	7				
075 [lead]	5/13/2006	R. palustris	Lead	4.2	C	5	5	7	4.8	7.0		
075 [lead]	5/13/2006	R. palustris	Lead	4.2	D	1	5	7				
075 [lead]	5/13/2006	R. palustris	Lead	4.2	D	2	6	7				
075 [lead]	5/13/2006	R. palustris	Lead	4.2	D	3	6	7				
075 [lead]	5/13/2006	R. palustris	Lead	4.2	D	4	5	7				
075 [lead]	5/13/2006	R. palustris	Lead	4.2	D	5	5	7	5.4	7.0	5.0	7.1
075 [lead]	5/13/2006	R. palustris	Lead	116	A	1	4	6				
075 [lead]	5/13/2006	R. palustris	Lead	116	A	2	5	7				
075 [lead]	5/13/2006	R. palustris	Lead	116	A	3	5	6				
075 [lead]	5/13/2006	R. palustris	Lead	116	A	4	5	6				
075 [lead]	5/13/2006	R. palustris	Lead	116	A	5	5	6	4.8	6.2		
075 [lead]	5/13/2006	R. palustris	Lead	116	B	1	5	7				
075 [lead]	5/13/2006	R. palustris	Lead	116	B	2	3	5				
075 [lead]	5/13/2006	R. palustris	Lead	116	B	3	5	6				
075 [lead]	5/13/2006	R. palustris	Lead	116	B	4	5	7				
075 [lead]	5/13/2006	R. palustris	Lead	116	B	5	5	7	4.6	6.4		
075 [lead]	5/13/2006	R. palustris	Lead	116	C	1	5	8				
075 [lead]	5/13/2006	R. palustris	Lead	116	C	2	5	6				
075 [lead]	5/13/2006	R. palustris	Lead	116	C	3	5	8				
075 [lead]	5/13/2006	R. palustris	Lead	116	C	4	5	7				
075 [lead]	5/13/2006	R. palustris	Lead	116	C	5	5	7	5.0	7.2		
075 [lead]	5/13/2006	R. palustris	Lead	116	D	1	5	7				
075 [lead]	5/13/2006	R. palustris	Lead	116	D	2	5	7				
075 [lead]	5/13/2006	R. palustris	Lead	116	D	3	5	7				
075 [lead]	5/13/2006	R. palustris	Lead	116	D	4	4	6				
075 [lead]	5/13/2006	R. palustris	Lead	116	D	5	4	6	4.6	6.6	4.8	6.6
075 [lead]	5/13/2006	R. palustris	Lead	298	A	1	4	6				
075 [lead]	5/13/2006	R. palustris	Lead	298	A	2	4	6				
075 [lead]	5/13/2006	R. palustris	Lead	298	A	3	4	6				
075 [lead]	5/13/2006	R. palustris	Lead	298	A	4	3	5				
075 [lead]	5/13/2006	R. palustris	Lead	298	A	5	-	-	3.0	4.8		
075 [lead]	5/13/2006	R. palustris	Lead	298	B	1	4	6				
075 [lead]	5/13/2006	R. palustris	Lead	298	B	2	5	6				
075 [lead]	5/13/2006	R. palustris	Lead	298	B	3	4	6				
075 [lead]	5/13/2006	R. palustris	Lead	298	B	4	4	6				
075 [lead]	5/13/2006	R. palustris	Lead	298	B	5	-	-	3.4	4.8		
075 [lead]	5/13/2006	R. palustris	Lead	298	C	1	3	5				
075 [lead]	5/13/2006	R. palustris	Lead	298	C	2	4	6				
075 [lead]	5/13/2006	R. palustris	Lead	298	C	3	4	6				
075 [lead]	5/13/2006	R. palustris	Lead	298	C	4	-	-				
075 [lead]	5/13/2006	R. palustris	Lead	298	C	5	-	-	2.2	3.4		
075 [lead]	5/13/2006	R. palustris	Lead	298	D	1	4	5				
075 [lead]	5/13/2006	R. palustris	Lead	298	D	2	5	7				
075 [lead]	5/13/2006	R. palustris	Lead	298	D	3	4	5				
075 [lead]	5/13/2006	R. palustris	Lead	298	D	4	-	-				
075 [lead]	5/13/2006	R. palustris	Lead	298	D	5	-	-	2.6	3.4	2.8	4.1

SubLethal Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Organism	Body Width (mm)	Body Length (mm)	Day 10 Average Body Width (mm)	Day 10 Average Body Length (mm)	Day 10 Average Treatment Body Width (mm)	Day 10 Average Treatment Body Length (mm)
076	7/1/2006	R. clamitans	Copper	4.2	A	1	4	6				
076	7/1/2006	R. clamitans	Copper	4.2	A	2	4	6				
076	7/1/2006	R. clamitans	Copper	4.2	A	3	4	6				
076	7/1/2006	R. clamitans	Copper	4.2	A	4	4	6				
076	7/1/2006	R. clamitans	Copper	4.2	A	5	4	6	4.0	6.0		
076	7/1/2006	R. clamitans	Copper	4.2	B	1	4	6				
076	7/1/2006	R. clamitans	Copper	4.2	B	2	4	6				
076	7/1/2006	R. clamitans	Copper	4.2	B	3	4	5				
076	7/1/2006	R. clamitans	Copper	4.2	B	4	-	-				
076	7/1/2006	R. clamitans	Copper	4.2	B	5	-	-	2.4	3.4		
076	7/1/2006	R. clamitans	Copper	4.2	C	1	4	6				
076	7/1/2006	R. clamitans	Copper	4.2	C	2	4	6				
076	7/1/2006	R. clamitans	Copper	4.2	C	3	4	6				
076	7/1/2006	R. clamitans	Copper	4.2	C	4	-	-				
076	7/1/2006	R. clamitans	Copper	4.2	C	5	-	-	2.4	3.6		
076	7/1/2006	R. clamitans	Copper	4.2	D	1	4	6				
076	7/1/2006	R. clamitans	Copper	4.2	D	2	4	6				
076	7/1/2006	R. clamitans	Copper	4.2	D	3	4	6				
076	7/1/2006	R. clamitans	Copper	4.2	D	4	4	6				
076	7/1/2006	R. clamitans	Copper	4.2	D	5	4	6	4.0	6.0		
076	7/1/2006	R. clamitans	Copper	4.2	E	1	4	6				
076	7/1/2006	R. clamitans	Copper	4.2	E	2	4	6				
076	7/1/2006	R. clamitans	Copper	4.2	E	3	4	6				
076	7/1/2006	R. clamitans	Copper	4.2	E	4	4	6				
076	7/1/2006	R. clamitans	Copper	4.2	E	5	4	6	4.0	6.0		
076	7/1/2006	R. clamitans	Copper	4.2	F	1	4	6				
076	7/1/2006	R. clamitans	Copper	4.2	F	2	4	6				
076	7/1/2006	R. clamitans	Copper	4.2	F	3	4	5				
076	7/1/2006	R. clamitans	Copper	4.2	F	4	4	6				
076	7/1/2006	R. clamitans	Copper	4.2	F	5	4	6	4.0	5.8		
076	7/1/2006	R. clamitans	Copper	4.2	G	1	4	6				
076	7/1/2006	R. clamitans	Copper	4.2	G	2	4	6				
076	7/1/2006	R. clamitans	Copper	4.2	G	3	4	6				
076	7/1/2006	R. clamitans	Copper	4.2	G	4	4	6				
076	7/1/2006	R. clamitans	Copper	4.2	G	5	4	6	4.0	6.0		
076	7/1/2006	R. clamitans	Copper	4.2	H	1	4	5				
076	7/1/2006	R. clamitans	Copper	4.2	H	2	4	6				
076	7/1/2006	R. clamitans	Copper	4.2	H	3	4	6				
076	7/1/2006	R. clamitans	Copper	4.2	H	4	4	6				
076	7/1/2006	R. clamitans	Copper	4.2	H	5	4	6	4.0	5.8	3.6	5.3
076	7/1/2006	R. clamitans	Copper	28.9	A	1	3	5				
076	7/1/2006	R. clamitans	Copper	28.9	A	2	4	6				
076	7/1/2006	R. clamitans	Copper	28.9	A	3	4	6				
076	7/1/2006	R. clamitans	Copper	28.9	A	4	4	6				
076	7/1/2006	R. clamitans	Copper	28.9	A	5	4	6	3.8	5.8		
076	7/1/2006	R. clamitans	Copper	28.9	B	1	4	6				
076	7/1/2006	R. clamitans	Copper	28.9	B	2	4	5				
076	7/1/2006	R. clamitans	Copper	28.9	B	3	4	6				
076	7/1/2006	R. clamitans	Copper	28.9	B	4	4	5				
076	7/1/2006	R. clamitans	Copper	28.9	B	5	4	5	4.0	5.4		
076	7/1/2006	R. clamitans	Copper	28.9	C	1	4	6				
076	7/1/2006	R. clamitans	Copper	28.9	C	2	4	6				
076	7/1/2006	R. clamitans	Copper	28.9	C	3	4	6				
076	7/1/2006	R. clamitans	Copper	28.9	C	4	4	6				
076	7/1/2006	R. clamitans	Copper	28.9	C	5	4	6	4.0	6.0		
076	7/1/2006	R. clamitans	Copper	28.9	D	1	4	6				
076	7/1/2006	R. clamitans	Copper	28.9	D	2	4	6				
076	7/1/2006	R. clamitans	Copper	28.9	D	3	4	6				
076	7/1/2006	R. clamitans	Copper	28.9	D	4	4	6				
076	7/1/2006	R. clamitans	Copper	28.9	D	5	4	6	4.0	6.0		
076	7/1/2006	R. clamitans	Copper	28.9	E	1	4	6				
076	7/1/2006	R. clamitans	Copper	28.9	E	2	4	6				
076	7/1/2006	R. clamitans	Copper	28.9	E	3	4	6				
076	7/1/2006	R. clamitans	Copper	28.9	E	4	4	6				
076	7/1/2006	R. clamitans	Copper	28.9	E	5	4	6	4.0	6.0		
076	7/1/2006	R. clamitans	Copper	28.9	F	1	4	6				
076	7/1/2006	R. clamitans	Copper	28.9	F	2	4	6				
076	7/1/2006	R. clamitans	Copper	28.9	F	3	4	6				
076	7/1/2006	R. clamitans	Copper	28.9	F	4	4	6				
076	7/1/2006	R. clamitans	Copper	28.9	F	5	-	-	3.2	4.8		
076	7/1/2006	R. clamitans	Copper	28.9	G	1	4	5				
076	7/1/2006	R. clamitans	Copper	28.9	G	2	4	6				
076	7/1/2006	R. clamitans	Copper	28.9	G	3	4	6				
076	7/1/2006	R. clamitans	Copper	28.9	G	4	4	6				
076	7/1/2006	R. clamitans	Copper	28.9	G	5	4	6	4.0	5.8		
076	7/1/2006	R. clamitans	Copper	28.9	H	1	4	6				
076	7/1/2006	R. clamitans	Copper	28.9	H	2	4	6				
076	7/1/2006	R. clamitans	Copper	28.9	H	3	4	6				
076	7/1/2006	R. clamitans	Copper	28.9	H	4	4	6				
076	7/1/2006	R. clamitans	Copper	28.9	H	5	4	6	4.0	6.0	3.9	5.7

SubLethal Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Organism	Body Width (mm)	Body Length (mm)	Day 10 Average Body Width (mm)	Day 10 Average Body Length (mm)	Day 10 Average Treatment Body Width (mm)	Day 10 Average Treatment Body Length (mm)
076	7/1/2006	R. clamitans	Copper	79.9	A	1	-	-				
076	7/1/2006	R. clamitans	Copper	79.9	A	2	-	-				
076	7/1/2006	R. clamitans	Copper	79.9	A	3	-	-				
076	7/1/2006	R. clamitans	Copper	79.9	A	4	-	-				
076	7/1/2006	R. clamitans	Copper	79.9	A	5	-	-	0.0	0.0		
076	7/1/2006	R. clamitans	Copper	79.9	B	1	3	5				
076	7/1/2006	R. clamitans	Copper	79.9	B	2	3	5				
076	7/1/2006	R. clamitans	Copper	79.9	B	3	3	5				
076	7/1/2006	R. clamitans	Copper	79.9	B	4	3	5				
076	7/1/2006	R. clamitans	Copper	79.9	B	5	3	5	3.0	5.0		
076	7/1/2006	R. clamitans	Copper	79.9	C	1	4	5				
076	7/1/2006	R. clamitans	Copper	79.9	C	2	3	5				
076	7/1/2006	R. clamitans	Copper	79.9	C	3	4	6				
076	7/1/2006	R. clamitans	Copper	79.9	C	4	3	5				
076	7/1/2006	R. clamitans	Copper	79.9	C	5	4	6	3.6	5.4		
076	7/1/2006	R. clamitans	Copper	79.9	D	1	2	3				
076	7/1/2006	R. clamitans	Copper	79.9	D	2	3	4				
076	7/1/2006	R. clamitans	Copper	79.9	D	3	-	-				
076	7/1/2006	R. clamitans	Copper	79.9	D	4	-	-				
076	7/1/2006	R. clamitans	Copper	79.9	D	5	-	-	1.0	1.4		
076	7/1/2006	R. clamitans	Copper	79.9	E	1	3	5				
076	7/1/2006	R. clamitans	Copper	79.9	E	2	3	5				
076	7/1/2006	R. clamitans	Copper	79.9	E	3	4	5				
076	7/1/2006	R. clamitans	Copper	79.9	E	4	4	5				
076	7/1/2006	R. clamitans	Copper	79.9	E	5	4	5	3.6	5.0		
076	7/1/2006	R. clamitans	Copper	79.9	F	1	-	-				
076	7/1/2006	R. clamitans	Copper	79.9	F	2	-	-				
076	7/1/2006	R. clamitans	Copper	79.9	F	3	-	-				
076	7/1/2006	R. clamitans	Copper	79.9	F	4	-	-				
076	7/1/2006	R. clamitans	Copper	79.9	F	5	-	-	0.0	0.0		
076	7/1/2006	R. clamitans	Copper	79.9	G	1	-	-				
076	7/1/2006	R. clamitans	Copper	79.9	G	2	-	-				
076	7/1/2006	R. clamitans	Copper	79.9	G	3	-	-				
076	7/1/2006	R. clamitans	Copper	79.9	G	4	-	-				
076	7/1/2006	R. clamitans	Copper	79.9	G	5	-	-	0.0	0.0		
076	7/1/2006	R. clamitans	Copper	79.9	H	1	-	-				
076	7/1/2006	R. clamitans	Copper	79.9	H	2	-	-				
076	7/1/2006	R. clamitans	Copper	79.9	H	3	-	-				
076	7/1/2006	R. clamitans	Copper	79.9	H	4	-	-				
076	7/1/2006	R. clamitans	Copper	79.9	H	5	-	-	0.0	0.0	1.4	2.1
077	7/1/2006	R. clamitans	Lead	2.6	A	1	4	6				
077	7/1/2006	R. clamitans	Lead	2.6	A	2	4	6				
077	7/1/2006	R. clamitans	Lead	2.6	A	3	4	6				
077	7/1/2006	R. clamitans	Lead	2.6	A	4	4	6				
077	7/1/2006	R. clamitans	Lead	2.6	A	5	4	6	4.0	6.0		
077	7/1/2006	R. clamitans	Lead	2.6	B	1	4	5				
077	7/1/2006	R. clamitans	Lead	2.6	B	2	4	6				
077	7/1/2006	R. clamitans	Lead	2.6	B	3	4	6				
077	7/1/2006	R. clamitans	Lead	2.6	B	4	4	6				
077	7/1/2006	R. clamitans	Lead	2.6	B	5	4	6	4.0	5.8		
077	7/1/2006	R. clamitans	Lead	2.6	C	1	4	5				
077	7/1/2006	R. clamitans	Lead	2.6	C	2	4	5				
077	7/1/2006	R. clamitans	Lead	2.6	C	3	3	4				
077	7/1/2006	R. clamitans	Lead	2.6	C	4	4	5				
077	7/1/2006	R. clamitans	Lead	2.6	C	5	4	5	3.8	4.8		
077	7/1/2006	R. clamitans	Lead	2.6	D	1	4	6				
077	7/1/2006	R. clamitans	Lead	2.6	D	2	4	6				
077	7/1/2006	R. clamitans	Lead	2.6	D	3	4	6				
077	7/1/2006	R. clamitans	Lead	2.6	D	4	4	6				
077	7/1/2006	R. clamitans	Lead	2.6	D	5	4	6	4.0	6.0		
077	7/1/2006	R. clamitans	Lead	2.6	E	1	4	6				
077	7/1/2006	R. clamitans	Lead	2.6	E	2	4	6				
077	7/1/2006	R. clamitans	Lead	2.6	E	3	4	6				
077	7/1/2006	R. clamitans	Lead	2.6	E	4	4	6				
077	7/1/2006	R. clamitans	Lead	2.6	E	5	4	6	4.0	6.0		
077	7/1/2006	R. clamitans	Lead	2.6	F	1	4	6				
077	7/1/2006	R. clamitans	Lead	2.6	F	2	4	6				
077	7/1/2006	R. clamitans	Lead	2.6	F	3	4	5				
077	7/1/2006	R. clamitans	Lead	2.6	F	4	4	5				
077	7/1/2006	R. clamitans	Lead	2.6	F	5	4	6	4.0	5.6		
077	7/1/2006	R. clamitans	Lead	2.6	G	1	4	6				
077	7/1/2006	R. clamitans	Lead	2.6	G	2	4	6				
077	7/1/2006	R. clamitans	Lead	2.6	G	3	3	5				
077	7/1/2006	R. clamitans	Lead	2.6	G	4	4	6				
077	7/1/2006	R. clamitans	Lead	2.6	G	5	-	-	3.0	4.6		
077	7/1/2006	R. clamitans	Lead	2.6	H	1	4	6				
077	7/1/2006	R. clamitans	Lead	2.6	H	2	4	6				
077	7/1/2006	R. clamitans	Lead	2.6	H	3	4	6				
077	7/1/2006	R. clamitans	Lead	2.6	H	4	4	6				
077	7/1/2006	R. clamitans	Lead	2.6	H	5	5	6	4.2	6.0	3.9	5.6

SubLethal Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Organism	Body Width (mm)	Body Length (mm)	Day 10 Average Body Width (mm)	Day 10 Average Body Length (mm)	Day 10 Average Treatment Body Width (mm)	Day 10 Average Treatment Body Length (mm)
077	7/1/2006	R. clamitans	Lead	116	A	1	4	6				
077	7/1/2006	R. clamitans	Lead	116	A	2	4	5				
077	7/1/2006	R. clamitans	Lead	116	A	3	4	6				
077	7/1/2006	R. clamitans	Lead	116	A	4	4	6				
077	7/1/2006	R. clamitans	Lead	116	A	5	4	6	4.0	5.8		
077	7/1/2006	R. clamitans	Lead	116	B	1	4	6				
077	7/1/2006	R. clamitans	Lead	116	B	2	4	6				
077	7/1/2006	R. clamitans	Lead	116	B	3	4	6				
077	7/1/2006	R. clamitans	Lead	116	B	4	4	6				
077	7/1/2006	R. clamitans	Lead	116	B	5	4	6	4.0	6.0		
077	7/1/2006	R. clamitans	Lead	116	C	1	4	6				
077	7/1/2006	R. clamitans	Lead	116	C	2	4	6				
077	7/1/2006	R. clamitans	Lead	116	C	3	4	6				
077	7/1/2006	R. clamitans	Lead	116	C	4	4	6				
077	7/1/2006	R. clamitans	Lead	116	C	5	4	6	4.0	6.0		
077	7/1/2006	R. clamitans	Lead	116	D	1	4	6				
077	7/1/2006	R. clamitans	Lead	116	D	2	4	5				
077	7/1/2006	R. clamitans	Lead	116	D	3	3	5				
077	7/1/2006	R. clamitans	Lead	116	D	4	4	6				
077	7/1/2006	R. clamitans	Lead	116	D	5	4	6	3.8	5.6		
077	7/1/2006	R. clamitans	Lead	116	E	1	4	6				
077	7/1/2006	R. clamitans	Lead	116	E	2	3	6				
077	7/1/2006	R. clamitans	Lead	116	E	3	4	6				
077	7/1/2006	R. clamitans	Lead	116	E	4	4	6				
077	7/1/2006	R. clamitans	Lead	116	E	5	4	6	3.8	6.0		
077	7/1/2006	R. clamitans	Lead	116	F	1	4	6				
077	7/1/2006	R. clamitans	Lead	116	F	2	4	6				
077	7/1/2006	R. clamitans	Lead	116	F	3	4	6				
077	7/1/2006	R. clamitans	Lead	116	F	4	4	6				
077	7/1/2006	R. clamitans	Lead	116	F	5	3	5	3.8	5.8		
077	7/1/2006	R. clamitans	Lead	116	G	1	4	6				
077	7/1/2006	R. clamitans	Lead	116	G	2	4	6				
077	7/1/2006	R. clamitans	Lead	116	G	3	4	5				
077	7/1/2006	R. clamitans	Lead	116	G	4	4	6				
077	7/1/2006	R. clamitans	Lead	116	G	5	4	6	4.0	5.8		
077	7/1/2006	R. clamitans	Lead	116	H	1	4	6				
077	7/1/2006	R. clamitans	Lead	116	H	2	4	6				
077	7/1/2006	R. clamitans	Lead	116	H	3	4	6				
077	7/1/2006	R. clamitans	Lead	116	H	4	4	6				
077	7/1/2006	R. clamitans	Lead	116	H	5	4	6	4.0	6.0	3.9	5.9
077	7/1/2006	R. clamitans	Lead	298	A	1	4	6				
077	7/1/2006	R. clamitans	Lead	298	A	2	4	5				
077	7/1/2006	R. clamitans	Lead	298	A	3	4	6				
077	7/1/2006	R. clamitans	Lead	298	A	4	4	5				
077	7/1/2006	R. clamitans	Lead	298	A	5	4	6	4.0	5.8		
077	7/1/2006	R. clamitans	Lead	298	B	1	4	5				
077	7/1/2006	R. clamitans	Lead	298	B	2	4	5				
077	7/1/2006	R. clamitans	Lead	298	B	3	4	5				
077	7/1/2006	R. clamitans	Lead	298	B	4	4	6				
077	7/1/2006	R. clamitans	Lead	298	B	5	4	5	4.0	5.2		
077	7/1/2006	R. clamitans	Lead	298	C	1	4	5				
077	7/1/2006	R. clamitans	Lead	298	C	2	3	5				
077	7/1/2006	R. clamitans	Lead	298	C	3	4	5				
077	7/1/2006	R. clamitans	Lead	298	C	4	3	5				
077	7/1/2006	R. clamitans	Lead	298	C	5	-	-	2.8	4.0		
077	7/1/2006	R. clamitans	Lead	298	D	1	4	5				
077	7/1/2006	R. clamitans	Lead	298	D	2	4	5				
077	7/1/2006	R. clamitans	Lead	298	D	3	4	5				
077	7/1/2006	R. clamitans	Lead	298	D	4	4	5				
077	7/1/2006	R. clamitans	Lead	298	D	5	4	6	4.0	5.2		
077	7/1/2006	R. clamitans	Lead	298	E	1	4	5				
077	7/1/2006	R. clamitans	Lead	298	E	2	4	5				
077	7/1/2006	R. clamitans	Lead	298	E	3	4	5				
077	7/1/2006	R. clamitans	Lead	298	E	4	3	4				
077	7/1/2006	R. clamitans	Lead	298	E	5	4	5	3.8	4.8		
077	7/1/2006	R. clamitans	Lead	298	F	1	3	5				
077	7/1/2006	R. clamitans	Lead	298	F	2	3	5				
077	7/1/2006	R. clamitans	Lead	298	F	3	3	5				
077	7/1/2006	R. clamitans	Lead	298	F	4	3	5				
077	7/1/2006	R. clamitans	Lead	298	F	5	4	5	3.2	5.0		
077	7/1/2006	R. clamitans	Lead	298	G	1	4	5				
077	7/1/2006	R. clamitans	Lead	298	G	2	4	5				
077	7/1/2006	R. clamitans	Lead	298	G	3	4	6				
077	7/1/2006	R. clamitans	Lead	298	G	4	3	5				
077	7/1/2006	R. clamitans	Lead	298	G	5	3	5	3.6	5.2		
077	7/1/2006	R. clamitans	Lead	298	H	1	4	6				
077	7/1/2006	R. clamitans	Lead	298	H	2	4	6				
077	7/1/2006	R. clamitans	Lead	298	H	3	4	6				
077	7/1/2006	R. clamitans	Lead	298	H	4	3	5				
077	7/1/2006	R. clamitans	Lead	298	H	5	4	5	3.8	5.6	3.7	5.1

SubLethal Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Organism	Body Width (mm)	Body Length (mm)	Day 10 Average Body Width (mm)	Day 10 Average Body Length (mm)	Day 10 Average Treatment Body Width (mm)	Day 10 Average Treatment Body Length (mm)
077	7/1/2006	R. clamitans	Lead	635	A	1	3	4				
077	7/1/2006	R. clamitans	Lead	635	A	2	3	4				
077	7/1/2006	R. clamitans	Lead	635	A	3	3	4				
077	7/1/2006	R. clamitans	Lead	635	A	4	3	4				
077	7/1/2006	R. clamitans	Lead	635	A	5	-	-	2.4	3.2		
077	7/1/2006	R. clamitans	Lead	635	B	1	3	4				
077	7/1/2006	R. clamitans	Lead	635	B	2	3	4				
077	7/1/2006	R. clamitans	Lead	635	B	3	4	5				
077	7/1/2006	R. clamitans	Lead	635	B	4	3	4				
077	7/1/2006	R. clamitans	Lead	635	B	5	-	-	2.6	3.4		
077	7/1/2006	R. clamitans	Lead	635	C	1	4	5				
077	7/1/2006	R. clamitans	Lead	635	C	2	3	5				
077	7/1/2006	R. clamitans	Lead	635	C	3	3	4				
077	7/1/2006	R. clamitans	Lead	635	C	4	3	4				
077	7/1/2006	R. clamitans	Lead	635	C	5	-	-	2.6	3.6		
077	7/1/2006	R. clamitans	Lead	635	D	1	3	4				
077	7/1/2006	R. clamitans	Lead	635	D	2	2	3				
077	7/1/2006	R. clamitans	Lead	635	D	3	3	4				
077	7/1/2006	R. clamitans	Lead	635	D	4	-	-				
077	7/1/2006	R. clamitans	Lead	635	D	5	-	-	1.6	2.2		
077	7/1/2006	R. clamitans	Lead	635	E	1	3	4				
077	7/1/2006	R. clamitans	Lead	635	E	2	3	5				
077	7/1/2006	R. clamitans	Lead	635	E	3	3	4				
077	7/1/2006	R. clamitans	Lead	635	E	4	3	4				
077	7/1/2006	R. clamitans	Lead	635	E	5	3	5	3.0	4.4		
077	7/1/2006	R. clamitans	Lead	635	F	1	3	4				
077	7/1/2006	R. clamitans	Lead	635	F	2	2	3				
077	7/1/2006	R. clamitans	Lead	635	F	3	2	3				
077	7/1/2006	R. clamitans	Lead	635	F	4	2	3				
077	7/1/2006	R. clamitans	Lead	635	F	5	-	-	1.8	2.6		
077	7/1/2006	R. clamitans	Lead	635	G	1	3	4				
077	7/1/2006	R. clamitans	Lead	635	G	2	3	4				
077	7/1/2006	R. clamitans	Lead	635	G	3	3	4				
077	7/1/2006	R. clamitans	Lead	635	G	4	2	3				
077	7/1/2006	R. clamitans	Lead	635	G	5	3	4	2.8	3.8		
077	7/1/2006	R. clamitans	Lead	635	H	1	2	3				
077	7/1/2006	R. clamitans	Lead	635	H	2	2	3				
077	7/1/2006	R. clamitans	Lead	635	H	3	2	3				
077	7/1/2006	R. clamitans	Lead	635	H	4	-	-				
077	7/1/2006	R. clamitans	Lead	635	H	5	-	-	1.2	1.8	2.3	3.1
103	5/12/2007	R. clamitans	Copper	3.2	A	1	4	6				
103	5/12/2007	R. clamitans	Copper	3.2	A	2	3	5				
103	5/12/2007	R. clamitans	Copper	3.2	A	3	3.5	5				
103	5/12/2007	R. clamitans	Copper	3.2	A	4	3.5	5				
103	5/12/2007	R. clamitans	Copper	3.2	A	5	4	6	3.6	5.4		
103	5/12/2007	R. clamitans	Copper	3.2	B	1	3.5	5				
103	5/12/2007	R. clamitans	Copper	3.2	B	2	3.5	5				
103	5/12/2007	R. clamitans	Copper	3.2	B	3	4	6				
103	5/12/2007	R. clamitans	Copper	3.2	B	4	3.5	5				
103	5/12/2007	R. clamitans	Copper	3.2	B	5	3.5	5	3.6	5.2		
103	5/12/2007	R. clamitans	Copper	3.2	C	1	4	6				
103	5/12/2007	R. clamitans	Copper	3.2	C	2	4	6				
103	5/12/2007	R. clamitans	Copper	3.2	C	3	4	6				
103	5/12/2007	R. clamitans	Copper	3.2	C	4	3.5	5				
103	5/12/2007	R. clamitans	Copper	3.2	C	5	3.5	5	3.8	5.6		
103	5/12/2007	R. clamitans	Copper	3.2	D	1	4	6				
103	5/12/2007	R. clamitans	Copper	3.2	D	2	4	6				
103	5/12/2007	R. clamitans	Copper	3.2	D	3	4	6				
103	5/12/2007	R. clamitans	Copper	3.2	D	4	4	6				
103	5/12/2007	R. clamitans	Copper	3.2	D	5	4	6	4.0	6.0		
103	5/12/2007	R. clamitans	Copper	3.2	E	1	3.5	6				
103	5/12/2007	R. clamitans	Copper	3.2	E	2	3.5	5				
103	5/12/2007	R. clamitans	Copper	3.2	E	3	4	6				
103	5/12/2007	R. clamitans	Copper	3.2	E	4	3.5	5				
103	5/12/2007	R. clamitans	Copper	3.2	E	5	4	6	3.7	5.8		
103	5/12/2007	R. clamitans	Copper	3.2	F	1	4	6				
103	5/12/2007	R. clamitans	Copper	3.2	F	2	5	6.5				
103	5/12/2007	R. clamitans	Copper	3.2	F	3	4	6				
103	5/12/2007	R. clamitans	Copper	3.2	F	4	4	6				
103	5/12/2007	R. clamitans	Copper	3.2	F	5	4	6	4.2	6.1	3.8	5.7
103	5/12/2007	R. clamitans	Copper	13	A	1	4.5	6				
103	5/12/2007	R. clamitans	Copper	13	A	2	4	6				
103	5/12/2007	R. clamitans	Copper	13	A	3	4	5				
103	5/12/2007	R. clamitans	Copper	13	A	4	4	5				
103	5/12/2007	R. clamitans	Copper	13	A	5	5	6	4.3	5.6		
103	5/12/2007	R. clamitans	Copper	13	B	1	5	7				
103	5/12/2007	R. clamitans	Copper	13	B	2	4.5	6				
103	5/12/2007	R. clamitans	Copper	13	B	3	4.5	6				
103	5/12/2007	R. clamitans	Copper	13	B	4	-	-				
103	5/12/2007	R. clamitans	Copper	13	B	5	-	-	2.8	3.8		
103	5/12/2007	R. clamitans	Copper	13	C	1	4	6				
103	5/12/2007	R. clamitans	Copper	13	C	2	4.5	6				
103	5/12/2007	R. clamitans	Copper	13	C	3	5	6.5				
103	5/12/2007	R. clamitans	Copper	13	C	4	4	6				
103	5/12/2007	R. clamitans	Copper	13	C	5	3.5	5	4.2	5.9		
103	5/12/2007	R. clamitans	Copper	13	D	1	5	6.5				
103	5/12/2007	R. clamitans	Copper	13	D	2	5					
103	5/12/2007	R. clamitans	Copper	13	D	3	4.5	6				
103	5/12/2007	R. clamitans	Copper	13	D	4	4.5	5.5				
103	5/12/2007	R. clamitans	Copper	13	D	5	-	-	3.8	3.6		
103	5/12/2007	R. clamitans	Copper	13	E	1	4.5	6				
103	5/12/2007	R. clamitans	Copper	13	E	2	4	5				
103	5/12/2007	R. clamitans	Copper	13	E	3	4	5.5				
103	5/12/2007	R. clamitans	Copper	13	E	4	4	5				
103	5/12/2007	R. clamitans	Copper	13	E	5	4	5	4.1	5.3		
103	5/12/2007	R. clamitans	Copper	13	F	1	4	5.5				
103	5/12/2007	R. clamitans	Copper	13	F	2	3	5				
103	5/12/2007	R. clamitans	Copper	13	F	3	4	6				
103	5/12/2007	R. clamitans	Copper	13	F	4	3.5	5				
103	5/12/2007	R. clamitans	Copper	13	F	5	4	5.5	3.7	5.4	3.8	4.9

SubLethal Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Organism	Body Width (mm)	Body Length (mm)	Day 10 Average Body Width (mm)	Day 10 Average Body Length (mm)	Day 10 Average Treatment Body Width (mm)	Day 10 Average Treatment Body Length (mm)
103	5/12/2007	R. clamitans	Copper	30.7	A	1	4	5				
103	5/12/2007	R. clamitans	Copper	30.7	A	2	4	5.5				
103	5/12/2007	R. clamitans	Copper	30.7	A	3	4	5.5				
103	5/12/2007	R. clamitans	Copper	30.7	A	4	4	6				
103	5/12/2007	R. clamitans	Copper	30.7	A	5	4	5	4.0	5.4		
103	5/12/2007	R. clamitans	Copper	30.7	B	1	4.5	5				
103	5/12/2007	R. clamitans	Copper	30.7	B	2	3.5	4.5				
103	5/12/2007	R. clamitans	Copper	30.7	B	3	3.5	5				
103	5/12/2007	R. clamitans	Copper	30.7	B	4	2.5	4				
103	5/12/2007	R. clamitans	Copper	30.7	B	5	3.5	4.5	3.5	4.6		
103	5/12/2007	R. clamitans	Copper	30.7	C	1	4	6				
103	5/12/2007	R. clamitans	Copper	30.7	C	2	4	5				
103	5/12/2007	R. clamitans	Copper	30.7	C	3	4	5.5				
103	5/12/2007	R. clamitans	Copper	30.7	C	4	3.5	4				
103	5/12/2007	R. clamitans	Copper	30.7	C	5	4	5	3.9	5.1		
103	5/12/2007	R. clamitans	Copper	30.7	D	1	2.5	4.5				
103	5/12/2007	R. clamitans	Copper	30.7	D	2	3.5	5.5				
103	5/12/2007	R. clamitans	Copper	30.7	D	3	-	-				
103	5/12/2007	R. clamitans	Copper	30.7	D	4	-	-				
103	5/12/2007	R. clamitans	Copper	30.7	D	5	-	-	1.2	2.0		
103	5/12/2007	R. clamitans	Copper	30.7	E	1	3.5	5				
103	5/12/2007	R. clamitans	Copper	30.7	E	2	3.5	5				
103	5/12/2007	R. clamitans	Copper	30.7	E	3	-	-				
103	5/12/2007	R. clamitans	Copper	30.7	E	4	-	-				
103	5/12/2007	R. clamitans	Copper	30.7	E	5	-	-	1.4	2.0		
103	5/12/2007	R. clamitans	Copper	30.7	F	1	4	6				
103	5/12/2007	R. clamitans	Copper	30.7	F	2	3.5	6				
103	5/12/2007	R. clamitans	Copper	30.7	F	3	3	4.5				
103	5/12/2007	R. clamitans	Copper	30.7	F	4	4	6				
103	5/12/2007	R. clamitans	Copper	30.7	F	5	4	5	3.7	5.5	3.0	4.1
103	5/12/2007	R. clamitans	Copper	75	A	1	-	-				
103	5/12/2007	R. clamitans	Copper	75	A	2	-	-				
103	5/12/2007	R. clamitans	Copper	75	A	3	-	-				
103	5/12/2007	R. clamitans	Copper	75	A	4	-	-				
103	5/12/2007	R. clamitans	Copper	75	A	5	-	-	0.0	0.0		
103	5/12/2007	R. clamitans	Copper	75	B	1	-	-				
103	5/12/2007	R. clamitans	Copper	75	B	2	-	-				
103	5/12/2007	R. clamitans	Copper	75	B	3	-	-				
103	5/12/2007	R. clamitans	Copper	75	B	4	-	-				
103	5/12/2007	R. clamitans	Copper	75	B	5	-	-	0.0	0.0		
103	5/12/2007	R. clamitans	Copper	75	C	1	3.5	5				
103	5/12/2007	R. clamitans	Copper	75	C	2	3.5	5				
103	5/12/2007	R. clamitans	Copper	75	C	3	-	-				
103	5/12/2007	R. clamitans	Copper	75	C	4	-	-				
103	5/12/2007	R. clamitans	Copper	75	C	5	-	-	1.4	2.0		
103	5/12/2007	R. clamitans	Copper	75	D	1	-	-				
103	5/12/2007	R. clamitans	Copper	75	D	2	-	-				
103	5/12/2007	R. clamitans	Copper	75	D	3	-	-				
103	5/12/2007	R. clamitans	Copper	75	D	4	-	-				
103	5/12/2007	R. clamitans	Copper	75	D	5	-	-	0.0	0.0		
103	5/12/2007	R. clamitans	Copper	75	E	1	-	-				
103	5/12/2007	R. clamitans	Copper	75	E	2	-	-				
103	5/12/2007	R. clamitans	Copper	75	E	3	-	-				
103	5/12/2007	R. clamitans	Copper	75	E	4	-	-				
103	5/12/2007	R. clamitans	Copper	75	E	5	-	-	0.0	0.0		
103	5/12/2007	R. clamitans	Copper	75	F	1	-	-				
103	5/12/2007	R. clamitans	Copper	75	F	2	-	-				
103	5/12/2007	R. clamitans	Copper	75	F	3	-	-				
103	5/12/2007	R. clamitans	Copper	75	F	4	-	-				
103	5/12/2007	R. clamitans	Copper	75	F	5	-	-	0.0	0.0	0.2	0.3
107	7/6/2007	X. laevis	Copper	3.2	A	1	4	5.5				
107	7/6/2007	X. laevis	Copper	3.2	A	2	5	7				
107	7/6/2007	X. laevis	Copper	3.2	A	3	4	5				
107	7/6/2007	X. laevis	Copper	3.2	A	4	5	7				
107	7/6/2007	X. laevis	Copper	3.2	A	5	-	-	3.6	4.9		
107	7/6/2007	X. laevis	Copper	3.2	B	1	4	5.5				
107	7/6/2007	X. laevis	Copper	3.2	B	2	5	6				
107	7/6/2007	X. laevis	Copper	3.2	B	3	4	5.5				
107	7/6/2007	X. laevis	Copper	3.2	B	4	5	6				
107	7/6/2007	X. laevis	Copper	3.2	B	5	-	-	3.6	4.6		
107	7/6/2007	X. laevis	Copper	3.2	C	1	5	6.5				
107	7/6/2007	X. laevis	Copper	3.2	C	2	4	5				
107	7/6/2007	X. laevis	Copper	3.2	C	3	5	6.5				
107	7/6/2007	X. laevis	Copper	3.2	C	4	4.5	6				
107	7/6/2007	X. laevis	Copper	3.2	C	5	4	4	4.5	5.6		
107	7/6/2007	X. laevis	Copper	3.2	D	1	3.5	5				
107	7/6/2007	X. laevis	Copper	3.2	D	2	3.5	4.5				
107	7/6/2007	X. laevis	Copper	3.2	D	3	-	-				
107	7/6/2007	X. laevis	Copper	3.2	D	4	-	-				
107	7/6/2007	X. laevis	Copper	3.2	D	5	-	-	1.4	1.9		
107	7/6/2007	X. laevis	Copper	3.2	E	1	5	6.5				
107	7/6/2007	X. laevis	Copper	3.2	E	2	4.5	5				
107	7/6/2007	X. laevis	Copper	3.2	E	3	4	5				
107	7/6/2007	X. laevis	Copper	3.2	E	4	-	-				
107	7/6/2007	X. laevis	Copper	3.2	E	5	-	-	2.7	3.3		
107	7/6/2007	X. laevis	Copper	3.2	F	1	4	5				
107	7/6/2007	X. laevis	Copper	3.2	F	2	4.5	5				
107	7/6/2007	X. laevis	Copper	3.2	F	3	5	5.5				
107	7/6/2007	X. laevis	Copper	3.2	F	4	5	6				
107	7/6/2007	X. laevis	Copper	3.2	F	5	4.5	6	4.6	5.5	3.4	4.3

SubLethal Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Organism	Body Width (mm)	Body Length (mm)	Day 10 Average Body Width (mm)	Day 10 Average Body Length (mm)	Day 10 Average Treatment Body Width (mm)	Day 10 Average Treatment Body Length (mm)
107	7/6/2007	X. laevis	Copper	13	A	1	5	5				
107	7/6/2007	X. laevis	Copper	13	A	2	5.5	7				
107	7/6/2007	X. laevis	Copper	13	A	3	5	5				
107	7/6/2007	X. laevis	Copper	13	A	4	5	6				
107	7/6/2007	X. laevis	Copper	13	A	5	4.5	5	5.0	5.6		
107	7/6/2007	X. laevis	Copper	13	B	1	5	7				
107	7/6/2007	X. laevis	Copper	13	B	2	5	6.5				
107	7/6/2007	X. laevis	Copper	13	B	3	5	6				
107	7/6/2007	X. laevis	Copper	13	B	4	5	7				
107	7/6/2007	X. laevis	Copper	13	B	5	5	6.5	5.0	6.6		
107	7/6/2007	X. laevis	Copper	13	C	1	5	5				
107	7/6/2007	X. laevis	Copper	13	C	2	4.5	5				
107	7/6/2007	X. laevis	Copper	13	C	3	5	6				
107	7/6/2007	X. laevis	Copper	13	C	4	4.5	5				
107	7/6/2007	X. laevis	Copper	13	C	5	5	5	4.8	5.2		
107	7/6/2007	X. laevis	Copper	13	D	1	4	5				
107	7/6/2007	X. laevis	Copper	13	D	2	4	5				
107	7/6/2007	X. laevis	Copper	13	D	3	4	5				
107	7/6/2007	X. laevis	Copper	13	D	4	4	4.5				
107	7/6/2007	X. laevis	Copper	13	D	5	4	5	4.0	4.9		
107	7/6/2007	X. laevis	Copper	13	E	1	4	5				
107	7/6/2007	X. laevis	Copper	13	E	2	4	5				
107	7/6/2007	X. laevis	Copper	13	E	3	5	6				
107	7/6/2007	X. laevis	Copper	13	E	4	5	5				
107	7/6/2007	X. laevis	Copper	13	E	5	5.5	5.5	4.7	5.3		
107	7/6/2007	X. laevis	Copper	13	F	1	3	3				
107	7/6/2007	X. laevis	Copper	13	F	2	5	6				
107	7/6/2007	X. laevis	Copper	13	F	3	4	6				
107	7/6/2007	X. laevis	Copper	13	F	4	4	6				
107	7/6/2007	X. laevis	Copper	13	F	5	4.5	6	4.1	5.4	4.6	5.5
107	7/6/2007	X. laevis	Copper	32	A	1	4.5	6				
107	7/6/2007	X. laevis	Copper	32	A	2	4	6				
107	7/6/2007	X. laevis	Copper	32	A	3	4	5.5				
107	7/6/2007	X. laevis	Copper	32	A	4	4	5.5				
107	7/6/2007	X. laevis	Copper	32	A	5	-	-	3.3	4.6		
107	7/6/2007	X. laevis	Copper	32	B	1	4.5	7				
107	7/6/2007	X. laevis	Copper	32	B	2	4.5	7				
107	7/6/2007	X. laevis	Copper	32	B	3	-	-				
107	7/6/2007	X. laevis	Copper	32	B	4	-	-				
107	7/6/2007	X. laevis	Copper	32	B	5	-	-	1.8	2.6		
107	7/6/2007	X. laevis	Copper	32	C	1	4.5	7				
107	7/6/2007	X. laevis	Copper	32	C	2	4	6				
107	7/6/2007	X. laevis	Copper	32	C	3	4	6				
107	7/6/2007	X. laevis	Copper	32	C	4	4	6				
107	7/6/2007	X. laevis	Copper	32	C	5	-	-	3.3	5.0		
107	7/6/2007	X. laevis	Copper	32	D	1	4	6				
107	7/6/2007	X. laevis	Copper	32	D	2	4	6				
107	7/6/2007	X. laevis	Copper	32	D	3	4	6				
107	7/6/2007	X. laevis	Copper	32	D	4	4	6				
107	7/6/2007	X. laevis	Copper	32	D	5	4	5	4.0	5.8		
107	7/6/2007	X. laevis	Copper	32	E	1	5	6.5				
107	7/6/2007	X. laevis	Copper	32	E	2	5	6.5				
107	7/6/2007	X. laevis	Copper	32	E	3	4.5	6				
107	7/6/2007	X. laevis	Copper	32	E	4	4	6				
107	7/6/2007	X. laevis	Copper	32	E	5	4	5.5	4.5	6.1		
107	7/6/2007	X. laevis	Copper	32	F	1	5	7				
107	7/6/2007	X. laevis	Copper	32	F	2	5	7				
107	7/6/2007	X. laevis	Copper	32	F	3	4	6				
107	7/6/2007	X. laevis	Copper	32	F	4	4	6				
107	7/6/2007	X. laevis	Copper	32	F	5	5	6	4.6	6.4	3.6	5.1
107	7/6/2007	X. laevis	Copper	85	A	1	3	4				
107	7/6/2007	X. laevis	Copper	85	A	2	3.5	5				
107	7/6/2007	X. laevis	Copper	85	A	3	3.5	5				
107	7/6/2007	X. laevis	Copper	85	A	4	3	5				
107	7/6/2007	X. laevis	Copper	85	A	5	-	-	2.6	3.8		
107	7/6/2007	X. laevis	Copper	85	B	1	-	-				
107	7/6/2007	X. laevis	Copper	85	B	2	-	-				
107	7/6/2007	X. laevis	Copper	85	B	3	-	-				
107	7/6/2007	X. laevis	Copper	85	B	4	-	-				
107	7/6/2007	X. laevis	Copper	85	B	5	-	-	0.0	0.0		
107	7/6/2007	X. laevis	Copper	85	C	1	-	-				
107	7/6/2007	X. laevis	Copper	85	C	2	-	-				
107	7/6/2007	X. laevis	Copper	85	C	3	-	-				
107	7/6/2007	X. laevis	Copper	85	C	4	-	-				
107	7/6/2007	X. laevis	Copper	85	C	5	-	-	0.0	0.0		
107	7/6/2007	X. laevis	Copper	85	D	1	-	-				
107	7/6/2007	X. laevis	Copper	85	D	2	-	-				
107	7/6/2007	X. laevis	Copper	85	D	3	-	-				
107	7/6/2007	X. laevis	Copper	85	D	4	-	-				
107	7/6/2007	X. laevis	Copper	85	D	5	-	-	0.0	0.0		
107	7/6/2007	X. laevis	Copper	85	E	1	-	-				
107	7/6/2007	X. laevis	Copper	85	E	2	-	-				
107	7/6/2007	X. laevis	Copper	85	E	3	-	-				
107	7/6/2007	X. laevis	Copper	85	E	4	-	-				
107	7/6/2007	X. laevis	Copper	85	E	5	-	-	0.0	0.0		
107	7/6/2007	X. laevis	Copper	85	F	1	-	-				
107	7/6/2007	X. laevis	Copper	85	F	2	-	-				
107	7/6/2007	X. laevis	Copper	85	F	3	-	-				
107	7/6/2007	X. laevis	Copper	85	F	4	-	-				
107	7/6/2007	X. laevis	Copper	85	F	5	-	-	0.0	0.0	0.4	0.6

SubLethal Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Organism	Body Width (mm)	Body Length (mm)	Day 10 Average Body Width (mm)	Day 10 Average Body Length (mm)	Day 10 Average Treatment Body Width (mm)	Day 10 Average Treatment Body Length (mm)
108	7/6/2007	X. laevis	Lead	1.9	A	1	3.5	5				
108	7/6/2007	X. laevis	Lead	1.9	A	2	4	4.5				
108	7/6/2007	X. laevis	Lead	1.9	A	3	2	3				
108	7/6/2007	X. laevis	Lead	1.9	A	4	3.5	4.5				
108	7/6/2007	X. laevis	Lead	1.9	A	5	4	5	3.4	4.4		
108	7/6/2007	X. laevis	Lead	1.9	B	1	3	4				
108	7/6/2007	X. laevis	Lead	1.9	B	2	-	-				
108	7/6/2007	X. laevis	Lead	1.9	B	3	-	-				
108	7/6/2007	X. laevis	Lead	1.9	B	4	-	-				
108	7/6/2007	X. laevis	Lead	1.9	B	5	-	-	0.6	0.8		
108	7/6/2007	X. laevis	Lead	1.9	C	1	4.5	6				
108	7/6/2007	X. laevis	Lead	1.9	C	2	4	5.5				
108	7/6/2007	X. laevis	Lead	1.9	C	3	4.5	6				
108	7/6/2007	X. laevis	Lead	1.9	C	4	4	5				
108	7/6/2007	X. laevis	Lead	1.9	C	5	3.5	5	4.1	5.5		
108	7/6/2007	X. laevis	Lead	1.9	D	1	-	-				
108	7/6/2007	X. laevis	Lead	1.9	D	2	-	-				
108	7/6/2007	X. laevis	Lead	1.9	D	3	-	-				
108	7/6/2007	X. laevis	Lead	1.9	D	4	-	-				
108	7/6/2007	X. laevis	Lead	1.9	D	5	-	-	0.0	0.0		
108	7/6/2007	X. laevis	Lead	1.9	E	1	4	5.5				
108	7/6/2007	X. laevis	Lead	1.9	E	2	-	-				
108	7/6/2007	X. laevis	Lead	1.9	E	3	-	-				
108	7/6/2007	X. laevis	Lead	1.9	E	4	-	-				
108	7/6/2007	X. laevis	Lead	1.9	E	5	-	-	0.8	1.1		
108	7/6/2007	X. laevis	Lead	1.9	F	1	5	6				
108	7/6/2007	X. laevis	Lead	1.9	F	2	5.5	6.5				
108	7/6/2007	X. laevis	Lead	1.9	F	3	5	6.5				
108	7/6/2007	X. laevis	Lead	1.9	F	4	-	-				
108	7/6/2007	X. laevis	Lead	1.9	F	5	-	-	3.1	3.8	2.0	2.6
108	7/6/2007	X. laevis	Lead	40	A	1	4	5.5				
108	7/6/2007	X. laevis	Lead	40	A	2	4.5	6				
108	7/6/2007	X. laevis	Lead	40	A	3	3	4				
108	7/6/2007	X. laevis	Lead	40	A	4	4	5				
108	7/6/2007	X. laevis	Lead	40	A	5	-	-	3.1	4.1		
108	7/6/2007	X. laevis	Lead	40	B	1	4	6				
108	7/6/2007	X. laevis	Lead	40	B	2	4	5.5				
108	7/6/2007	X. laevis	Lead	40	B	3	4	6				
108	7/6/2007	X. laevis	Lead	40	B	4	4.5	6				
108	7/6/2007	X. laevis	Lead	40	B	5	-	-	3.3	4.7		
108	7/6/2007	X. laevis	Lead	40	C	1	5	7				
108	7/6/2007	X. laevis	Lead	40	C	2	4	6				
108	7/6/2007	X. laevis	Lead	40	C	3	4	6				
108	7/6/2007	X. laevis	Lead	40	C	4	4	5.5				
108	7/6/2007	X. laevis	Lead	40	C	5	-	-	3.4	4.9		
108	7/6/2007	X. laevis	Lead	40	D	1	5	6				
108	7/6/2007	X. laevis	Lead	40	D	2	4	4.5				
108	7/6/2007	X. laevis	Lead	40	D	3	3	4				
108	7/6/2007	X. laevis	Lead	40	D	4	5	6				
108	7/6/2007	X. laevis	Lead	40	D	5	4	5	4.2	5.1		
108	7/6/2007	X. laevis	Lead	40	E	1	4	5				
108	7/6/2007	X. laevis	Lead	40	E	2	5	6				
108	7/6/2007	X. laevis	Lead	40	E	3	4	6				
108	7/6/2007	X. laevis	Lead	40	E	4	4	5				
108	7/6/2007	X. laevis	Lead	40	E	5	-	-	3.4	4.4		
108	7/6/2007	X. laevis	Lead	40	F	1	4.5	6				
108	7/6/2007	X. laevis	Lead	40	F	2	4	5				
108	7/6/2007	X. laevis	Lead	40	F	3	4	5.5				
108	7/6/2007	X. laevis	Lead	40	F	4	4	5.5				
108	7/6/2007	X. laevis	Lead	40	F	5	4	5.5	4.1	5.5	3.6	4.8
108	7/6/2007	X. laevis	Lead	77	A	1	4	5				
108	7/6/2007	X. laevis	Lead	77	A	2	4	5				
108	7/6/2007	X. laevis	Lead	77	A	3	4	5.5				
108	7/6/2007	X. laevis	Lead	77	A	4	4	4.5				
108	7/6/2007	X. laevis	Lead	77	A	5	4.5	6	4.1	5.2		
108	7/6/2007	X. laevis	Lead	77	B	1	5.5	6				
108	7/6/2007	X. laevis	Lead	77	B	2	4	5				
108	7/6/2007	X. laevis	Lead	77	B	3	4.5	5.5				
108	7/6/2007	X. laevis	Lead	77	B	4	-	-				
108	7/6/2007	X. laevis	Lead	77	B	5	-	-	2.8	3.3		
108	7/6/2007	X. laevis	Lead	77	C	1	4	5				
108	7/6/2007	X. laevis	Lead	77	C	2	4	5				
108	7/6/2007	X. laevis	Lead	77	C	3	4.5	5.5				
108	7/6/2007	X. laevis	Lead	77	C	4	4	5				
108	7/6/2007	X. laevis	Lead	77	C	5	-	-	3.3	4.1		
108	7/6/2007	X. laevis	Lead	77	D	1	5	6				
108	7/6/2007	X. laevis	Lead	77	D	2	5	6				
108	7/6/2007	X. laevis	Lead	77	D	3	5	5.5				
108	7/6/2007	X. laevis	Lead	77	D	4	5	6.5				
108	7/6/2007	X. laevis	Lead	77	D	5	4.5	5.5	4.9	5.9		
108	7/6/2007	X. laevis	Lead	77	E	1	4	6				
108	7/6/2007	X. laevis	Lead	77	E	2	4	6				
108	7/6/2007	X. laevis	Lead	77	E	3	4	6				
108	7/6/2007	X. laevis	Lead	77	E	4	4.5	6				
108	7/6/2007	X. laevis	Lead	77	E	5	-	-	3.3	4.8		
108	7/6/2007	X. laevis	Lead	77	F	1	4	6				
108	7/6/2007	X. laevis	Lead	77	F	2	4	6				
108	7/6/2007	X. laevis	Lead	77	F	3	4	5				
108	7/6/2007	X. laevis	Lead	77	F	4	3	4.5				
108	7/6/2007	X. laevis	Lead	77	F	5	3	4	3.6	5.1	3.7	4.7

SubLethal Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Organism	Body Width (mm)	Body Length (mm)	Day 10 Average Body Width (mm)	Day 10 Average Body Length (mm)	Day 10 Average Treatment Body Width (mm)	Day 10 Average Treatment Body Length (mm)
108	7/6/2007	X. laevis	Lead	140	A	1	4	5				
108	7/6/2007	X. laevis	Lead	140	A	2	4	5				
108	7/6/2007	X. laevis	Lead	140	A	3	4	5				
108	7/6/2007	X. laevis	Lead	140	A	4	4	5				
108	7/6/2007	X. laevis	Lead	140	A	5	4	5	4.0	5.0		
108	7/6/2007	X. laevis	Lead	140	B	1	4	5				
108	7/6/2007	X. laevis	Lead	140	B	2	4	5.5				
108	7/6/2007	X. laevis	Lead	140	B	3	5	6				
108	7/6/2007	X. laevis	Lead	140	B	4	4.5	5				
108	7/6/2007	X. laevis	Lead	140	B	5	-	-	3.5	4.3		
108	7/6/2007	X. laevis	Lead	140	C	1	4	6				
108	7/6/2007	X. laevis	Lead	140	C	2	4	6				
108	7/6/2007	X. laevis	Lead	140	C	3	4	5				
108	7/6/2007	X. laevis	Lead	140	C	4	4	6				
108	7/6/2007	X. laevis	Lead	140	C	5	3.5	5.5	3.9	5.7		
108	7/6/2007	X. laevis	Lead	140	D	1	3.5	5				
108	7/6/2007	X. laevis	Lead	140	D	2	4	5.5				
108	7/6/2007	X. laevis	Lead	140	D	3	3	4.5				
108	7/6/2007	X. laevis	Lead	140	D	4	3	4.5				
108	7/6/2007	X. laevis	Lead	140	D	5	3	4.5	3.3	4.6		
108	7/6/2007	X. laevis	Lead	140	E	1	4	6				
108	7/6/2007	X. laevis	Lead	140	E	2	5	7				
108	7/6/2007	X. laevis	Lead	140	E	3	4	5.5				
108	7/6/2007	X. laevis	Lead	140	E	4	-	-				
108	7/6/2007	X. laevis	Lead	140	E	5	-	-	2.6	3.7		
108	7/6/2007	X. laevis	Lead	140	F	1	4	6				
108	7/6/2007	X. laevis	Lead	140	F	2	4	5				
108	7/6/2007	X. laevis	Lead	140	F	3	4.5	6				
108	7/6/2007	X. laevis	Lead	140	F	4	4	5.5				
108	7/6/2007	X. laevis	Lead	140	F	5	3.5	4.5				
108	7/6/2007	X. laevis	Lead	140	F	6	3	4.5	3.8	5.3	3.5	4.8
108	7/6/2007	X. laevis	Lead	340	A	1	3.5	4.5				
108	7/6/2007	X. laevis	Lead	340	A	2	3.5	5.5				
108	7/6/2007	X. laevis	Lead	340	A	3	3.5	5				
108	7/6/2007	X. laevis	Lead	340	A	4	4	6				
108	7/6/2007	X. laevis	Lead	340	A	5	3	4	3.5	5.0		
108	7/6/2007	X. laevis	Lead	340	B	1	3	4.5				
108	7/6/2007	X. laevis	Lead	340	B	2	3	4.5				
108	7/6/2007	X. laevis	Lead	340	B	3	3	4.5				
108	7/6/2007	X. laevis	Lead	340	B	4	2.5	4				
108	7/6/2007	X. laevis	Lead	340	B	5	-	-	2.3	3.5		
108	7/6/2007	X. laevis	Lead	340	C	1	4	5				
108	7/6/2007	X. laevis	Lead	340	C	2	3	5				
108	7/6/2007	X. laevis	Lead	340	C	3	3	4				
108	7/6/2007	X. laevis	Lead	340	C	4	3	4				
108	7/6/2007	X. laevis	Lead	340	C	5	3.5	4.5	3.3	4.5		
108	7/6/2007	X. laevis	Lead	340	D	1	4	5.5				
108	7/6/2007	X. laevis	Lead	340	D	2	3	4				
108	7/6/2007	X. laevis	Lead	340	D	3	3	4.5				
108	7/6/2007	X. laevis	Lead	340	D	4	3.5	4.5				
108	7/6/2007	X. laevis	Lead	340	D	5	3	4	3.3	4.5		
108	7/6/2007	X. laevis	Lead	340	E	1	4	5.5				
108	7/6/2007	X. laevis	Lead	340	E	2	3.5	4.5				
108	7/6/2007	X. laevis	Lead	340	E	3	3	4				
108	7/6/2007	X. laevis	Lead	340	E	4	3.5	4.5				
108	7/6/2007	X. laevis	Lead	340	E	5	4	5.5	3.6	4.8		
108	7/6/2007	X. laevis	Lead	340	F	1	4	6				
108	7/6/2007	X. laevis	Lead	340	F	2	4	6.5				
108	7/6/2007	X. laevis	Lead	340	F	3	3.5	5				
108	7/6/2007	X. laevis	Lead	340	F	4	4	6				
108	7/6/2007	X. laevis	Lead	340	F	5	-	-	3.1	4.7	3.2	4.5
108	7/6/2007	X. laevis	Lead	750	A	1	-	-				
108	7/6/2007	X. laevis	Lead	750	A	2	-	-				
108	7/6/2007	X. laevis	Lead	750	A	3	-	-				
108	7/6/2007	X. laevis	Lead	750	A	4	-	-				
108	7/6/2007	X. laevis	Lead	750	A	5	-	-	0.0	0.0		
108	7/6/2007	X. laevis	Lead	750	B	1	2.5	3.5				
108	7/6/2007	X. laevis	Lead	750	B	2	2	3				
108	7/6/2007	X. laevis	Lead	750	B	3	-	-				
108	7/6/2007	X. laevis	Lead	750	B	4	-	-				
108	7/6/2007	X. laevis	Lead	750	B	5	-	-	0.9	1.3		
108	7/6/2007	X. laevis	Lead	750	C	1	3	4				
108	7/6/2007	X. laevis	Lead	750	C	2	2.5	3.5				
108	7/6/2007	X. laevis	Lead	750	C	3	3	4				
108	7/6/2007	X. laevis	Lead	750	C	4	-	-				
108	7/6/2007	X. laevis	Lead	750	C	5	-	-	1.7	2.3		
108	7/6/2007	X. laevis	Lead	750	D	1	-	-				
108	7/6/2007	X. laevis	Lead	750	D	2	-	-				
108	7/6/2007	X. laevis	Lead	750	D	3	-	-				
108	7/6/2007	X. laevis	Lead	750	D	4	-	-				
108	7/6/2007	X. laevis	Lead	750	D	5	-	-	0.0	0.0		
108	7/6/2007	X. laevis	Lead	750	E	1	2.5	3.5				
108	7/6/2007	X. laevis	Lead	750	E	2	2.5	3.5				
108	7/6/2007	X. laevis	Lead	750	E	3	2.5	3.5				
108	7/6/2007	X. laevis	Lead	750	E	4	3	4				
108	7/6/2007	X. laevis	Lead	750	E	5	2	3	2.5	3.5		
108	7/6/2007	X. laevis	Lead	750	F	1	-	-				
108	7/6/2007	X. laevis	Lead	750	F	2	-	-				
108	7/6/2007	X. laevis	Lead	750	F	3	-	-				
108	7/6/2007	X. laevis	Lead	750	F	4	-	-				
108	7/6/2007	X. laevis	Lead	750	F	5	-	-	0.0	0.0	0.9	1.2

SubLethal Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Organism	Body Width (mm)	Body Length (mm)	Day 10 Average Body Width (mm)	Day 10 Average Body Length (mm)	Day 10 Average Treatment Body Width (mm)	Day 10 Average Treatment Body Length (mm)
114	9/28/2007	X. tropicalis	Lead	1.9	A	1	3	3				
114	9/28/2007	X. tropicalis	Lead	1.9	A	2	2.5	3				
114	9/28/2007	X. tropicalis	Lead	1.9	A	3	3	3.5				
114	9/28/2007	X. tropicalis	Lead	1.9	A	4	2	3				
114	9/28/2007	X. tropicalis	Lead	1.9	A	5	2	3	2.5	3.1		
114	9/28/2007	X. tropicalis	Lead	1.9	B	1	2	2				
114	9/28/2007	X. tropicalis	Lead	1.9	B	2	1.5	2				
114	9/28/2007	X. tropicalis	Lead	1.9	B	3	2	2.5				
114	9/28/2007	X. tropicalis	Lead	1.9	B	4	2	2				
114	9/28/2007	X. tropicalis	Lead	1.9	B	5	-	-	1.5	1.7		
114	9/28/2007	X. tropicalis	Lead	1.9	C	1	2.5	2.5				
114	9/28/2007	X. tropicalis	Lead	1.9	C	2	2	2.5				
114	9/28/2007	X. tropicalis	Lead	1.9	C	3	-	-				
114	9/28/2007	X. tropicalis	Lead	1.9	C	4	-	-				
114	9/28/2007	X. tropicalis	Lead	1.9	C	5	-	-	0.9	1.0		
114	9/28/2007	X. tropicalis	Lead	1.9	D	1	2	2				
114	9/28/2007	X. tropicalis	Lead	1.9	D	2	2	2.5				
114	9/28/2007	X. tropicalis	Lead	1.9	D	3	2	2.5				
114	9/28/2007	X. tropicalis	Lead	1.9	D	4	-	-				
114	9/28/2007	X. tropicalis	Lead	1.9	D	5	-	-	1.2	1.4		
114	9/28/2007	X. tropicalis	Lead	1.9	E	1	2.5	3				
114	9/28/2007	X. tropicalis	Lead	1.9	E	2	2	2.5				
114	9/28/2007	X. tropicalis	Lead	1.9	E	3	2	3				
114	9/28/2007	X. tropicalis	Lead	1.9	E	4	2	2				
114	9/28/2007	X. tropicalis	Lead	1.9	E	5	2.5	3	2.2	2.7		
114	9/28/2007	X. tropicalis	Lead	1.9	F	1	2.5	3				
114	9/28/2007	X. tropicalis	Lead	1.9	F	2	3	3				
114	9/28/2007	X. tropicalis	Lead	1.9	F	3	2.5	3				
114	9/28/2007	X. tropicalis	Lead	1.9	F	4	2.5	2.5				
114	9/28/2007	X. tropicalis	Lead	1.9	F	5	2.5	3	2.6	2.9	1.8	2.1
114	9/28/2007	X. tropicalis	Lead	40	A	1	2	2.5				
114	9/28/2007	X. tropicalis	Lead	40	A	2	2	2.5				
114	9/28/2007	X. tropicalis	Lead	40	A	3	2	2.5				
114	9/28/2007	X. tropicalis	Lead	40	A	4	2	2.5				
114	9/28/2007	X. tropicalis	Lead	40	A	5	2	3	2.0	2.6		
114	9/28/2007	X. tropicalis	Lead	40	B	1	2.5	2.5				
114	9/28/2007	X. tropicalis	Lead	40	B	2	2.5	3				
114	9/28/2007	X. tropicalis	Lead	40	B	3	2	2.5				
114	9/28/2007	X. tropicalis	Lead	40	B	4	2	2.5				
114	9/28/2007	X. tropicalis	Lead	40	B	5	2	2.5	2.2	2.6		
114	9/28/2007	X. tropicalis	Lead	40	C	1	2	2				
114	9/28/2007	X. tropicalis	Lead	40	C	2	-	-				
114	9/28/2007	X. tropicalis	Lead	40	C	3	-	-				
114	9/28/2007	X. tropicalis	Lead	40	C	4	-	-				
114	9/28/2007	X. tropicalis	Lead	40	C	5	-	-	0.4	0.4		
114	9/28/2007	X. tropicalis	Lead	40	D	1	2	2.5				
114	9/28/2007	X. tropicalis	Lead	40	D	2	-	-				
114	9/28/2007	X. tropicalis	Lead	40	D	3	-	-				
114	9/28/2007	X. tropicalis	Lead	40	D	4	-	-				
114	9/28/2007	X. tropicalis	Lead	40	D	5	-	-	0.4	0.5		
114	9/28/2007	X. tropicalis	Lead	40	E	1	-	-				
114	9/28/2007	X. tropicalis	Lead	40	E	2	-	-				
114	9/28/2007	X. tropicalis	Lead	40	E	3	-	-				
114	9/28/2007	X. tropicalis	Lead	40	E	4	-	-				
114	9/28/2007	X. tropicalis	Lead	40	E	5	-	-	0.0	0.0		
114	9/28/2007	X. tropicalis	Lead	40	F	1	2.5	2.5				
114	9/28/2007	X. tropicalis	Lead	40	F	2	2	2.5				
114	9/28/2007	X. tropicalis	Lead	40	F	3	2	2				
114	9/28/2007	X. tropicalis	Lead	40	F	4	-	-				
114	9/28/2007	X. tropicalis	Lead	40	F	5	-	-	1.3	1.4	1.1	1.3
114	9/28/2007	X. tropicalis	Lead	77	A	1	2	2.5				
114	9/28/2007	X. tropicalis	Lead	77	A	2	2	2.5				
114	9/28/2007	X. tropicalis	Lead	77	A	3	-	-				
114	9/28/2007	X. tropicalis	Lead	77	A	4	-	-				
114	9/28/2007	X. tropicalis	Lead	77	A	5	-	-	0.8	1.0		
114	9/28/2007	X. tropicalis	Lead	77	B	1	2	2.5				
114	9/28/2007	X. tropicalis	Lead	77	B	2	2	3				
114	9/28/2007	X. tropicalis	Lead	77	B	3	2	2				
114	9/28/2007	X. tropicalis	Lead	77	B	4	2.5	3				
114	9/28/2007	X. tropicalis	Lead	77	B	5	-	-	1.7	2.1		
114	9/28/2007	X. tropicalis	Lead	77	C	1	-	-				
114	9/28/2007	X. tropicalis	Lead	77	C	2	-	-				
114	9/28/2007	X. tropicalis	Lead	77	C	3	-	-				
114	9/28/2007	X. tropicalis	Lead	77	C	4	-	-				
114	9/28/2007	X. tropicalis	Lead	77	C	5	-	-	0.0	0.0		
114	9/28/2007	X. tropicalis	Lead	77	D	1	2.5	3				
114	9/28/2007	X. tropicalis	Lead	77	D	2	2.5	3				
114	9/28/2007	X. tropicalis	Lead	77	D	3	3	3				
114	9/28/2007	X. tropicalis	Lead	77	D	4	2	3				
114	9/28/2007	X. tropicalis	Lead	77	D	5	-	-	2.0	2.4		
114	9/28/2007	X. tropicalis	Lead	77	E	1	2.5	3				
114	9/28/2007	X. tropicalis	Lead	77	E	2	2.5	3				
114	9/28/2007	X. tropicalis	Lead	77	E	3	2	3				
114	9/28/2007	X. tropicalis	Lead	77	E	4	2.5	3				
114	9/28/2007	X. tropicalis	Lead	77	E	5	-	-	1.9	2.4		
114	9/28/2007	X. tropicalis	Lead	77	F	1	3	3				
114	9/28/2007	X. tropicalis	Lead	77	F	2	2.5	2.5				
114	9/28/2007	X. tropicalis	Lead	77	F	3	3	3.5				
114	9/28/2007	X. tropicalis	Lead	77	F	4	2.5	3				
114	9/28/2007	X. tropicalis	Lead	77	F	5	-	-	2.2	2.4	1.4	1.7

SubLethal Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Organism	Body Width (mm)	Body Length (mm)	Day 10 Average Body Width (mm)	Day 10 Average Body Length (mm)	Day 10 Average Treatment Body Width (mm)	Day 10 Average Treatment Body Length (mm)
114	9/28/2007	X. tropicalis	Lead	140	A	1	2	2				
114	9/28/2007	X. tropicalis	Lead	140	A	2	2	2				
114	9/28/2007	X. tropicalis	Lead	140	A	3	2	2				
114	9/28/2007	X. tropicalis	Lead	140	A	4	-	-				
114	9/28/2007	X. tropicalis	Lead	140	A	5	-	-	1.2	1.2		
114	9/28/2007	X. tropicalis	Lead	140	B	1	2	2				
114	9/28/2007	X. tropicalis	Lead	140	B	2	2	2				
114	9/28/2007	X. tropicalis	Lead	140	B	3	-	-				
114	9/28/2007	X. tropicalis	Lead	140	B	4	-	-				
114	9/28/2007	X. tropicalis	Lead	140	B	5	-	-	0.8	0.8		
114	9/28/2007	X. tropicalis	Lead	140	C	1	-	-				
114	9/28/2007	X. tropicalis	Lead	140	C	2	-	-				
114	9/28/2007	X. tropicalis	Lead	140	C	3	-	-				
114	9/28/2007	X. tropicalis	Lead	140	C	4	-	-				
114	9/28/2007	X. tropicalis	Lead	140	C	5	-	-	0.0	0.0		
114	9/28/2007	X. tropicalis	Lead	140	D	1	-	-				
114	9/28/2007	X. tropicalis	Lead	140	D	2	-	-				
114	9/28/2007	X. tropicalis	Lead	140	D	3	-	-				
114	9/28/2007	X. tropicalis	Lead	140	D	4	-	-				
114	9/28/2007	X. tropicalis	Lead	140	D	5	-	-	0.0	0.0		
114	9/28/2007	X. tropicalis	Lead	140	E	1	2	2				
114	9/28/2007	X. tropicalis	Lead	140	E	2	1.5	2				
114	9/28/2007	X. tropicalis	Lead	140	E	3	-	-				
114	9/28/2007	X. tropicalis	Lead	140	E	4	-	-				
114	9/28/2007	X. tropicalis	Lead	140	E	5	-	-	0.7	0.8		
114	9/28/2007	X. tropicalis	Lead	140	F	1	2	2				
114	9/28/2007	X. tropicalis	Lead	140	F	2	2	2				
114	9/28/2007	X. tropicalis	Lead	140	F	3	2	2				
114	9/28/2007	X. tropicalis	Lead	140	F	4	-	-				
114	9/28/2007	X. tropicalis	Lead	140	F	5	-	-	1.2	1.2	0.7	0.7
114	9/28/2007	X. tropicalis	Lead	340	A	1	-	-				
114	9/28/2007	X. tropicalis	Lead	340	A	2	-	-				
114	9/28/2007	X. tropicalis	Lead	340	A	3	-	-				
114	9/28/2007	X. tropicalis	Lead	340	A	4	-	-				
114	9/28/2007	X. tropicalis	Lead	340	A	5	-	-	0.0	0.0		
114	9/28/2007	X. tropicalis	Lead	340	B	1	2	2				
114	9/28/2007	X. tropicalis	Lead	340	B	2	-	-				
114	9/28/2007	X. tropicalis	Lead	340	B	3	-	-				
114	9/28/2007	X. tropicalis	Lead	340	B	4	-	-				
114	9/28/2007	X. tropicalis	Lead	340	B	5	-	-	0.4	0.4		
114	9/28/2007	X. tropicalis	Lead	340	C	1	-	-				
114	9/28/2007	X. tropicalis	Lead	340	C	2	-	-				
114	9/28/2007	X. tropicalis	Lead	340	C	3	-	-				
114	9/28/2007	X. tropicalis	Lead	340	C	4	-	-				
114	9/28/2007	X. tropicalis	Lead	340	C	5	-	-	0.0	0.0		
114	9/28/2007	X. tropicalis	Lead	340	D	1	-	-				
114	9/28/2007	X. tropicalis	Lead	340	D	2	-	-				
114	9/28/2007	X. tropicalis	Lead	340	D	3	-	-				
114	9/28/2007	X. tropicalis	Lead	340	D	4	-	-				
114	9/28/2007	X. tropicalis	Lead	340	D	5	-	-	0.0	0.0		
114	9/28/2007	X. tropicalis	Lead	340	E	1	-	-				
114	9/28/2007	X. tropicalis	Lead	340	E	2	-	-				
114	9/28/2007	X. tropicalis	Lead	340	E	3	-	-				
114	9/28/2007	X. tropicalis	Lead	340	E	4	-	-				
114	9/28/2007	X. tropicalis	Lead	340	E	5	-	-	0.0	0.0		
114	9/28/2007	X. tropicalis	Lead	340	F	1	-	-				
114	9/28/2007	X. tropicalis	Lead	340	F	2	-	-				
114	9/28/2007	X. tropicalis	Lead	340	F	3	-	-				
114	9/28/2007	X. tropicalis	Lead	340	F	4	-	-				
114	9/28/2007	X. tropicalis	Lead	340	F	5	-	-	0.0	0.0	0.1	0.1
113	9/28/2007	X. tropicalis	Copper	3.2	A	1	2	2.5				
113	9/28/2007	X. tropicalis	Copper	3.2	A	2	-	-				
113	9/28/2007	X. tropicalis	Copper	3.2	A	3	-	-				
113	9/28/2007	X. tropicalis	Copper	3.2	A	4	-	-				
113	9/28/2007	X. tropicalis	Copper	3.2	A	5	-	-	0.4	0.5		
113	9/28/2007	X. tropicalis	Copper	3.2	B	1	-	-				
113	9/28/2007	X. tropicalis	Copper	3.2	B	2	-	-				
113	9/28/2007	X. tropicalis	Copper	3.2	B	3	-	-				
113	9/28/2007	X. tropicalis	Copper	3.2	B	4	-	-				
113	9/28/2007	X. tropicalis	Copper	3.2	B	5	-	-	0.0	0.0		
113	9/28/2007	X. tropicalis	Copper	3.2	C	1	2	2.5				
113	9/28/2007	X. tropicalis	Copper	3.2	C	2	2	2				
113	9/28/2007	X. tropicalis	Copper	3.2	C	3	2	2				
113	9/28/2007	X. tropicalis	Copper	3.2	C	4	2	2				
113	9/28/2007	X. tropicalis	Copper	3.2	C	5	-	-	1.6	1.7		
113	9/28/2007	X. tropicalis	Copper	3.2	D	1	2	2				
113	9/28/2007	X. tropicalis	Copper	3.2	D	2	2	2				
113	9/28/2007	X. tropicalis	Copper	3.2	D	3	2	2				
113	9/28/2007	X. tropicalis	Copper	3.2	D	4	-	-				
113	9/28/2007	X. tropicalis	Copper	3.2	D	5	-	-	1.2	1.2		
113	9/28/2007	X. tropicalis	Copper	3.2	E	1	2	2.5				
113	9/28/2007	X. tropicalis	Copper	3.2	E	2	2	2				
113	9/28/2007	X. tropicalis	Copper	3.2	E	3	2	2.5				
113	9/28/2007	X. tropicalis	Copper	3.2	E	4	-	-				
113	9/28/2007	X. tropicalis	Copper	3.2	E	5	-	-	1.2	1.4		
113	9/28/2007	X. tropicalis	Copper	3.2	F	1	2	2				
113	9/28/2007	X. tropicalis	Copper	3.2	F	2	2	2				
113	9/28/2007	X. tropicalis	Copper	3.2	F	3	-	-				
113	9/28/2007	X. tropicalis	Copper	3.2	F	4	-	-				
113	9/28/2007	X. tropicalis	Copper	3.2	F	5	-	-	0.8	0.8	0.9	0.9

SubLethal Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Organism	Body Width (mm)	Body Length (mm)	Day 10 Average Body Width (mm)	Day 10 Average Body Length (mm)	Day 10 Average Treatment Body Width (mm)	Day 10 Average Treatment Body Length (mm)
113	9/28/2007	X. tropicalis	Copper	13	A	1	2	2				
113	9/28/2007	X. tropicalis	Copper	13	A	2	2	2				
113	9/28/2007	X. tropicalis	Copper	13	A	3	2	2				
113	9/28/2007	X. tropicalis	Copper	13	A	4	-	-				
113	9/28/2007	X. tropicalis	Copper	13	A	5	-	-	1.2	1.2		
113	9/28/2007	X. tropicalis	Copper	13	B	1	2	2.5				
113	9/28/2007	X. tropicalis	Copper	13	B	2	2	2				
113	9/28/2007	X. tropicalis	Copper	13	B	3	2	2				
113	9/28/2007	X. tropicalis	Copper	13	B	4	2	2				
113	9/28/2007	X. tropicalis	Copper	13	B	5	-	-	1.6	1.7		
113	9/28/2007	X. tropicalis	Copper	13	C	1	2	2				
113	9/28/2007	X. tropicalis	Copper	13	C	2	-	-				
113	9/28/2007	X. tropicalis	Copper	13	C	3	-	-				
113	9/28/2007	X. tropicalis	Copper	13	C	4	-	-				
113	9/28/2007	X. tropicalis	Copper	13	C	5	-	-	0.4	0.4		
113	9/28/2007	X. tropicalis	Copper	13	D	1	2	2				
113	9/28/2007	X. tropicalis	Copper	13	D	2	-	-				
113	9/28/2007	X. tropicalis	Copper	13	D	3	-	-				
113	9/28/2007	X. tropicalis	Copper	13	D	4	-	-				
113	9/28/2007	X. tropicalis	Copper	13	D	5	-	-	0.4	0.4		
113	9/28/2007	X. tropicalis	Copper	13	E	1	2.5	3.5				
113	9/28/2007	X. tropicalis	Copper	13	E	2	2.5	3				
113	9/28/2007	X. tropicalis	Copper	13	E	3	-	-				
113	9/28/2007	X. tropicalis	Copper	13	E	4	-	-				
113	9/28/2007	X. tropicalis	Copper	13	E	5	-	-	1.0	1.3		
113	9/28/2007	X. tropicalis	Copper	13	F	1	-	-				
113	9/28/2007	X. tropicalis	Copper	13	F	2	-	-				
113	9/28/2007	X. tropicalis	Copper	13	F	3	-	-				
113	9/28/2007	X. tropicalis	Copper	13	F	4	-	-				
113	9/28/2007	X. tropicalis	Copper	13	F	5	-	-	0.0	0.0	0.8	0.8
113	9/28/2007	X. tropicalis	Copper	32	A	1	2	2				
113	9/28/2007	X. tropicalis	Copper	32	A	2	-	-				
113	9/28/2007	X. tropicalis	Copper	32	A	3	-	-				
113	9/28/2007	X. tropicalis	Copper	32	A	4	-	-				
113	9/28/2007	X. tropicalis	Copper	32	A	5	-	-	0.4	0.4		
113	9/28/2007	X. tropicalis	Copper	32	B	1	2	2				
113	9/28/2007	X. tropicalis	Copper	32	B	2	-	-				
113	9/28/2007	X. tropicalis	Copper	32	B	3	-	-				
113	9/28/2007	X. tropicalis	Copper	32	B	4	-	-				
113	9/28/2007	X. tropicalis	Copper	32	B	5	-	-	0.4	0.4		
113	9/28/2007	X. tropicalis	Copper	32	C	1	2	2				
113	9/28/2007	X. tropicalis	Copper	32	C	2	2	2.5				
113	9/28/2007	X. tropicalis	Copper	32	C	3	-	-				
113	9/28/2007	X. tropicalis	Copper	32	C	4	-	-				
113	9/28/2007	X. tropicalis	Copper	32	C	5	-	-	0.8	0.9		
113	9/28/2007	X. tropicalis	Copper	32	D	1	2	2				
113	9/28/2007	X. tropicalis	Copper	32	D	2	-	-				
113	9/28/2007	X. tropicalis	Copper	32	D	3	-	-				
113	9/28/2007	X. tropicalis	Copper	32	D	4	-	-				
113	9/28/2007	X. tropicalis	Copper	32	D	5	-	-	0.4	0.4		
113	9/28/2007	X. tropicalis	Copper	32	E	1	-	-				
113	9/28/2007	X. tropicalis	Copper	32	E	2	-	-				
113	9/28/2007	X. tropicalis	Copper	32	E	3	-	-				
113	9/28/2007	X. tropicalis	Copper	32	E	4	-	-				
113	9/28/2007	X. tropicalis	Copper	32	E	5	-	-	0.0	0.0		
113	9/28/2007	X. tropicalis	Copper	32	F	1	2	2				
113	9/28/2007	X. tropicalis	Copper	32	F	2	2	2				
113	9/28/2007	X. tropicalis	Copper	32	F	3	-	-				
113	9/28/2007	X. tropicalis	Copper	32	F	4	-	-				
113	9/28/2007	X. tropicalis	Copper	32	F	5	-	-	0.8	0.8	0.5	0.5
119	2/19/2008	X. laevis	Lead	1.9	A	1	3	4.5				
119	2/19/2008	X. laevis	Lead	1.9	A	2	3	4.5				
119	2/19/2008	X. laevis	Lead	1.9	A	3	3	4.5				
119	2/19/2008	X. laevis	Lead	1.9	A	4	3	4.5				
119	2/19/2008	X. laevis	Lead	1.9	A	5	3	4.5	3.0	4.5		
119	2/19/2008	X. laevis	Lead	1.9	B	1	-	-				
119	2/19/2008	X. laevis	Lead	1.9	B	2	-	-				
119	2/19/2008	X. laevis	Lead	1.9	B	3	-	-				
119	2/19/2008	X. laevis	Lead	1.9	B	4	-	-				
119	2/19/2008	X. laevis	Lead	1.9	B	5	-	-	0.0	0.0		
119	2/19/2008	X. laevis	Lead	1.9	C	1	3	4.5				
119	2/19/2008	X. laevis	Lead	1.9	C	2	3	4.5				
119	2/19/2008	X. laevis	Lead	1.9	C	3	2.5	4				
119	2/19/2008	X. laevis	Lead	1.9	C	4	3	4				
119	2/19/2008	X. laevis	Lead	1.9	C	5	2.5	4	2.8	4.2		
119	2/19/2008	X. laevis	Lead	1.9	D	1	-	-				
119	2/19/2008	X. laevis	Lead	1.9	D	2	-	-				
119	2/19/2008	X. laevis	Lead	1.9	D	3	-	-				
119	2/19/2008	X. laevis	Lead	1.9	D	4	-	-				
119	2/19/2008	X. laevis	Lead	1.9	D	5	-	-	0.0	0.0		
119	2/19/2008	X. laevis	Lead	1.9	E	1	-	-				
119	2/19/2008	X. laevis	Lead	1.9	E	2	-	-				
119	2/19/2008	X. laevis	Lead	1.9	E	3	-	-				
119	2/19/2008	X. laevis	Lead	1.9	E	4	-	-				
119	2/19/2008	X. laevis	Lead	1.9	E	5	-	-	0.0	0.0		
119	2/19/2008	X. laevis	Lead	1.9	F	1	2.5	4.5				
119	2/19/2008	X. laevis	Lead	1.9	F	2	-	-				
119	2/19/2008	X. laevis	Lead	1.9	F	3	-	-				
119	2/19/2008	X. laevis	Lead	1.9	F	4	-	-				
119	2/19/2008	X. laevis	Lead	1.9	F	5	-	-	0.5	0.9	1.1	1.6

SubLethal Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Organism	Body Width (mm)	Body Length (mm)	Day 10 Average Body Width (mm)	Day 10 Average Body Length (mm)	Day 10 Average Treatment Body Width (mm)	Day 10 Average Treatment Body Length (mm)
119	2/19/2008	X. laevis	Lead	140	A	1	3	4				
119	2/19/2008	X. laevis	Lead	140	A	2	-	-				
119	2/19/2008	X. laevis	Lead	140	A	3	-	-				
119	2/19/2008	X. laevis	Lead	140	A	4	-	-				
119	2/19/2008	X. laevis	Lead	140	A	5	-	-	0.6	0.8		
119	2/19/2008	X. laevis	Lead	140	B	1	2.5	3.5				
119	2/19/2008	X. laevis	Lead	140	B	2	-	-				
119	2/19/2008	X. laevis	Lead	140	B	3	-	-				
119	2/19/2008	X. laevis	Lead	140	B	4	-	-				
119	2/19/2008	X. laevis	Lead	140	B	5	-	-	0.5	0.7		
119	2/19/2008	X. laevis	Lead	140	C	1	3.5	4.5				
119	2/19/2008	X. laevis	Lead	140	C	2	2	3.5				
119	2/19/2008	X. laevis	Lead	140	C	3	2.5	4				
119	2/19/2008	X. laevis	Lead	140	C	4	2	3.5				
119	2/19/2008	X. laevis	Lead	140	C	5	2.5	3.5	2.5	3.8		
119	2/19/2008	X. laevis	Lead	140	D	1	2	3				
119	2/19/2008	X. laevis	Lead	140	D	2	2	3				
119	2/19/2008	X. laevis	Lead	140	D	3	-	-				
119	2/19/2008	X. laevis	Lead	140	D	4	-	-				
119	2/19/2008	X. laevis	Lead	140	D	5	-	-	0.8	1.2		
119	2/19/2008	X. laevis	Lead	140	E	1	2.5	4				
119	2/19/2008	X. laevis	Lead	140	E	2	-	-				
119	2/19/2008	X. laevis	Lead	140	E	3	-	-				
119	2/19/2008	X. laevis	Lead	140	E	4	-	-				
119	2/19/2008	X. laevis	Lead	140	E	5	-	-	0.5	0.8		
119	2/19/2008	X. laevis	Lead	140	F	1	2	3				
119	2/19/2008	X. laevis	Lead	140	F	2	-	-				
119	2/19/2008	X. laevis	Lead	140	F	3	-	-				
119	2/19/2008	X. laevis	Lead	140	F	4	-	-				
119	2/19/2008	X. laevis	Lead	140	F	5	-	-	0.4	0.6	0.9	1.3
119	2/19/2008	X. laevis	Lead	340	A	1	2	4				
119	2/19/2008	X. laevis	Lead	340	A	2	2	4				
119	2/19/2008	X. laevis	Lead	340	A	3	2	4				
119	2/19/2008	X. laevis	Lead	340	A	4	2	4				
119	2/19/2008	X. laevis	Lead	340	A	5	2	3.5	2.0	3.9		
119	2/19/2008	X. laevis	Lead	340	B	1	3	5				
119	2/19/2008	X. laevis	Lead	340	B	2	2.5	3.5				
119	2/19/2008	X. laevis	Lead	340	B	3	2.5	3.5				
119	2/19/2008	X. laevis	Lead	340	B	4	-	-				
119	2/19/2008	X. laevis	Lead	340	B	5	-	-	1.6	2.4		
119	2/19/2008	X. laevis	Lead	340	C	1	2	3.5				
119	2/19/2008	X. laevis	Lead	340	C	2	2	3.5				
119	2/19/2008	X. laevis	Lead	340	C	3	2	3.5				
119	2/19/2008	X. laevis	Lead	340	C	4	2	3.5				
119	2/19/2008	X. laevis	Lead	340	C	5	-	-	1.6	2.8		
119	2/19/2008	X. laevis	Lead	340	D	1	2.5	4.5				
119	2/19/2008	X. laevis	Lead	340	D	2	3	4.5				
119	2/19/2008	X. laevis	Lead	340	D	3	3	4.5				
119	2/19/2008	X. laevis	Lead	340	D	4	2.5	4				
119	2/19/2008	X. laevis	Lead	340	D	5	2.5	4	2.7	4.3		
119	2/19/2008	X. laevis	Lead	340	E	1	3	5				
119	2/19/2008	X. laevis	Lead	340	E	2	3	4.5				
119	2/19/2008	X. laevis	Lead	340	E	3	2.5	4.5				
119	2/19/2008	X. laevis	Lead	340	E	4	2.5	4				
119	2/19/2008	X. laevis	Lead	340	E	5	-	-	2.2	3.6		
119	2/19/2008	X. laevis	Lead	340	F	1	2.5	4				
119	2/19/2008	X. laevis	Lead	340	F	2	3	4.5				
119	2/19/2008	X. laevis	Lead	340	F	3	2.5	3.5				
119	2/19/2008	X. laevis	Lead	340	F	4	2	3.5				
119	2/19/2008	X. laevis	Lead	340	F	5	2	3.5	2.4	3.8	2.1	3.5
119	2/19/2008	X. laevis	Lead	750	A	1	3	3.5				
119	2/19/2008	X. laevis	Lead	750	A	2	-	-				
119	2/19/2008	X. laevis	Lead	750	A	3	-	-				
119	2/19/2008	X. laevis	Lead	750	A	4	-	-				
119	2/19/2008	X. laevis	Lead	750	A	5	-	-	0.6	0.7		
119	2/19/2008	X. laevis	Lead	750	B	1	2.5	4				
119	2/19/2008	X. laevis	Lead	750	B	2	3	4				
119	2/19/2008	X. laevis	Lead	750	B	3	-	-				
119	2/19/2008	X. laevis	Lead	750	B	4	-	-				
119	2/19/2008	X. laevis	Lead	750	B	5	-	-	1.1	1.6		
119	2/19/2008	X. laevis	Lead	750	C	1	3	4				
119	2/19/2008	X. laevis	Lead	750	C	2	2	3.5				
119	2/19/2008	X. laevis	Lead	750	C	3	-	-				
119	2/19/2008	X. laevis	Lead	750	C	4	-	-				
119	2/19/2008	X. laevis	Lead	750	C	5	-	-	1.0	1.5		
119	2/19/2008	X. laevis	Lead	750	D	1	-	-				
119	2/19/2008	X. laevis	Lead	750	D	2	-	-				
119	2/19/2008	X. laevis	Lead	750	D	3	-	-				
119	2/19/2008	X. laevis	Lead	750	D	4	-	-				
119	2/19/2008	X. laevis	Lead	750	D	5	-	-	0.0	0.0		
119	2/19/2008	X. laevis	Lead	750	E	1	1	2.5				
119	2/19/2008	X. laevis	Lead	750	E	2	-	-				
119	2/19/2008	X. laevis	Lead	750	E	3	-	-				
119	2/19/2008	X. laevis	Lead	750	E	4	-	-				
119	2/19/2008	X. laevis	Lead	750	E	5	-	-	0.2	0.5		
119	2/19/2008	X. laevis	Lead	750	F	1	-	-				
119	2/19/2008	X. laevis	Lead	750	F	2	-	-				
119	2/19/2008	X. laevis	Lead	750	F	3	-	-				
119	2/19/2008	X. laevis	Lead	750	F	4	-	-				
119	2/19/2008	X. laevis	Lead	750	F	5	-	-	0.0	0.0	0.5	0.7

SubLethal Results

Test Number	Test Start Date	Species	Metal	Concentration (mg/kg)	Replicate	Organism	Body Width (mm)	Body Length (mm)	Day 10 Average Body Width (mm)	Day 10 Average Body Length (mm)	Day 10 Average Treatment Body Width (mm)	Day 10 Average Treatment Body Length (mm)
119	2/19/2008	X. laevis	Lead	1900	A	1	-	-				
119	2/19/2008	X. laevis	Lead	1900	A	2	-	-				
119	2/19/2008	X. laevis	Lead	1900	A	3	-	-				
119	2/19/2008	X. laevis	Lead	1900	A	4	-	-				
119	2/19/2008	X. laevis	Lead	1900	A	5	-	-	0.0	0.0		
119	2/19/2008	X. laevis	Lead	1900	B	1	-	-				
119	2/19/2008	X. laevis	Lead	1900	B	2	-	-				
119	2/19/2008	X. laevis	Lead	1900	B	3	-	-				
119	2/19/2008	X. laevis	Lead	1900	B	4	-	-				
119	2/19/2008	X. laevis	Lead	1900	B	5	-	-	0.0	0.0		
119	2/19/2008	X. laevis	Lead	1900	C	1	-	-				
119	2/19/2008	X. laevis	Lead	1900	C	2	-	-				
119	2/19/2008	X. laevis	Lead	1900	C	3	-	-				
119	2/19/2008	X. laevis	Lead	1900	C	4	-	-				
119	2/19/2008	X. laevis	Lead	1900	C	5	-	-	0.0	0.0		
119	2/19/2008	X. laevis	Lead	1900	D	1	-	-				
119	2/19/2008	X. laevis	Lead	1900	D	2	-	-				
119	2/19/2008	X. laevis	Lead	1900	D	3	-	-				
119	2/19/2008	X. laevis	Lead	1900	D	4	-	-				
119	2/19/2008	X. laevis	Lead	1900	D	5	-	-	0.0	0.0		
119	2/19/2008	X. laevis	Lead	1900	E	1	-	-				
119	2/19/2008	X. laevis	Lead	1900	E	2	-	-				
119	2/19/2008	X. laevis	Lead	1900	E	3	-	-				
119	2/19/2008	X. laevis	Lead	1900	E	4	-	-				
119	2/19/2008	X. laevis	Lead	1900	E	5	-	-	0.0	0.0		
119	2/19/2008	X. laevis	Lead	1900	F	1	-	-				
119	2/19/2008	X. laevis	Lead	1900	F	2	-	-				
119	2/19/2008	X. laevis	Lead	1900	F	3	-	-				
119	2/19/2008	X. laevis	Lead	1900	F	4	-	-				
119	2/19/2008	X. laevis	Lead	1900	F	5	-	-	0.0	0.0	0.0	0.0