ESTCP
Cost and Performance Report

(ER-0514)

Demonstration and Certification of Amphibian Ecological Risk Assessment Protocol

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ENVIRONMENTAL SECURITY
TECHNOLOGY CERTIFICATION PROGRAM

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COST & PERFORMANCE REPORT
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TABLE OF CONTENTS

Page

TABLE OF CONTENTS (continued)

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

ACRONYMS AND ABBREVIATIONS

AFB	Air Force Base
AFCEE	Air Force Center for Engineering and the Environment
APG	Aberdeen Proving Ground
ASTM	American Society of Testing and Material
AVS	acid volatile sulfides
BTAG	Biological Technical Assistance Group
CEC	cation exchange capacity
DNT	2,4-dinitrotoluene
DOC	dissolved organic carbon
DoD	Department of Defense
DOE	Department of Energy
Eco-SSL	Ecological Soil Screening Level
ERA	Ecological Risk Assessment
ESTCP	Environmental Security Technology Certification Program
FCETL	Fort Collins Environmental Toxicology Laboratory
FETAX	Frog Embryo Teratogenesis Assay-Xenopus
FFA	Federal Facilities Agreement
LANTDIV	Naval Facilities Engineering Command Atlantic Division
LC50	median lethal concentration
LOAEC	Lowest Observed Adverse Effect Concentration
mg/kg	milligram per kilogram
NAVFAC	Naval Facilities Engineering Command
NOAEC	No Observed Adverse Effect Concentration
NFESC	Naval Facilities Engineering Service Center
NPL	National Priorities List
NWS	Naval Weapons Station
OSHA	Occupational Safety and Health Administration
OU	Operable Unit
PAH	polycyclic aromatic hydrocarbon
PCB	polychlorinated biphenyl
QA/QC	quality assurance/quality control
QAPP	Quality Assurance Project Plan

ACRONYMS AND ABBREVIATIONS (continued)

RDX	cyclotrimethylenetrinitramine
SEM	simultaneously extracted metals
SERDP	Strategic Environmental Research and Development Program
SETAC	Society of Environmental Toxicology and Chemistry
SOP	standard operating procedure
SVOC	semi-volatile organic compound
TEC	Threshold Effect Concentration
TNT	2,4,6-trinitrotoluene
TOC	total organic carbon
USACHPPM	U.S. Army Center for Health Promotion and Preventive Medicine
USEPA	U.S. Environmental Protection Agency
USFWS	U.S. Fish and Wildlife Service
USGS	U.S. Geological Survey
VOC	volatile organic compounds
XRF	x-ray fluorescence

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EXECUTIVE SUMMARY

BACKGROUND

This Environmental Security Technology Certification Program (ESTCP) project, *Demonstration and Certification of Amphibian Ecological Risk Assessment Protocol*, *ER-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 Department of Defense (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 soil protocol evaluates impacts to adult salamanders, and the sediment protocol evaluates impacts to larval tadpoles.

When selecting appropriate receptors to derive ERA-based remedial goals, amphibians should be considered since these species play a key ecological role in wetlands and are an important link in ecological food chains, serving both as predators and prey items. However, there is a relative lack of available toxicity data for amphibians. As a result, remedial decisions at sites are often based on data from aquatic or terrestrial species that are not typical of wetlands. These species may be more or less sensitive to chemical stressors than amphibians. This project presents a methodology for evaluating potential risks to amphibians in wetlands and for deriving remediation goals based on these important ecological receptors.

OBJECTIVES OF THE DEMONSTRATION

The demonstration 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 the amphibian ERA framework presented in *Development of a Standardized Approach for Assessing Potential Risks to Amphibians Exposed to Sediments and Hydric Soils* (Naval Facilities Engineering Command [NAVFAC], 2004) at a DoD site to evaluate whether or not it provides valuable risk management information
- Evaluate the validity of previously developed lead and copper screening values designed to be protective of amphibians (these values were developed during the laboratory validation phase of this project [NAVFAC, 2007b]).

These objectives were met by evaluating sediment and hydric soil samples collected from two DoD sites. Based on the results of the toxicity testing and the evaluation of the analytical data, the protocols were deemed appropriate for use at both demonstration sites. In addition, they were sensitive enough to detect lethal and sublethal impacts due to firing range contaminant exposure. The ERA framework and lead and copper screening values were also applicable at both demonstration sites.

DEMONSTRATION RESULTS

Traditional ERA methods include the use of nonamphibian benchmarks and toxicity tests to evaluate potential risks to amphibians in wetland environments. The tiered amphibian ERA framework and the soil and sediment exposure protocols were developed to provide a more appropriate assessment of potential risks to amphibians. The amphibian ERA framework is designed to be a part of wetland site investigations and incorporates a variety of field and laboratory methods. These include comparing media concentrations to benchmarks, conducting laboratory toxicity tests, and performing field surveys to evaluate habitat and amphibian populations. Not all methods will be employed at all sites.

Travis Air Force Base (AFB) in California and the Aberdeen Proving Ground (APG) in Maryland were selected as the demonstration sites. Both have amphibian habitat colocated with contamination and associated with firing ranges. The field demonstration focused on lead because copper levels at the selected sites were not expected to be present at levels high enough to result in significant adverse impacts to amphibians.

Soil and sediment exposure tests were conducted with samples containing lead concentrations up to approximately 17,000 milligrams per kilogram (mg/kg). The test results demonstrated that field-collected soils and sediments were substantially less toxic than the laboratory-spiked soils and sediments. Therefore, the ecological screening levels derived based on the laboratory validation testing with spiked soils and sediments would have the potential to be overly conservative in assessing risks to amphibians exposed to lead under field conditions.

The application of the tiered amphibian ERA framework, incorporating 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 ERA methods (e.g., use of nonamphibian benchmarks and toxicity tests).

The performance objectives for the field demonstration effort were met. The field demonstrations indicate that the sediment exposure protocol and the amphibian ERA framework are both applicable tools for potential impact investigation to amphibians at wetland sites. 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.

IMPLEMENTATION ISSUES

Implementation of this technology will provide an appropriate methodology for evaluating potential risks to amphibians in wetlands and for deriving more appropriate remediation goals. The costs for this demonstration indicated that implementation of the sediment exposure protocol will be within ±20% of the costs of testing with more traditionally used species (i.e., benthic invertebrates). 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.

Limitations exist for the application of these toxicity testing protocols. 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 (generally spring, late fall, and winter for frog eggs and February through May for salamanders). The availability of frog eggs caused a delay in the start of the sediment toxicity tests for both demonstration sites. These seasonal limitations are known to be potential concerns when using field-collected or laboratory-spawned test organisms. For example, amphibian testing conducted as part of the GE/Housatonic River Site evaluation was limited by a lack of frogs in reference areas during breeding season and difficulties in fertilizing and culturing egg masses in the laboratory (Weston Solutions, 2004). These types of seasonal limitations need to be considered when developing a sampling and testing program in support of a site investigation.

In addition to seasonal limitations on the availability of salamanders, the soil exposure protocol requires several dozen adult test organisms and could lead to local extirpation of populations. Based on these limitations, as well the expense of the assay and supporting parameters, the soil exposure protocol is not likely to be feasible for most site investigations. However, the protocol may be appropriate for controlled toxicological investigations designed to derive safe soil levels.

The transition of the technology to stakeholders and end users is already in progress. First, the sediment exposure protocol has recently been approved as an American Society of Testing and Materials (ASTM) standard (ASTM E2591-07 Standard Guide for Conducting Whole Sediment Toxicity Tests with Amphibians). Second, the tiered amphibian ERA approach was described in a 2004 technical report (NAVFAC, 2004). Third, the soil exposure protocol was discussed in a recent peer-reviewed article. Fourth, the results from this program have been reported at multiple international conferences and symposia. These endorsements should facilitate regulatory (e.g., U.S. Environmental Protection Agency [USEPA]) acceptance.

Although copper and lead were the focus of this ESTCP project, it is anticipated that these methodologies would be applicable to many different wetland contaminants.

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1.0 INTRODUCTION

1.1 BACKGROUND

This Environmental Security Technology Certification Program (ESTCP) project, *Demonstration and Certification of Amphibian Ecological Risk Assessment Protocol, ER-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 innovative technology programs, which developed 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 or sediments.

Amphibians should be considered when selecting appropriate receptors to derive ERA-based remedial goals. These species play a key ecological role in wetlands and are an important link in ecological food chains, serving as both predators and prey items. However, due to a relative lack of available toxicity data, remedial decisions at many sites may be based on data from aquatic or terrestrial species that are not typical of wetlands. These species may be more or less sensitive to chemical stressors than amphibians. When inappropriate receptors and methods are used to derive ERA-based remediation goals, site risks can be overestimated, resulting in the unnecessary excavation and destruction of wetlands. This project presents a more appropriate methodology for evaluating potential risks to amphibians in wetlands and for deriving more appropriate remediation goals.

1.2 OBJECTIVES OF THE DEMONSTRATION

The objectives of the demonstration were to:

- Demonstrate and validate use of the soil and sediment exposure protocols at two DoD sites with potential amphibian risk assessment concerns
- Apply the amphibian ERA framework presented in Development of a Standardized Approach for Assessing Potential Risks to Amphibians Exposed to Sediments and Hydric Soils (Naval Facilities Engineering Command [NAVFAC], 2004) at a DoD site to evaluate whether or not it provides valuable risk management information
- Evaluate the validity of previously developed lead and copper screening values designed to be protective of amphibians (these values were developed during the laboratory validation phase of this project (NAVFAC, 2007b)).

These objectives were met by evaluating samples collected from two demonstration sites.

1.3 REGULATORY DRIVERS

As a component of site investigation activities, regulatory agencies are increasingly requesting that amphibians be evaluated as part of the ERA process. Because limited ecotoxicity data are available for amphibians, it can be difficult to evaluate effectively potential impacts to these receptors. The soil and sediment exposure protocols demonstrated during this project were designed to help address regulatory agency's requests to assess potential impacts to amphibians.

2.0 TECHNOLOGY

The field demonstration was designed to validate and demonstrate the use of the soil and sediment exposure protocols, assess the validity of the lead and copper ecological screening values developed during a previous phase of this project (NAVFAC, 2007b), and apply the amphibian ERA framework (NAVFAC, 2004) at two DoD sites with potential amphibian risk assessment concerns.

2.1 TECHNOLOGY DESCRIPTION

The sediment and the soil exposure protocol technologies are laboratory bioassays developed to represent model systems for the evaluation of amphibian risks on a nationwide basis. Although copper and lead were the focus of this ESTCP project, it is anticipated that these methodologies would be broadly applicable to the evaluation of many different contaminants found in wetlands (e.g., explosives, other metals, organic contaminants).

2.1.1 Sediment and Soil Exposure Protocols

The sediment exposure protocol is a 10-day laboratory toxicity test designed to evaluate potential risks to early-life-stage frogs and toads due to exposure to contaminants in 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, the Department of Energy (DOE), the U.S. Environmental Protection Agency (USEPA), the U.S. Geological Survey (USGS), and the U.S. Fish and Wildlife Service (USFWS) representatives.

Sediment tests are conducted with recently hatched tadpoles (i.e., *Rana pipiens*, Gosner stages 17 and 20). Young tadpoles are placed in beakers containing sediment and overlying water (Figure 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 sublethal (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. Longer duration studies (i.e., 28 days or until complete metamorphosis) may also be conducted to evaluate potential impacts on tadpole development.

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

The soil exposure protocol assesses adult salamander (i.e., *Plethodon cinereus*) exposure to mesic soils by evaluating effects on salamander growth, survival, and target organs following 28 days of test exposure.

In the soil exposure protocol, each test organism is placed into an individual petri dish containing treatment-specific soil (Figure 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.

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

At test termination, surviving salamanders are weighed, anesthetized, and euthanized. Growth, mortality, and health criteria (blood parameters and histological organ evaluation, including quantification of liver melanomacrophages) are evaluated as the endpoints for this assay. The liver melanomacrophages are a nonspecific indicator of stress, and show potential as biomarkers for a wide variety of chemical stressors.

Although sublethal endpoints measured in the blood and histopathology may not result in other adverse impacts during the laboratory study, they are likely to affect individuals in field exposures over longer durations. Ecological interactions in the field can affect exposure and therefore the probability for adverse effect. Changes in blood parameters and in the observations recorded at specific concentrations show effects that could easily translate to those exhibiting profound ecological consequences. For example, anemia can result in lethargy, which can affect territorial vigor, mate acquisition, subsequent reproduction (fitness) and predator vigilance (predation), the latter leading directly to mortality. The importance of these measurements to population- and community-level influences should be considered and assumed given the constraints of this controlled testing regime and of the importance of these ecological outcomes to populations and communities described in the literature.

2.1.2 Amphibian Risk Assessment Methodology

The tiered amphibian ERA framework is designed to assess potential risks to amphibians as part of site investigations conducted at wetland sites managed by the DoD or other entities. The amphibian ERA framework incorporates a variety of methods to evaluate potential risks to amphibians. These include comparing media concentrations to benchmarks, conducting laboratory toxicity tests, and performing field surveys to evaluate habitat and amphibian populations.

If amphibian habitat is identified and an evaluation of available analytical data indicates the potential for risk, then additional evaluation is recommended. This evaluation could include toxicity testing, amphibian population surveys, or collection of amphibian tissues for analysis. It is recognized that site-specific amphibian field population studies, such as those conducted as part of the GE/Housatonic River Site (Woodlot Alternatives, 2003), can be very involved and labor intensive. Depending on the nature of the study, these field surveys may include chorusing surveys or more quantitative methods to assess population numbers and diversity, observations of courtship and breeding behavior, and observations of metamorphosis and exodus from water bodies. The toxicity testing program may be conducted prior to other phases of investigation (i.e., field surveys, tissue collection) in order to avoid conducting additional studies if toxicity is not observed. If the toxicity testing does not identify the potential for adverse impacts, then additional field surveys would not be warranted. Therefore, a critical component of the site-specific ERAs warranted at many wetland sites with overlapping amphibian habitat and contamination will be the performance of appropriate toxicity tests.

The implementation of the soil and sediment exposure protocols requires both field and laboratory components. The initial field component includes an assessment of the wetland under investigation for applicable amphibian habitat as well as the collection of samples for chemical and toxicological analyses. If limited analytical data are available, a field reconnaissance sampling effort may be warranted prior to the collection of field samples for testing. This reconnaissance sampling may be conducted using field analyses (e.g., X-ray fluorescence [XRF] survey, field kits for detection of polycyclic aromatic hydrocarbons [PAH] in sediment), or samples may be submitted to an analytical laboratory for analysis. The chemical analyses used to characterize the soil and sediment samples (e.g., metals, organic compounds, total organic carbon [TOC]) are not unusual for chemistry labs accustomed to analyzing samples from environmental sites.

The purpose of the reconnaissance sampling is to identify appropriate sampling locations for the toxicity testing. In many cases, this will mean identifying a range of locations that will achieve a gradient of contaminant concentrations. In other cases, testing may only be desired within a particular footprint. In all cases, it is important to collect at least one reference sample from a location outside the area of impact. This sample should have similar physical conditions (e.g., grain size, organic content) to the impacted samples. Additional samples such as field duplicates and equipment blanks should be collected to ensure the quality of the data collected (see the Quality Assurance Project Plan [QAPP] presented in Appendix B of the Field Demonstration Plan (NAVFAC, 2007a) for additional detail on data quality).

Mobilization of a field crew for sample collection may be conducted concurrent with other site investigation activities. Sample collection for toxicity testing does not necessarily require special training beyond typical sample collection, chain-of-custody, and safety training conducted for field crews. Training requirements may vary depending on the nature of the site (e.g., Superfund site, active firing range) or the sampling methods (e.g., sediment sampling from boat, hydric soil sampling in a wetland). In general, it is possible to collect samples from multiple locations within a single sampling day (i.e., 16 field demonstration samples were collected in two field days at Travis AFB).

Toxicity testing procedures are described in detail in Appendix A of the Field Demonstration Plan (NAVFAC, 2007a). Training of laboratory staff is required prior to the use of these protocols since not all environmental laboratories have experience with these test organisms. Existing laboratory health and safety plans should address any potential safety issues associated with the protocols (e.g., appropriate handling of test material). The qualifications of the toxicity testing laboratory should be investigated prior to conducting the soil and sediment protocols to confirm that the tests will be conducted properly and that laboratory controls will meet test acceptability criteria. Neither protocol is technically more difficult to set up or conduct than other existing toxicity testing protocols. However, the histological and blood parameters measured at the termination of the soil exposure protocol will require the use of a laboratory that is familiar with these analyses. This level of experience may not be typical of most environmental toxicity laboratories.

2.1.3 Chronological Summary of Technology Development

Both the soil and sediment exposure protocols are mature technologies, with little remaining development or refinement warranted. Extensive laboratory and data analysis efforts were conducted during the past 4 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 (NAVFAC, 2007b).

The sediment exposure protocol was developed under the Navy's YO817 program. It was presented in a guidance manual designed for risk assessment staff and state and federal regulators involved in the review and approval of risk assessment work plans and reports (NAVFAC, 2004). The guidance manual included a standard operating procedure (SOP) for conducting the sediment exposure toxicity test as well as recommendations for field survey methodologies and a framework for conducting amphibian ERAs. An initial phase of this ESTCP project included a number of laboratory assays designed to validate and refine the sediment exposure protocol with lead and copper prior to the field demonstration. The laboratory validation phase of testing evaluated 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.

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.

2.2 ADVANTAGES AND LIMITATIONS OF THE TECHNOLOGY

The use of the sediment and soil exposure protocols to assess potential impacts to amphibians in wetlands will typically be more appropriate than using existing toxicity tests with alternative non-wetland species. Conducting toxicity tests with 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 soils. Use of these alternative species may over- or under-estimate potential impacts to amphibians.

In addition, the sediment and soil exposure protocols were designed to provide methodologies that include the use of native North American amphibian species in wetland ERAs. Historically, the FETAX (Frog Embryo Teratogenesis Assay-Xenopus) protocol was the only American Society of Testing and Material (ASTM) method used in the evaluation of toxicants on amphibians. However, this methodology is limited since it uses a non-native species (the African clawed frog [*Xenopus laevis*]) and generally evaluates a short-term water exposure (96 hours) to assess mortality, growth, and malformations in larvae. As described in Section 8.3, difficulties were encountered when attempting to use *Xenopus* species in a sediment-exposure assay, and information in the literature indicates that this species may be among the least sensitive test organisms (Birge, et al. 2000; Hoke and Ankley, 2005). Therefore, the approval of the sediment exposure protocol as an ASTM standard (ASTM E2591-07 Standard Guide for Conducting Whole Sediment Toxicity Tests with Amphibians) provides investigators with an important new tool that can more appropriately evaluate risks to amphibians within wetlands by including indigenous species in the testing program.

However, limitations exist for the application of these testing protocols. 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. Testing with laboratory-raised organisms such as fish and earthworms are not likely to be subject to these seasonal limitations.

Another possible limitation of the technology is that 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 the soil exposure 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 costly, 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 not feasible for wide-scale use in determining toxicity from mixtures at individual sites in support of environmental restoration.

The chemical analyses used to characterize the soil and sediment samples are typical for most site investigations designed to derive safe soil levels for particular compounds. However, training of laboratory staff is required prior to the use of these protocols since not all environmental laboratories have experience with these test organisms. Neither protocol is technically more difficult to set up or conduct than other existing toxicity testing protocols. However, the histological and blood parameters measured at the termination of the soil exposure protocol will require the use of a laboratory that is familiar with these analyses.

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

3.0 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. Table 1 presents the performance objectives for evaluating the field demonstration effort and indicates whether the objectives were met.

Table 1. Performance Objectives.

Type of Performance Objective	Primary Performance Criteria	Expected Performance Metric	Performance Metric Met?
Qualitative	Sediment protocol is applicable to evaluating copper and lead in palustrine wetlands	Correlation between sediment concentrations and lethal or sublethal results	Yes
	Soil protocol is applicable to evaluating copper and lead in forested uplands	Correlation between mesic soil concentrations and lethal or sublethal results	Yes
	Collection and biological evaluation of native salamanders is applicable for evaluating potential impacts due to metals	Correlation between mesic soil concentrations and histopathological evaluation	No, native salamanders were not collected. Evaluation of laboratory exposed salamanders found no histopathological effects.
	Regulatory acceptance of toxicity test protocols	Results are accepted by agency as component of ERA	Yes, although neither demonstration site is currently under agency review, use of the protocols at other regulated sites has yielded positive results.
	Versatility of the overall ERA protocol	Application of the ERA protocol at both field demonstration sites	Yes
	Technology transferred to other potential end users	Presentation at conference or in journal; ASTM certification	Yes
Quantitative – Sediment Exposure Protocol	Sediment toxicity test is valid and acceptable	Mean survival in laboratory control is >80%	Yes
	Lethal endpoint indicates toxicity or lack of toxicity	Statistical difference between survival in control or reference samples and site samples	Yes
	Sublethal endpoints indicate toxicity or lack of toxicity	Statistical difference between sublethal endpoints in control or reference samples and site samples (may include growth, abnormalities, behavior, metamorphic stage, or other measurements)	Yes

Table 1. Performance Objectives (continued)**.**

Type of Performance Objective	Primary Performance Criteria	Expected Performance Metric	Performance Metric Met?
Quantitative – Soil Exposure Protocol	Soil toxicity test is valid and acceptable	Mean survival in laboratory control is >80%	Yes
	Lethal endpoint indicates toxicity or lack of toxicity	Statistical difference between survival in control and site samples	Yes, lack of toxicity indicated
	Growth endpoints indicate toxicity or lack of toxicity	Statistical difference between growth endpoints in control or reference samples and site samples	Yes
	Blood parameters indicate toxicity or lack of toxicity	Statistical difference between blood parameters measured in control or reference samples and site samples	Yes, lack of toxicity indicated

4.0 SITE DESCRIPTION

4.1 SITE LOCATION

Travis Air Force Base (AFB) and Aberdeen Proving Ground (APG) were selected as preferred locations for the demonstration of the amphibian risk assessment protocol. This section provides a summary of the sites and presents the site selection criteria used to identify these locations.

Section 5 provides additional information on the selected demonstration sites, including sampling maps and analytical data presenting the distribution of copper and lead. Site geology was not a significant selection criterion for the demonstration sites. Hydrogeology was relevant in that wetland locations were preferred sampling locations.

4.1.1 Travis Air Force Base

Travis AFB in Fairfield, California, is located midway between Sacramento and San Francisco in northern California. 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. Travis AFB contains several well-documented palustrine wetland complexes and vernal pools that are in close proximity to firing ranges. In addition, there is a documented vernal pool complex in close proximity to an active skeet range.

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. Suitable habitat for amphibians was not present within the area of the firing range that was surveyed for lead and copper soil concentrations. A vernal pool located approximately 800 ft 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). The vernal pool associated with the skeet shooting range was selected as the primary study area at Travis AFB.

4.1.2 Aberdeen Proving Ground

APG in 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, it has not been possible to provide aerial images of the proposed study area.

Army personnel identified an on-site small arms range adjacent to a palustrine wetland as the specific area of study. The selection of this location was based primarily on the wetlands present on site, the observations of amphibian populations, and the likelihood of contamination due to the adjacent small arms range.

4.2 TEST SITE SELECTION CRITERIA

The two 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 colocated and are often found at military sites and ranges. Travis AFB in California and APG in Maryland were selected based on the following criteria:

Preferred Chemical Parameters

- Presence of copper ranging from 150 milligrams per kilogram (mg/kg) to <3,000 mg/kg in mesic soil and/or palustrine hydric soil
- Presence of lead ranging from 100 mg/kg to <6,000 mg/kg in mesic soil and/or palustrine hydric soil
- Presence of colocated copper and lead in mesic soil and/or palustrine hydric soil
- Lack of chemical stressors other than lead and copper, which may confound interpretation of results (i.e., chemical stressors absent or present below ecological screening values).

Preferred Ecological Parameters

- Presence of Plethodontid salamanders and/or habitat on site
- Presence of Anurans (frogs or toads) and/or habitat on site
- Lack of non-chemical stressors (i.e., physical stressors such as bridges, roadways, drainage ditches, etc.) which may confound interpretation of results
- Federal- or state-listed threatened and endangered amphibians occur at the site.

Site Historical and Logistical Parameters

- Previous site investigations conducted with analytical and habitat investigations completed
- Site is currently being investigated to determine whether remedial response actions are required to address potential risks to amphibians

- Study area had firing range or other similar activity which generated lead and copper contamination
- Demonstration sites are located in varying geographic regions
- Accessible facility locations
- Site access available within proposed schedule and cost framework
- Site provides facilities for sampling personnel (e.g., electricity, running water)
- No significant health and safety concerns.

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5.0 TEST DESIGN

5.1 CONCEPTUAL EXPERIMENTAL DESIGN

The field demonstration was designed to collect sediment and hydric soils from two DoD sites and submit samples for testing with both the soil and sediment testing protocols. The objectives were to demonstrate that the testing protocols were able to detect lethal and sublethal impacts due to firing range contaminant exposure. In addition, the amphibian ERA framework (NAVFAC, 2004) was applied at each site to determine whether or not the methodology would be useful for risk management purposes. Finally, the results of the field demonstration toxicity tests were evaluated relative to the lead and copper screening values developed during the laboratory validation phase of this project (NAVFAC, 2007b) to determine whether the screening values would be sufficiently protective of amphibians.

5.2 BASELINE CHARACTERIZATION

As described in Section 4.1, a site reconnaissance visit was conducted at Travis AFB to identify suitable habitat for amphibians that was likely to be impacted by the skeet shooting range. A site reconnaissance visit was not conducted at the APG site since one of the ESTCP team partners was already intimately familiar with the overlap between amphibian habitat and lead-contaminated ranges.

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).

Prior to the sample collection activities for testing, an XRF survey was conducted at each demonstration site to identify an appropriate range of copper and lead levels. The XRF survey information was used to select sampling locations that represented a concentration gradient bracketing and containing the concentrations suspected to result in lethal and sublethal responses in amphibians, based on the previously conducted work (including the laboratory refinement phase of this ESTCP project).

The XRF surveys indicated that the leads levels in the Travis AFB samples ranged from 20 mg/kg to nearly 3,000 mg/kg and the APG samples ranged from approximately 30 mg/kg to 12,000 mg/kg.

5.3 TREATABILITY OR LABORATORY STUDY RESULTS

Treatability studies and laboratory confirmation testing was not conducted as part of the field demonstration. Previous testing of each protocol was described in the June 2007 Test Refinement Interim Report (NAVFAC, 2007b).

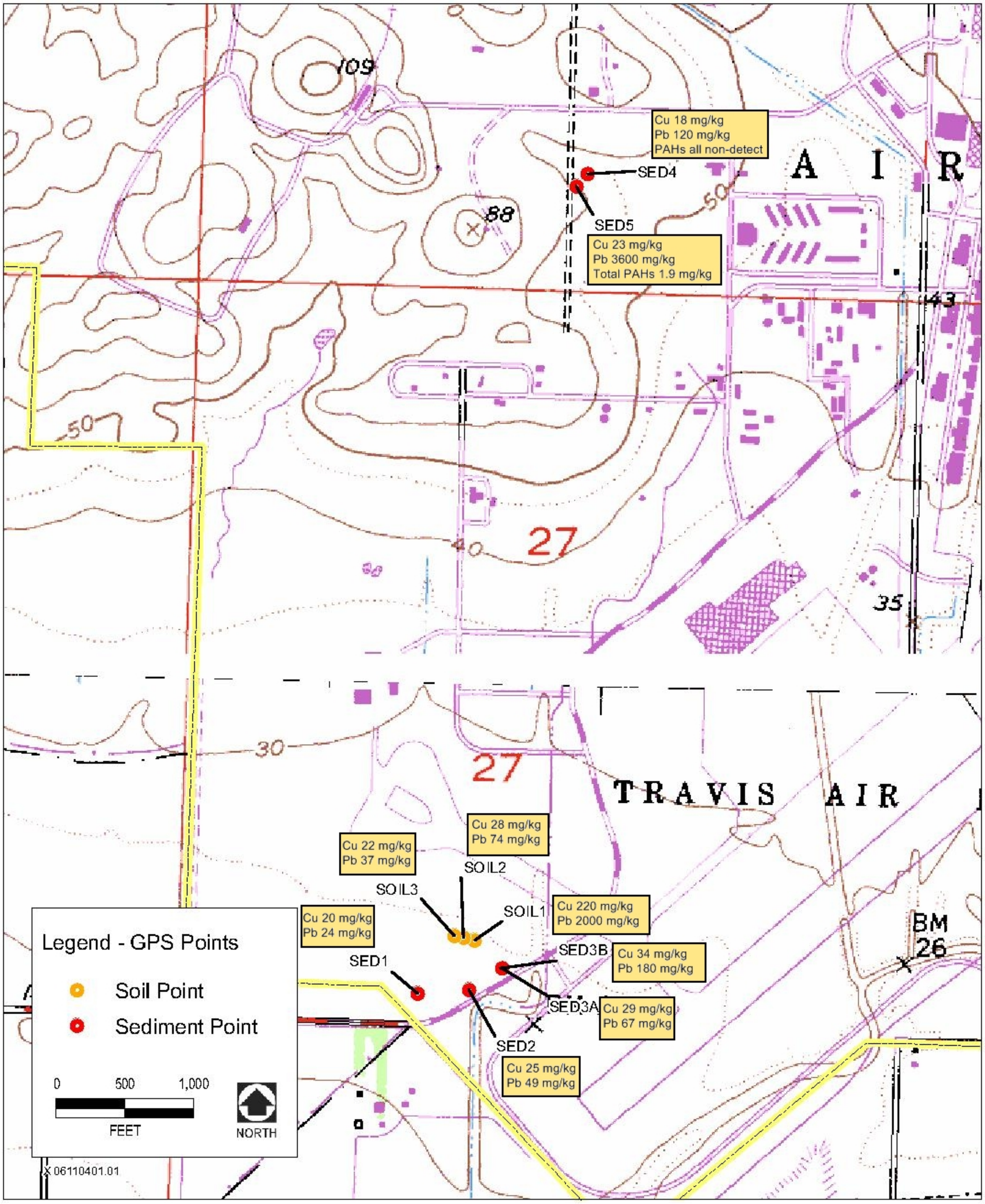

Figure 3. Travis Air Force Base Site Reconnaissance Sampling Locations and Analytical Data.

5.4 FIELD TESTING

The field sampling efforts at each demonstration site were conducted in two phases with an initial XRF survey conducted prior to the actual sample collection efforts. Following the field effort, sediment and soil samples were submitted to the appropriate laboratories for chemical analyses and toxicity testing.

At the Travis AFB study area, 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 for chemical analyses were collected from the vernal pool and the reference location on March 27, 2006. 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 U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM) personnel on April 10, 2006, 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 commercially available frog eggs, there was a delay in the initiation of the sediment toxicity testing program. The sediment testing was conducted by AECOM’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. 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.

5.5 SAMPLING METHODS

Based on the results of the XRF survey, soil and sediment samples were collected from nine Travis AFB locations within the vernal pool study area and one reference location (Figure 4). Based on the water content of the material, five samples, including the reference, were identified as sediment and the remaining four were identified as soils.

All Travis AFB sediment and soil samples were analyzed for copper, lead, TOC, grain size, cation exchange capacity (CEC), simultaneously extracted metals (SEM), and acid volatile sulfides (AVS). 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. Surface water samples were collected from two 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.

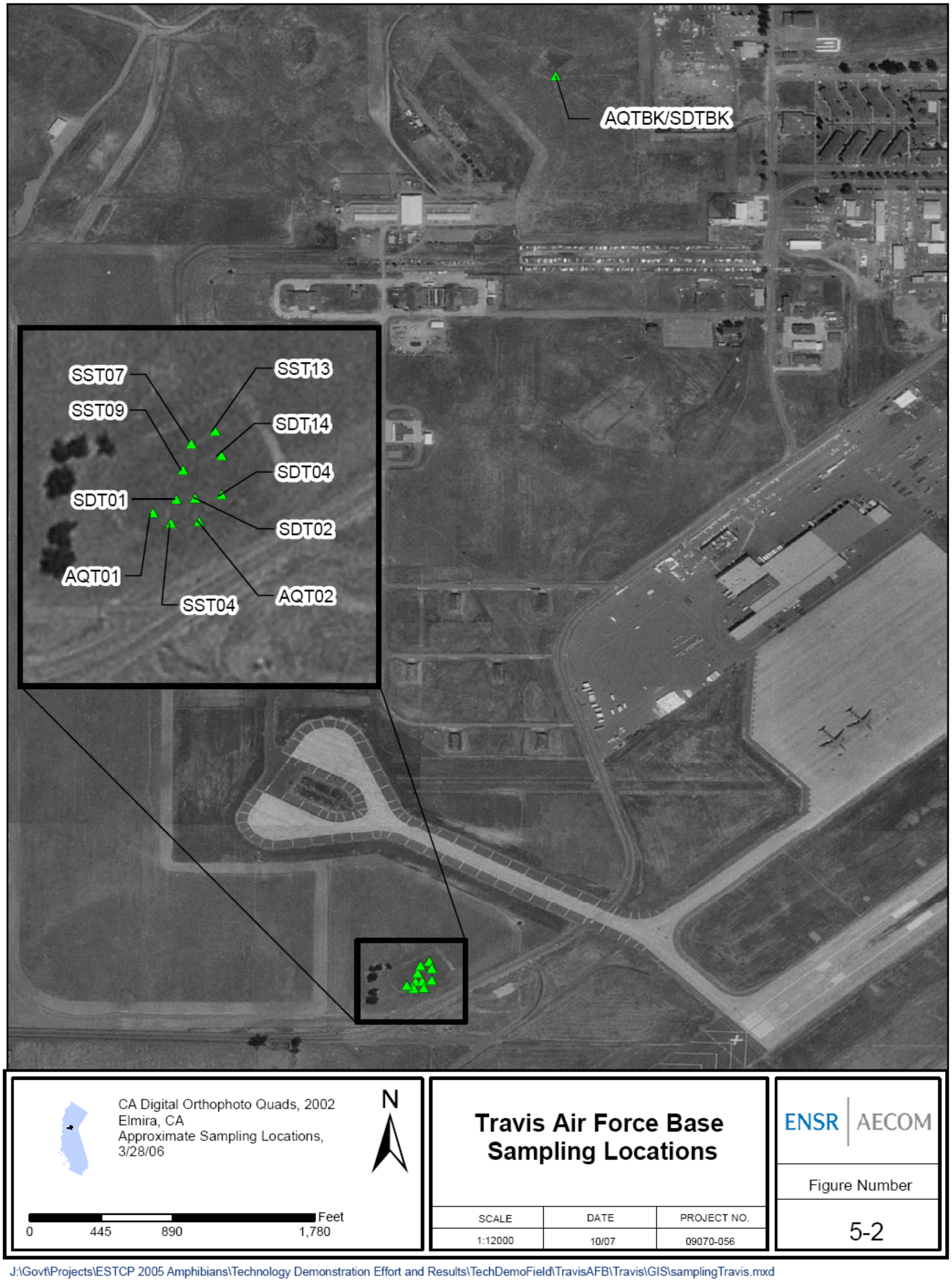

Figure 4. Travis Air Force Base Sampling Locations.

Based on the results of the XRF survey, samples were collected from 10 APG locations within the palustrine wetland study area and one reference location (Figure 5). Based on the water content of the material, eight samples, including the reference, were identified as sediment for the sediment exposure protocol, and the remaining three were identified as soils for the soil exposure protocol.

All APG sediment and soil samples were analyzed for copper, lead, TOC, grain size, CEC, SEM, and AVS. Two samples and a field duplicate were also analyzed for a full suite of 23 metals, 21 pesticides, and PCB Aroclors. 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.

5.5.1 Sample Collection

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 subsampling 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.

A completed chain-of-custody form accompanied samples. Shipping containers were secured with strapping tape and sealed with custody seals. Samples were shipped daily on ice from the field to the chemistry and toxicity testing laboratories using an overnight courier.

Chemical analyses were conducted by Paragon Analytics of Fort Collins, Colorado; Mitkem Corporation of Warwick, Rhode Island; STL-Burlington of Colchester, Vermont; and GeoTesting Express of Boxborough, Massachusetts. The sediment toxicity testing was conducted at AECOM’s FCETL of Fort Collins and the soil toxicity testing was conducted at USACHPPM’s Aberdeen laboratory.

Laboratory quality assurance/quality control (QA/QC) measures were performed by the analytical laboratories to ensure that all environmental efforts to produce the data were technically sound and legally defensible. Measures to ensure representativeness, completeness, comparability, accuracy, and precision of the data were presented in the QAPP (NAVFAC, 2007a). Toxicity testing was conducted according to the QA/QC plans in place at the AECOM and the USACHPPM toxicity laboratories and the protocols presented in Appendix A of the Field Demonstration Plan (NAVFAC, 2007a).

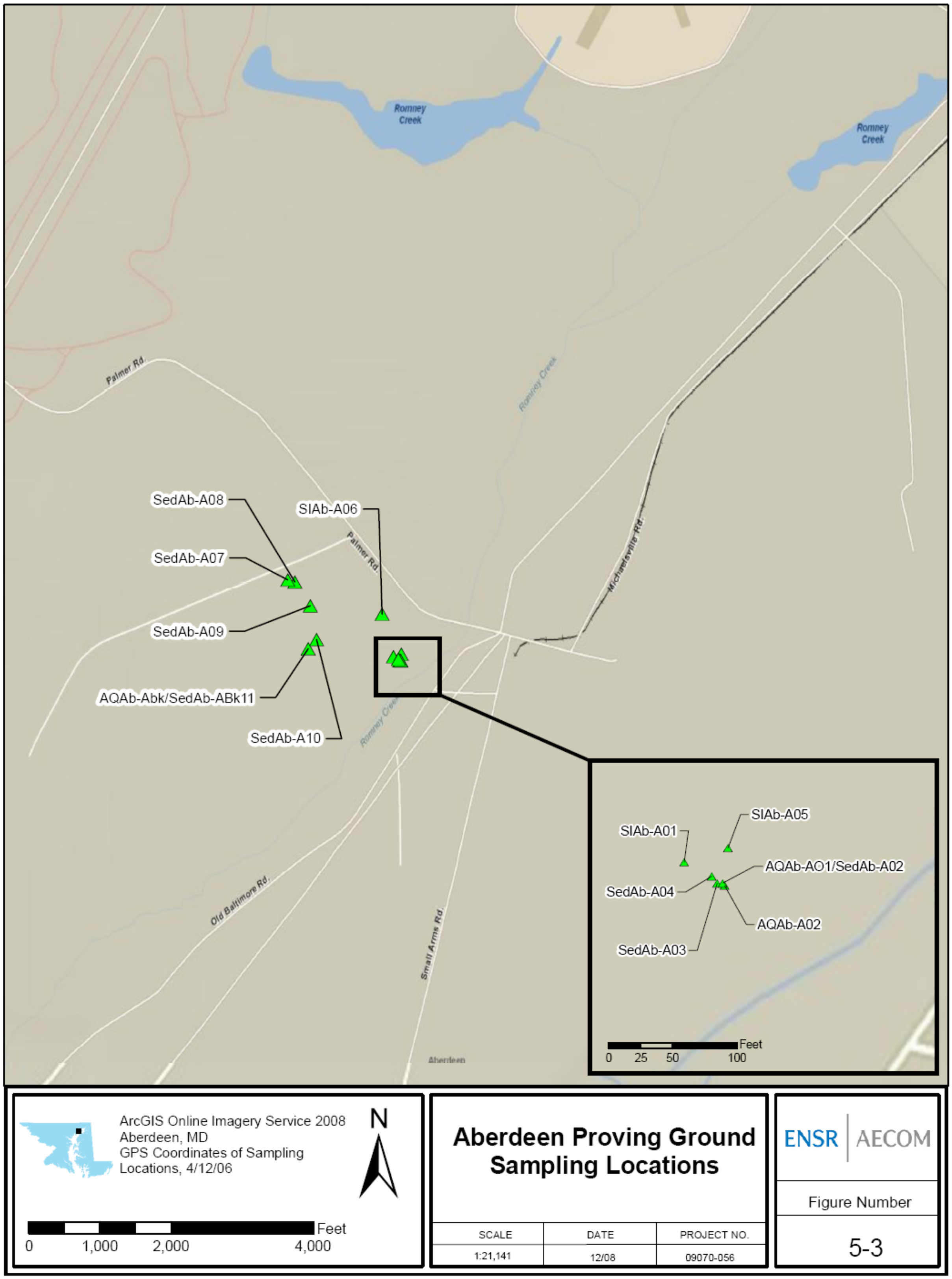

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Figure 5. Aberdeen Proving Ground Sampling Locations.

5.6 SAMPLING RESULTS

A summary of the soil exposure results for both demonstration sites is presented in Table 2. Table 3 presents a summary of the sediment exposure results. Detailed analytical results for the soil and sediment samples from both demonstration sites can be found in the Final Technical Report (NAVFAC, 2009). The data are also discussed in Section 6 relative to the performance objectives presented in Section 3.

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 Lowest Observed Adverse Effect Concentrations (LOAECs) and No Observed Adverse Effect Concentrations (NOAECs) for both survival and sublethal endpoints.

To derive these values, the survival or sublethal 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 nontoxic response. LOAECs and NOAECs were estimated by identifying the concentration of each analyte at the demarcation between toxic and nontoxic samples, as indicated by the statistical evaluation. The NOAEC represents the tested sample with the highest concentration of a constituent of potential concern 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 (NAVFAC, 2007b), and 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 2. Summary of Soil Exposure Results.

			Mean Survival	Mean Change in Weight from Day 0 (%)				Average Erythrocyte Counts (10x4 cells/µL)	Average Leukocyte Counts (10x3 cells/µL)	Average Hemoglobin (g/dL)
Location ID	Lead [1] (mg/kg)	TOC (%)	Day 28	Day 7	Day 14	Day 21	Day 28	Day 28	Day 28	Day 28
Travis Air Force Base										
SDTBK	10.8	1.5	100%	1.244	0.255	2.727	2.308	9.73	4.06	9.4
SST09	1,430	1.6	100%	0.811	-0.250	0.461	-1.947	9.40	4.20	8.8
SST13	2,710	2.3	100%	-2.975	1.882	4.360	3.412	11.44	4.12	8.6

Table 2. Summary of Soil Exposure Results (continued).

Location ID	Lead [1] (mg/kg)	TOC (%)	Mean Survival	Mean Change in Weight from Day 0 (%)				Average Erythrocyte Counts (10x4 cells/µL)	Average leukocyte counts (10x3 cells/µL)	Average Hemoglobin (g/dL)
			Day 28	Day 7	Day 14	Day 21	Day 28	Day 28	Day 28	Day 28
Aberdeen Proving Ground										
SlAb-A06	28	1.3	100%	0.658	-0.073	0.748	0.755	9.30	4.01	8.3
SlAb-A05	260	0.41	100%	2.174	-0.447	1.461	-0.289	8.95	4.09	9.3
SlAb-A01	16,967	0.088	100%	5.764	-1.025	-2.878	**-6.565**	8.98	4.80	8.4

Table 3. 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	1,700	1.8	100	4.9	7.1
SST07	17	2,100	1.6	**40** [2]	**2.5** [3]	**3.5** [3]
SDT14	21	2,800	1.9	100	5.2	7.3
SST13	13	3,700	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	2,912	0.72	**30** [2]	**1.3** [3]	**2.1** [3]
SedAb-A3A [M2]	306	4,270	0.84	**35** [2]	**1.5** [3]	**2.4** [3]
SedAb-A3A [M3]	604	8,513	1.2	**12** [2]	**0.4** [3]	**0.6** [3]
SedAb-A3A	1,200	17,000	2.0	**15** [2]	**0.4** [3]	**0.7** [3]

[1] Samples re-analyzed by Paragon 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 estimated based on the analytical results for the samples included in the dilution

6.0 PERFORMANCE ASSESSMENT

6.1 PERFORMANCE DATA

For the soil exposure protocol, 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.

As indicated in Table 2, no blood parameter effect results (i.e., average erythrocyte and leukocyte counts and average hemoglobin) were statistically different from associated soil reference sample results. One sample from APG exhibited reduced growth on Day 28. The final histology report concluded that there was no toxicity associated with the field-collected (aged) soil exposures. No test article-related histopathologic findings were found.

A summary of the sediment exposure results for both demonstration sites is provided in Table 3. Copper concentrations were analyzed to rule out another possible chemical stressor. Reductions in survival were observed at one Travis AFB sample and four APG samples. Sublethal effects were observed in two Travis AFB samples and five APG samples. At APG, the impacted samples were all associated with elevated lead levels. However, this was not the case at Travis AFB where samples with more elevated lead levels exhibited no toxicity.

Detailed analytical results for the soil and sediment samples from both demonstration sites can be found in the Final Technical Report (NAVFAC, 2009).

6.2 PERFORMANCE CRITERIA

Adherence to the data collection methods and analyses presented in the Field Demonstration Plan (NAVFAC, 2007a) ensured that reliable data were collected. 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 presents the primary and secondary performance criteria established to evaluate the field demonstration technology, the method used to confirm performance, the expected performance results, and the actual results.

Table 4. 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 sublethal results	Statistical evaluation conducted	Some samples from each demonstration site were statistically different from the reference samples

Table 4. Performance and Performance Confirmation Methods (continued)**.**

Performance Criteria	Expected Performance Metric	Performance Confirmation Method	Actual
Soil protocol is applicable to evaluating copper and lead in forested uplands	Correlation between mesic soil concentrations and lethal or sublethal results	Statistical evaluation conducted	Some samples 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	Demonstration site 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, and the use of the amphibian testing protocol has been approved by USEPA and state agencies at other sites.
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
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 conducted	Statistical evaluation indicated significant mortality in some samples
Sediment Exposure Protocol—sublethal endpoints indicate toxicity or lack of toxicity	Statistical difference between sublethal endpoints in control or reference samples and site samples[1]	Statistical evaluation conducted	Statistical evaluation indicated significant growth reduction (i.e., body width and length) in some samples
Primary Criteria (Quantitative)			
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 conducted	No lethal toxicity observed in any sample
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 some samples relative to reference sample results

[1] Sublethal endpoints may include growth, abnormalities, behavior, metamorphic stage, or other measurements.

Table 4. Performance and Performance Confirmation Methods (continued)**.**

Performance Criteria	Expected Performance Metric	Performance Confirmation Method	Actual
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 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 conducted	Native salamanders were not collected at either site so criteria could not be evaluated. No effects noted in lab exposed salamanders.
Technology transferred to other potential end users	Presentation at conference or in journal	Results or protocols presented	Peer-reviewed articles are under development to present soil exposure results. Sediment exposure protocol has been accepted as ASTM guide. Multiple technical presentations have been given by team members at national and international meetings and symposia.
Primary Criteria (Qualitative)			
Sediment protocol is applicable to evaluating copper and lead in palustrine wetlands	Correlation between sediment concentrations and lethal or sublethal results	Statistical evaluation conducted	Some samples 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 sublethal results	Statistical evaluation conducted	Some samples 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	Demonstration site 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, and the use of the amphibian testing protocol has been approved by USEPA and state agencies at other sites.
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. 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 conducted	Statistical evaluation indicated significant mortality in some samples
Sediment Exposure Protocol—sublethal endpoints indicate toxicity or lack of toxicity	Statistical difference between sublethal endpoints in control or reference samples and site samples[2]	Statistical evaluation conducted	Statistical evaluation indicated significant growth reduction (i.e., body width and length) in some samples
Primary Criteria (Quantitative)			
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 conducted	No lethal toxicity observed in any sample
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 some samples 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 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 conducted	Native salamanders were not collected at either site so criteria could not be evaluated. No effects noted in lab exposed salamanders.
Technology transferred to other potential end users	Presentation at conference or in journal	Results or protocols presented	Peer-reviewed articles are under development to present soil exposure results. Sediment exposure protocol has been accepted as ASTM guide. Multiple technical presentations have been given by team members at national and international meetings and symposia.

[2] Sublethal endpoints may include growth, abnormalities, behavior, metamorphic stage, or other measurements.

6.3 DATA ASSESSMENT

The results of the field demonstration show that the field-collected soils and sediments were substantially less toxic than similar levels of lead in the laboratory spiked soils and sediments tested in the laboratory validation phase of testing (Table 5). These results indicate that using screening values derived from studies conducted with laboratory-spiked soils and sediment may be overly protective of amphibians exposed to lead under field conditions; however, use of these screening values in the context of a preliminary or screening level ERA is appropriately conservative.

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, which appears to be impacted (i.e., is less available) by weathering as well as grain size composition. Further, 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.

It is unclear whether lead is the stressor responsible for the observed toxicity in the Travis AFB sediment sample since samples with higher lead levels did not show a significant reduction in survival. Additionally, it is possible that, in addition to the lead, copper concentrations in the APG sediment samples also contributed to observed toxicity.

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 sublethal impacts due to exposure to firing range contaminants. The tiered amphibian ERA protocol was determined to be useful for conducting both screening level and more sophisticated ERA analyses. The use of the sediment exposure protocol provides a site-specific assessment of the bioavailability and toxicity of lead, or other stressors, on larval amphibians that might be present in the wetland.

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 (Table 5). At both demonstration sites, the use of technologies developed and refined through the ESTCP 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 5. Comparison of Demonstration Testing Results and Screening Values.

Medium	Source	Value	Receptor	Lead (mg/kg)	
				Travis Air Force Base	Aberdeen Proving Ground
Soil screening values	Literature	Eco-SSL	Terrestrial invertebrate	1,700	1,700
		Eco-SSL	Vertebrate [bird]	11	11
	Validation testing	Survival NOAEC	Salamander	1,700	1,700
		Sublethal NOAEC	Salamander	1,700	1,700
	Demonstration testing	Survival NOAEC	Salamander	2,710	16,967
		Sublethal NOAEC	Salamander	2,710	260
Sediment screening values	Literature	TEC	Benthic invertebrate	35.8	35.8
	Validation testing	Survival NOAEC	Tadpole	1,200	1,200
		Sublethal NOAEC	Tadpole	100	100
	Demonstration testing	Survival NOAEC	Tadpole	1,700	960
		Sublethal NOAEC	Tadpole	286	410

Validation testing values were presented in Tables 3-3 and 3-4 of the Final Technical Report (NAVFAC, 2009).
Demonstration testing values were presented in Tables 4-5 and 4-7 of the Final Technical Report (NAVFAC, 2009).
Eco-SSL - Ecological-Soil Screening Level (USEPA, 2005; 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).

7.0 COST ASSESSMENT

Developing an understanding of cost performance is equally important as assessing the technical performance of the amphibian testing protocols. Cost considerations 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.

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.

7.1 COST MODEL

Table 6 presents a simple cost model for implementation of the ERA framework and the sediment exposure protocol at several sites of various sizes. The soil exposure protocol has not been included in the cost comparison since this method is not recommended to determine toxicity from mixtures at individual sites in support of environmental restoration. However, this protocol may be appropriate to use in controlled toxicological investigations designed to derive safe soil levels of particular constituents (i.e., TNT, DNT, RDX). Costs associated with conducting the soil exposure protocol are approximately $14,400 for the first sample (which includes costs for an associated laboratory control) and $7,200 for each additional sample. Costs for histology and chemistry account for approximately 25% of the costs.

Table 6. Tier I and Tier II Amphibian ERA Implementation Costs.

Cost Category	Sub Category	Details	Estimated 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 two 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
Tier II ERA Costs					
Direct operating costs	Capital equipment rentals	XRF analyzer	$600	$1,200	$3,000
	Toxicity testing	Sediment protocol ($1,200/sample)	$4,800	$10,800	$21,600
	Supervision	Labor and travel for one person	$500	$1,145	$3,080
	Operator labor	Labor and travel for two 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 6. Tier I and Tier II Amphibian ERA Implementation Costs (continued).

Cost Category	Sub Category	Details	Estimated Costs Site A	Site B	Site C
Indirect operating costs	Environmental and safety training	OSHA[1] 40-hour training for two 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 of Tier I and Tier II ERA			**$48,780**	**$82,275**	**$141,090**

[1] OSHA - Occupational Safety and Health Administration
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
Field staff rate = $100/hour
Supervisor rate = $150/hour
Supervisor in the field 50% of the time
XRF rental fee is $600/day

Travel assumptions:
No airfare included
Hotel = $150/night
Car + mileage = $90/day
Meals = $50/day

Table 7 presents the incremental implementation costs on both a per-acre and a per-toxicity testing sample basis. A more detailed analysis of the costs is discussed in Sections 7.2 and 7.3.

Table 7. 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 6.

7.2 COST DRIVERS

Table 6 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 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 de minimis 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 to or correlated with field observations.

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 2009 dollars) range from approximately $750 to $1,500 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 cost 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 sublethal 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 provides 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 on labor and shipping costs. Labor is likely to be relatively consistent from site to site. However, shipping costs and 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 6 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, and 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 7.

7.3 COST ANALYSIS

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 underestimate 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, *H. azteca*, or the midge, *C. 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 (i.e., excavating a larger footprint than warranted if a site-specific evaluation was conducted) and the environmental costs associated with the preventable loss of valuable wetland resources (i.e., the loss of functional habitat) .

In addition, as indicated in Table 5, the results of the field demonstration resulted in higher soil and sediment no-effect values (i.e., the NOAECs) than the literature-based values for earthworms and benthic invertebrates. These site-specific values could be used as cleanup levels, thus reducing the footprint requiring remediation. For example, if a wetland was contaminated with lead and an ERA was not conducted, the sediment TEC of 35.8 mg/kg (based on impacts to benthic invertebrates) could be selected as a cleanup level. At the APG, the site-specific cleanup level based on the sublethal NOAEC from the sediment exposure test would be 410 mg/kg. Depending on the distribution of the contamination, the change from a cleanup level of 35.8 mg/kg to 410 mg/kg could significantly reduce the amount of wetland that would need to be remediated, resulting in both a financial savings (by reducing the costs to remove soil and remediate the area) and an environmental savings (by retaining the intrinsic value of an existing functional 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 NWS 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 ERA 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, volatile organic compounds (VOC), and semi-volatile organic compounds (SVOC). 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 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, selenium, and silver) found in a palustrine scrub/shrub wetland. The toxicity testing program included testing with green frog tadpoles (*R. clamitans*), the amphipod (*H. 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.

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 cleanup goals, the remediation activities, the wetland restoration activities, and the more intangible disturbance associated with disturbing the wetland.

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 on 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. 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. The ecological costs associated with the disturbance of the wetland habitat need to be considered when reviewing the costs of remediating or restoring a wetland.

7.3.1 Cost Comparison

The expected costs to implement the 10-day sediment exposure protocol (ASTM E2591-07 Standard Guide for Conducting Whole Sediment Toxicity Tests with Amphibians) generated through this ESTCP program is expected to be similar to other ASTM and USEPA assays. Actual unit costs for these benthic invertebrate assays (in 2009 dollars) range from approximately $750 to $1,500 per 10-day test, depending on site-specific circumstances, whereas longer term tests are typically proportionately scaled. It is anticipated that the sediment exposure protocol market costs will be within ±20% of the invertebrate costs. As indicated in Section 7.1, the costs associated with the soil exposure protocol are much higher at approximately $14,400 for the first sample and $7,200 for each additional sample (including blood and histopathological parameters). This is one reason that it is not likely to be feasible to include the soil exposure protocol in site investigations designed to evaluate site-specific toxicity.

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 sublethal 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.

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 6 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, and 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 7.

The use of these organisms has the potential to overestimate potential risks and increase project costs, or alternatively to underestimate potential risks and thereby result in a less costly, but less protective, risk management decision. 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.

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8.0 IMPLEMENTATION ISSUES

8.1 COST OBSERVATIONS

As previously discussed (Section 7.0), 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 decisions.

Implementing the amphibian sediment testing protocol could be as much as 20% more costly than these traditional methods (depending on 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.

8.2 PERFORMANCE OBSERVATIONS

The primary objective of the field demonstration was to show that the soil and sediment exposure protocols were effective tools for identifying potential risks to amphibians to exposure to contaminated soil or sediment. Overall, the performance criteria set in the Field Demonstration Plan (NAVFAC, 2007a) were successfully achieved at both field demonstration sites.

Both exposure protocols were sensitive enough to identify lethal or sublethal responses that could generally be correlated with increasing lead levels. The tiered amphibian ERA methodology was also an applicable framework for evaluating potential impacts to amphibians at both demonstration sites. Since neither demonstration site is currently under investigation, the results of these studies have not been submitted to regulators. However, it is anticipated that regulatory acceptance of the sediment exposure protocol would be likely given the recent acceptance of the methodology as an ASTM guide, and given the acceptance of amphibian toxicity testing using this protocol on other sites regulated by the USEPA and state agencies.

As discussed previously (Section 2.2), the soil exposure protocol is more appropriate for scientific studies designed to derive safe levels of contaminants in soil for salamanders than for regular use in site investigations. Concerns about the extirpation of salamander populations have excluded the soil exposure protocol from being considered as a testing procedure in the development of an ASTM protocol to conduct whole sediment toxicity tests with amphibians.

8.3 OTHER SIGNIFICANT OBSERVATIONS

It is generally recommended that samples be analyzed for a full suite of contaminants to avoid the possibility of unknown contaminants impacting the results of a toxicity test. This might not be necessary if sufficient information is available regarding a limited set of possible contaminants.

The use of a limited analyte list could make it difficult to elucidate the cause of toxicity in some samples. For example, in the Travis AFB demonstration, significant toxicity (40% survival) was observed in a sample containing 2,100 mg/kg lead. However, samples with higher lead levels (3,700 mg/kg lead) showed no impacts on survival (95% survival). Since levels of copper, TOC, CEC, SEM, and AVS were similar between the samples, some other unmeasured stressor may have resulted in the observed toxicity.

The soil and sediment toxicity tests re-affirm the importance of analyzing environmental samples for factors like TOC, which may influence bioavailability. Compounds such as lead may be significantly less toxic to amphibians in wetland systems with elevated levels of organic matter than they would be in more sandy, less organic systems.

The implementation of the sediment exposure protocol is somewhat seasonally dependent. The availability of *R. pipiens* eggs caused a delay in the start of the sediment toxicity tests for both demonstration sites. The use of alternative species may ameliorate this limitation. However, the end user should be aware of the differential sensitivity of the test organism relative to the amphibian species present in the wetland under investigation. For example, use of a less sensitive frog species would not be an appropriate choice for evaluating a wetland containing a sensitive endangered anuran species.

To assess the differential sensitivities of larval amphibians to sediment spiked with either copper or lead, ESTCP funded an additional set of laboratory studies. 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 sediment. A total of 24 10-day sediment toxicity tests were conducted using 11 larval amphibian species with survival, body width, and body length measured at test termination (NAVFAC, 2009). Figure 6 presents the median lethal concentrations (LC50) following 10 days of exposure to lead or copper in the sediment.

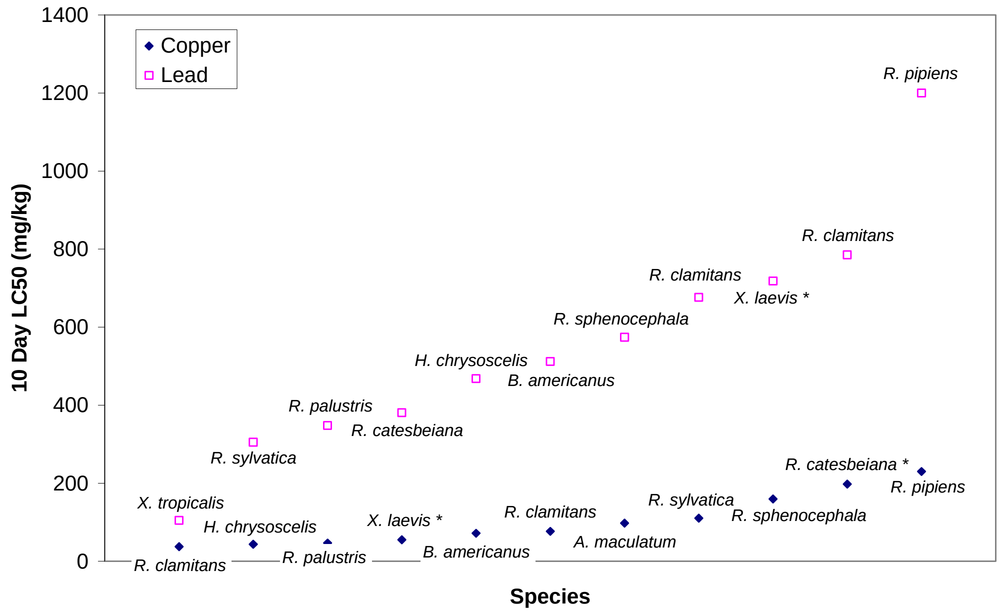
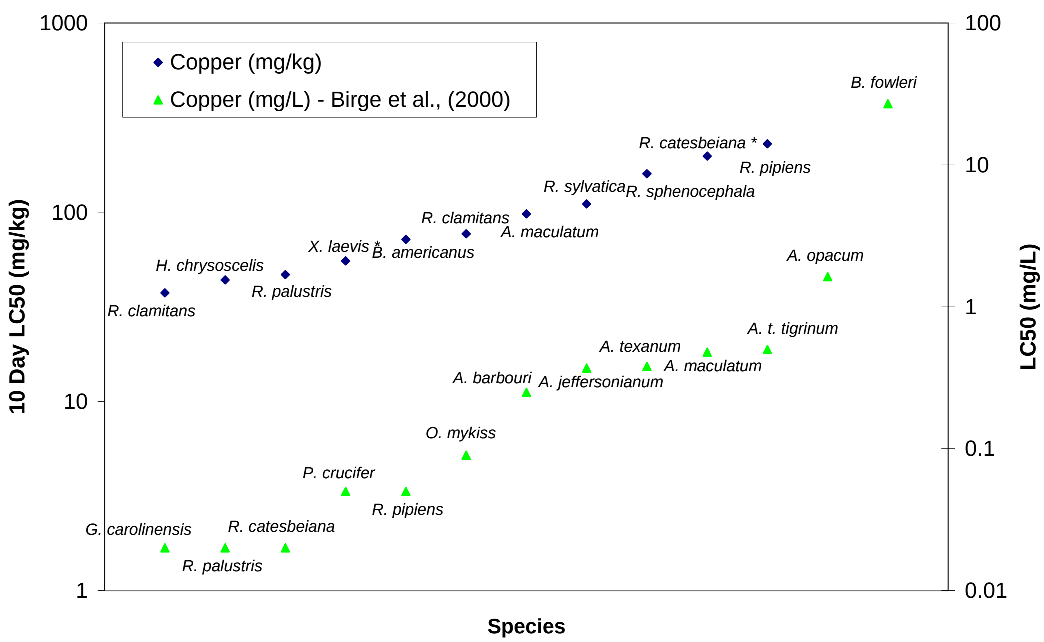


Figure 6. LC50 Values Following 10-Day Exposure to Copper or Lead.

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 range of copper LC50s from the species sensitivity testing is much smaller than the range of LC50s for metals presented by Birge et al (2000) (Figure 7), and a comparison of the four species in common between the two data sets 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)

The testing indicated that, 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.



*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 7. Comparison of Copper LC50 Values.

8.4 END-USER ISSUES

ERAs are often warranted in wetland areas 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. In general, regulators and environmental managers understand that other methodologies are needed in order to evaluate potential impacts to amphibians. This demonstration effort shows that the recently developed toxicity testing methods and the amphibian ERA protocol are an appropriate option for assessing risks to amphibians.

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

8.5 APPROACH TO REGULATORY COMPLIANCE AND ACCEPTANCE

One of the primary qualitative objectives of this demonstration was to have the results of the toxicity tests accepted by regulatory agencies in support of an ERA. However, since neither demonstration site is currently under regulatory review, this has not been accomplished. However, the sediment exposure protocol has been accepted as part of ERAs conducted under state and federal programs, and the soil exposure protocol is similar to a methodology used by DoD to generate toxicity data for the development of soil screening levels for other compounds (e.g., TNT, DNT, RDX).

The acceptance of the sediment exposure protocol as an ASTM guide is likely to expand the audience that is aware of this technology and to promote regulatory acceptance of the methodology.

Results from this program have been presented at national and international conferences and symposia. 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. Presenting the ESTCP project in these venues is an important part of publicizing the work and achieving regulatory acceptance. 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/Strategic Environmental Research and Development Program (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
- Battelle's Fifth International Conference on Remediation of Contaminated Sediments in February 2009.

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 Air Force Center for Engineering and the Environment (AFCEE) Technology Transfer Newsletter that is distributed to more than 75,000 regulators, consultants, and members of DoD and in the Winter 2009 issue of the Navy's magazine *Currents*. 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 articles discussing the response of the salamanders to lead exposures and the results of the sediment testing program are in progress.

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9.0 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.

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.

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.

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.

MacDonald, D.D., C.G. Ingersoll, 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. Available at http://web.ead.anl.gov/ecorisk/index.cfm.

NAVFAC. 2005. *Laboratory Validation Plan for ESTCP Project ER-0514, Demonstration and Certification of Amphibian Ecological Risk Assessment Protocol.* December.

NAVFAC. 2007a. *Field Demonstration Plan for ESTCP Project ER-0514, Demonstration and Certification of Amphibian Ecological Risk Assessment Protocol.* May.

NAVFAC. 2007b. *Test Refinement Interim Report for ESTCP Project ER-0514, Demonstration and Certification of Amphibian Ecological Risk Assessment Protocol.* June.

NAVFAC. 2009. *Demonstration and Certification of Amphibian Ecological Risk Assessment Protocol.* Final Report. May.

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

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.

Weston Solutions. 2004. Ecological Risk Assessment for General Electric (GE)/Housatonic River Site, Rest of River. Volume 5, Appendix E: Assessment Endpoint – Amphibians. November.

Woodlot Alternatives, Inc. 2003. Amphibian Reproductive Success Within Vernal Pools Associated With The Housatonic River - Pittsfield to Lenoxdale, Massachusetts. Prepared for USEPA Region 1. May.

APPENDIX A

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