DRAFT TECHNOLOGY DEMONSTRATION PLAN

The Effect of Soil Properties on Metal Bioavailability: Field Scale Validation to Support Regulatory Acceptance

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TABLE OF CONTENTS

List of Figures

List of Tables

1 INTRODUCTION

1.1 BACKGROUND

There are thousands of metal-contaminated sites on DoD lands awaiting remediation and closure. The toxic metals Pb, As, Cr, and Cd are of particular concern since these metals often control risk-based remedial decisions for soils at DoD sites (Exponent, 2001). Ingestion of contaminated soil by children is the exposure pathway that generally controls remediation goals (Paustenbach, 1989) (Sheehan et al., 1991). With the exception of Pb-contaminated soils, the risk posed by soil ingestion is currently calculated from the total metal concentration and the allowed reference dose (non-carcinogen) or cancer slope factor (carcinogen). Reference doses and cancer slope factors are available for most metals and are typically derived from studies of very soluble metal species. In other words, with the exception of Pb, EPA's risk assessment guidance implicitly assumes a default relative bioavailability of 100%. The toxicity assessment for Pb is unique and is based on a pharmacokinetic model of blood Pb. The default bioavailability assumptions in EPA's blood-Pb model are 50% for food and water and 30% for soil, thus yielding a relative bioavailability in soil of 60% (30%/50%).

Metals in soil, however, can be relatively insoluble and sometimes require aggressive digestion procedures for complete analytical metal recovery. As a result, reference doses developed from studies using soluble metal species may overstate the risk posed by less soluble metals in soils. The generally low bioavailability of Pb and As in mining areas has been well documented. Numerous studies, for example, have shown that Pb in soil (Freeman et al., 1994; Casteel et al., 1997), mining waste (Dieter et al., 1993; Polak et al., 1996) and aggregate (Cheng et al., 1991; Preslan et al., 1996) is much less bioavailable than more soluble Pb species such as Pb oxide, nitrate, or acetate commonly used in toxicological studies. As a result, Pb in mining environments often exhibits limited bioavailability, and children in Pb mining communities often have lower blood Pb levels than in other areas of the country (Rieuwerts and Farago, 1995). Relatively low Pb bioavailability is a consequence of Pb speciation and the corresponding solubility constraints (Davis et al., 1993) and of kinetically-controlled dissolution due to limited residence times in the gastrointestinal (GI) tract (Ruby et al., 1992). Risk assessments based on data from studies using soluble metal salts overestimate the risk posed by these soils (Davis et al., 1992). In mining-impacted areas, low soil-metal bioavailability is most likely due to the presence of residual low-solubility metal.

Recent SERDP research on certain DOE and DoD hazardous waste and firing range contaminated soils found that nearly all soil-bound Pb was bioaccessible (an in vitro surrogate for oral bioavailability). These data were in agreement with highly labile Pb in Pb-spiked soils from around the country that suggested Pb bioaccessibility remained high despite the fact that it was thoroughly adsorbed to various mineral constituents in the soils (Yang et al., 2003). Molecular speciation analyses using x-ray absorption spectroscopy (XAS) suggested that Pb(II) was weakly associated with the soil via electrostatic interactions (Fig. 1). Apparently in these systems, weak surface bonds between Pb and soil are easily disrupted by the acidic conditions encountered in the stomach. This makes Pb much more bioavailable relative to Pb in mining soils where it most likely exists as sparingly-soluble PbS. However, not all DoD soils have

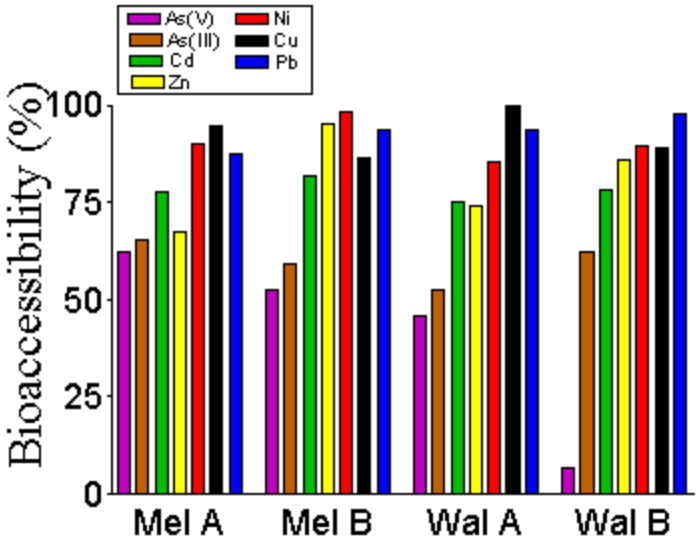
highly bioaccessible Pb, as molecular speciation suggests that the Pb is metallic or precipitated as sparingly soluble species (Fendorf, Stanford University, unpublished data).

The reference dose for As is based on human epidemiological studies of As in drinking water. However, soluble As in drinking water is much more bioavailable than insoluble As in soils, the latter being primarily excreted through the feces without absorption in the GI tract (Freeman et al., 1995). Estimates of risk due to ingestion of As-contaminated soils from some areas will be overstated unless the lower bioavailability of As in these soils is considered (Davis et al., 1996). Rodriguez et al. (1999) found that the *in vivo* relative bioavailability of As in soils from various mining and smelter sites ranged from 3 to 43%. They further found that a physiologically-based *in vitro* bioaccessibility method correlated extremely well with the *in vivo* method that used immature swine as a model for the gastrointestinal function of children.

Recent SERDP research has also shown that reference dose criteria used for soil As and Cr is often highly conservative because the indigenous metal-sequestering properties of many soils can significantly lower the bioavailability of ingested toxic metals relative to commonly used default values (Yang et al., 2002; Stewart et al., 2003; Stewart et al., 2003; Yang et al., 2003). We used a relative bioaccessibility factor to show that numerous DoD soils throughout the U.S. can effectively sequester Cr(III/VI) and As(III/V), significantly decreasing metal bioavailability (Figs. 2 and 3). Certain soil physical and chemical properties (e.g., Fe-oxide content, organic matter content, and pH) were highly correlated with decreased metal bioaccessibility, and statistical models were formulated to estimate metal bioaccessibility. We also used high-resolution spectroscopic techniques, such as XAS, to characterize the chemical environment and speciation of sequestered metals and to verify the modeling results (Figs. 2 and 3). Studies conducted at DOE's Stanford Synchrotron Radiation Laboratory confirmed that numerous DoD soils contain natural soil constituents that could reduce mobile Cr(VI) to the less toxic Cr(III) species, and oxidize highly mobile As(III) to the less mobile As(V) species. These redox transformations significantly decreased toxic metal bioaccessibility. Nevertheless, certain soil conditions were also found to enhance bioavailability of these metals. For example, when the soil Fe-oxide content for a particular DoD soil fell below 0.5% on a mass basis, the bioaccessibility of As increased dramatically, particularly for alkaline soils (Fig. 2) (Yang et al., 2002; Yang et al., 2003). Likewise, for DoD soils low in organic and inorganic carbon, the bioaccessibility of Cr(III) and Cr(VI) is significantly higher relative to soils that possessed these mineral constituents (Stewart et al., 2003; Stewart et al., 2003) (Jardine et al., 1999; Fig. 3).

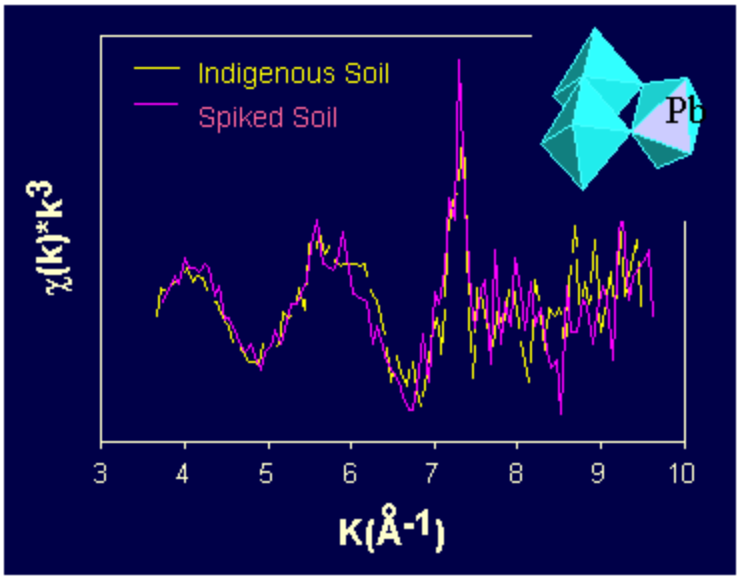
Unlike Pb and As, most studies of Zn, Cu, Cd, and Ni bioavailability in soils have focused on ecological bioavailability, primarily plant uptake. However, Schroder et al. (2003) reported strong correlation ($P<0.001$) between Cd bioaccessibility measured using a modified in vitro method of Rodriguez et al. (1999) and Cd bioavailability determined from an immature swine dosing trial. Removal of the "dough" dosing vehicle in Rodriguez et al. (1999) was necessary to obtain a correlation between bioaccessible Cd under gastric conditions and Cd bioavailability (Schroder et al., 2003). Plant uptake studies have shown that these metals are largely immobilized by soils, and only a small fraction is bioavailable. Banjoko et al. (1991) found that most of the zinc (78%) present in soil existed in the recalcitrant residual fraction and was not available to maize grown in the soils. When Zn was added to the soil, the Ca-exchangeable fraction decreased to zero within a few days, reflecting the increasing strength of the metal-soil

Figure 1



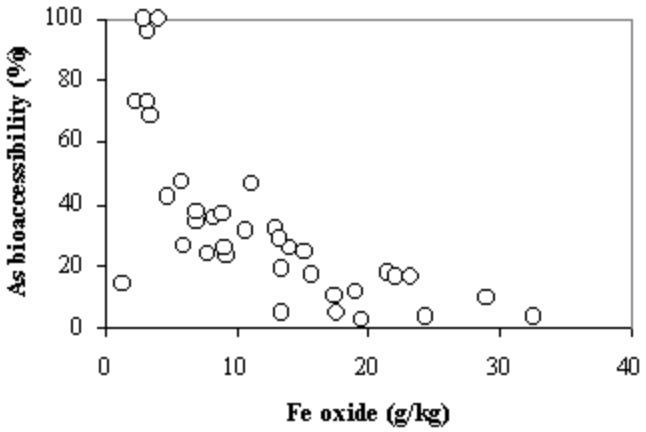
An example of toxic metal bioaccessibility on four soils relevant to DoD sites. Divalent cations, such as Pb, can be highly bioaccessible due to weak interactions with the soil.

EXAFS Spectra of Pb-contaminated Soils

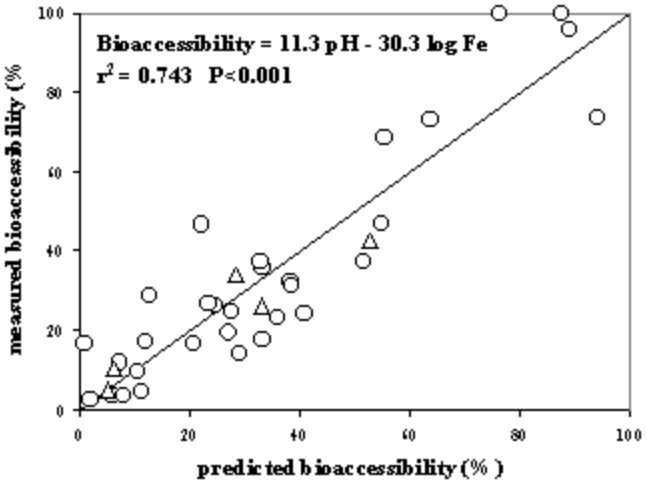


An example EXAFS spectra for Pb contaminated McClellan Air Force Base soil showing that Pb(II) is weakly associated with the soil via electrostatic interactions, thus supporting its high bioaccessibility.

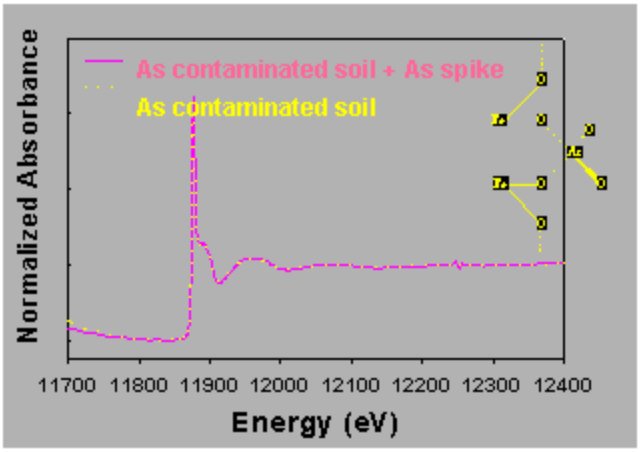
Figure 2



Arsenic bioaccessibility sharply increases in DoD soils that are lacking in Fe-oxides.



Arsenic bioaccessibility was strongly correlated with both soil pH and Fe-oxide content. As an example, the model was able to independently predict bioaccessibility in five DoD soils (triangles).



An example XAS spectra of As(V)-contaminated McClellan Air Force Base soil showing the strong inner sphere complexes with Fe-oxides, which is consistent with the above empirical model.

bond over time. Pierzynski (1993) found that uptake of Zn by soybeans correlated not with total soil Zn, but with more readily available fractions. Similarly, only a readily-available fraction of Cu, Cd, and Ni (Krishnamurti et al., 1995; Sloan et al., 1997; Hamon et al., 1998; Luo and Christie, 1998) is typically bioavailable in soils. In addition, when metal-scavenging manganese (Boularbah et al., 1996) or iron (Chlopecka and Adriano, 1996) oxyhydroxides are added to soil, metal bioavailability decreases. Recent SERDP research in our group, using a physiologically-based *in vitro* bioaccessibility method to simulate the human GI tract, has shown that DoD soil-bound metals such as Pb^{2+} and Cd^{2+} sometimes remain highly bioaccessible even though they are sequestered by the soil solid phase (e.g., Fig. 1). Although these toxic metals were effectively bound to the surfaces of mineral constituents in the soil, their weak surface bonds were easily disrupted by the acidic conditions encountered in the simulated stomach environment, allowing them to be much more bioaccessible. These findings are consistent with several bioavailability studies documented by the National Environmental Policy Institute (NEPI, 2000) that confirm soils decrease the bioaccessibility of Cd, but not nearly to the extent as is observed for metals such as As and Cr. Schroder et al. (2003) reported mean bioaccessible Cd of 63.0% using an in vitro gastrointestinal method and mean Cd relative bioavailability of 63.4% in contaminated soils from dosing trials using immature swine. Based on these findings, measurements of key soil properties could be used as indicators to determine whether site remediation is necessary or if more definitive site-specific *in vivo* metal bioavailability studies are warranted. However, site-specific use of bioavailability estimates from soil properties is impeded by the lack of regulatory acceptance. This is rational due to the lack of site-specific investigations that couple *in vivo* bioavailability and *in vitro* bioaccessibility studies with soil properties and microscopic interrogation of the solid phase metals. Several studies have shown good correlations between the *in vitro* Physiologically-Based Extraction Test (PBET) or In Vitro Gastrointestinal (IVG) methods and *in vivo* swine feeding studies for soil Pb (Ruby et al., 1996), soil As (Rodriguez et al., 1999), and soil Cd (Schroder et al., 2003). However, none were designed to investigate DoD site-specific soils or considered the role of soil properties in controlling metal bioavailability.

On DoD sites where human exposure is not the main cleanup driver or ecological risk assessment (ERA) is required, metal bioavailability must be estimated by methods other than PBET or IVG extractions in order to assess exposure for wildlife, soil invertebrates, and plants. Although these extraction techniques may serve to estimate dietary metal exposure in mammalian wildlife, they would not suffice for exposure estimates for soil invertebrates and plants. Similar to human exposure estimates, bioavailability is not currently considered in ecological risk assessments and exposure dose is measured as total metal levels. Instead of references doses, toxicity reference values (TRVs) and ecological soil screening levels (EcoSSLs) have been developed by the US EPA (US EPA 2005) for screening soil metal levels for wildlife, soil invertebrates, and plants. These values have been developed considering soils in which metals are maximally bioavailable (sandy, low pH and low organic carbon content). However, site-specific bioavailability adjustments are possible if site metal levels are found to exceed these screening values. A number of techniques are available for making bioavailability adjustments for metals exposure to soil invertebrates and plants. Weak salt extractions (e.g., $Ca(NO_3)_2$ or $CaCl_2$) offer a reasonable alternative to total metal levels, and diffusion gradient

thin films (DGT) are currently being employed as an additional method for estimating the bioaccessible fraction of metals in soils.

1.2 OBJECTIVES OF THE DEMONSTRATION

(1) To validate the use of soil properties coupled with *in vitro* bioaccessibility methods as a screening tool for estimating *in vivo* toxic metal bioavailability in DoD soils.

(2) To provide DoD with a scientifically and technically sound procedure for estimating human and ecological risk associated with metal-contaminated soils, thus reducing or eliminating the need for more-detailed, site-specific bioavailability (e.g., animal dosing) studies.

(3) To obtain regulatory and end-user acceptance of the use of bioaccessibility values derived from *in vitro* methods in human health and ecological risk assessments.

1.3 REGULATORY DRIVERS

Several recently published studies have summarized the current regulatory climate in regards to these issues. For example, Ehlers and Luthy (2003) summarized the results of the recent NRC report "Bioavailability of Contaminants in Soils and Sediments." There is neither a national policy nor legal recognition of incorporating bioavailability considerations in site cleanup. To help fill this void, the EPA is developing guidance and hosted an expert panel discussion in April 2003 on metal bioavailability in soils. Several factors must be aligned at a site to make bioavailability of a contaminant an important consideration: 1) the contaminant whose bioavailability is being investigated is the risk driver; 2) default assumptions of 100% bioavailability are unrealistic; and 3) substantial quantities of contaminated soil and sediment are involved. Bioavailability arguments should also only be used where site conditions are unlikely to change over time. The report advocates long-term monitoring of contaminant sequestration. A range of tools is available to study bioavailability, from microscopy, to chemical extractions, to bioassays. Tools that promote mechanistic understanding and lead to the development of a predictive capability are preferred over empirical approaches. Although the report provides a nice ranking of tools, no single tool achieves the highest ranking in all categories. The report thus advocates a "weight-of-evidence" approach to tool selection. The default assumption is typically 100% contaminant bioavailability, which is usually a conservative assumption, because most toxicity tests intentionally use forms of chemicals that are readily absorbed. Bioavailability assessments can be used to help better prioritize site cleanup. Most previous assessments have usually come from industry-funded studies at specific sites.

Studies have also focused on the application of these techniques specifically to DoD sites (Battelle and Exponent, 2000; Kelley et al., 2002). Except for Pb, the EPA's human health risk assessment guidance implicitly assumes a default relative bioavailability of 100%. Bioavailability data can be incorporated into risk assessments at the screening level (Tier IB) as well as in the baseline risk assessment (Tier II). The results of the Tier IB assessment can be used to remove sites from further consideration or for early identification as to whether or not a bioavailability adjustment is potentially useful in the baseline risk assessment. Bioavailability

adjustments should be considered in the following situations: a) a risk estimate slightly exceeds an acceptable level and triggers required remediation; b) risk-based cleanup goals require extensive remediation; c) remediation is not technically feasible; and d) remediation will adversely impact the environment. If more than three chemicals are risk drivers at a given site, the chances that bioavailability adjustments of a few would significantly affect the required cleanup levels are lessened. Factors that significantly affect whether or not a bioavailability study should be considered include: a) whether the studies can be completed within the required timeframe; b) the cost of the bioavailability study relative to cleanup; c) whether or not existing data support the likelihood of reduced bioavailability.

1.4 STAKEHOLDER/END-USER ISSUES

A workshop was held in San Diego, California on September 15, 2005 to which state regulators, DoD site end-users, EPA officials, and scientists familiar with soil metal bioavailability were invited to help address several technical and regulatory issues associated with ESTCP project ER-0517. The workshop focused on past, current, and future research on soil metal bioavailability including the possible use of *in vitro* bioaccessibility values for human health and ecological risk assessments. The workshop agenda included presentations by experts in the field and discussion sessions that addressed four challenge questions. Part of the first challenge question addressed soil selection relative to metal concentration. The stated question asked "For the four metals in question (As, Cr, Cd, and Pb), what is the range of concentrations for which bioavailability adjustments may affect site decisions?" Among the 35 participants, there was no general agreement on the As, Cr, Cd, and Pb concentrations relevant to bioavailability adjustments in risk assessment decisions. The lack of guidance and policy coupled with time constraints on moving forward with cleanups was deemed a regulatory barrier. The lack of guidance was thought to stem from insufficient published data to support the use of bioavailability adjustments in risk assessments. Data shortfalls are many and the group felt that the following should be considered:

(1) More data is needed for all metal concentration ranges, including low concentrations to justify back-extrapolation of dose/response curves,

(2) Data quantifying speciation effects on bioavailability and toxicity is needed,

(3) More data is needed to select/justify *in vivo* models, such as swine and plant models (indigenous plants vs. lettuce), and accumulation rather than toxicity should be measured.

2 TECHNOLOGY DESCRIPTION

2.1 TECHNOLOGY DEVELOPMENT AND APPLICATION

The project seeks to provide field-validated evidence that *in vitro* bioaccessibility methods can serve as time- and cost-effective predictive indices of toxic metal bioavailability (*in vivo*) in DoD soils relative to *in vivo* feeding studies. By quantifying the extent to which soil properties control metal bioavailability, we will show that the models developed in SERDP projects CU-1166 and CU-1210 can be used with reasonable confidence to predict site-specific metal bioavailability for DoD soils throughout the United States. By coupling *in vitro* and *in vivo* methods at numerous DoD field scale facilities with upfront regulator and end user input, our goal is to obtain regulatory acceptance of *in vitro* methods and predictive tools for assessing toxic metal bioavailability in contaminated DoD soils as it relates to human and ecological risk.

The purpose of this demonstration is to validate the ability of soil chemical and bioassay methods to predict metal bioavailability for human and ecological risk assessment. Soil properties, total metal content, and metal bioaccessibility and bioavailability (as measured by various *in vitro* and *in vivo* methods, respectively) will be determined for metal contaminated soils collected from the four DoD sites for the human health models. A similar approach will be taken for the in vitro ecological model and it will be made more robust by considering an additional 8 DoD soils (total of 12 contaminated and 12 control soils for the ecological models).

Metal bioaccessibility and metal bioavailability for the four study soils will be calculated using soil property-driven models developed from CU-1166 and CU-1210 studies, respectively. Calculated bioaccessibility values will be compared with measured bioaccessibility values using *in vitro* gastrointestinal methods for study soils. The physiologically based extraction test (PBET) developed by Ruby et al. (1999), will be utilized at a variety of pH conditions to estimate metal bioaccessibility for a variety of stomach environments indicative of food intake, or lack thereof. Using the method of Stewart et al. (2003; 2003), additional soil property-driven models will be constructed using the PBET method at these pH values. This is particularly important for Pb contaminated soils since Pb bioaccessibility decreases with an increase in pH (Yang et al., 2002; Yang et al., 2005). In contrast, As(V) bioaccessibility was minimally influenced by changing pH environments. In addition to PBET, the OSU-IVG method will be used to measure bioaccessible As, Cd, and Pb. The ability of the OSU-IVG method to predict contaminant bioavailability will be determined.

For ecological risk estimates, metal bioavailability will be estimated from multiple regression and path analysis models developed using toxicity and bioaccumulation data from 26 soils (CU-1210 and the US EPA-NCEA study (Dayton et al., 2005; Bradham et al., 2006). Additionally, 8 selected DoD sites will be tested in addition to the four soils proposed above. This is necessary to enhance the robustness of the ecological model (CU-1210; Dayton et al., 2005; Bradham et al., 2006)as has already been done for the human-based model in CU-1166. In the ecological investigations, metal concentrations from *in vitro* DoD soil metal extractions coupled with DoD soil chemical and physical properties will be compared to existing statistical relationships for estimating metal bioavailability to plants and soil invertebrates. Initially, statistical relationships

developed for metal availability from a set of 26 soils will be used to estimate the chemical availability of metals in DoD soils, based upon total metal levels and soil physical/chemical characteristics. This will be followed by extraction of the DoD soils using several wet chemical methods (e.g., extraction with dilute salts such as $Ca(NO_3)_2$; Basta and Gradwohl, 2000; Bradham et al., 2006; Dayton et al., 2006; Dayton, 2003) to actually measure metal availability in DoD soils. These measurements will be compared to predicted chemical availability estimated by the models to determine the ability of the models to predict metal availability. Toxicity predictions for soil invertebrates and plants will be made assuming additive toxicity of individual metals. Finally, bioassays will be conducted with DoD soils to determine actual toxicity and these results will be compared to the model predictions. Comparison of the actual toxicity from bioassays with predicted toxicity from *in vitro* models will be used to quantify the ability of *in vitro* models to predict actual ecotoxicity in field DoD soils. This will be the basis for validation of the in vitro methods for field DoD soils.

2.2 PREVIOUS TESTING OF THE TECHNOLOGY

Within SERDP CU-1166, a predictive model, the Soil BioAccessibility Tool (SBAT) (Heuscher et al., 2004) was developed to assess the relative bioavailability of toxic metals in soils. The model was built on the premise that key soil physical and chemical properties (e.g., Fe-oxide content, organic matter content, pH) were statistically correlated with decreased metal bioaccessibility (as measured by *in vitro*, PBET technique). Model results were found to be in good agreement with molecular level metal speciation studies and *in vivo* swine feeding studies (Yang et al., 2002; Yang et al., 2005). Nevertheless, model validation using *in vivo* studies on actual DoD field samples is lacking. Such an endeavor is critical if the model is ever to obtain end-user and regulatory acceptance.

In addition, recent publications within our group, investigating the bioavailability of As in soil have found that the *in vitro* bioaccessibility method (PBET) correlated extremely well with the *in vivo* method that used non-DoD soils and immature swine as a model for the gastrointestinal function of children (Rodriguez and Basta, 1999). Similar findings have been reported for soil bound Pb and Cd where the *in vitro* PBET method correlated very well with *in vivo* swine feeding studies (Ruby et al., 1996; Schroder et al., 2003). The Ohio State University IVG (OSU-IVG) method has been shown to be correlated with As (Rodriguez et al., 1999; Basta et al., 2006), Pb (Schroder et al., 2004), and Cd (Schroder et al., 2003) Such information has lead to partial regulatory acceptance in England, where the *in vitro* methods have been used to assess field scale metal bioavailability issues. Our research team members also belong to the Bioavailability Research Group of Europe (BARGE) where we have established an international collaboration that seeks to demonstrate the appropriateness of *in vitro* methods for assessing risk associated with soil metal bioavailability. The UK and several countries within the EU have used our data (United States) of coupled *in vitro* and *in vivo* soil metal bioavailability to convince the regulatory community, in their respective countries, that *in vitro* measurements of soil metal bioaccessibility are acceptable estimates of *in vivo* soil metal bioavailability. However, regulators in the United States remain uncertain that the *in vitro* methods can adequately predict soil metal bioavailability in humans.

Prior ecotoxicological studies within our group have also been completed that show soil properties similarly affect the bioavailability of As, Cd, Pb, and Zn for soil invertebrates and plants . Measures of metal exposure based upon soil extraction techniques, such as dilute salts (Basta and Gradwohl, 2000; Dayton, 2003; Bradham et al., 2006; Dayton et al., 2006), have been coupled with soil chemical and physical properties to develop statistical relationships for estimating metal bioavailability for soil organisms. These statistical models are the first step in the development of models capable of predicting the toxicity of metals to soil invertebrates and plants.

Based on our previous scientific and technical advances in the area of *in vitro* and *in vivo* metal bioavailability in soils, we believe that it is timely to apply these techniques to DoD site-specific problems. Such an effort will validate bioaccessibility and bioavailability estimates based on *in vitro* methods and soil properties for DoD sites. Close cooperation with regulators and end users should lead us closer to regulatory acceptance of *in vitro* methods for assessing toxic metal bioavailability in soils and use of the validated predictive tool SBAT.

Our team has also been involved in research addressing the ecological risk of metals in soil systems. Basta, Dayton and Lanno conducted soil ecotoxicological research for a US EPA-NCEA research project "An Integrated Soil Chemical and Toxicological Approach for the Development of Ecological Screening Levels for Heavy Metals in Soil" (NCEA-ORD Award # CR 827230-01-0) which involved developing methods for determining metal exposure in soil to earthworms and plants using chemical analysis methods other than total metals. Experiments were conducted in 22 soils differing in physical/chemical characteristics to develop statistical models relating soil characteristics to bioavailable levels of metals and toxicity in plants and earthworms. This project was followed by CU-1210 (Determining the Bioavailability, Toxicity, and Bioaccumulation of Organic Chemicals and Metals for the Development of Ecological Soil Screening Levels) that examined in greater detail the factors affecting the bioavailability, bioaccumulation, and toxicity of As, Cd, Pb, and Zn to soil invertebrates and plants. The basic understanding garnered from these two collaborative efforts has resulted in the generation of sufficient data that we feel will allow us to begin to predict the toxicity of metals to plants and invertebrates in soil. Application of the statistical models generated during these studies to the DoD soils in the proposed study would provide a validation of the models. The results of our research have also lead to studies examining the physiological partitioning of metals in soil invertebrates and collaborations with researchers at RIVM (Bilthoven, The Netherlands) and the Vrije Univeristeit (Amsterdam, The Netherlands) in the development of a Biotic Ligand Model (BLM) for predicting the toxicity of metals to invertebrates in soil systems.

2.3 FACTORS AFFECTING COST AND PERFORMANCE

The results from this project will provide site managers and risk assessors with tools to make better initial estimates of site risk that can then be used to prioritize sites and to justify, on the basis of the projected cost savings from the revised Environmentally Acceptable Endpoints (EAEs), more definitive site-specific *in vivo* bioavailability studies. EAEs are concentrations of a chemical or other measures of contamination (e.g., biological responses) that are judged acceptable by a regulatory agency or an appropriate entity, either a priori (e.g., screening level or guideline) or following an analysis of site-specific or chemical-specific information and/or

testing (Linz and Nakles, 1997). In this context, measures of metal bioavailability can be used to eliminate sites or portions of sites from further risk assessment procedures during screening or Phase I procedures. Two types of approaches could be used: 1) where background data on the site such as total metal levels and soil properties are available, direct application of the models developed from CU-1166 and CU-1210 would provide estimates of the hazard posed by metals at the site; 2) for sites where little information is available, chemical data such as an *in vitro* GI extraction for human risk or a weak salt extraction for ecological risk would be meaningful for making a decision regarding the site. These values would be compared to screening criteria to determine whether any further assessment is warranted. These concepts are quite unique in that site risks are based on bioavailability estimates versus the current standard of basing site risk on traditional total soil metal analyses; concepts that could save DoD huge expenses in unnecessary remedial costs.

Estimated costs of *in vitro* studies are $5K-15K and $50K-200K for *in vivo* studies (Battelle and Exponent, 2000). Soil excavation and landfilling costs have been estimated at US $730 m^{-2} to a 60 cm depth (Vangronsveld and Cunningham, 1998). Remediation costs associated with soil excavation and replacement exceeding $10,000,000 per site are not uncommon. Many times, excavation is performed because risk assessments assume the contaminant is highly bioavailable (i.e., 60% for Pb, 100% for As, Cd, and Cr). Use of in vitro methods to assess contaminant bioavailability will identify soils that have low contaminant bioavailability and/or little/acceptable risk and not require remediation via excavation/replacement. *In vitro* methods will help focus prudent use of limited fiscal resources for contaminant remediation and cleanup on DoD sites. Regulatory acceptance of *in vitro* methods will produce cost savings in the range of billions of dollars.

2.4 ADVANTAGES AND LIMITATIONS OF THE TECHNOLOGY

The proposed initiative seeks to provide field validated evidence that *in vitro* bioaccessibility methods can serve as predictive indices of toxic metal bioavailability (*in vivo*) in DoD soils relative to the more costly and time intensive *in vivo* feeding studies. By quantifying the extent that soil properties control metal bioavailability, we will show that the predictive models developed in CU-1166 and CU-1210 can be used with a reasonable level of confidence to predict site-specific metal bioavailability for DoD soils throughout the United States. By coupling in vitro and in vivo methods at numerous DoD field scale facilities with upfront regulator and end user input, our goal is to obtain regulatory acceptance of in vitro methods and the SBAT tool for assessing toxic metal bioavailability in contaminated DoD soils as it relates to human and ecological risk.

The lack of regulatory acceptance of the *in vitro* methods is the largest technical limitation. This may be an issue because we are investigating 24 soils(12 contaminated and 12 control soils) at 12 DoD sites and regulators may wish to see more data before making a decision. The problem then becomes expense. *In vivo* feeding trials and ecological bioassays are expensive and time consuming; however, they are far less expensive than actually having to remediate a site, particularly when remediation was not needed on the site in the first place. We believe that this upfront investment by ESTCP to compare *in vitro* methods with *in vivo* investigations will

minimize these technical limitations and potentially save DoD significant remedial cost in the long term.

3 DEMONSTRATION DESIGN

3.1 PERFORMANCE OBJECTIVES

The bioavailability screening tool for DoD soils (SBAT from CU-1166; soil extractions from CU-1210) will be tested by determining the chemical speciation, bioaccessibility, bioavailability, and toxicity of metals (Pb, As, Cd, Cr) in DoD soils as measured by biological models used to evaluate ecological risk (e.g., plants, earthworms) and human risk (e.g., immature swine model). Since ingestion is often the primary human risk driver at contaminated sites (Exponent, 2001), human risk by ingestion will be evaluated rather than dermal pathways. Only four sites are considered for the *in vivo* swine dosing studies due to the experimental cost. The use of *in vitro* ecological models will be further verified by comparison with *in vivo* ecological bioassay studies of approximately 12 DoD soils (12 contaminated, 12 control). Many of these soils will be the same as those used for the human-based models in SERDP project CU-1166. In the latter study, over 40 DoD soils were screened using the PBET method, yielding data to guide our choice of DoD sites for initial and future *in vivo* studies. This project will also take advantage of the significant prior investment by SERDP and ESTCP in projects CU-1165 and CU-0222, respectively. Both of these projects have goals complementary to those of ER-0517, and we plan to collaborate with the PIs in an effort to leverage our efforts. At the workshop, the research strategy was discussed among scientists, regulators, EPA, and end-users to advance the acceptance of *in vitro* methods in human health and ecological risk assessment and policy.

An important component of the technical approach is to validate and demonstrate the ability of soil property models (Yang et al., 2002; Stewart et al., 2003; Stewart et al., 2003; Yang et al., 2005) and *in vitro* techniques to predict metal bioavailability and risk (e.g., ecological, human). Results obtained from methods developed for assessing metal risk-based endpoints for human (CU-1166) and ecological receptors (CU-1210) will be compared with results from well-established standard methods used to determine human risk (U.S. EPA Risk Assessment Guidance for Superfund--RAGS) and ecological risk (U.S. EPA Ecological Risk Assessment).

3.2 SELECTING TEST SITES

Four (4) DoD facilities with different soil properties, but with common metal contamination problem with regards to Cr, As, Pb, and Cd were desired for the swine dosing trials and 12 soils were desired for ecological bioassay studies. Both soil types hypothesized to strongly sequester metals and soil types thought to have poor metal sequestering potential were desired. Example of such DoD sites are Hill AFB – UT, Travis AFB - CA, Deseret Chemical Depot – UT, Aberdeen Proving Ground – MD, Redstone Arsenal – AL, Naval Station Newport – RI, and Fallon Naval Air Station – NV, all of which have significant problems with metal contaminated soils. Select chemical, physical, and mineralogical, properties of some of the possible study soils had previously been quantified in our laboratory as described by Stewart et al. (Stewart et al., 2003; Stewart et al., 2003). Soils at Hill, Deseret, and Fallon Naval Air Station are Aridisols that are sandy, high pH soils with a limited capacity to sequester metals. These soils were expected to have high metal bioaccessibility. Soils from Aberdeen and Travis are silty, neutral pH soils with

Table 3-1

Performance Objectives ER-0517

Type of Objectives	Primary Performance Metrics	Expected Performance Metric	Actual Performance Objective Met?
Quantitative – Ecological Bioassays vs. in vitro protocol	Statistical correlation	Significant multiple correlation	TBD*
	Consistent with speciation	Physical significance of model confirmed	TBD
	Estimated risk	Adequate risk assessment	TBD
Quantitative – Swine bioassays vs. in vitro protocol	Statistical correlation	Significant multiple correlation	TBD
	Consistent with speciation	Physical significance of model confirmed	TBD
	Estimated risk	Adequate risk assessment	TBD
Technology Transfer	Agency acceptance	Results considered acceptable by state or federal regulatory agency for site evaluation	TBD
Qualitative – Ecological bioavailability protocol	Protocol is applicable for evaluating Pb, Cd, Cr, As in soil	Validated statistical model	TBD
	Agency acceptance	Results considered acceptable by state or federal regulatory agency for site evaluation	TBD
Qualitative – Human bioavailability protocol	Protocol is applicable for evaluating Pb, Cd, Cr, As in soil	Validated statistical model	TBD
	Agency acceptance	Results considered acceptable by state or federal regulatory agency for site evaluation	TBD

* TBD = To be Determined

good to excellent metal sequestering properties. These soils were expected to have low metal bioaccessibility. Redstone and Naval Station Newport are acidic, Fe-oxide rich Ultisols and Inceptisols that have excellent sequestering properties for As, and potentially poor sequestering properties for Cd, Pb, and Cr(VI), thus the latter metals being highly bioaccessible.

During the September 2005 workshop, the second challenge question specifically addressed the use of soil properties in human and ecological risk, and these criteria were considered in site selection. The stated question asked "How can soil properties be used to adjust risk estimates? At what stage of a risk assessment are they best applied?" and "How can soil property data be best utilized to make risk assessment adjustments (i.e., how can we account for site variability in soil properties)?" It was suggested that in collecting new data, it would be helpful to have a list of ideal soil property ranges to guide sample collection, and samples should be well-homogenized. Translating soil properties into field-scale risk assessment adjustments will require consideration of future site uses that may alter soil characteristics and the subsurface environment and hence, bioavailability.

Site selection considered the suggestions brought forth by the workshop where a wide range of soil properties and contaminant concentrations were considered. Site selection will also be strongly influenced by site access issues and ability to remove contaminated soil from the site. Many of the sites are RCRA metal contaminated sites and regulatory issues may not allow the site to release contaminated soil due to liability issues.

The final criteria for selecting test sites varied considerably depending upon the risk endpoint. Initial soil criteria were arranged by risk endpoint, including plant studies (Table 3-2), earthworms (Table 3-3), and swine studies (human health, Table 3-4).

3.3 TEST SITE HISTORY/CHARACTERISTICS

Based on the factors outlined above, letters were sent to a variety of DoD facilities throughout the country requesting permission to acquire fifty-five gal of contaminated soil from their respective sites. As of April 2006, sixteen (16) DoD sites have been identified and contacted with regard to contaminated soil. Of the 16, 14 sites listed below have been deemed accectable for use in project ER-0517. A description of each site was discussed earlier in section 3.0 of this document. The sites differ drastically with regard to geographical location, metal concentrations, and soil properties. Such differences are advantageous for this project in that they allow us to test the influence of soil properties on *in vivo* metal bioavailability and cross-correlations with *in vitro* bioaccessibility measurements. Table 4 illustrates the number of As, Cr, Cd, and Pb contaminated soils available thus far, and the range in contaminant concentration and soil properties most likely to influence metal bioavailability. Soils with appreciable Cd contamination are the only ones lacking in sufficient number at the present time. Arsenic contaminated soils range from 100 to 1000 mg kg^{-1} and have a range in pH of 5-8 and Fe-oxide content of 0.4 to 10 g kg^{-1}. Since soil As bioavailability is thought to be largely controlled by pH and Fe-oxides (Yang et al., 2002; Yang et al., 2005), the large variation in soil pH and Fe-oxide content is advantageous for testing the influence of soil properties on As bioavailability and the validation of previous *in vitro* models. Likewise, chromium contaminated soils range from 100

Table 3-2 Soil-metal site selection criteria for plant studies

As	50 to 500
Cd	< 100
Cr	open
Cu	50 to 500
Pb	200 to 1500
Zn	100 to 500
pH	4 to 8
EC	< 4 dS/m

Table 3-3 Soil-metal site selection criteria (mg/kg) for earthworm studies

As	250
Cd	100
Cr	1000
Cu	200
Ni	250
Pb	1000
Zn	300
pH	4 to 8

Table 3-4 Soil-metal site selection criteria (mg/kg) for swine studies

As	300-500+
Cd	1000-1500+
Cr	1000+
Pb	1500-2500+

to 3500 mg kg^{-1} and have a range in pH of 5 to 8, an organic matter content of 0.5 to 10 %, and a clay content of 5 to 40%. Since Cr bioavailability is largely controlled by organic matter, pH, and clay content (Stewart et al., 2003; Stewart et al., 2003), significant variations in these soil properties will benefit the evaluation of soil Cr bioavailability and the validation of previous *in vitro* models. Cadmium and Pb contaminated soils range from 60 to 350 mg kg^{-1} and 200 to 5500 mg kg^{-1}, respectively. Soil properties such as pH and clay content range from 4 to 8 and 5 to 40 %, respectively, allowing for adequate evaluation of the influence of soil properties on soil Cd and Pb bioavailability.

Based on the above analysis, the following four sites have been selected for the swine dosing studies.

McClellan Air Force Base

Marine Corp Air Station Cherry Point

Deseret Chemical Depot

Former Sugarcane Fields

The following sites will be used for the ecological bioavailability and *in vitro* bioaccessibility studies that include the four sites chosen for the swine dosing studies.

McClellan Air Force Base

Hill Air Force Base

Marine Corp Air Station Cherry Point

Travis Air Force Base

Anniston Army Depot

Portsmouth Naval Shipyard

Naval Support Activity Mechanicsburg

Concord Naval Weapons Site

Naval Base Point Loma

Naval Complex, Pearl Harbor, HI

A summary of these soils, with their physical and chemical properties are described below and in Tables 3-5 to 3-7.

Table 3-5 Test Sites

Site Name	Site Location	Soil Type
Travis AFB	Fairfield, CA	Alfisol
McClellan AFB	Sacramento, CA	Alfisol
Hill AFB	Ogden, UT	Entisol
Portsmouth Naval Shipyard	Kittery, ME	Inceptisol
NSA	Mechanicsburg, PA	Ultisol
MCAS Cherry Point	Cherry Point, NC	Entisol
Deseret Chemical Depot	Tooele, UT	Aridisol
Concord Naval Weapons Site	Concord, CA	Vertisol
Naval Base Point Loma	San Diego, CA	Entisol
Naval Complex, Pearl Harbor	Honolulu, HI	Mollisol
Former Sugar Cane fields	Hilo, HI	Andisol
ORNL Firing Range	Oak Ridge, TN	Ultisol

Table 3-6 Metal concentrations at the various sites in mg kg^{-1}

	As	Cr	Cd	Pb
Optimal values	**300-1000**	**1000**	**1000**	**1000-3000**
Travis AFB	19.7	89		4672
McClellan AFB	14	1155	63	315
Hill AFB	99	3480	360	1098
Portsmouth Naval Shipyard				1260
Naval Support Activity Mechanicsburg				2000
MCAS Cherry Point	31	452	64	3190
Deseret Chemical Depot	700	64		17
Concord Naval Weapons Site	1000			
Naval Base Point Loma	1000			1000
Naval Complex, Pearl Harbor	600			1000
Former Sugar Cane Fields	500			
ORNL Firing Range				1200

Table 3-7 Select physical and chemical properties of the soils

Site Name	Fe (g/kg)	TC (%)	TOC (%)	TIC (%)	pH	Clay (%)	Silt (%)	Sand (%)
Travis AFB	19.28	1.83	1.3	0.53	6.14	13.0	28.0	59.0
McClellan AFB	0.5	0.25	*	*	6.1	39.4	31.4	29.2
Hill AFB	7.9	7.68	8.02	0	7.43	5.0	14.0	81.0
Portsmouth Naval Shipyard	1.4	*	3.1	*	4.2	24.7	56.3	19
NSA	2.7	*	0.18	*	5.2	36	50.3	13.7
MCAS Cherry Point	109.7	8.03	9.8	0	8.07	33	26	41
Deseret Chemical Depot	7.62	3.74	0.44	3.3	8.24	16.7	41.4	41.9
Concord Naval Weapons Site	*	*	0.4	*	6.3	34	53	13
Naval Base Point Loma	0.4	*	0.62	*	7.8	10.3	38.8	50.9
Naval Complex, Pearl Harbor	*	*	*	*	*	*	*	*
Former Sugar Cane Fields	*	*	*	*	*	*	*	*
ORNL Firing Range	21	0.1	0.1	0	4.5	24	42	34

* To be determined.

Hill Air Force Base

Hill Air Force Base is located in Ogden, UT. The contaminated area was historically used as sludge drying beds (SDBs) during the treatment of water for potable use. Soils are Entisols and contain chromium, cadmium, and lead. The sand fraction constitutes more than 80% of the soil which has a pH of 7.5 and a TOC level near 8%.

Travis Air Force Base

Travis Air Force Base is located in Fairfield, CA. Soils from a former small arms range that operated from 1957 until 1977 contain elevated concentrations of lead and antimony. Soils consist of silt and clay loam with clay or clay loam subsoil. The Fe-oxide content of these soils can be quite high (e.g. 2% w/w).

Marine Corp Air Station, Cherry Point

The Marine Corp Air Station is located in Cherry Point, NC. Soils from a former incinerator site contain elevated concentrations of chromium. The soils are poorly developed Entisols that have a very high organic matter content and have a high pH.

Naval Support Activity, Mechanicsburg

The Naval Support Activity is located in Mechanicsburg, PA. The soils are silty clay Ultisols with low organic matter and an acidic pH. Soil from Site 11, which has functioned as a lead ingot stockpile location from the early 1950s until recent years, are heavily contaminated with lead.

Portsmouth Naval Shipyard

The Portsmouth Naval Shipyard is located in Kittery, Maine. The soils are Inceptisols consisting primarily of silt and sand with significant organic matter. Soils from Site 6, an area impacted by particulate deposition from historical land use as a temporary storage area of a variety of materials, including lead battery cell plates, will be used.

McClellan Air Force Base

McClellan Air Force Base is located in Sacramento, CA. Soils from a former wastewater treatment lagoon are contaminated with high concentrations of lead, chromium, and cadmium. The soils are fine-grained Alfisols with slightly acidity and significant organic carbon.

Deseret Chemical Depot

The Deseret Chemical Depot is located in Tooele, UT. Soils from an area that was contaminated with mine tailings from flooding during the 1930s. Soils contain very high concentrations of arsenic. The soils are Aridisols with significant silt and sand with a pH of ~8.

Concord Naval Weapons Station

The Concord Naval Weapons Site is located in Concord, CA. Soils from a site that contains elevated arsenic from pesticide applications will be utilized. Soils are silty clay Vertisols that have a near neutral pH.

Naval Base Point Loma

Naval Base Point Loma is located in San Diego, CA. Soils are contaminated with high concentrations of lead and arsenic. Soils are poorly developed sandy Entisols with a high pH.

Former Sugar Cane Fields

Former sugar cane fields located in Hilo on the big island of Hawaii contain high concentrations of arsenic. The use of arsenic based pesticides during the 1920-1940s is believed to be the source of the contaminant. The soil is of the Andisol order and consists of fine crystalline colloidal materials such as allophanes, imogolite, and ferrihydrite and thus capable of significant As(III/V) sequestration.

Naval Complex, Pearl Harbor

Soils located at the Pearl City Fuel Annex contain high levels of arsenic and lead. The source of arsenic at this site is thought to be historic pesticide or rodenticide use. The soils are of the Mollisol order and have thick organic rich surface horizons, near neutral pH and high base cation saturation.

Firing Range, Oak Ridge National Laboratory

Soils located on the small arms firing range contain elevated concentrations of lead. The soils are highly weathered acidic ultisols with abundant silt and clay that contain 2-4% crystalline Fe-oxides.

3.4 PRESENT OPERATIONS

Sampling has been conducted or arranged so as to avoid or minimize interference with present and ongoing operations at these sites.

3.5 PRE-DEMONSTRATION TESTING AND ANALYSIS

It is anticipated that 10 to 12 plastic buckets with approximately 25 kg of soil will be collected from each site. A portable X-Ray Fluorimeter will be used to identify the contaminant concentration of the collection area but the metal contaminant concentration can vary greatly between and within each collected bucket. Therefore, all soil collected from one site (e.g., 10 to 12 buckets) will have to be thoroughly mixed to produce one homogenous sample for all investigators to study and evaluate. Reference soil, the same soil series but uncontaminated (i.e., natural background levels of Cd, Pb, As) were also collected at each of the study sites for each of the contaminated soils. Soil samples collected from DoD sites will be homogenized at Ohio State University. Each field soil collected will be air-dried and then homogenized using a large modified cement mixer.

3.6 TESTING AND EVALUATION PLAN

3.6.1 Demonstration Set-Up and Start-Up

Contacting site personnel

Using the site selection criteria obtained in section 3.2, various DoD installations throughout the country are being contacted via phone and email. The site Environmental Officer is typically the site contact individual. They are briefed concerning the objectives and needs of our project and site legal personnel are than informed of the situation to ensure there are no violations concerning soil removal.

Scheduling and planning site visits

Site visits by ORNL staff are planned in detail to ensure all analytical and soil sampling equipment, personal protective gear, and containment buckets are available and ready for shipment. Every attempt is made to access more than one site for any given trip in an effort to keep travel and shipping cost to a minimum. ORNL staff work under existing and rigorously reviewed Research Safety Summaries (RSS) for working with hazardous materials and equipment (Field portable X-Ray Fluorimeter – XRF). The XRF has three sealed sources and is shipped as hazardous materials under specific DOT regulations. One of the sources is classified as a Reportable Quantity which requires the inclusion of hazard identification documentation within the package for emergency first responders.

Soil sampling protocol and off-site shipping

Once personnel and materials have arrived at a site, the vegetative layer is removed if applicable. The XRF is used to map out the location and concentrations of contaminants at the site. The XRF is pre-calibrated in the laboratory and we have found that its precision and accuracy are excellent for the metals of interest (As, Pb, Cd, and Cr). Soil is typically removed with hand tools and placed in eleven 5 gal plastic buckets (high density polyethylene) for a total of 55 gal. Each bucket is lined with a plastic bag which is sealed when full of soil so as to eliminate air dispersion of contaminated soils during shipment. ORNL staff wear steel-toed boots, nitrile gloves, dust masks, and clothes that provide minimal skin exposure. When soil extraction is complete, all PPE are stored in plastic bags to avoid air dispersion of contaminated soil. Contaminated soils targeted for this study contain metal concentrations that are below levels that would classify them as DOT hazardous materials. The soils are labeled appropriately, and are shipped to Ohio State University. Ohio State University and ORNL both have active USDA compliance agreements to allow them to receive soils from quarantined areas.

Soil homogenization and sieving

Soil samples collected from DoD sites will be homogenized at Ohio State University. The homogenized soil will be distributed to investigators to conduct bioassays and for contaminant characterization. Equipment and facilities will be in place for the processing of large quantities

of the project soils. It is anticipated that 10 to 12 20-L plastic buckets with approximately 25 kg of soil each will be collected from each site. A portable field X-Ray Fluorimeter will be used to identify target metal concentrations in the collection area prior to sampling. Since the metal concentration in soil can vary greatly between and within sample buckets, all soil collected from one site (e.g., 10 to 12 buckets) will be thoroughly mixed to produce one homogenous composite sample for all investigators to study and evaluate.

Soils are initially air dried prior to homogenization. When dry the soils are homogenized by placing all eleven buckets of a given soil (55 gal) into a large, heavy duty electric powered mixer that has a 9 cu ft. plastic drum. A large cement mixer will be modified to allow simultaneous homogenization and sieving (<2 mm) of large amounts (250+ kg) of contaminated soil. The cement mixer will be modified by using a steel cone attachment fitted with a 2-mm sieve to allow dust-free soil processing. The steel cone attachment will be custom built for the cement mixer. The novel cone attachment will allow (i) greatly improved homogenization, (ii) improved safety by greatly reducing exposure to contaminated dust from the project soils, and (iii) improved efficiency and recovery of homogenized soil. Virtually no soil will be lost during processing using the modified cement mixer whereas a large amount of soil would have been lost by conventional methods, soil that would have needed to be disposed of as hazardous waste.

The mixer is equipped with a dust trap to avoid air dispersion of the material. Soils are mixed for six hours. For soils where clumping is an issue, hardened ceramic balls, of a size that can fit in the palm of ones hand, are placed in the mixer with the soil in order to enhance aggregate breakup. Soils are next sieved to < 2 mm with a subsample sieved to < 270 um. The < 2mm samples are used in the in vitro and in vivo plant and earthworm model studies whereas the < 270 um samples are used in the in vitro and in vivo swine model studies, and for interfacial surface spectroscopy interrogation. To verify that soil samples are homogeneous, numerous subsamples (10 or more) are acid digested using USEPA method 3051a followed by Cr, As, Cd, and Pb analysis. Soils will be archived at Ohio State University where in vitro and in vivo plant and earthworm model investigations will occur. A soil storage area in the School of Environment and Natural Resources at Ohio State University will be modified (shelving, etc) to store collected soils (several hundred 20-L buckets). The soil storage area is a locked facility with very limited controlled access.

Shipping soil to various institutions for in vitro and in vivo studies

Homogeneous sieved soils will be shipped from Ohio State University to (1) University of Missouri for swine feeding trails, (2) Vanderbilt University for interfacial surface spectroscopy interrogation, (3) Oak Ridge National Laboratory for physical and chemical characterization and in vitro PBET investigations. All institutions have USDA permits for receiving and shipping quarantined soils.

Soil properties and total metal content will be determined for homogenized soils *before* samples are sent to investigators for study to assure that target metal concentrations are attained. Analyses will be conducted on sub-samples of homogenized soils and results will be summarized

and distributed to the study investigators for review. The soils that meet the study criteria will be sent to investigators to conduct bioassays.

In vitro investigations

OSU-IVG

The Ohio State University In Vitro Gastrointestinal method (IVG) will be performed on all soils at OSU at a variety of pH conditions as described below. These investigations are relevant to the swine model. Standard operating procedures are in place and all equipment necessary to perform experiments are available and ready for project use.

PBET

The Physiological-Based Extraction Test (PBET) will be preformed all soils at ORNL at a variety of pH conditions as described below. These investigations are relevant to the swine model. Standard operating procedures are in place and all equipment necessary to perform experiments are available and ready for project use.

Dilute salt and chelate

The dilute salt and DTPA chelate extraction tests will be preformed all soils at ORNL and OSU at a variety of pH conditions as described below. These investigations are relevant to the plant and earthworm models. Standard operating procedures are in place and all equipment necessary to perform experiments are available and ready for project use.

In vivo investigations

Plant Basta

Chemical and physical properties of the collected soils will be determined prior to conducting plant bioassays. This data will be used to select 12 soils for study.

Soil invertebrate

Determining the toxicity or bioaccumulation of metals by soil invertebrates

Toxicity and bioaccumulation bioassays will be conducted with earthworms (Lumbricidae - *Eisenia fetida*), potworms (Enchytraeidae - *Enchytraeus albidus*, and/or *E. minutus* or *E. crypticus* for lower pH soils), and springtails (Collembola - *Folsomia candida*). Testing will be conducted according to standard protocols (ASTM, 1999; ASTM International, 2004). Although some of the details will differ, the general experimental set up for each of the soil invertebrate bioassays is similar. Two weeks prior to the beginning of each test, approximately twice as many adult (clitellate) oligochaetes (*E. fetida* and *E. albidus*) as are needed for the bioassays will be selected from laboratory cultures and placed in an aliquot of the appropriate reference soil

corresponding to each metal-contaminated soil. At the same time, the same number of organisms will be counted into a laboratory reference soil for conditioning. A laboratory reference soil test is conducted with every bioassay in our laboratory as a QC measure to compare organism responses over time in our lab. At the commencement of each test, five replicates of each soil (metal-contaminated and corresponding reference soil) will be hydrated to the appropriate moisture content (% of water-holding capacity) and 10 organisms will be randomly selected for each replicate from either those pre-conditioned in reference soil (oligochaetes) or from laboratory cultures (Collembola). Initial responses will be observed at 1, 2, 4, 7, and 14 days to determine if an acute (mortality) response is present. If an acute response is not observed, the bioassay will proceed into a reproduction test. This consists of leaving the invertebrates in the soils for an additional time period (from 1-2 weeks, depending upon species) and then removing and counting adults in the test replicates. The soils are then allowed to incubate for an additional time (1-3 weeks, depending upon species) to allow cocoons or eggs to hatch and juveniles to attain a size that allows accurate counting. If no acute effects are evident, bioaccumulation tests will also be conducted with each of the invertebrate species. These tests will be designed to examine metal uptake kinetics. Replicates will be set up in the manner described for acute and reproduction tests, with for three replicates for each sampling time. Organisms will be sampled at 1, 2, 4, 8, 16, 32, and 64 days and total metal burdens measured to determine uptake kinetics curves.

Swine

Assessment of Arsenic and Chromium Bioavailability in Designated Test Soils

The example of arsenic assessment is provided for methodology demonstration. Pigs will be housed individually in metabolic cages (cages designed to collect and separate urine and feces) with groups of randomly selected animals (N= 4 or 5) given oral doses of test material or the soluble reference material, sodium arsenate (Na_2HAsO_4), for a total of 14 days, with the dose for each day being administered in two equal portions given at 9:00 AM (after an overnight fast) and 3:00 PM (two hours before feeding). Dose material will be placed in the center of a small portion (about 5 grams) of moistened feed (referred to as a "doughball"), and administered to the animals by hand. All missed doses will be recorded and the time-weighted average dose calculation for each animal will be adjusted downward accordingly.

Samples of urine (48 hour composites) will be collected from each animal on days 6-7, 9-10, and 12-13 during the study. Urine will be collected by placing a stainless steel pan beneath each cage, which drains into a plastic storage bottle. Each collection pan will be fitted with a nylon screen to minimize contamination with feces, spilled food, or other debris.

In addition to urine collections chromium bioavailability studies will include blood collections on study days 0, 8 and 14. 6 to 8 ml of blood will be collected from the cranial vena cava and placed into vacutainer tube for later analysis of chromium concentration.

Assessment of Lead and Cadmium Bioavailability in Designated Test Soils

Intact male pigs weighing about 10 kg initially, will be housed individually in stainless steel cages and fed low-metal feed. All doses will be delivered daily for 15 days in a low-metal

vehicle according to the diurnal schedule. For lead studies, blood samples (6-8 mls) will be drawn (following SOP #9) from each animal on days 0, 2, 4, 7, 9, 12, and 15, into a new plastic lead-free syringe by venipuncture of the anterior vena cava. The blood will be immediately transferred into lead-free Vacutainer^{R} tubes containing EDTA. In each case, blood samples will be drawn 17 hours after the second dosing of the previous day. Animal weights will be recorded and doses and feed adjusted on days -1, 2 and every third day thereafter until study termination. Blood samples will be prepared as per SOP #11.

Animals will be fed according to the regular daily schedule outlined in the Project Notebook.

On study day #15, pigs will be humanely sacrificed and representative samples of liver, kidney, and bone will be collected and prepared for analysis as per SOP #11.

3.6.2 Period of Operation

Soil collection and characterization

Soil collection and characterization will occur during the first two full years of the project. Soils will be obtained from as many DoD installations as financially possible and as time permits.

XAS

Metal speciation using high resolution surface spectroscopy techniques will be conducted starting January 2007 and ending December 2007

In vitro

In vitro investigations will be conducted starting January 2007 and ending July 2007

In vivo

Plant investigations will be conducted starting January 2007 and July 2007

Earthworm investigations will be conducted starting January 2007 and July 2007

Swine investigations will be conducted starting September 2006 and ending September 2007

3.6.3 Amount/Treatment Rate of Material to be Treated

Soil is not actually treated during this demonstration because the technology to be demonstrated is not a treatment system. However, if the scientific and technical information demonstrated by the project indicates that certain sites based on their soil properties and low metal bioavailability may be exempt from remediation, then a substantial active treatment effort may be avoided at numerous DoD sites.

3.6.4 Residuals Handling

Bulk soils will be archived at OSU and treated as material for research and scientific investigation rather than hazardous waste. There is no desire to dispose of unused soil in the near future. Soil that is subject to in vitro and plant and earthworm studies will be disposed of as hazardous waste. Residual from in vitro is minimum and less than a kilogram. Soil from plant and earthworm studies is on the order of 4 kilograms. Residual soil from surface spectroscopy studies is on the order of < 0.5 kg. Residual solutions from in vitro, IVG, PBET, and salt/chelate studies are also considered hazardous waste and will be on the order of tens of liters. Prior to disposal all wastes will be thoroughly characterized and disposed of according to state and federal regulations regarding the storage and disposal of waste materials.

3.6.5 Operating Parameters for the Technology

Site access and soil collection – Site access involves ORNL technical staff contacting DoD site Environmental officials and describing project objectives and needs. Sometimes this can become a time consuming process due to legal issues, lack of interest, or technical logistics. The next step is trip planning and preparation as well as shipping supplies. The complete process often requires about 5 days of total effort for one individual and 3 days for another individual per site. ORNL technical staff that travel to the sites are educated in analytical chemistry and soil science, thus highly qualified.

Homogenization and sieving – Homogenization and sieving will by done by OSU staff. Homogenizing and sieving soils involves the initial fabrication of apparati to contain air borne dust from the mixer and the sieving of large quantities of soil. It is expected that this procedure will take 4 months to complete, with two people's continuous labor.

3.6.6. Experimental Design

Soil physical and chemical characterization

Select, yet the most pertinent, soil chemical and physical properties will be quantified using established analytical procedures. Properties such as total metal analysis, total organic and inorganic carbon, amorphous and crystalline Fe-oxide content, Mn-oxide content, particle size analysis (sand, silt, clay content), and soil pH will be quantified on all soils. These are soil analysis parameters that the PIs of the project are quite familiar with and perform on a routine basis. This information is used in pending statistical models that will assess the influence of soil properties on metal bioavailability as measured by in vitro and in vivo techniques.

Metal speciation and chemical environment

In an effort to validate the physical significance of the soil property models used to describe the bioaccessibility of metals in the DoD soils, the mechanisms of enhanced metal sequestration and solid-phase metal speciation will be quantified with a variety of high-resolution surface spectroscopy techniques. Such techniques will include Scanning Electron Microscopy with Energy Dispersive Spectroscopy (SEM-EDS) and X-ray Absorption Spectroscopy (XAS). Bulk SEM-EDS measurements will be conducted at the nation's premier facility for determining the environmental speciation of metals, located at DOE's Environmental Molecular Science Laboratory (EMSL), Pacific Northwest Laboratory, Richland, WA., which will provide direct quantification of the mineralogical nature of solid phase contaminants that are present. These facilities are state-of-the-art with a field emission SEM having resolutions of at least 1.5 nm at 30 KeV and 4.0 nm at 1.0 KeV. This technique is useful for determining the crystalline domains of the solids, and with associated energy dispersive spectroscopy, elemental composition. Our research group has extensive experience in the use of this interfacial interrogation technique for monitoring changes in the mineralogy of toxic metals in heterogeneous media and we have a good working relationship with the EMSL staff.

Metal speciation will be assessed on the four DoD soil types using the synchrotron-generated X-ray source at Argonne National Laboratory (ANL). X-ray absorption spectroscopy will be utilized to determine the oxidation state (from near-edge structure, XANES) and atomic coordination environment (from extended fine structure, EXAFS) of the target metals in the soil samples. Least-squares fitting algorithms of the EXAFS function will be applied to determine nearest and second-nearest neighbor atomic identities, coordination numbers, and distances from each target metal. Comparison with theoretical models generated using the *ab initio* computer code FEFF8 and with spectra from relevant model compounds will enable distinction between adsorption and substitution/coprecipitation modes of metal sequestration.

The approach will be to first identify particular soil grains within the samples that are elevated in the target metals using X-ray fluorescence, followed by microbeam XAS on the targeted regions. By using microbeam techniques, we will address problems that are frequently encountered for bulk analyses of heterogeneous materials, such as (a) low bulk concentrations of the target element, and (b) spectral signals with contributions from different metal environments within the sample.

This effort will provide an improved conceptual understanding of the molecular-level speciation of Pb, Cd, Cr, and As in the soils, and how the molecular speciation influences the resulting bioaccessibility. All of the elements that are the focus of this research have core electron excitation energies between 8 and 26 KeV, making them ideal for synchrotron research. High-intensity synchrotron x-ray sources permit such analysis of undisturbed samples and with new available focused beams allow spatial heterogeneity to be appreciated. XAS is one of the few atomic techniques for obtaining molecular level information that can be conducted in unaltered samples, which is crucial for examining the true *in situ* molecular-level speciation of these metals. The detection limits for synchrotron-generated XAS vary depending on the matrix, but samples with concentrations greater than 10 mg/kg should yield good results. Our research group

has extensive experience in the use of XAS to monitor changes in molecular speciation of toxic metals in heterogeneous media and we have a good working relationship with the ANL staff. The metal speciation results will be used to confirmed macroscopic observations of metal bioavailability for both the *in vitro* and *in vivo* methods (Yang et al., 2002; Stewart et al., 2003; Stewart et al., 2003; Yang et al., 2005).

In vitro investigations to assess human health risks

IVG: *In-vitro* Gastrointestinal Method

Incidental soil ingestion is an important exposure pathway for assessing public health risks associated with contaminated soils (Dudka and Miller, 1999; Ryan et al., 2004). The bioavailability of Pb, As, and Cd in soils can be determined by conducting dosing trials using animal models. Immature swine have been successfully used as an animal model for the gastrointestinal (GI) function of children (Weis, 1991; Casteel et al., 2001; Ryan et al., 2004). However, conducting *in vivo* animal trials is lengthy and expensive.

To overcome the difficulty and expense associated with *in vivo* trials, research effort has been directed toward the development of *in vitro* methods to simulate human gastrointestinal conditions. Several of these methods have been reviewed (Rodriguez and Basta, 1999; Ruby et al., 1999; Oomen et al., 2002).The OSU-IVG is a rapid, inexpensive and reliable screening tool for determining the potential bioavailability (i.e., bioaccessible) of soil contaminants including As (Rodriguez and Basta, 1999), Cd (Schroder et al., 2003), and Pb (Schroder et al., 2004). The OSU IVG method simulates important parameters of the human GI tract under fasting conditions. The amount of contaminant extracted by the OSU-IVG is assumed to be available for absorption across the intestinal membrane (i.e., bioaccessible) and incorporation into systemic circulation (Ruby et al., 1999). Contaminant bioaccessiblity is expressed as a percentage of the total contaminant content of the test sample.

PBET – Physiologically-Based Extraction Test

The physiologically-based extraction test (PBET) developed by Ruby et al. 1996, 1999, will be utilized at a variety of pH conditions to estimate metal bioaccessibility for a variety of stomach environments indicative of food intake, or lack thereof. Using the method of Stewart et al. 2003a,b additional soil property-driven models will be constructed using the PBET method at these pH values. This is particularly important for Pb contaminated soils since Pb bioaccessibility decreases with an increase in pH (Yang et al., 2002; Yang et al., 2005). In contrast, As(V) bioaccessibility was minimally influenced by changing pH environments. Triplicate samples of 0.3 g dry soil are placed in 50 mL polyethylene tubes to which 30 mL 0.4 M glycine at pH 1.5, 2.0., or 3.0 and are added. The slurries are quickly placed in a rotating water bath of 37^0C and agitated at 30 ± 2 rpm for 1 hr. After 1 hour the samples are rapidly cooled in an ice bath. Supernatant is separated from the solid via centrifugation. The pH of the supernatant is measured to ensure that the final pH is within ± 0.5 pH units of the initial pH.

Metal bioaccessibility and metal bioavailability for the four study soils will be calculated using soil property-driven models developed from CU-1166 and CU-1210 studies, respectively. Calculated bioaccessibility values will be compared with measured bioaccessibility values using *in vitro* gastrointestinal methods for study soils.

In vitro investigations to assess ecological risks

Dilute salt and chelate

For ecological risk estimates, metal bioavailability will be estimated from multiple regression and path analysis models developed using toxicity and bioaccumulation data from 26 soils (CU-1210; previous US EPA-NCEA project). Additionally, 12 selected DoD sites (24 soils) from CU-1166 will be tested in addition to the four soils proposed above. This is necessary to enhance the robustness of the ecological model from CU-1210 as has already been done for the human-based model in CU-1166. In the ecological investigations, data from *in vitro* DoD soil metal extraction coupled with DoD soil chemical and physical properties will be compared to existing statistical relationships for estimating metal bioavailability to plants and soil invertebrates. Initially, statistical relationships developed for metal availability from a set of 26 soils will be used to estimate the chemical availability of metals in DoD soils, based upon total metal levels and soil physical/chemical characteristics. This will be followed by extraction of the DoD soils using several wet chemical methods (e.g., extraction with chelates (DTPA) or dilute salts ($Ca(NO_3)_2$); (Basta and Gradwohl, 2000; Dayton, 2003) to actually measure the chemical availability of metals in DoD soils. These measurements will be compared to predicted chemical availability estimated by the models to determine the ability of the models to predict metal availability. The statistical models will also be used to predict the toxicity of the DoD soils to earthworms and plants, assuming additivity of the toxicity of individual metals. Bioassays will be conducted with DoD soils to determine actual toxicity and these results will be compared to the model predictions. Comparison of the actual toxicity from bioassays with predicted toxicity from in vitro models will be used to quantify the ability of in vitro models to predict actual ecotoxicity in field DoD soils. This will be the basis for validation of the in vitro methods for field DoD soils.

In vivo investigations

Plant

Plant bioassays with Perennial ryegrass, *Lolium perrene*; and Lettuce, *Lactuca sativa*, will be conducted according to Dayton et al. (Dayton et al., 2006) with contaminated soils from DoD to provide plant risk-based endpoints of germination, dry matter growth, and tissue metal concentrations.

Soil Invertebrate

Metal bioavailability and ecotoxicity in contaminated soils collected from DoD sites will be assessed using soil invertebrate bioassays with earthworms (*Eisenia fetida*), potworms (*Enchytraeus albidus*), and collembola (*Folsomia candida*) according to standard protocols (ASTM, 1999; ASTM International, 2004). Bioassay endpoints will include mortality, reproduction, and internal concentration of metals (bioaccumulation).

Swine

Metal bioaccessibility calculated by CU-1166 in vitro methods using DoD soils will be correlated with metal bioavailability using *in vivo* immature swine dosing trials. The pig has been used as an animal model in a number of research fields including gastroenterology, nutrition, and metabolism. Specific justification for the use of swine in chemical bioavailability studies with soil matrices revolves primarily around biological (anatomical, physiological, biochemical) similarities to humans. There is an extensive database of information on the use of the swine model. Standard operating procedures (SOPs) using the immature swine model developed by Dr. Stan Casteel, University of Missouri-Columbia Veterinary Medical Diagnostic Laboratory, have been approved by the USEPA Region 8 for measuring the bioavailability of Pb from incidental ingestion of soils by children. During the past 10 years, the swine model has served well as a surrogate for study of systemic bioavailability of soil Pb in a sensitive population of humans. More than 30 Superfund Site soils from locations across the nation have been tested. The swine model uses relative bioavailability data as measured by comparing oral absorption of the metal of interest in test soils to oral absorption of some fully soluble form of the metal. The fraction of the absorbed dose of a metal can be measured using concentrations in blood and tissues such as liver, kidney, and bone. For the special case of As, the urinary excretion fraction is most appropriate for estimating relative bioavailability. It has been shown by Weis *et al.* that preliminary site-specific estimates of soil Pb relative bioavailability in 20 soils of concern to the USEPA ranged from 6% to greater than 85%, relative to the absorption measured for Pb from lead acetate. The model has also been used successfully to assess the bioavailability of Cd and As.

An example of the general study design for the Pb-contaminated soils dosing trial is shown in Table 3-8 where two different contaminated soils are shown with their soluble control at three different dosing levels and five replications.

Live male pigs weighing 10-12 kg will be housed individually in lead-free cages and fed low-lead feed. All doses will be delivered daily for 15 days in a low-lead vehicle according to the diurnal schedule. One blood sample (6-8 ml) will be drawn (following SOP #9) from each animal on days 0, 1, 2, 3, 5, 7, 9, 12, and 15, into a new plastic lead-free syringe by venipuncture of the anterior vena cava. The blood will be immediately transferred into lead-free Vacutainer^{R} tubes containing EDTA. In each case, blood samples will be drawn 17 hours after the second dosing of the previous day. Animal weights will be recorded and doses and feed adjusted on days -1, 2, and every third day thereafter until study termination. Blood samples will be prepared as per SOP #11. Animals will be fed according to the regular daily schedule outlined in the

Table 3-8 Pb swine dosing study design

Group	N	Treatment	Acetate/Soil Lead mg/day	Lead intake ug/kg/day
1	5	$Pb(Ac)_2 \cdot 3H_2O$	weight adjusted	25
2	5	$Pb(Ac)_2 \cdot 3H_2O$	weight adjusted	75
3	5	$Pb(Ac)_2 \cdot 3H_2O$	weight adjusted	225
4	5	$Site_1$ media	mass & weight adjusted	75
5	5	$Site_1$ media	mass & weight adjusted	225
6	5	$Site_1$ media	mass & weight adjusted	675
7	5	$Site_2$ media	mass & weight adjusted	75
8	5	$Site_2$ media	mass & weight adjusted	225
9	5	$Site_2$ media	mass & weight adjusted	675
10	3	Negative Control	oral vehicle	0

experimental protocol. On study day #15, pigs will be humanely sacrificed and representative samples of liver, kidney, and bone will be collected and prepared for analysis as per SOP #11.

Statistics

A multiple regression technique will be used to derive model functions that relate metal bioavailability to common soil properties and the correlation of in vitro measurements with in vivo ecological and mammal based studies. Models will be run using forward stepwise regression to determine the most salient soil properties for calculating bioavailability for the various metals. Multiple linear regression will then be employed to determine the linear equations to use when computing toxic metal bioavailability based on the important soil properties previously ascertained.

The bioassay results will be compared to results from the various soil property-driven models in an effort to show that the cost-effective *in vitro* methods can serve as a screening tool for estimating toxic metal bioavailability. This information can in turn be used to prioritize DoD sites in terms of their potential ecological risk and the need for more-detailed, and costly, site-specific bioavailability (e.g., animal dosing, plant and invertebrate) studies.

Risk estimates from incidental ingestion of contaminated soils will be calculated using metal bioavailability values derived from CU-1166 methods (total metal content and soil properties). Adjustments to ecological risk-based endpoints (bioaccumulation, ecotoxicity) based upon study soil properties will be calculated using methods developed in CU-1210 and by Dayton et al. (2005) and Bradham et al. (2006). As is being done in CU-1350, neural network models will be implemented in a spreadsheet program to compute health risk due to ingestion of one or more metals of interest and any given soil properties. The program will compute confidence limits on risk estimates due to combined effects of intrinsic model uncertainty and to uncertainty in soil properties (e.g., as estimated from tabulated data for various soil types). The results from this task will provide a tool to evaluate risk reduction due to toxic metal sequestration in soils to support DoD's performance/risk assessment and decision-making process for military base site restoration.

3.6.7 Sampling Plan

For each metal-contaminated site, soil sub-samples (10 to 12) will be collected and combined into one homogenized composite sample. This number of samples will represent a range of lead, cadmium, zinc, chromium, and/or arsenic concentrations selected to bracket the effects range for each species in their respective bioassays and generate sufficient data for statistical assessment, while still being manageable from a cost and level of effort perspective. Similarly, 10 to 12 sub-samples will be collected from uncontaminated soils at each site. These samples will represent reference conditions (i.e., no significant metal contamination). A field XRF unit will be used to verify metal levels in soils on-site prior to the collection of samples. Sufficient soil sample volumes will be collected for soil characterization and to conduct bioassays.

3.6.8 Sample Collection

Soil samples will only be collected once from the sites for the field demonstration. Sampling of test organisms (i.e., plant, earthworms, swine) will be conducted during bioassays. The Quality Assurance Project Plan (QAPP) (Appendix C) contains the details of the sampling of test organisms that will be conducted during the bioassays.

3.6.9 Sample Analysis

Soil properties and contaminant content will be determined for the soil collected from each site. Homogenized soil samples will be sent to Dr. Stan Casteel at the Veterinary Diagnostic Laboratory at the University of Missouri-Columbia for dosing trials using immature swine. Soil samples will be used at Ohio State University for plant and earth bioassays. Sample analysis of test organisms (i.e., plant, earthworms, swine) will be conducted during bioassays. Detailed methods used for bioassay tissue analysis are described in Appendix A: Analytical Methods Supporting The Experimental Design. The Quality Assurance Project Plan (QAPP) (Appendix C) contains the details of the sample analysis of test organisms that will be conducted during the bioassays.

The PBET supernatant will be measured for total As, Pb, Cd, and Cr using calibrated Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Standard curves containing a minimum of three standards which bracket the concentration range of the samples are analyzed at the beginning of each analytical run. The full set of calibration standards are analyzed as unknowns at the beginning and end of each run. In addition, check standards are analyzed after every 10 samples to ensure accuracy and precision during a run. The PBET supernatant will also be measured for Cr(VI) and total Cr(III/VI). Cr(VI) is measured using a modified s-diphenylcarbohydrazide colormetric method (Bartlett and James, 1979) using a UV-VIS spectrophotometer at wavelength 540 um. Analysis of Cr(VI) is performed immediately on rapidly cooled PBET solutions to avoid possible reduction of Cr(VI) to Cr(III) by glycine. Independent studies revealed that Cr(VI) reduction by glycine at 37 ^{0}C and 1 hr was insignificant. Cr(VI) standardization is achieved in the same method as detailed for the ICP-MS. Total Cr is measured by ICP-MS and Cr(III) is calculated as the difference between Cr total and Cr(VI).

3.6.10 Experimental Controls

Uncontaminated soil collected at sites will serve as negative controls for both the ecological bioassays (i.e., plants, earthworms) and the *in vitro* studies. These controls represent soil without significant levels of metal contaminants. For earthworms and plants, tests with a laboratory reference soil will also be conducted. Webster clay loam has been used over the past two years as a reference soil with each soil invertebrate bioassay conducted in order to monitor test organism performance over time. Results are compared to control chart values and if an endpoint values

falls outside the 95% confidence limits for organism performance in Webster soil, this will trigger an examination of why performance was different and corrective action would be taken.

3.6.11 Data Quality Parameters

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

Measures to ensure representativeness, completeness, comparability, accuracy and precision of the data are discussed below and in the QAPP (Appendix C).

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

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

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

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

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

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

Accuracy - Accuracy is the closeness of a measured value to an accepted true value. Standard reference materials (SRMs; i.e., plant, tissue, soil SRM) with certified analyte concentrations and certified check standards will be used to provide the accepted true value for analytical laboratory methods.

Precision - Precision is a measure of the degree of agreement among replicate analyses of a sample usually expressed as the standard deviation. Precision will be measured through the calculation of relative standard deviation (RSD) derived from replicated sample or control analyses.

3.6.12 Calibration Procedures, Quality Control (QC) Checks, and Corrective Action

In Vitro

The PBET studies will be performed in triplicate. Should the analysis of the supernatant solutions vary more than 15%, the samples will be re-diluted and analyzed again. If a dilution error is eliminated as the cause of variability, the experiment will be repeated.

The PBET supernatant will be measured for total As, Pb, Cd, and Cr using calibrated Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Standard curves containing a minimum of three standards which bracket the concentration range of the samples are analyzed at the beginning of each analytical run. The full set of calibration standards are then analyzed as unknowns at the beginning and end of each run. In addition, check standards and system blanks are analyzed after every 10 samples to ensure accuracy and precision during a run. Should the check standards vary more than 15%, the analysis will be re-done. The solutions will also be measured for Cr(VI) using a modified s-diphenylcarbohydrazide colormetric method (Bartlett and James, 1979) using a UV-VIS spectrophotometer at wavelength 540 um. Cr(VI) standardization and corrective actions are achieved in the same method as detailed for the ICP-MS.

Standard procedures to ensure Quality Control /Quality Assurance of analytical data produced from laboratory analyses of metal contaminants from *in-vitro* (OSU-IVG) are described in detail in The Quality Assurance Project Plan (QAPP) (Appendix C). Replicated analyses (duplicate or triplicate) of all sample digests or extracts will be conducted. Data reduction of analytical results will include reporting of analyte concentrations of all replicates and statistical calculation of analyte mean and median.

In Vivo

Plant

If excessive plant phytotoxicity (i.e., no growth) occurs during the plant bioassay in the control soil, then the test data should be carefully examined to determine if it is acceptable. Standard procedures to ensure Quality Control /Quality Assurance of analytical data produced from laboratory analyses of metal contaminants in bioassay tissue (i.e., plant, earthworms) and soil samples are described in detail in The Quality Assurance Project Plan (QAPP) (Appendix C). Replicated analyses (duplicate or triplicate) of all sample digests or extracts will be conducted. Data reduction of analytical results will include reporting of analyte concentrations of all replicates and statistical calculation of analyte mean and median.

Soil invertebrate

All soil invertebrate tests will also be conducted using laboratory reference soils, Webster clay loam for E. fetida and F. candida and Sassafras sandy loam for E. albidus. Every toxicity bioassays we conduct in our laboratory is accompanied by a test in a laboratory reference soil, so we have generated and expected response in the lab reference soils that meets validity criteria for an acceptable toxicity bioassay (e.g., minimum of 20 cocoons produced per replicate over 21 days). This allows us to ensure that, over time, test organisms from our laboratory cultures are

responding in a consistent manner during testing and to differentiate between soil matrix effects in site reference soils and effects caused by the presence of metals in the site soils.

Swine

All swine studies will be conducted according to the laboratory project manual for systemic availability of environmental contaminants to young swine from subchronic administration of metal-contaminated test materials.

3.6.13 Data Quality Indicators

Accuracy of laboratory analytical methods will be calculated as follows:

Accuracy (%) = [(measured SRM or check standard value)/certified true value] x 100%

Analyses with ≤ 10% will be high accuracy and values ≤ 15% will be acceptable accuracy.

Precision of laboratory analytical methods will be calculated from RSD as follows:

RSD (%) = [(standard deviation / mean) x 100%]

Laboratory methods with ≤ 10% will be high precision and values with ≤ 15% will be acceptable precision.

3.6.14 Demobilization

Sampling and homogenization equipment will be washed and decontaminated between samples and prior to demobilization. Sampling equipment will be shipped back to the appropriate point of origin.

3.6.15 Health and Safety Plan

The Health and Safety Plan for this field demonstration is included in Appendix D of this workplan.

3.7 SELECTION OF ANALYTICAL/TESTING METHODS

We will use standard published analytical methods in this study. These include:

Total Metal Concentration in Soils

Method 3050B (or equivalent), EPA (1998). Test methods for evaluating solid waste, physical/chemical methods. SW-846. Washington, DC, United States Environmental Protection Agency (EPA, 1998).

Soil Properties

Methods of soil analysis. 1996. D.L. Sparks et al. (ed.) Part 3 Soil Science Society of America Book Series 5 Soil Sci. Soc. America, Madison, WI (Sparks, 1996).

Trace Metal Speciation

Manceau, A., Marcus, M.A., and Tamura, N. (2002) Quantitative speciation of heavy metals in soils and sediments by synchrotron X-ray techniques. *in* Vol. 49 Reviews in Mineralogy and Geochemistry, Washington, D.C. (Fenter et al., eds.)

Physiologically-Based Extraction Test

Kelley, M. E., S. E. Brauning, R. A. Schoof and M. V. Ruby (2002). Assessing Oral Bioavailability of Metals in Soil. Columbus, OH, Battelle Press (Kelley et al., 2002).

Ohio State University In Vitro Gastrointestinal (OSU IVG) Method

Basta, N.T., J. N. Foster, E.A. Dayton, R. R. Rodriguez, and S.W. Casteel. 2006. The Effect of Dosing Vehicle on Arsenic Bioaccessibility in Smelter-contaminated Soils. J. Environ. Health Sci. Part A. *Invited manuscript for the special JEHS publication "B*ioaccessibility and human bioavailability of soil contaminants." (Basta, 2006).

Plant Bioassay

Dayton, E.A, N.T. Basta, M.E. Payton, K.D. Bradham, J.L. Schroder, and R.P. Lanno. 2006. Evaluating the contribution of soil properties to modifying lead phytoavailability and phytotoxicity. Environ. Toxicol. Chem. 25(3):719-725. Invited manuscript for the special ET&C publication "Assessing Risks of Metals added to Soils in Europe and North America. (Dayton et al., 2006)

Earthworm Bioassay

Bradham, K.D., E.A. Dayton, N.T. Basta, J. Schroder, M. Payton, and R.P. Lanno. 2006. Effect of soil properties on lead bioavailability and toxicity to earthworms. Environ. Toxicol. Chem. 25(3):769-775. Invited manuscript for the special ET&C publication "Assessing Risks of Metals added to Soils in Europe and North America. (Bradham et al., 2006)

3.8 SELECTION OF ANALYTICAL/TESTING LABORATORY

The analytical/testing methods will be conducted in the PI/co-PI's laboratories by the PI/co-PI or under their direct supervision. The following laboratories will be utilized.

The Veterinary Medical Diagnostic Laboratory

The Veterinary Medical Diagnostic Laboratory at the University of Missouri is one of 35 labs accredited by the American Association of Veterinary Laboratory Diagnosticians within the United States. This lab is fully equipped, staffed, and available for protection of test animals during tissue sample collections. All ancillary animal health diagnostic services are available including histopathology, bacteriology, serology, virology, and molecular diagnostics. An incinerator is also present for carcass incineration.

Plant and Soil Invertebrate Bioassay Facilities

The facilities for conducting plant and soil invertebrate bioassays are housed in the Department of Entomology and the School of Natural Resources at Ohio State University. Complete controlled environment systems are available for conducting the bioassays. Soil invertebrate cultures (earthworms) have been maintained for three years providing a constant supply of test organisms. Analytical facilities include two wet chemical and analytical instrumentation laboratories totaling approximately 2100 ft^2. Analytical instrumentation in these laboratories is new (3 yr old) including High Resolution Varian Vista MPX Simultaneous Inductively Coupled

Plasma Atomic Emission Spectrophotometer (ICP) with SPS-5 Auto Sampler, and VGA77 Hydride Unit; Perkin-Elmer Flame and Graphite Furnace Atomic Absorption Spectrophotometers (HGA). All labs are supplied with high purity Type I deionized water. Metal contaminant determinations will be performed by using a Varian Vista MPX ICP or Perkin Elmer HGA. The Varian Vista is a new generation of ICP instruments that has superior optics that minimize inter-element interferences and provides highly accurate measurement for difficult elements (e.g., arsenic). This instrument is operated by a highly qualified, full-time research specialist, Shane Whitacre, and by Dr. Elizabeth Dayton, the co-PI on this project. Dr. Dayton has 10 years of experience working in analytical chemistry and with advanced analytical chemistry instrumentation.

ORNL Solute Analysis Facilities

The ESD has laboratory facilities to enable the detection and quantification of virtually any solute used in subsurface science research. We summarize below general analytical equipment available for use in this project with an ensuing description of specialized equipment.

- Dedicated gas chromotographs (GC) and high pressure liquid chromatographs for the analysis of organic compounds and dissolved gases.
- Ion chromatographs equipped with electrical conductivity, spectral array, and fluorescence detectors for anion analysis and the detection of chelated metals.
- Perkin-Elmer 8000 AAnalyst atomic absorption spectrophotometer with graphite furnace.
- Perkin-Elmer Elan 6000 inductively coupled argon plasma mass spectrometer (ICP-MS).
- Chemchek kinetic phosphorescence analyzers (KPA) for the determination of U(VI).
- Ranishaw micro-Raman spectrometer.
- Nicolet Fourier transform infrared (FTIR) spectrometer.
- PTI time-resolved laser-induced fluorescence spectrometer.
- Spex Fluorog-32 steady-state fluorescence spectrometer.
- Brookhaven 90Plus/ZetaPlus dynamic light scattering instrument.
- Total carbon analyzers (organic and inorganic).
- Capillary electrophoresis equipment with UV/vis detectors for anion, cation, and chelated metal detection.
- Atomic force microscope.
- Variety of UV-Vis spectrophotometers.
- Coy anaerobic chambers for controlled atmosphere experiments and sample processing.
- Coulter N4MD photon correlation spectrometer.
- Coulter DELSA 440 microelectrophoresis instrument.

The division also houses an array of standard analytical and support equipment such as: sonicators, furnaces, ovens, centrifuges, freeze-drying equipment, Milli-Q and reverse osmosis water purification systems, pH meters, balances, refrigerators, and freezers.

X-Ray Fluorescence Analyzer: ESD has a Niton XLp 700 Series portable x-ray fluorescence (xrf) analyzer for the non-destructive analyses of elements with atomic numbers greater than 20 (calcium). A dual-isotope (^{109}Cd and ^{241}Am) excitation source allows quantitative detection of heavy metal contaminants (As, Cd, Cr, Pb, and Hg) in addition to U and Th in soil and water

samples in the field. Gross field-scanning of soil and rock cores and samples can also be preformed which greatly simplifies selection of specific materials for further investigation. The same instrument, when operated in a controlled laboratory environment, functions well for the quantitative non-destructive analyses of elements in almost any solid or liquid material.

3.9 MANAGEMENT AND STAFFING

This project will involve the collaborative efforts of the following performers: Amy Hawkins, Naval Facilities Engineering Service Center will coordinate regulatory and end-user involvement; Dr. Philip Jardine, Oak Ridge National Laboratory will lead the effort for the demonstration and validation of models to predict bioaccesibility; Dr. Roman Lanno, Dr. Nicholas Basta, and Dr. Elizabeth Dayton, Ohio State University will be responsible for soil characterization and in vivo ecological bioassays; Dr. Stan Casteel, University of Missouri, Columbia will conduct the in vivo swine dosing trials for model validation; Dr. Kaye Savage, Vanderbilt University will provide metals speciation for use in the models; and Dr. Mark Barnett, Auburn University will serve as project technical liaison and provide expertise on solid-phase metal bioavailability in soils and its relationship to human-health risk assessment.

Amy Hawkins is a biologist in the consultation/information management branch of the Naval Facilities Engineering Service Center (NFESC). As a member of the Ecological Risk Technical Assistance Team (ERTAT) her duties include providing review of ecological risk assessments, site-specific application of Navy policy, and management of risk assessment-related research and development projects. She has provided ecological risk assessment technical support at more than 30 Navy sites. She has presented various technologies as part of NAVFAC's Remedial Innovative Technology Seminars (RITS) and now serves on the technical review team for the RITS. Ms. Hawkins has also managed various projects implementing new technologies through the Navy's Broad Agency Announcement.

Dr. Philip Jardine is a Distinguished Research Staff Scientist at ORNL. He specializes in subsurface science research that deals with time-dependent, multi-process fate and transport issues at multiple scales. His current research activities include chemical and microbial controls on contaminant fate and transport, experimental and theoretical aspects of subsurface contaminant migration at laboratory and field scales, quantifying the bioavailability of toxic metals in contaminated soils. He has garnered twelve national and international scientific awards, including a coveted TOYA, and published over 100 peer-reviewed publications.

Dr. Roman Lanno is an Associate Professor of Entomology at Ohio State University. He has over 18 years experience in ecotoxicology with research the last seven years focusing on issues of chemical bioavailability, toxicity, and bioaccumulation of organic chemicals and metals in soil invertebrates, specifically using the earthworm as a model. His bioavailability research has involved examining abiotic and biotic modifying factors in determining the bioavailability of chemicals via dermal and intestinal routes of exposure. He has been invited to give presentations on the bioavailability and toxicity of chemicals in the environment by such agencies as the US EPA (Metals Bioavailability White Paper), National Environmental Policy Institute (NEPI), and the United Nations European Council on the Economy (UN ECE). He has been involved in the preparation and review of the US EPA "Framework for Metals Risk Assessment". He has participated in a number of SETAC Pellston Workshops in soil and aquatic toxicology and is

editor of “Assessing Contaminated Soils: From Soil-Contaminant Interactions to Ecosystem Management”, the publication resulting from one of these workshops.

Dr. Nicholas Basta is Professor of Soil and Environmental Chemistry in the School of Environment and Natural Resources at The Ohio State University. Dr. Basta has active research and instruction programs that focus on environmental soil chemistry including the risk-based environmental chemistry of organic and inorganic pollutants in contaminated soils with emphasis on bioavailability and contaminant transmission to human and ecological receptors. He has authored or co-authored > 65 manuscripts in refereed journals, 7 book chapters, and 150 abstracts and proceedings of presentations at scientific meetings. He has served as co-Chair, CSREES Technical Committee, "Chemistry and Bioavailability of Waste Constituents in Soils," International Society for Trace Element Biogeochemistry, Steering Committee, is an active member of the Bioavailability Research Group of Europe, and Contaminated Soil Advisory Group, Society for Environmental Toxicology and Chemistry.

Dr. Elizabeth Dayton is Research Scientist of Soil and Environmental Chemistry in the School of Environment and Natural Resources at The Ohio State University. Dr. Dayton is actively involved in supervising and conducting research focused on environmental soil chemistry including the risk-based environmental chemistry of organic and inorganic pollutants in soil. She conducted plant bioassay and soil chemical analyses for the previous SERDP CU-1210 project and has published these results in peer-reviewed scientific journals and presented these findings at national and international scientific meetings. She has authored or co-authored 8 manuscripts in refereed journals, and 22 abstracts and proceedings of presentations at scientific meetings.

Dr. Stan Casteel: Professor and Director of the Veterinary Medical Diagnostic Laboratory at the University of Missouri’s College of Veterinary Medicine. Solving animal disease problems, teaching veterinary students, and doing research in environmental risk assessment represent the body of his work. As a diagnostician and researcher Dr. Casteel has given more than 150 presentations at scientific meetings and has authored more than 150 abstracts and scientific papers and 29 book chapters. Major funding and current research efforts focus on an understanding of the biokinetics of lead, arsenic and cadmium from contaminated industrial matrices using juvenile and adult-pregnant swine as models for children and pregnant women. This effort is specifically directed toward improving the understanding of the absorption of arsenic, lead and cadmium from contaminated media from various EPA Superfund sites, many of which are on the National Priority List. This is an important departure from EPA's default assumptions regarding heavy metal bioavailability. The impetus for this departure is to provide additional scientific evidence in support of EPA's integrated exposure uptake biokinetic model and site-specific data generated from Superfund Site test soils.

Dr. Kaye Savage is an Assistant Professor of Earth and Environmental Sciences at Vanderbilt University. She has a Ph.D. in Geological and Environmental Sciences from Stanford University. Her research background includes the environmental chemistry of sulfide and sulfate minerals, spectroscopic studies of trace elements in rocks and sediments, arsenic geochemistry in mine environments and associated waters. She was awarded a “Science to Achieve Results” (U.S.-E.P.A) graduate fellowship, a Mineralogical Society of America student research grant, and outstanding student paper awards at American Geophysical Union and Stanford Synchrotron

Radiation Laboratory conferences. She has published five peer-reviewed journal articles in which synchrotron radiation techniques were used for trace metal speciation.

Dr. Mark Barnett is an Associate Professor of Civil Engineering at Auburn University. His research background is the soil property controls on metal bioavailability in soils and the speciation, transformation, and transport of toxic metals in the subsurface. He has 10+ years of environmental engineering experience at DoD and DOE facilities, including serving as a key project scientist for the remediation of a X-contaminated Superfund site in Oak Ridge, TN. The speciation and bioavailability results adopted at this site resulted in millions of dollars in cost savings. The work was cited by the EPA Regional Administrator and awarded an ORNL Corp. President's Award (1995). He has published over 20 peer-reviewed journal articles.

Investigator-task responsibilities. The activities in this demonstration will be coordinated between the investigators and institutions. Task assignments (Table 3-9) are based on the strengths of the investigators and availability of funds. To enhance organization of data and consistency across the project a secure website will be set up where data can be easily shared within the group.

3.10 DEMONSTRATION SCHEDULE

The overall project schedule is shown in Table 3-10.

Soil Collection: to be completed November, 2006

Soil Processing and Characterization: August 2006 to January, 2007

In vitro gastrointestinal testing: January, 2007; Finish July 07

Plant Bioassays: Start January, 2007; finish July 2007

Earthworm Bioassays: Start January, 2007; finish July 2007

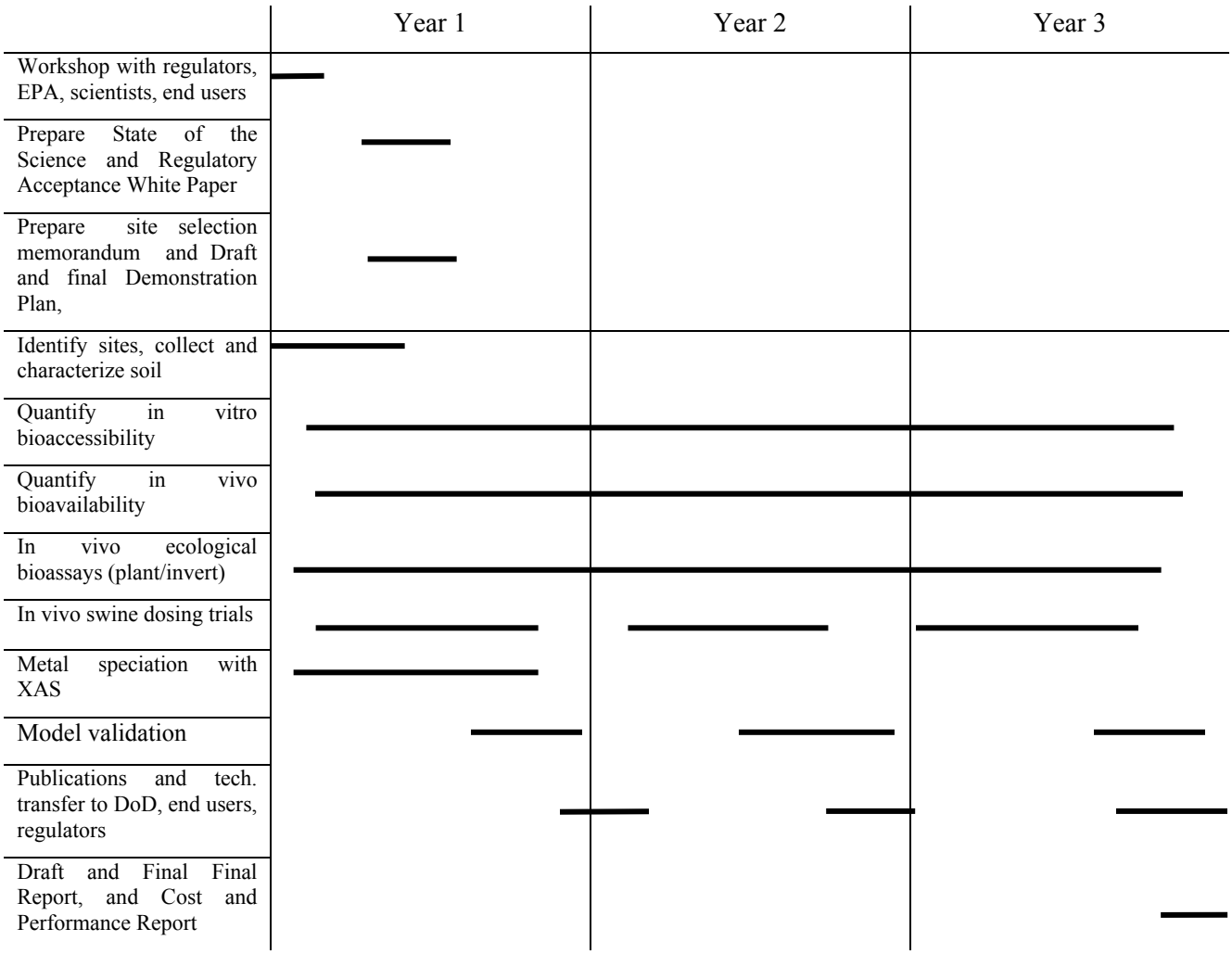
Swine Dosing: Started Sept, 2006; finish Sept 2007

Data Reduction and Model Validation: July 2007 to July 2008

Table 3-9 Investigator Task Responsibilities

	Task I: In vitro methods and soil property models to predict metal bioaccessibility and bioavailability in study soils			Task II: In vivo methods for validation of in vitro methods for predicting bioavailability		Task III: Human and ecological risk assessment
	Soil collection and characterization	In vitro bioaccessibility studies	Contaminant speciation with high resolution spectroscopy	In vivo ecological bioasssys (plant/invert)	In vivo swine dosing trials	Model validation, risk assessment and tech transfer
Hawkins (NFESC)						Y
Jardine (ORNL)	Y	Y				Y
Basta and Dayton (Ohio State)	Y	Y		Y		Y
Lanno (Ohio State)				Y		Y
Casteel (U. Missouri)					Y	Y
Savage (Vanderbilt)			Y			Y
Barnett (Auburn)						Y

Table 3-10 Project Schedule



4 PERFORMANCE ASSESSMENT

4.1 PERFORMANCE CRITERIA

Comparison of bioavailability assessment technologies developed by CU-1166 and CU-1210 with bioavailability endpoints in the current U.S. EPA risk assessment framework will satisfy a critical element of the ESTCP proposal requirements to "provide data and support to achieve regulatory and end-user acceptance." The approach to validate and demonstrate the use of the SBAT tool and in vitro methods to derive risk-based data for end users at the DoD study sites follows.

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. At this early stage of the project, performance objectives are identified; however, they will not be evaluated until the demonstration of the technology is complete. In general, the quantitative performance objectives for typical remediation-related ESTCP projects (e.g. end-point criteria, remediation time, and analytical sensitivity) are indirectly associated with the performance objectives of this project (e.g. ecological and human soil metal bioavailability performance objectives).

Table 4-1: Performance Criteria

Performance Criteria	Description	Primary or Secondary
In-vitro gastrointestinal protocol is applicable for evaluation of metal contaminant bioavailability in soil.	Describe whether or not there is a statistical relationship between the in-vitro bioaccessible and in-vivo bioavailable.	Primary
Using the approach developed in SERDP project CU-1210 to estimate plant endpoints for metal contaminated soils.	Describe whether or not there is a relationship between CU-1210 soil measurements (soil properties, soil extractable metal) and plant endpoints (dry matter growth, bioaccumulation.	Primary
Factors Affecting Technology Performance	Limited soils Limited budget	Primary
Reliability	Lack of statistical significance	Primary
Ease of Use	In vitro vs. in vivo	Secondary
Versatility	Model applicability to numerous soil types	Secondary

4.2 PERFORMANCE CONFIRMATION METHODS

Expected performance and performance confirmation methods are shown in Table 4.2.

Table 4.2: Expected Performance and Performance Confirmation Methods

Performance Criteria	Expected Performance Metrics	Performance Confirmation Method	Actual Performance Objective Met?
Quantitative – Ecological Bioassays vs. in vitro protocol	Statistical correlation	Significant multiple correlation	TBD*
	Consistent with speciation	Physical significance of model confirmed	TBD
	Estimated risk	Adequate risk assessment	TBD
Quantitative – Swine bioassays vs. in vitro protocol	Statistical correlation	Significant multiple correlation	TBD
	Consistent with speciation	Physical significance of model confirmed	TBD
	Estimated risk	Adequate risk assessment	TBD
Technology Transfer	Agency acceptance	Results considered acceptable by state or federal regulatory agency for site evaluation	TBD
Qualitative – Ecological bioavailability protocol	Protocol is applicable for evaluating Pb, Cd, Cr, As in soil	Validated statistical model	TBD
	Agency acceptance	Results considered acceptable by state or federal regulatory agency for site evaluation	TBD
Qualitative – Human bioavailability protocol	Protocol is applicable for evaluating Pb, Cd, Cr, As in soil	Validated statistical model	TBD

Table 4.2: Expected Performance and Performance Confirmation Methods (cont'd)

In-vitro gastrointestinal protocol is applicable for evaluation of metal contaminant bioavailability in soil.	Correlation between the in-vitro bioaccessible and in-vivo bioavailable is statistically significant at ($P < 0.1$).	Statistical evaluation to be conducted	TBD
Using the approach developed in SERDP project CU-1210 to estimate plant and soil invertebrate endpoints for metal contaminated soils.	Describe whether or not there is a relationship between CU-1210 soil measurements (soil properties, soil extractable metal) and plant endpoints (dry matter growth, bioaccumulation) and soil invertebrate (reproduction, mortality, bioaccumulation) endpoints.	Statistical evaluation to be conducted	TBD

4.3 DATA ANALYSIS, INTERPRETATION AND EVALUATION

Multiple regression analyses and/or neural network models will be utilized to develop predictive relationships between soil properties and metal bioavailability and to quantify the prediction uncertainty (e.g., confidence limits). The models must utilize input parameters that are physically meaningful in terms of known biogeochemistry.

The primary product of the work proposed herein will be new knowledge. This knowledge will be documented and disseminated in peer-reviewed publications, both as ESTCP reports and open scientific literature publications. Peer review, both from EPA and non-EPA peer scientists, will be the ultimate arbiter of the success of this project. The PIs have a strong track record of producing peer-reviewed publications, which is essential to ensure technical and regulatory acceptance of the results. Performances of conventional processes for bioavailability measurements and adjustments have been well documented. The performance and practical feasibility of this technical demonstration will be evaluated and benchmarked against various conventional processes.

5 COST ASSESSMENT

5.1 COST REPORTING

Table 5.1 Cost Reporting

Cost Category	**Sub Category**	**Details**
Start-Up Costs	Soil Collection	Travel to DoD sites, labor, shipping, supplies
	Soil Homogenization	Equipment, labor, shipping, laboratory analysis.
	Soil Chemistry	Laboratory analysis, labor, consumable supplies
Plant Bioassay Costs	Experimental	Labor, laboratory analysis, consumable supplies.
	Data interpretation	Labor
Earthworm Bioassay Costs	Experimental	Labor, laboratory analysis, consumable supplies.
	Data interpretation	Labor
Swine Dosing Costs	Experimental	Labor, laboratory analysis, consumable supplies.
	Data interpretation	Labor
In vitro Costs	Extraction and analytics	Labor, laboratory analysis, consumable supplies.
	Data interpretation	Labor
IVG Costs	Extraction and analytics	Labor, laboratory analysis, consumable supplies.
	Data interpretation	Labor
XAS Costs	Sample prep and analysis	Labor
	Modeling and data interpretation	Labor
Modeling Costs	Multiple regression	Labor
	Neural network	Labor

5.2 COST ANALYSIS

Factors that significantly affect whether or not a bioavailability study should be considered include: a) whether the studies can be completed within the required timeframe; b) the cost of the bioavailability study relative to cleanup; c) whether or not existing data support the likelihood of reduced bioavailability (Battelle and Exponent, 2000). Estimated costs of *in vitro* studies are $5-15 K and $50-200 K for *in vivo* studies.(Kelley et al., 2002). Thus it is clear that in vitro studies are more economical than in vivo studies (Ruby et al., 1999).

Cost Comparison

The costs for modeling and *in vitro* studies will be compared to the costs for *in vivo* studies. The impact on final remediation decisions will also be considered.

6 IMPLEMENTATION ISSUES

6.1 ENVIRONMENTAL CHECKLIST

The research conducted by ORNL staff is covered under a NEPA generic category exclusion for R&D conducted by the Environmental Sciences Division of ORNL, CX2657X. ORNL's UDSA compliance agreement TN01-018 was issued on 9/6/00 by the Tennessee Dept. of Agriculture and USDA compliance agreements apply.

There are no project-specific required permits or regulations pertinent to the completion of this work. No hazardous waste will be generated in this project. Since the soils are collected in the field, are being used for it's intended purpose and is not coming from a RCRA TSDF, the soils are not waste and are not subject to RCRA waste treatability study requirements. Contaminated soils targeted for this study contain metal concentrations that are below levels that would classify them as DOT hazardous materials.

6.2 OTHER REGULATORY ISSUES

Regulatory barriers for using bioavailability adjustments in ecological and human health risk assessments are complex and not easily resolvable. Regulatory acceptance of *in vitro* bioavailability in the near term will be on a case-by-case basis with most decisions based on site-specific data. This technical demonstration will contribute to this effort by providing significantly more complete and coupled data sets that link *in vivo* and *in vitro* bioavailability with soil characterization and metal speciation data. As part of this effort, we will contribute to developing standardized *in vitro* methods and determining if swine are an appropriate model for *in vivo* studies. Choices of ecological models will also be examined, e.g., indigenous plant types vs. lettuce.

6.3 END-USER ISSUES

The lack of guidance and policy coupled with time constraints on moving forward with cleanups present a regulatory barrier. The lack of guidance stems from insufficient published data to support the use of bioavailability adjustments in risk assessments. Data shortfalls are many:

a. More data is needed for all metal concentration ranges, including low concentrations to justify back-extrapolation of dose/response curves,

b. Data quantifying speciation effects on bioavailability and toxicity is needed,

c. More data is needed to select/justify *in vivo* models, such as swine and plant models (indigenous plants vs. lettuce), and accumulation rather than toxicity should be measured, and

d. More thought should be given to the statistical tools used to design experiments and analyze data.

The mechanisms that drive bioavailability are not well understood, so before soil properties can be used to adjust risk estimates, more research must be done to understand the mechanisms. Using existing data as well as collecting as much new data as possible, will allow scientists to create robust data sets and utilize multiple regression statistical techniques or neural net modeling to predict soil property effects on bioavailability. Translating soil properties into field-scale risk assessment adjustments will require consideration of future site uses that may alter soil characteristics and the subsurface environment and hence, bioavailability.

At present, *in vitro* data alone is generally not sufficient to make risk adjustments. More robust data sets are needed that correlate *in vitro* and *in vivo* data. Researchers must collect and publish data in peer-reviewed journals, including information on which *in vitro* tests work and which do not. Regulators should be involved every step of the way to facilitate information transfer and improve regulators' comfort level with *in vitro* test results. Standardizing methods for bioavailability testing would aid regulatory acceptance of bioavailability-based risk assessments. At present, focus should be on case-by-case acceptance of bioavailability data until enough data can be collected to justify broader acceptance.

Ultimately, acceptance of the results of this project will be accomplished by publishing in peer-reviewed journals and establishing an advisory panel of multi-disciplinary individuals including regulators, site end-users and researchers, that meets biannually to discuss the progress of this technical demonstration. Keeping regulators and site end-users abreast of these research findings will ultimately pave the way for an enhanced appreciation of *in vitro* methods as tools to estimate metal bioavailability on contaminated DoD sites.

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APPENDIX A: ANALYTICAL METHODS SUPPORTING THE EXPERIMENTAL DESIGN

Plant Bioassays

Soil (800 g) will be mixed with 50%, by volume, vermiculite in 1-L pots. Twenty plant seeds will be planted per pot. Three replicates of each soil (Pb-contaminated and control) will be grown in a completely randomized design in a controlled environment growth chamber with 18h of light/day, daytime temperatures of 20°C, and night temperatures of 18.5°C. To ensure soil has adequate nutrition, macronutrients will be tested and adjusted. Plant available phosphorus (P) and potassium (K) will be determined using the Mehlich 3 extraction with subsequent analysis by ICP-AES (Mehlich, 1984). Plant available nitrogen (NO_3-N and NH_4-N) will be determined by a 1*M* KCl extraction followed by automated flow injection analysis (Mulvaney, 1996). Percent germination will be determined at 7 days. Pots will be thinned to 5 plants per pot at 14 days. Plants will be harvested at maturity (ca. 40 days), rinsed in deionized water, dried at 70°C for 48 h and crushed by hand. The dried material will be weighed to determine dry matter growth (DMG). Dried plant tissue (0.25 g) will be predigested for 4 h in 10 mL of nitric acid. Predigested samples will be digested at 140°C for 4h, or until clear (Zarcinas et al. 1987). Filtered (0.45 μm) solutions will be analyzed for metals by ICP-AES. To account for differences in plant endpoints due to differences in soil quality (i.e., acidity, texture), dry matter growth (DMG) and germination (G) will be presented relative to their respective control soils.

Earthworm bioassays

Earthworm bioassays will be conducted using mature (clitellate) manure worms (*E. andrei*) according to ASTM (1999) protocol. Testing will be conducted in an environmental chamber set to 20^{o}C with constant light, with five replicates in each treatment. Each replicate will contain 200 g of soil (10 earthworms). Twenty-four hours prior to the addition of earthworms to the test soils, the earthworms will be removed from in-house culture containers, rinsed with distilled water, and placed on moist filter paper, during which time they will depurate most of the culture medium from their intestinal tract. At the start of the toxicity test, earthworms will be removed from the filter paper, rinsed, and separated into replicates (usually groups of 10). Each replicate group will be blotted dry, weighed, and added to a randomly determined soil replicate. Replicates will be monitored at 4, 8, 12, 24, and 48 h, 7 and 14 days. Should significant mortality occur at any time during the 14-d period, the test will be conducted again and adult worms sampled immediately before the expected time of mortality in order to determine metal residues. At 14 days, each replicate will be checked for cocoon production and cocoons enumerated and placed in a plastic petri dish containing distilled water and 1 g of reference soil (to maintain solution osmotic balance) for incubation. At 28 days, replicates will again be checked for

cocoons. Any remaining adult worms will be removed, rinsed thoroughly with distilled, deionized water, placed individually in plastic vials, and frozen at -40°C for storage.

Earthworms will be analyzed for metals using a combination of procedures developed by Honeycutt et al. (1995), Jenkins and Mason (1988), and Stafford and McGrath (1986). Each individual earthworm will be thawed, placed in a preweighed glass centrifuge tube containing 5 mL 0.05M HCl at a pH>7.0, and homogenized with a teflon-tipped Polytron homogenizer. The homogenate will then be centrifuged for 20 min at 10,000 x g, with temperature maintained at 4°C. The supernatant will then be transferred to another preweighed centrifuge tube. Both supernatant and pellet will be digested in 2 mL concentrated HNO_3 (trace metal grade) which will be boiled to dryness and subsequently resolubilized in 5 mL 0.5M HNO_3. For total metal levels, some worms will be will be predigested for 4 h in 10 mL of nitric acid. Predigested samples will be digested at 140°C for 4h, or until clear (Zarcinas et al. 1987). All samples will be filtered (0.45 μm) and analyzed for metals by ICP-OES or ICP-MS.

Enchytraeid bioassays

Enchytraeid (potworm) bioassays will be conducted using mature (clitellate) worms (*Enchytraeus albidus*) according to Rombke and Moser (2002) protocol. Testing will be conducted in an environmental chamber set to 15°C with constant light, with five replicates at each concentration. Each replicate will contain 20 g of soil (20 potworms). At the start of the toxicity test, potworms will be removed from the culture medium, rinsed, and counted into the test and reference soils. The bioassay can be divided into two steps: (a) an acute toxicity test in which mortality is the primary endpoint and will be assessed at 4, 12, 24, 48, 96h, and 7 and 14 d. Should significant mortality occur at any time during the test, the test will be conducted again and adult worms sampled immediately before the expected time of mortality in order to determine metal residues. The potworms will be removed from the soil, rinsed thoroughly with distilled, deionized water, placed as pooled samples in plastic vials, and frozen at -40°C for storage. Live potworms present at the termination of the bioassays will be rinsed, weighed, and stored as described above; (b) If 100% survival of adults is observed at 14 d, the bioassay will be continued as a reproduction bioassay in which the total number of juveniles produced per parent animal and the survival of parent animals are assessed. The duration of the reproduction test is 6 weeks. After the first 3 weeks, the adult enchytraeids are removed and morphological changes, if any, are recorded. After an additional 3 weeks, the number of offspring hatched from cocoons is counted. Adults will be sampled at 3 weeks as described above for metals analysis.

Pooled potworm samples will be predigested for 4 h in 10 mL of nitric acid. Predigested samples will be digested at 140°C for 4h, or until clear (Zarcinas et al. 1987). Filtered (0.45 μm) solutions will be analyzed for metals by ICP-AES.

Collembola bioassays

Collembola (springtail) bioassays will be conducted using *Folsomia candida* according to the ISO (1999) protocol. Testing will be conducted in an environmental chamber set to 20°C with constant light, with five replicates at each concentration. Each replicate will contain 30 g of soil.

At the start of the toxicity test, ten mature (14±1 days) *Folsomia candida* will be added to each test jar using a fine moistened paintbrush, the lids replaced, and the jars placed in an incubator ($20^oC \pm 1$) for 28 days. Two mg of dried active Baker's yeast will be provided as food. The lids will be removed twice per week to allow air exchange and the insides of the jars will be lightly misted with distilled water to maintain 100% humidity. At the end of the experiment (28 days), all the soil will be emptied into Tullgren funnels until all the collembola are extracted (approx. 48 h). Adult and juvenile collembola will be killed by freezing and counted under a dissecting microscope. Collembola samples from all replicates will be pooled in an attempt to determine metal body burdens, but this may not be possible due to the small mass of these organisms. Metals analysis will be conducted as described previously for Enchytraeids.

OSU-IVG method.

Basta, N.T., J. N. Foster, E.A. Dayton, R. R. Rodriguez, and S.W. Casteel. 2006. The Effect of Dosing Vehicle on Arsenic Bioaccessibility in Smelter-contaminated Soils. J. Environ. Health Sci. Part A. *Invited manuscript for the special JEHS publication "B*ioaccessibility and human bioavailability of soil contaminants." (Basta, 2006).

The OSU-IVG method used was first described by Rodriguez et al.(1999). This method is a soil extraction that simulates gastric then small intestinal conditions in sequence. The gastric solution is 0.15 M NaCl and 1% porcine pepsin (Sigma Chemical Company, St. Louis, MO, Cat. No. P7000). Each soil sample (1 g, < 250 μm) is mixed with 150 mL of gastric solution paddle stirrers in open beakers in a water bath at body temperature (37ºC). Results are expressed as gastric extractable (GE) arsenic. The pH of the gastric solution is adjusted to 1.8 using trace metal grade HCl to simulate the gastric phase of the digestive tract. The pH is continuously monitored and adjusted to 1.8 ± 0.1 every 5 min for 1 h. After 1 h, 10 mL of gastric solution is removed for analysis. To simulate the intestinal phase, the pH of the remaining solution is adjusted to 6.1 ± 0.1 by adding saturated Na_2CO_3 solution followed by the addition of 0.563 g of porcine bile extract (Sigma Chemical Company, St. Louis, MO, Cat. No. B8631) and 0.563 g of porcine pancreatin (Cat. No. P1500). After 1 h of mixing, 10 mL of intestinal solution is collected for analysis. Results are expressed as intestinal extractable (IE) arsenic. Each sample is centrifuged (11,160 g, 15min) and filtered through 0.45 μm membrane filters immediately after collection. Solutions are refrigerated for preservation and subsequent analysis by a high resolution Varian Vista-MPX inductively coupled plasma atomic emission spectrophotometer (ICP-AES). Arsenic is measured at an analytical wavelength of 188.980 nm.
Bioaccessible As was calculated as the percentage of the total As content extracted during the *in vitro* gastric or intestinal phase as shown as follows.

$$\%\ Bioaccessible\ As = \frac{[In\ vitro\ Extractable\ As \quad (mg\ kg^{-1})]}{[Total\ As\ Content \quad (mg\ kg^{-1})]} \text{ x } 100$$

Total As content is determined using USEPA Method 3050B or an equivalent method. All results are expressed on a dry weight basis.

ASTM. 1999. Standard Guide for Conducting a Laboratory Soil Toxicity or Bioaccumulation Tests With the Lumbricid Earthworm *Eisenia foetida*. E 1676-97. American Society for Testing and Materials, Philadelphia PA. ASTM Standards Vol. 11.04 (ASTM, 1999).

Honeycutt, M.E., Roberts, B.L. Roane, D.S., 1995. Cadmium disposition in the earthworm *Eisenia fetida*. Ecotox. Environ. Safety 30, 143-150 (Honeycutt et al., 1995).

ISO. 1999. Soil quality – Inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants. 11267. International Organization for Standardization, Geneva, Switzerland. (ISO, 1999)

Jenkins, K.D., Mason, A.Z., 1988. Relationships between subcellular distributions of cadmium and perturbations in reproduction in the polychaete *Neanthes arenaceodentata*. Aquat. Toxicol. 12, 229-244 (Jenkins and Mason, 1988).

Mehlich A. 1984. Mehlich 3 soil test extracting: A modification of Mehlich 2 extracting. *Commun. Soil Sci. Plant Anal*. 15:1409-1416 (Mehlich, 1984).

Mulvaney, R.L. 1996. Nitrogen-Inorganic forms. P. 1123-1184. In D.L. Sparks et al. (ed) *Methods of Soil Analysis*. Part 3 Chemical methods. SSSA Book Ser. 5. SSSA. Madison, WI (Mulvaney, 1996).

Rombke, J., Moser, Th., 2002. Validating the Enchytraeid reproduction test: Organisation and results of an international ring test. Chemosphere. 46-1117-1140 (Rombke and Moser, 2002).

Stafford, E.A., McGrath, S.P., 1986. The use of acid insoluble residue to correct for the presence of soil-associated metals in the gut of earthworms used as bio-indicator organisms. Environ. Pollut. 42, 233-246 (Stafford and McGrath, 1986).

Zarcinas BA, Cartwright B, Spouncer LR. 1987. Nitric acid digestion and multi-element analysis of plant material by inductively coupled plasma spectrometry. *Commun. Soil Sci. Plant Anal*. 18:131-146 (Zarcinas et al., 1987).

APPENDIX B: ANALYTICAL METHODS SUPPORTING THE SAMPLING PLAN

APPENDIX C: QUALITY ASSURANCE PROJECT PLAN

C.1 PURPOSE AND SCOPE OF THE PLAN

To adequately protect or restore soil ecosystems, it is necessary to accurately characterize soils suspected or presumed to be contaminated with heavy metals and define levels of metals in these soils that constitute a hazard to soil organisms. Currently there are no peer-reviewed, ecologically-based screening levels for soil in the United States. Metal toxicity is often not directly related to the total concentration of metals present due to a number of modifying factors that depend, in part, on soil chemical properties. Soil organic matter, pH, cation exchange capacity (CEC), and clay content are soil chemical properties that influence metal toxicity to earthworms.

The purpose of this demonstration is to validate the ability of soil chemical and bioassay methods predict contaminant bioavailability. Soil properties, total metal content, and metal bioaccessibility and bioavailability (as measured by various *in vitro* and *in vivo* methods, respectively) will be determined for metal contaminated soils collected from the four DoD sites for the human health models. A similar approach will be taken for the in vitro ecological model and it will be made more robust by considering an additional 8 DoD soils (total of 12 contaminated and 12 control soils for the ecological models).

C.2 QUALITY ASSURANCE RESPONSIBILITIES

Soil invertebrate and plant bioassays will be conducted at Ohio State University in the laboratories of Dr. Roman Lanno and Dr. Nicholas Basta, respectively. Other key personnel include Dr. Elizabeth Dayton, Research Scientist and co-PI, post-doctoral researchers and graduate students directly involved in conducting the research. The following is a list of the key personnel and their corresponding responsibilities involved with the demonstration:

1. Dr. Roman Lanno, Associate Professor, Department of Entomology

Dr. Lanno will be the QA officer for and manage the overall co-ordination of the soil invertebrate bioassays, including data collection, and be responsible for the timely preparation of reports. He will be responsible for overseeing bioassays conducted with earthworms (*Eisenia andrei*), potworms (*Enchytraeus albidus*), and collembola (*Folsomia candida*) and associated chemical analysis involving the determination of metals by ICP-OES.

2. Dr. Nick Basta (PI), Professor, and Dr. Elizabeth Dayton (Co-PI) School of Environment and Natural Resources.

Dr. Basta will be the QA officer for and manage the overall co-ordination of (i) soil chemistry, characterization and preparation and plant bioassays, including data collection, and be responsible for the timely preparation of reports. He and Dr. Dayton will be responsible for overseeing plant bioassays conducted with perennial ryegrass (*Lolium perrene*), lettuce (*Lactuca*

sativa), and associated chemical analysis involving the determination of metals by ICP-OES, soil pH, soil texture, organic carbon analysis, and Fe and Al-oxides.

Drs. Lanno, Basta, and Dayton will be responsible for project management and ensuring QA/QC procedures are implemented for their specific responsibilities in the project, communications with Amy Hawkins (PI) and for the preparation, editing, and submission of the final report.

3. Dr. Dayton, a post-doctoral researcher (Dr. Astrid Voigt; Lanno) and a PhD student (Richard Anderson; Basta), specializing in environmental soil toxicology, will conduct the toxicity tests, bioavailability estimates, soil chemical and physical characteristics, and metals analysis.

Dr. Lanno, Basta, and Dayton will be responsible for project management and ensuring QA/QC procedures are implemented for their specific responsibilities in the project, communications with Amy Hawkins (PI) and for the preparation, editing, and submission of the final report.

C.3 DATA QUALITY PARAMETERS

The biological (soil invertebrate and plant bioassays) and chemical (extractions, DGT measurements, metal residues in biological tissues) data generated during this research will be used to assess the bioavailability of metals from soils. Part of this research focuses on the application of a relatively new technique for estimating bioavailability (i.e., DGT). For this reason there are no standardized protocols or guidance for their application and estimates of their precision and accuracy are unavailable. We intend to maximize the precision and accuracy of our laboratory chemical analysis in order to ensure that the majority of the variation in our data analysis originates from the differences in the bioavailability of metals in the test soils. Soil invertebrate and plant toxicity and bioaccumulation bioassays are critical ecological measures for which accuracy cannot be assessed, since it is not possible to determine the true expected toxicity or bioaccumulation factor for metals in a specific soil. The essence of the project is to use models, existing data, and soil physical/chemical characteristics to predict the toxicity and bioaccumulation potential of the metals in the test soils and then conduct the bioassays to see how accurate the estimates were. Bioassay techniques for soil invertebrates and plants will follow standardized protocols to maximize precision of the tests. Table 1 provides a summary of the parameters that will be measured. Furthermore, the analytical program is designed to generate definitive data of sufficient quality and sensitivity to meet the project objectives. Measures to ensure representativeness, completeness, comparability, accuracy and precision of the data are discussed below and in the QAPP (Appendix B).

Representativeness – Representativeness expresses the degree to which data accurately and precisely represent a characteristic of a population, parameter variations at a sampling point, a process condition, or an environmental condition within a defined spatial and/or temporal

boundary. Using the proper analytical procedures, appropriate methods, meeting sample holding times, and analyzing and assessing blank samples ensure representativeness in the laboratory.

Completeness - Completeness is a measure of the amount of valid data obtained from a measurement system compared to the amount of data expected to be obtained under normal conditions. "Normal conditions" are defined as the conditions expected if the sampling plan was implemented as planned. Laboratory completeness is a measure of the amount of valid measurements obtained from all the measurements taken in the project. The laboratory completeness objective is greater than 95 percent.

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

Accuracy - Accuracy is the closeness of a measured value to an accepted true value. Standard reference materials (SRMs; i.e., plant, tissue, soil SRM) with certified analyte concentrations and certified check standards will be used to provide the accepted true value for analytical laboratory methods.

Precision - Precision is a measure of the degree of agreement among replicate analyses of a sample usually expressed as the standard deviation. Precision will be measured through the calculation of relative standard deviation (RSD) derived from replicated sample or control analyses.

C.4 CALIBRATION PROCEDURES, QUALITY CONTROL CHECKS, AND CORRECTIVE ACTION

4.1 Instrument/Equipment Testing, Inspection and Maintenance

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

4.2 Instrument/Equipment Calibration and Frequency

Calibration procedures for laboratory instruments will consist of initial calibrations, initial calibration verifications, and continuing calibration verification. The SOP for each analysis performed in the laboratory describes the calibration procedures, their frequency, acceptance criteria, and the conditions that will require calibration. This information is summarized in laboratory QA manuals. General calibration and QC procedures for metals analysis and bioassays are presented below.

Chemical Analysis Quality Control

The following contains the general QA/QC plan for metals analysis using Inductively-Coupled Plasma-arc Optical Emission Spectroscopy (ICP-OES).

1. General Chemistry.

   A. Bench records - Bench records that document data associated with the analysis, date, time, analyst, method, amounts, calculation, sample matrix, and sample identification number will be maintained and readily available for inspection.

   B. Calibration data - Calibration data, including calibration curve or coefficient of the linear equation which describes the calibration curve, concentration/response data for standards, percent recovery of a calibration check standard, and laboratory sample identification number of the samples run with this curve, will be maintained and readily available for inspection.

   C. Maintenance logbooks - Maintenance log books that include maintenance records, description of repairs, preventative maintenance, malfunctions, and other actions or events affecting performance will be maintained for each instrument and readily available for inspection.

   D. Standard Operating Procedures (SOPs) - Although general standard guidance for chemical analysis is available (EPA, ASTM, OECD), the specific manner in which routine testing is carried out in each laboratory may differ slightly. SOPs will be prepared that detail the specific process or analysis to assure that is carried out in a consistent manner by all laboratory personnel performing the task.

   E. Temperature logs - Temperature logs will be maintained for all instruments where temperature control is important and will be readily available for reference or inspection. The thermometer used to monitor temperature in these instruments will be calibrated daily with a precision thermometer certified by the National Institute of Standards and Technology.

   F. Weight logs - Most chemical and biological assays require weights to be determined during the execution of the protocol. This requires that weight logs be maintained for each balance. Weight logs will be checked with the appropriate range of class S weights on a weekly basis before use.

Metals analysis

A. Initial ICP-OES parameters will be selected from specified protocols and procedures according the element that is being quantified.

   B. Calibration of standards will be performed at the following times:

      1. At the start of a new project

      2. Each time the instrument is turned on

3. As the procedure requires

C. Calibration curve will be created using a minimum of three concentrations of standard solutions. Accuracy of the curve will be checked against a reference standard.

D. A bound maintenance log will be kept to record all calibration curves and sample runs.

Analytical procedures

A. Samples will be preserved, prepared, and stored according to specific methods.

B. The apparatus used (ICP-OES, ICP-MS) will be determined by the procedures for the specific analyte and desired detection limit.

C. Any necessary dilutions will be made with deionized water and ultra pure, low-metal content acids.

D. Stock standards will be made from pure or certified standards.

E. When filtering is required, low ash and metal content filters (e.g. Gelman GN-6 metricel membrane filters) pore size 45 micron, will be used.

Toxicity Test Quality Control

1. Test Organism Quality

A. Monitor and record the health of the soil invertebrate cultures. If excessive mortalities are observed during routine culture maintenance, then animals from that culture will not be used.

B. Conduct biannual reference toxicant tests (pentachlorophenol or NaCl) and maintain control charts. Hold suspect or discard any data that is generated during a time when control chart shows reference toxicant values outside of the acceptable limits.

C. Plant seeds will be purchased from only one distributor, be of one variety, and the lot number will be recorded for reference.

2. Test Organism (earthworms (*Eisenia andrei*), potworms (*Enchytraeus albidus*), collembola (*Folsomia candida*), perennial ryegrass (*Lolium perrene*), Lettuce (*Lactuca sativa*)

Use and Handling

A. Ensure that organism handling is done carefully and consistently. Use organisms from groups of consistent size and age. Earthworms will be adult, clitellate, and 300-600 mg in mass. Enchytraeids will be adults with a prominent clitellum. Collembola will be the largest adults available in the cultures. Plant seeds will be sieved to ensure

uniformity in size, and sorted to remove broken or discolored seeds, and seed hulls.

B. Soil organisms and plant seeds will be randomly assigned to test chambers one at a time, using an intermediate vessel to hold the required number of organisms/seeds prior to placement in test soils. Every 5th sample will be independently verified to assure counting accuracy.

C. All equipment used for transfer or manipulation of organisms (forceps, probes, spatulas) will be rinsed and cleaned between different soil samples to prevent cross-contamination during organism transfer and counting.

D. For each soil tested, controls will include an uncontaminated site soil similar in physical/chemical characteristics and an uncontaminated reference soil for which specific performance data (e.g., number of cocoons produced in 28 days) exists in our lab.

3. Standard Operating Procedures (SOPs)

To ensure that all personnel involved in conducting bioassays are aware of the proper procedures, SOPs have been developed (see example Appendix A) and will be followed for all general routine practices in soil organism and plant bioassays. These include, but are not limited to, sample preparation, preparation of foods for the test and culture organisms, and calibration and standardization for all measurements (temperature, pH, moisture content, organic matter content).

Data reduction, validation, and reporting

A. All samples will be logged into a bound sample record book

B. The data will be reported on a standardized form

C. Calibration curves will be checked against past performance

D. A linear regression will be estimated and recorded for each calibration curve generated

E. Duplicates will be noted as such but reported as separate samples, not averaged

Internal checks

A. Minimum of three concentrations of standards for a calibration curve.

B. One continuing calibration check standard after every ten samples tested. Any variation >10% from the calibration check standard shall be deemed unacceptable and any samples analyzed between acceptable and unacceptable calibration check standards shall be reanalyzed.

C. One blank per 20 samples tested.

D. One spike per 20 samples tested.

E. One duplicate or spike duplicate per 20 samples.

F. All absorbance and energy values for a specific element will be recorded in a laboratory notebook that is accessible for verification.

F. Any dilutions made from an original sample will be accompanied by a transferable label from container to container during preparation.

C.5 DEMONSTRATION PROCEDURES

Each laboratory has a QC program in place to ensure the reliability and validity of the analysis performed at the laboratory. All analytical procedures are documented in writing as SOPs and each SOP includes the minimum requirements for the procedure. The QC program for the individual laboratories are described in their respective QA manuals.

Laboratory quality control will be measured by analysis of the following types of samples:

1) Method blanks – used to define the level of laboratory background and reagent contamination
2) Lab control spikes – used to determine method accuracy
3) Matrix spikes - used to indicate the appropriateness of the method for the matrix
4) Duplicate samples – used to verify laboratory consistency and precision
5) Calibrations – necessary for accurate quantification

For toxicity tests, site reference soil tests will be conducted, laboratory reference soil and reference toxicant tests will be conducted and results will meet validity criteria for the specific test.

C.6 CALCULATION OF DATA QUALITY INDICATORS

The laboratory data collected during this investigation with be used to achieve the objectives identified in the demonstration plan. The QC results associated with each analytical parameter for each matrix (soil and biological tissues) will be compared to the measurement objectives presented in Section 3.0. Only data generated in association with QC results meeting the stated acceptance criteria (i.e., data determined to be valid) will be considered for decision-making purposes.

6.1 Accuracy Assessment

Accuracy of laboratory analytical methods will be calculated as follows:

Accuracy (%) = [(measured SRM or check standard value)/certified true value] x 100%

Analyses with $\leq$ 10% will be high accuracy and values $\leq$ 15% will be acceptable accuracy.

Precision of laboratory analytical methods will be calculated from RSD as follows:

RSD (%) = [(standard deviation / mean) x 100%]

Laboratory methods with ≤ 10% will be high precision and values with ≤ 15% will be acceptable precision.

C.7 PERFORMANCE AND SYSTEM AUDITS

Performance and systems audits

A. Performance Audit for evaluation of the system with a known sample will make use of certified reference materials.

   1. Reference check samples will be run at the beginning of each day and/or before a new sample set

   2. Corrective action (if necessary) will be identified

B. Systems audits (qualitative evaluations of all components of QC measurement systems) will be carried out at the start of every new project:

   1. Develop lab QA/QC project plan

   2. Develop reporting format and forms for data and QA/QC

Lab audits would include:

1) QA organization and procedures
2) Personnel training and qualifications
3) Sample log-in procedures
4) Sample storage facilities
5) Analyst technique
6) Adherence to laboratory SOPs and project QAPP
7) Compliance with QA/QC objectives
8) Instrument calibration and maintenance
9) Data recording, reduction, review, and reporting
10) Cleanliness and housekeeping

A written report will summarize audit findings and suggest corrective action, if any.

C.8 QUALITY ASSURANCE REPORTS

QA reports will be submitted to the QA officer to ensure that any problems during bioassays or chemical analysis are identified and to ensure that proper corrective measures are taken in response. The QA reports will include:

1) All results of laboratory audits

2) Significant QA/QC problems, recommended corrective actions, and the outcome of corrective actions.

QA reports will be prepared by the QA officer and submitted on an as-need basis.

C.9 DATA FORMAT

Project personnel will maintain records in which they will record all procedures, raw data, observations, test organism information, test conditions, calibration records, QC checks, results of tests, summaries of data, instrument printouts, etc., relevant to the experimental work. All results will be recorded in indelible ink, and when corrections are made, values will not be erased, but a single line will be drawn through the error, initialed by the responsible person, and clearly rewritten. Any change in entries should not be obscured and should indicate the reason for the change, and should be signed and dated at the time of the change. Laboratory data will be recorded in bound laboratory notebooks and tracked with the sample login number. Original copies of the data will remain in these notebooks and stored in Aronoff Laboratory 434 and Kottman Hall 409. Xeroxed copies of these records will be kept in a file folder and stored in a file cabinet located in Aronoff Laboratory 492 and Kottman Hall 410 during the course of the study. Records will be identified by the sample login number and filed according to that number. All records will be maintained for a period of at least five years from the completion date of the project.

To use the raw data, much of it will be transferred to computer for compiling into tables and charts. Worksheets and computer reports will continue to use the same sample identification numbers and will contain appropriate cross-references to lab and field notebook pages for sample manipulations, observations, analytical methods used, standards used, and any problems that may have been encountered. Someone other than the person entering the data will carefully check the transcription of raw data to worksheets and reports. A checklist indicating person entering the data, person checking for errors, error correction, and final copy preparation will be used to detect and correct paperwork errors. This tracking will also prevent loss of data during data reduction, data reporting, and data summarization.

Several statistical packages may be used in the analysis of the data. SAS will be used for hypothesis testing to determine whether treatment effects exist using ANOVA or t-tests, and for correlation and multivariate analysis. Data will be backed up weekly or immediately after major data entry events, as needed, on memory sticks dedicated to the project.

C.10 DATA STORAGE AND ARCHIVING PROCEDURES

All raw data, documentation, records, protocols, and reports generated as a result of the demonstration will be retained. Electronic archives and hard-copy archives will be retained at the institution of the PI responsible for that activity. Archived data will reside on a computer hard drive and be backed up on a memory stick.

Electronic archives and hard-copy archives for bioassay data will reside in Aronoff Laboratory 492 and Kottman Hall 410 on the Ohio State University campus. Researchers at the Oak Ridge National Laboratory will maintain data archives in Building 1505, rms. 342, 360 and 367.

Table C-1: Examples of Data Parameters

Critical measurement	Method	Reference	Precision[1]	Accuracy[2]	Completeness	MDL[3]
Total soil metal levels	US EPA Method 3050	US EPA 1996	NA	NA	NA	NA
Earthworm, potworm, Collembola toxicity & bioaccumulation	Standard toxicity test protocol	ASTM 1999 ASTM International 2004	ND	NA	ND	NA
Plant toxicity & bioaccumulation	Standard toxicity test protocol	Dayton et al. 2006	ND	NA	ND	NA
Metals in plants and earthworms	ICP	General digestion methods	NA	NA	NA	NA
Soil moisture	Gravimetric	Topp 1993	NA	NA	NA	NA
Soil pH	Water and $CaCl_2$ method	Thomas 1996	10%	NA	NA	NA
Soil organic matter	Heated dichromate oxidation	Nelson and Sommers 1996	10%	90-110%	NA	0.01 %
CEC	Cation displacement	Sumner and Miller 1996	10%	75-125%	NA	0.5 cmol kg^{-1}
Clay content	Pipet method	Gee and Bauder 1986	5%	90-110%	NA	0.2%

NA - Not available; ND - Not determined

[1]As relative percent of lab duplicates

[2]As percent recovery of lab matrix samples.

[3]MDL(method detection limit) as reported in the reference.

APPENDIX D: HEALTH AND SAFETY PLAN

The Health and Safety Plan for Ohio State University covers the mixing and homogenization of soils, conducting plant and soil invertebrate bioassays with site soils, and routine chemical analysis of the test soils for metals and soil physical/chemical characteristics. Each laboratory at Ohio State University is required to have a lab safety plan kept in the laboratory that includes general safety procedures, specific SOPs for routine analysis conducted in the laboratory, MSDS information on the chemicals used in the lab, and safety course certificates. Each faculty member, student, technician, and post-doc that works in the lab is required to take a 10-hour safety training course and pass an examination to assure that each individual has been provided with basic lab safety information. Conducting bioassays and routine metals and soil parameter analysis is covered by the lab safety plan. In addition, each lab has to have a Chemical Hygiene Plan as specified by OSU Environmental Health and Safety (http://www.ehs.ohio-state.edu/index.asp?PAGE=research.chp) dealing specifically with ensuring that lab personnel are aware of specific potential dangers of the chemicals they are working with.

There is the potential for exposure to dust from soil containing metals during the mixing and homogenization stage of the project. Safety precautions reducing or eliminating operator exposure to fine soil particles will be taken by modifying a large cement mixer using a steel cone attachment fitted with a 2-mm sieve to allow dust-free soil processing. The steel cone attachment will be custom built for the cement mixer. The novel cone attachment will allow (i) greatly improved homogenization, (ii) improved safety by greatly reducing exposure to contaminated dust from the project soils, and (iii) improved efficiency and recovery of homogenized soil. Virtually no soil will be lost during processing using the modified cement mixer whereas a large amount of soil would have been lost by conventional methods, soil that would have needed to be disposed of as hazardous waste.

Additional safety regulations and procedures are available for all personnel conducting research at Ohio State University on the College of Biological Science Safety Plan home page (http://www.biosci.ohio-state.edu/safety/safety/CHP.htm).

In accordance with the Department of Energy's Integrated Safety Management requirements, all work conducted at Oak Ridge National Laboratory must undergo a review process prior to authorization. ORNL's responsibilities in this project have been reviewed by a number of subject matter experts from transportation, industrial hygiene, waste management, radiological protection and facility support organizations. The review process has defined the hazards associated with the research activities and established the necessary controls in order to ensure that the work is conducted in a manner that is protective of both worker health and the environment as well as compliant to applicable state and federal regulations.

Each laboratory at ORNL is assigned a Laboratory Space Manager (LSM). It is the responsibility of the LSM to oversee activities and operation in this space. A Laboratory Operations Manual is maintained for each lab which contains a copy of the safety review for each project conducting work in that space, evacuation information, waste management

documentation, ORNL's chemical hygiene plan, and standard operation procedures routinely performed in the lab. Each lab worker must be trained by the LSM on the specific hazards associated with work conducted in the lab prior to conducting research. In addition to the lab specific training, ORNL requires a number of training courses on hazard communication, waste awareness and generation, ORNL's MSDS system and safe laboratory practices.