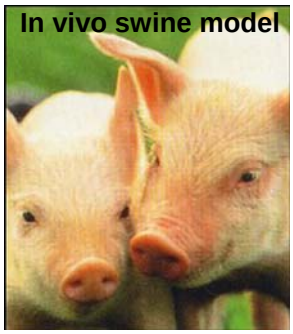
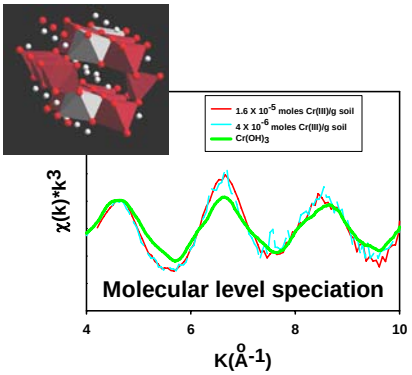
The Use of *In vitro* Soil Metal Bioavailability Methodologies to Adjust Human and Ecological Risk Assessment

ESTCP ER-0517

Workshop Summary
September 15, 2005
San Diego California





The Use of *In vitro* Soil Metal Bioavailability Methodologies to Adjust Human and Ecological Risk Assessment

(Summary of a workshop held September 15, 2005 in San Diego California)
Prepared March 2006

Workshop Overview

The following white paper describes a workshop held in San Diego, California on September 15, 2005 to which state regulators, DoD site end-users, state 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. There was also a representative from the Interstate Technology Regulatory Council (ITRC), which is a state-led industry and stakeholders coalition that seeks to facilitate regulatory acceptance of environmental technologies. Unfortunately, the ITRC was unable to provide further assistance due to large FY 06 budget cuts. 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 (Appendix A) included presentations by experts in the field and discussion sessions that addressed four challenge questions:

Challenge Question Set #1: Regulatory Acceptance

What are the regulatory and/or logistical barriers to the use of bioavailability adjustments in ecological/human health risk assessments?

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?

Challenge Question Set #2: Use of soil properties in human and ecological risk

How can soil properties be used to adjust risk estimates? At what stage of a risk assessment are they best applied?

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)?

Challenge Question Set #3: Use of *in vitro* (extract) data on human and ecological risk

Which in vitro data can be used to adjust risk estimates? At what stage of a risk assessment are they best applied?

How can in vitro data be best utilized to make risk assessment adjustments (i.e., how can we account for site variability in in vitro results)?

Challenge Question Set #4: Contributions of ESTCP project

How can this ESTCP project contribute to the implementation of bioavailability-based risk assessments that are acceptable in a regulatory context and are decision-focused?

How can the bioavailability research community at large contribute to this goal? What role is there for the ITRC, groups similar to the Bioavailability Research Group of Europe, or other groups?

Three invited speakers gave presentations on soil metal bioavailability as it relates to human and ecological risk. The first was the U.S. EPA's Dr. Jim Ryan, an internationally recognized expert on soil toxic-metal bioavailability in human risk assessment. The second speaker was Dr. Mark Sprenger from the U.S. EPA, an internationally recognized expert on soil toxic-metal bioavailability in terrestrial ecological risk assessment. The third speaker was Dr. Loren Lund of the DoD, who discussed incorporating *in vitro* bioavailability data coupled with soil property data into cleanup goals in a DoD site Remedial Action Plan. Following the invited presentations, Amy Hawkins (DoD) and Mark Barnett (Auburn University) gave an overview of ESTCP project ER-0517. After lunch, the workshop resumed, and an open, informal dialogue was initiated to address the challenge questions. The results of these discussions are described below. Thirty-four individuals from a variety of disciplines and institutional settings attended the workshop (Appendix B).

This document is organized as follows. First, a brief project description of ESTCP ER-0517 objectives, goals, and deliverables is given. Next are summaries of the three invited presentations. These are followed by the concerns and suggestions of workshop participants in response to the challenge questions. Details of the discussions are provided as well as summaries of key points. Lastly, our path forward coupling science, regulatory acceptance, and policy suggestions is described.

Project Description

Project Title: **The Effect of Soil Properties on Decreasing Toxic Metal Bioavailability: Field Scale Validation to Support Regulatory Acceptance**

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Dr. Stan Casteel, Professor of Toxicology and Director of Veterinary Medical Diagnostic Laboratory

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Dr. Scott Fendorf, Associate Professor, Geological and Environmental Science

Background and Relevant Past Research

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 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 (Pausterbach, 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. Reference doses 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.

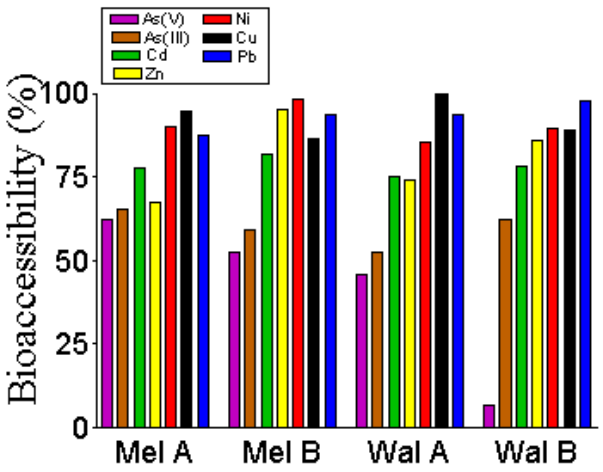
Recent SERDP research on certain DoD contaminated soils and DOE firing range soils found that nearly all soil-bound Pb was bioaccessible (Fig. 1) even at very high solid phase Pb concentrations (near 1% on a mass basis). These data were in agreement with highly liable Pb-spiked soils from around the 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; Barnett et al., unpublished data). 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 high bioaccessible Pb as molecular speciation suggest 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 mining 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, 2003; Stewart et al., 2003 a,b). 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 found to be 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

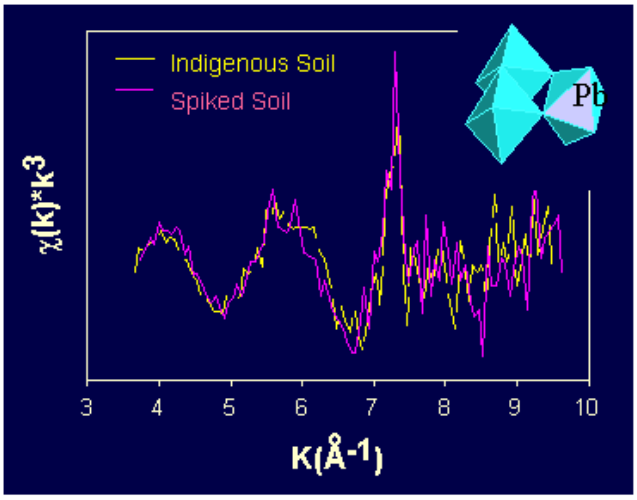
were also found to enhance bioavailability of these metals. For example, when the soil Fe-oxide content for a particular DoD soil fell below 0.5% on a mass basis, the bioaccessibility of As increased dramatically, particularly for alkaline soils (Yang et al., 2002, 2003; Fig. 2). Likewise, for DoD soils 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 a,b; Jardine et al., 1999; Fig. 3).

Figure 1



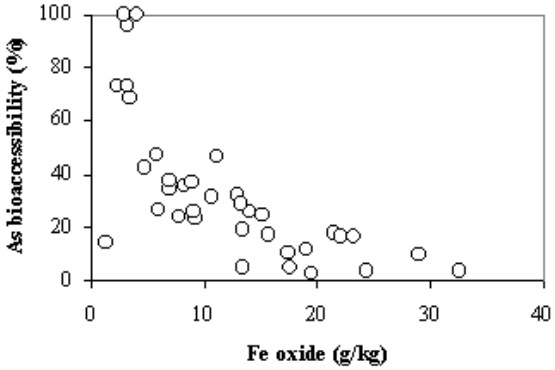
An example of toxic metal bioaccessibility on four soils relevant to DoD sites. Divalent cations, such as Pb, can be highly bioaccessible due to weak interactions with the soil.

EXAFS Spectra of Pb-contaminated Soils

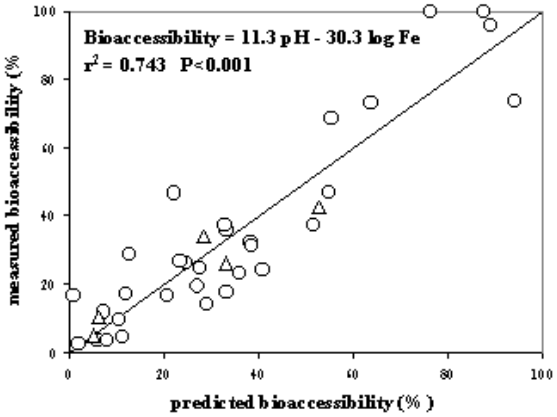


An example EXAFS spectra for Pb contaminated McClellan Air Force Base soil showing that Pb(II) is weakly associated with the soil via electrostatic interactions, thus supporting its high bioaccessibility.

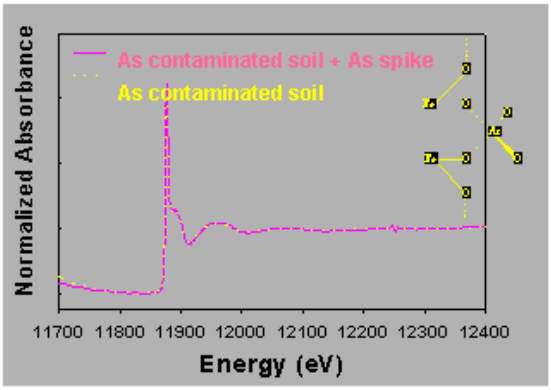
Figure 2



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).

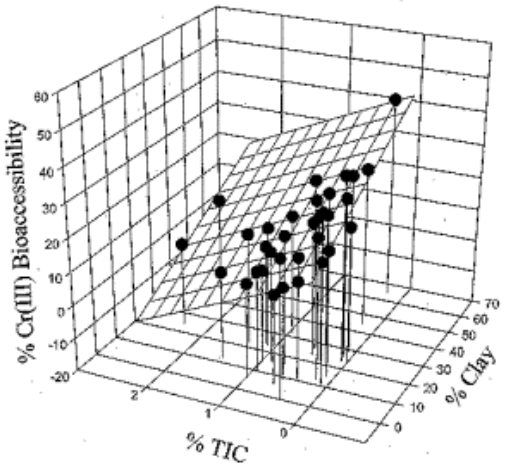


An example XAS spectra of As(V)-contaminated McClellan Air Force Base soil showing the strong inner sphere complexes with Fe-oxides, which is consistent with the above empirical model.

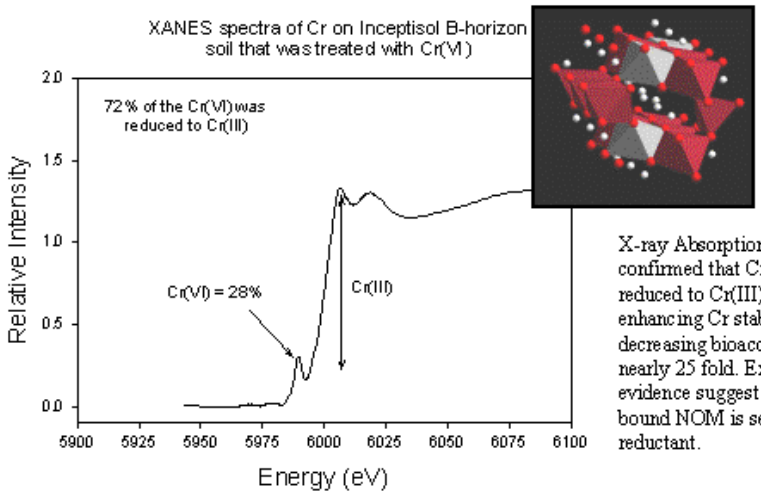
Figure 3

% Cr(III) bioaccessibility = 16.019 + (0.426 * % clay) - (9.564 * % TIC)

$r^2 = 0.722$



The bioaccessibility of Cr(III) and Cr(VI) in DoD soils was found to be strongly correlated with the clay content and the inorganic and organic C content. The modeling results agreed with XAS and EXAFS analyses suggesting that redox transformations and precipitation reactions contributed to the strong sequestration and decreased bioaccessibility of Cr in these soils.



X-ray Absorption Spectroscopy confirmed that Cr(VI) was reduced to Cr(III) thereby enhancing Cr stabilization and decreasing bioaccessibility by nearly 25 fold. Experimental evidence suggest that surface bound NOM is serving as the reductant.

Unlike Pb and As, most studies of Zn, Cu, Cd, and Ni bioavailability in soils have focused on ecological bioavailability, primarily 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 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 stomach digestive system, 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.

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* PBET or 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.

Project Objectives for ER-0517

(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 method for estimating human and ecological risk associated with metal-contaminated soils, thus 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.

ER-0517 Project Description

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 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 the SBAT tool for assessing toxic metal bioavailability in contaminated DoD soils as it relates to human and ecological risk.

Project Approach

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 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. More soils may be considered as additional funding becomes available. The use of *in vitro* ecological models will be further verified by comparison with *in vivo* ecological bioassay studies of approximately 10 DoD soils (10 contaminated, 10 control). 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. Appendix C provides more information on CU-1165 and CU-0222. 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 organizational chart of the study is given in Figure 4.

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 a) and *in vitro* techniques to predict metal bioavailability and risk (e.g., ecological, human). Results obtained from methods developed for accessing 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) (Fig. 5).

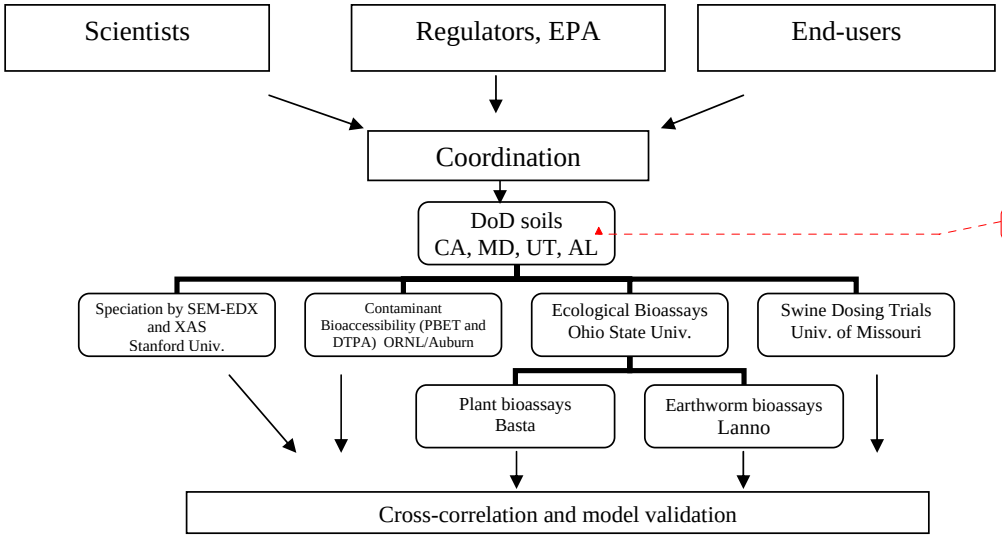


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Figure 4. Overview of the experimental design for Project ER-0517.

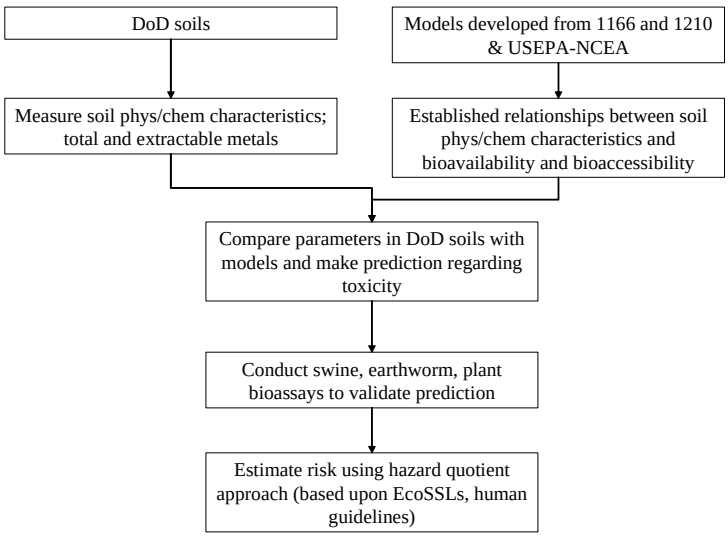

Figure 5. Validation of *in vitro* methods developed in CU-1166 and CU-1210 for estimating human and ecological risk in contaminated field DoD soils.

Summary of Presentations

Presentation #1: "Soil metal bioavailability: The use of in vitro soil metal bioavailability methodologies to adjust human and ecological risk assessment," by Dr. J. A. Ryan, U.S. EPA.

Dr. Ryan emphasized that total metal content in soil is not a good indicator of exposure and risk. He suggested that soil chemistry is very important in controlling metal bioavailability and phytoavailability. Metal speciation, as well as its chemical environment, can strongly affect metal adsorption. Dr. Ryan also stressed that one cannot assume an increase in concentration within the food chain equates to an increased transfer through the food chain. Predicting the potential transfer of soil metals requires a holistic evaluation of soil, plant, animal, and human processes that may increase or reduce the transfer (bioavailability). Dr. Ryan discussed efforts to clarify the Exposure-Dose-Response Continuum. Pharmacokinetic/Pharmacodynamic (PBPK/PBPD) models can be utilized to describe absorption, distribution, metabolism, and excretion within animals after metals enter the central compartment (e.g., blood). Currently, efforts are underway to expand these traditional approaches and include novel technologies derived from computational chemistry, molecular biology and systems biology in toxicological risk assessment. However, no consolidated effort to understand the relationships between external environmental exposure (fate and effects) and route of exposure on the transfer to the central compartment of the exposed organism exists. This important process is relegated to a simple term (bioavailability) without clarification of how to measure it or what affects it. Finally, Dr. Ryan showed results from a field demonstration where bioavailability of soil lead was not a simple function of total soil lead. Furthermore, lead bioavailability could be measured by swine, rat, human, and *in vitro* methods, with the magnitude of the absolute bioavailability depending on the method. Dr. Ryan showed that soil lead bioavailability can be changed by addition of various P sources to the soil since geochemistry of the soil lead is significantly altered.

Presentation #2: "The use of in vitro soil metal bioavailability methodologies to adjust human and ecological risk assessment," by Dr. Mark Sprenger, U.S. EPA.

Dr. Mark Sprenger discussed metal bioavailability within the context of U.S. EPA's terrestrial ecological risk assessment framework. He described this framework in detail and its use in a field study of metal bioavailability. He stressed the importance of:

(1) separating physical impact from chemical risk,
(2) accounting for essentiality,
(3) incorporating availability and chemical form,
(4) determining if there are critical exposure pathways,
(5) linking terrestrial and aquatic systems,
(6) acknowledging the natural risk level in an area, and
(7) realizing the solution may not be concentration reduction.

He described how risk is not a constant and, in fact, depends on many factors. For example, one can have periods of little risk if critically exposed organisms are not present

year-round (e.g., birds). Some critical exposure pathways may be temporal (e.g., insect emergence when birds are nesting; worms abundant in the spring). Dr. Sprenger stressed that average exposure models and assessment will not capture this. What and when one collects data is important and may make huge differences in the conclusions reached.

Presentation #3: "Site-specific bioavailability for lead at small arms firing ranges and the development of an Air Force protocol document," by Dr. Loren Lund, PARSONS.

Dr. Lund spoke on the use of *in vitro* bioavailability studies coupled with soil property characterization and metal speciation to assist with remediation decisions at several Pb-contaminated DoD sites. He discussed investigation timeframe guidance documents and issue papers published over the past decade, as well as research he has undertaken to incorporate site-specific bioavailability data into risk assessments. His group quantified soil particle size and Pb concentrations, determined *in vitro* bioavailability of soil Pb using the PBET technique, and performed speciation of the soil Pb using an electron microprobe. The results of these efforts were well received by DoD personnel at Travis AFB where site-specific bioavailability data were used to derive cleanup goals in the Remedial Action Plan (RAP). The RAP cleanup value was selected as the Pb final cleanup goal in the final Record of Decision (ROD).

Challenge Questions and Discussions

Challenge Question Set #1: Regulatory Acceptance

What are the regulatory and/or logistical barriers to the use of bioavailability adjustments in ecological/human health risk assessments?

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?

Stephen Tyahla, a Remedial Program Manager for the Navy who deals with this issue on a daily basis, provided a written response to the first question in Set #1. He considers regulatory acceptance key to using bioavailability data in adjusting ecological/human health risk assessments. The subjectivity in applying bioavailability to risk assessments is a major problem. Additional policy and guidance is needed. In the meantime, regulatory acceptance of bioavailability data must be argued on a case-by-case basis.

Jim Ryan of the U.S. EPA also submitted a written response to the first question in Set #1. He does not think this question can be answered at the level of this workshop. Rather, a regulatory policy solution is required. The lack of guidance and policy coupled with time constraints on moving forward with cleanups present regulatory barriers. Dr. Ryan believes the job of scientists is to advance scientific understanding, integrating metal speciation, chemical extraction, *in vitro* bioaccessibility, and measures of bioavailability. He also states, "Chemical solubility is the important issue. All animal

models will provide a biological measure indicative of the change in solubility. If the issue is kinetics of dissolution or animal physiology, then there may be some differences between responses of different animal models." Dr. Ryan points out the lack of data to determine which animal model is most representative of humans (swine, monkey, etc.) and adds that there is also no consensus on the best model/pathway for ecological concerns. He warns against designing experiments to resolve regulatory concerns without considering the broader scientific understanding. Finally, he encourages scientists to publish in peer-reviewed journals in an effort to effectively share information.

Roman Lanno, co-PI on project ER-0517, started discussions on the second question of Set #1 by providing the group with a handout of soil contaminant loading levels developed through toxicity testing experience and eco-SSLs (ecological soil screening levels) for plants and invertebrates (Appendix D). He stated that they were the maximum desired concentrations. Stan Casteel, also project co-PI, commented that the swine numbers on the handout were based on detectable levels and not necessarily based on DoD site remedial requirements. These concentrations were considered the minimum desired concentrations for use in the swine feeding studies.

Mark Sprenger pointed out that using concentrations near the effect threshold will not yield a good dose/response curve. When looking at soils pre-remediation, everything dies; when looking at soils post-remediation, everything lives. As a result, there is little data in between, and bioavailability assessment becomes statistically bimodal, which is undesirable. Bioavailability studies should include doses that do not elicit a toxic response. Chromium may not be the best metal to use because speciation often eliminates any concern. Site risk models must be examined to ensure that risk assumptions are compatible with bioavailability assumptions. Dr. Sprenger suggested that accumulation is more important than toxicity, and other workshop participants agreed.

Loren Lund suggested that there should be a way to include non-linear responses because not everything fits linear response models. This could be accomplished if different ranges of environmentally relevant concentrations (including background) are used to develop and test multi-stage-response models at lower and higher concentrations. Stan Casteel agreed and mentioned that blood has a limited capacity for lead; the blood either becomes saturated with Pb or the rate of uptake matches the rate of removal. Jim Ryan pointed out that the plateau might also be a function of the Pb source (e.g., speciation and chemical environment in the soil).

Marc Greenberg further suggested that in order to test whether certain soil characteristics are influencing toxic metal bioavailability, the range of concentrations should encompass the range relevant in risk decisions. Concentrations should fall between the NOEC (no observed effect concentration) and LOEC (lowest observable effects concentration) for ecological risk, cancer risk between 10^{-4} – 10^{-6}, and hazard quotients for chronic human risk between 0.1 and 10. Adhering to such standards would ensure that the tests are decision-oriented and in line with intended site remedial strategies.

Loran Lund recognized that the As concentrations listed under "Soil Requirements" on the handout were well above most cleanup levels. Arsenic is regulated at concentrations close to background levels at many contaminated DoD sites. He noted that detection limits for As in plant tissue might be higher than the regulatory As concentration but thought that working in the range where decisions are made was important.

Jim Ryan pointed out, however, that a soil concentration of 1000 mg/kg may be acceptable even though it seems too high. He suggested that much higher concentrations may not be a concern based on solubility/availability. Dr. Ryan challenged extrapolation to lower concentrations. Loren Lund wondered if we could provide evidence to support back-extrapolation (for Stan Casteel's data) to lower levels that may be more environmentally realistic and relevant. Nick Basta and Stan Casteel argued that the evidence could be produced. Steven DiZio stated that we need a pragmatic tool and should not focus too much on environmentally relevant doses. The group agreed.

Jim Ryan made the point that different metals have different toxicological significance for different receptor species (e.g., human vs. ecological) and that we must look at both metal concentration and speciation. When metal concentrations at a site are high, the metal may not be bioavailable, but the soil may be removed because of the high concentration. This is a very important point because metal speciation and the chemical environment within soils frequently control bioavailability. Phil Jardine cited a specific example of Cr speciation in soils and in the gut. While Cr(VI) is a highly toxic and mobile species in the environment, Cr(III) is sparingly soluble with low toxicity. However, toxicity levels for Cr(III) are rarely achieved (78,000 ppm on the solid phase), and knowledge of Cr speciation in soils is advantageous from a remedial perspective. Furthermore, there are numerous constituents in soil and the gut that can chemically reduce toxic Cr(VI) to Cr(III) thus limiting bioavailability and human and ecological risk. Dr. Ryan further reinforced the importance of metal speciation by mentioning that the soil Pb bioavailability values might be influenced by the form of Pb, and the range of concentrations may capture the difference in availability of different Pb forms.

Loren Lund cautioned that we should consider only plant species that are most relevant to DoD sites, and Nick Basta agreed to try to accommodate that advice. Toni Palazzo also recommended that plant studies include many replicates with uniform plant material. This would account for heterogeneity in plant genetics that could affect the experimental results. He cited an example in which 14 different species of plants showed Zn toxicity at 200 mg/kg soil but not at 500 mg/kg. The observed phenomenon may be related to Zn speciation or chemical environment differences between the soils rather than genetic influences. Nick Basta assured the group that these concerns have been met in the project by using many plants per pot and one type of lettuce. The group thought that rye grass may be more appropriate for arid soils since lettuce clearly is not an arid-climate plant. Dr. Lund commented that RA managers would appreciate data on plant species that grow on contaminated sites and are (receptor-wise) relevant.

As the discussion of this challenge question came to an end, Yvette Lowney stated that several important logistical barriers to bioavailability studies include: What is a robust data set (i.e., how many soils are needed)? What is an adequate correlation? Using the r^2 statistic doesn't tell how good the fit is for any one soil. What are the correct statistical tools? She also suggested that the study design might be a practical challenge. The group agreed completely. Mark Sprenger added, "When making decisions, it is easy to accept a decision of risk and difficult to accept a decision of no risk." Jim Ryan added "Apply common sense! Any value that is being used as an average estimate today will not be the same tomorrow. There is no guarantee."

Summary of Discussion on Challenge Question Set #1

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:

(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, and
(4) More thought should be given to the statistical tools used to design experiments and analyze data.

There was no general agreement on the As, Cr, Cd, and Pb concentrations relevant to bioavailability adjustments in risk assessment decisions. A handout (Appendix D) provided some numbers for discussion, but workshop participants pointed to the need for data at both higher and lower concentrations than those in the handout.

Challenge Question Set #2: Use of soil properties in human and ecological risk

How can soil properties be used to adjust risk estimates? At what stage of a risk assessment are they best applied?

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)?

Loren Lund opened up discussions by asking, "Which soil properties are we most interested in?" Roman Lanno mentioned that most data sets are simply total metal content of the soil. Often these data sets do not even include pH, Eh, organic matter, CEC, or clay content. He suggested that each of these chemical or physical parameters should be measured. Jim Ryan added that toxic metal bioavailability studies should measure every soil property imaginable (or affordable) when trying to extrapolate soil parameters to bioavailability. Through careful consideration of the mechanisms by which

a particular soil may interact with a metal, whether it is physical, geochemical, or microbiological, one can more carefully assess the soil properties that control metal bioavailability. Nick Basta mentioned that his group will quantify pH, salinity, sorbent phases, organic content, and clay content. Elizabeth Dayton mentioned that her group has existing data to make a more robust data set and to look at correlations, properties, and statistics. Phil Jardine added that multiple regression statistical techniques or neural net modeling are good methods for predicting soil property effects on metal bioavailability. These methods can warn of colinearity effects between soil properties and can provide a solid predictive framework for assessing future metal bioavailability scenarios. It is important that the models have physical significance and their parameters make sense scientifically. Multiple regression techniques have been previously successful in CU-1166 and CU-1210. Finally, Jim Ryan suggested that researchers use existing animal data and attempt to characterize soil properties and metal speciation to develop correlations. Mark Barnett has done this in CU-1166 for As(V) and As(III) contaminated soils, and the findings were exciting. He used soil properties and *in vitro* PBET statistics to show that he could predict *in vivo* As bioavailability in swine and monkey with ~15% relative error. The current ESTCP project will consider this approach as well by acquiring existing *in vivo* data from Stan Casteel of the University of Missouri for various Army soils he has worked with and DoD soils utilized by Yvette Lowney of Exponent.

Mark Sprenger mentioned that acceptance at the end of the study is complex because mechanisms are complex. The soil source (native or industrial) has an impact because 10% organic matter could mean, for example, 10% oil or coal. The origins of organic matter also have a dramatic impact on earthworm nutrition. Mark Bricka added that the difficulty in many studies is defining the mechanisms that drive bioavailability. Soil selection is important because soil organic carbon should not be anthropogenic (oil and grease). He mentioned that industrial samples are quite different than most DoD contaminated sediments. Also, there is very little information on the nutritional quality of sediments. He suggested that soil sampling should consider other "soil quality requirements" and the nutritional status. Phil Jardine agreed, commenting that most, if not all, of the proposed DoD sites had contaminated soils rather than sludge or sediments. Nine of the twelve soil orders were targeted such that physicochemical properties and the nutritional status of the soils could be estimated through the extensive NRCS database prior to site-specific data collection.

Loren Lund recommended that a soil property list be developed similar to the list of soil metal concentrations. He suggested that the list focus on the ideal or range of soil properties for our study and said that he knew of possible demo sites and may have data on the properties there. Phil Jardine mentioned that a list of possible sites with known contaminant concentrations and soil properties has already been considered (see the final section of this paper). Mark Bricka commented that previously, studies have been driven by asking where the problem is and studying those areas. For example, most DoD firing ranges are in the Southeast so they study those soils. Phil Jardine agreed and suggested that problem DoD soils were the driver of this ESTCP project.

Mark Sprenger suggested incorporating elements like Zn that compete with other toxic metals. Nick Basta replied that this is already being done. For example, Zn is measured when Cd is studied, and likewise with phosphate and As.

Loren Lund observed that there is a lot of soil property variation at a given site. He asked how to account for variability when applying studies to various DoD sites. Jim Ryan opined that the variability of contaminant distributions and soil properties at a particular site cannot be addressed within the framework of this study. The present ESTCP project seeks to determine what is driving the variability in bioavailability. Phil Jardine added that the site sampling protocol will involve use of a hand-held X-ray fluorometer to find appropriate soil contamination levels and is not totally random. Also, there are several skilled soil chemists on the project that are experts in soil characterization and soil properties. Nick Basta suggested that reference (control) soils are necessary to correct for other soil quality - driven effects for both the plant and invertebrate studies. Elizabeth Dalton added that the project ideally would include both contaminated and clean (control) soils from each site or adjacent sites.

Mark Bricka suggested that homogenizing large soil samples is very important and often difficult, especially when anthropogenic materials (e.g., bullet fragments) are present. He mentioned, however, that there is less variability with smaller particle sizes. Mark Sprenger warned that homogenization will affect bioassay protocols because the samples will be highly processed with long holding times. Stan Casteel added that mixing was used throughout the swine study. He noted that smaller particle size and smaller volumes used in the swine studies make it easier. Nick Basta said that BARGE was producing standard material for *in vivo* tests. We may need to find a larger facility for our blending. The current ESTCP project includes a large-volume mixing protocol employing an industrial mixer.

Michael Anderson made an interesting point that investigators need to be looking at future site use; current risk and bioavailability estimates might not apply in the future if, for example, soil properties change. Michael Wade mentioned that a former industrial site developed into a subdivision may involve changes in soil properties and the subsurface environment, adding temporal uncertainty to toxic metal bioavailability. Michael Anderson reiterated his concern that we will be looking at bioavailability at a single moment in time, without consideration of future land use and the use of soil amendments. If however, metal speciation is unaffected by changes in land use, which is often the case, and metal stability in the soil is maintained over geologic time scales, this concern is of little consequence.

Dr. Ryan concluded the discussion by asking what should be done if modeling shows risk lower than background. Average numbers from studies may change over time as soil properties and the subsurface environment change. For example, one form of galena is not absorbed and could show risk below background, but galena can change into an absorbable form with different subsurface conditions.

Summary of Discussion on Challenge Question Set #2

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, scientists need to create robust data sets and utilize multiple regression statistical techniques or neural net modeling to predict soil property effects on bioavailability. 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.

Challenge Question Set #3: Use of *in vitro* (extract) data on human and ecological risk

Which in vitro data can be used to adjust risk estimates? At what stage of a risk assessment are they best applied?

How can in vitro data be best utilized to make risk assessment adjustments (i.e., how can we account for site variability in in vitro results)?

Jim Ryan provided a written response to Question Set #3. He believes that *in vitro* data must be integrated with *in vivo* data and soil characterization data. He states, "If *in vitro* studies are going to make an impact on risk adjustments, more robust data sets will be needed."

Yvette Lowney reiterated the need for more robust data sets. She has seen good correlations between her *in vitro* and *in vivo* data, and others have likewise reported good correlations (Basta, Barnett, Casteel). However, she states that it is important to identify the reason why certain data do not fit. Yvette does not feel confident in generalizing the correlations until more comprehensive data sets are available. She thinks a large data set with good correlation for all samples is needed and is not yet available. The group agreed, and Nick Basta and Jim Ryan stressed that no soil test works for everything. The key is to publish results in the peer-reviewed literature so that data is accessible throughout the world. Also, it is important to define under what conditions tests do and do not work.

Jim Ryan offered his thoughts on why there is a lack of robust soil metal bioavailability data sets. He suggested that the problem is a lack of funds because most bioavailability money is spent on toxico-kinetics studies rather than on studies of toxic exposure and effects. The data (bioavailability and toxic effects) must be linked together to make rational decisions. The EPA and the public are concerned with health effects, but we are not spending money on exposure. In the meantime, a weight-of-evidence approach is needed.

Steven DiZio argued that the question is not which methods should be used (i.e., *in vitro* vs. *in vivo*), but rather how each method should be used. The major regulatory barrier is the paucity of studies relating *in vitro* and *in vivo* data. Workshop participants agreed that regulators and scientists must consider (1) how *in vitro* data can be used, and (2) whether a specified risk confidence level (e.g., 95% probability) computed from *in vitro* data will be accepted by regulators. An agreement between regulators and scientists is important, as well as a mechanistic understanding to support it. The group felt there was a need for more robust data sets to develop values that are useful for remedial action. Risk assessors, such as Stephen Tyahla, need a validated tool that they can place in their toolbox to close contaminated sites. An easy, validated *in vitro* test such as the PBET (which is typically conservative) is desirable to support remedial action at contaminated sites. Tyahla stated that he wants a test to move away from the assumption of 100% bioavailability, but the test must be relatively easy to "sell" to regulators. Phil Jardine thinks this is doable as long as some robust data sets are generated and models are validated.

Jim Ryan stated that he has no problem using extraction data to predict bioavailability. The question is how they are to be used. He asked whether we can rapidly move from an assumption of 100% metal bioavailability to one of less than 100%. He mentioned that in the past, nobody wanted to look at mechanistic data. At present, there needs to be agreement so there is some confidence that extraction data will even be considered. A robust data set is very important. Loren Lund suggested that bioavailability values less than 50% may warrant an adjustment to screening levels. Phil Jardine thought that was a good idea. A measure that is based on sound scientific experiments may help to communicate risk, or lack thereof, to the public.

Loren Lund was concerned with how long it may take and what it will take to satisfy decision-makers to use *in vitro* data. He reminded the group that it has taken years to get buy-in on *in vitro* tests for one metal (Pb). What is it going to take to convince decision-makers to use surrogate measures of bioavailability for other toxic metals such as As, Cd and Cr? Nick Basta mentioned that the UK looks at arsenic soil bioavailability based on *in vitro* (PBET) tests and adjusts the screening numbers accordingly. He reminded us that much of their data sets come from studies done in the United States and published in peer-reviewed journals.

Mark Sprenger pointed out that if regulators and risk assessors will accommodate a small shift in the screening levels, than this may have a huge impact in defining clean-up and/or remediation goals at many DoD sites. He felt that this concept would not be useful for eliminating contaminants of potential concern (COPCs) but that it may be useful in determining cleanup goals after the environmental risk assessment is complete. Stephen Tyahla mentioned that this could be useful at his site with 500 acres of elevated As. He was not sure if this would be useful at a smaller site. Loren Lund mentioned that the EPA dermal superfund workgroup looked at bioavailability and decided that for GI absorption of 50% or greater, bioavailability would be assumed to be 100%. If it was less than 50%, bioavailability could be adjusted. He also mentioned that U.S. EPA Region

VIII accepts *in vitro* data only along with other evidence. This is a nice example of incorporating bioavailability into human risk assessment and supports the importance of robust data sets that correlate *in vitro* and *in vivo* data.

Steve Geiger suggested setting up an advisory group and said that regulators need to be involved in this ESTCP project. Regulators need to be comfortable with values defined using *in vitro* data. The entire group agreed. Jim Ryan stated that this approach will accelerate the movement of good science to the agency. Yvette Lowney stated that she wanted us to meet again and perhaps form a metal bioavailability work group.

Summary of Discussion on Challenge Question Set #3

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. Workshop participants discussed how *in vitro* data should be used, but there was no consensus.

Challenge Question Set #4: Contributions of ESTCP project

How can this ESTCP project contribute to the implementation of bioavailability-based risk assessments that are acceptable in a regulatory context and are decision-focused?

How can the bioavailability research community at large contribute to this goal? What role is there for the ITRC, groups similar to the Bioavailability Research Group of Europe, or other groups?

Michael Anderson began discussions by suggesting that involvement of a standard-setting organization such as the ASTM is a good way to achieve standardization. It was suggested that the work of Annie Weisbrodt, of the SETAC bioavailability work group promote this concept since they feel that the use of a certified method assists in gaining regulatory acceptance.

Mark Sprenger of EPA stated that U.S. regulatory agencies deal with values and apply them differently than other countries. Regulatory acceptance is different than legislation or written guidelines. Loren Lund asked, "What are Canada's criteria? Are the criteria based on literal application?" Mark replied, "Regarding the difference between USA and Canada in acceptance of bioavailability values: in the United States, defined values are actually being used (e.g., cleanup values); however, in other countries, ideas are in the regulations but defined numbers do not exist and therefore are not being used (e.g., for cleanup). Canada mandates a literal use of bioavailability."

Loren Lund mentioned that a NRC statement suggested that there is no clear guidance or consensus on the level and lines of evidence necessary. It would be interesting to see which criteria led to regulatory acceptance in other countries. Will our data lead to acceptance? Terry Walker with the Army replied that "universal acceptance may be a pipe dream, but it is worth collecting the data and going forward." Mark Sprenger stated that "regulatory acceptance can imply acceptance on a case-by-case basis or legislatively." Amy Hawkins remarked that this project is focusing on case-by-case acceptance.

Summary of Discussion on Challenge Question Set #4

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.

The Path Forward for ESTCP ER-0517

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. ESTCP project ER-0517 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, researchers need to develop standardized *in vitro* methods and determine if swine are an appropriate model for *in vivo* studies. Choices of ecological models also need to be examined, e.g., indigenous plant types vs. lettuce.

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. This 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 ESTCP project ER-0517 progress. The following individuals will be invited to participate as advisors to the project:

Suggested Advisory Board

Randy Wentzel or Anne Fairbrother (U.S. EPA)
Jim Ryan (U.S. EPA)
Steve DiZio (DTSC HERD)
Mike Beringer (U.S. EPA)
Ron Checkai (ARMY)
Steve Geiger (RETEC)

To determine what drives bioavailability variability, four DoD facilities with markedly different soil properties, but with common metal contamination problems (Cr, As, Pb, and Cd) will be utilized in swine dosing trials. Ten facilities will be sampled in ecological bioassay studies. Soil types hypothesized to strongly sequester metals will be compared to soil types thought to have poor metal sequestering potential. Examples of such DoD sites are Hill AFB (Utah), Travis AFB (California), Deseret Chemical Depot (Utah), Aberdeen Proving Ground (Maryland), Redstone Arsenal (Alabama), Naval Station Newport (Rhode Island), and Fallon Naval Air Station (Nevada), all of which have significant problems with metal-contaminated soils. Selected chemical, physical, and mineralogical properties of the soils have been quantified at ORNL as described by Stewart et al. (2003a). 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 are expected to have high metal bioaccessibility. Soils from Aberdeen and Travis are silty, neutral-pH soils with good to excellent metal sequestering properties. These soils are 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), making the latter metals highly bioaccessible. Reference soils, i.e., the same soil series but with natural (background) levels of Cr, As, Pb, and Cd, will also be collected at each of the study sites.

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.

Much work remains to be done before the project goals can be realized. Well-designed experiments integrated across disciplines, together with physically meaningful analysis is essential to the success of this project. Communication among the researchers *and* between researchers, regulators and end-users is key.

Appendix A

Workshop Agenda

September 15, 2005
Holiday Inn on the Bay

8:00 – 8:30	Check-in and continental breakfast
8:30 – 8:45	Welcome and Introduction (Amy Hawkins)
8:45 – 9:25	Metal Bioavailability in the U.S. EPA Human Risk Assessment Framework (Dr. James Ryan)
9:25 – 10:05	Metal Bioavailability in the U.S. EPA Terrestrial Ecological Risk Assessment Framework (Dr. Mark Sprenger)
10:05 – 10:20	Break
10:20 – 11:00	DoD Case Study: Application of Bioavailability Assessment (Dr. Loren Lund)
11:00 – 11:45	ESTCP Project Overview (Dr. Mark Barnett and Amy Hawkins)
11:45 – 12:45	Lunch (catered on-site)
12:45 – 12:55	Presentation of Challenge Questions (Dr. Roman Lanno)
12:55 – 1:35	Challenge Question Set #1 Discussion
1:35 – 2:15	Challenge Question Set #2 Discussion
2:15 – 2:30	Break
2:30 – 3:10	Challenge Question Set #3 Discussion
3:10 – 3:50	Challenge Question Set #4 Discussion
3:50 – 4:00	Summary and Wrap-Up

Appendix B

Workshop Attendees

Dr. Michael Anderson	DTSC HERD	Manders7@dtsc.ca.gov	916-225-6625
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Appendix C

SERDP CU-1165

Development of Extraction Tests for Determining the Bioavailability of Metals in Soil

Background:

Considerable research and other evaluative efforts have been under way in recent years to identify environmentally acceptable endpoints (EAE) in soil, to develop protocols that can be used to determine EAEs, and to make site-specific decisions using EAE data. When applied effectively, these efforts have provided useful descriptions of risk. The effectiveness of these methods can be expanded by research directed at problems particularly relevant at Department of Defense (DoD) installations. EAEs for soil most commonly are defined as concentrations of chemicals or other measures of contamination (e.g., biological response or leachability) that are judged acceptable by a regulatory agency or an appropriate entity and are derived either from standard guidelines or following an analysis of site-specific or chemical-specific information and/or testing. There is a need to supplement the current lack of information regarding metals-contaminated soils.

Objective:

The primary objective of this project is to develop a suite of simple and easy-to-use extraction tests to predict human and ecological exposures to metals in soil. Such tests will provide inexpensive and rapid tools for establishing the bioavailability of metals in soils at hazardous waste sites. Soils used in the project will be characterized for metal species and soil parameters to provide a mechanistic basis for any differences in metals bioavailability among the samples. Therefore, results from the project will also provide an understanding of how various species of a metal may differ in bioavailability and how various soil properties may affect metals bioavailability and the stability of the measured bioavailability estimates.

Summary of Process/Technology:

This project will be framed around specific metals (i.e., arsenic, cadmium, copper, lead, nickel, and zinc) that are cost drivers for soil remediation at DoD sites and will focus on the most important receptors and exposure pathways for these metals. Historically, oral exposures to humans and terrestrial receptors have dominated risk assessments. Recently, dermal exposures have become more important in human health risk assessments as the U.S. Environmental Protection Agency adopts default dermal absorption values for some metals. A second aspect of the project will focus on assessing dermal absorption of arsenic and cadmium from soil. Dermal absorption of these metals from weathered soils has not been demonstrated to date. Initial studies will include animal studies and *in vitro* studies using human cadaver skin. After testing dermal absorption of these metals from weathered soils, development of a simple extraction test for dermal absorption will begin.

Benefit:

The research is designed to yield a suite of simple extraction tests that are inexpensive to perform, produce reliable results, and predictive of metals bioavailability from soil to human and ecological receptors. These tools will then be available to DoD personnel for site-specific evaluation of metals bioavailability from soil at field sites and will result in more accurate exposure and risk estimates that are still protective of human health and the environment.

Accomplishments:

This is an FY 2001 New Start project.

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ESTCP CU-0222

Validation of a Rapid and Low Cost Method for Prediction of the Oral Bioavailability of Lead from Small Arms Range Soils

Purpose:

Numerous small arms ranges are contaminated with lead from bullets. Models of risk assessment in these soils require a relative bioavailability term. The default value of 60 percent can be replaced with a more meaningful site-specific value by using an *in vivo* swine-feeding study. Although the swine model has been used to successfully adjust the default bioavailability at some Superfund sites, the uniqueness of small arms ranges, in terms of their small size, potential number, and main pollutant being lead, make it feasible to use an alternative and more cost-effective estimate of potential toxicity. The *in vitro* surrogate of mammalian digestion is intended to provide a rapid, cost-effective and robust alternative to the *in vivo* swine model for use at small arms ranges.

Description:

The relative bioavailability of lead in small arms range soils will be compared using both the *in vivo* and *in vitro* models. Soil samples from eight small arms ranges will be collected, dried and sieved to a particle size of less than 250 micrometers. For the *in vivo* model, weanling pigs will be orally dosed over a 15-day period and blood lead measurement will be used to assess absorption from the gastrointestinal tract. Absorption will be normalized to a lead acetate treatment group, giving the *in vivo* relative bioavailability. For the *in vitro* method, lead will be extracted in a simulated gastric solution with a pH of 1.5 at 37 degrees Celsius for 60 minutes, filtered, and analyzed for lead. This extractable lead will be normalized to the total lead in the sample, giving the *in vitro* relative bioavailability. A correlation coefficient of 0.8 or greater will be considered good evidence that the methods agree and that this technology can be applied to small arms range sites.

Benefit:

The use of a low-cost, rapid, *in vitro* test for lead in small arms range soils will enable site-specific bioavailability testing at these sites. Since the number of small arms ranges is thought to be in the hundreds, significant decrements in risk assessment costs would accrue by using the *in vitro* method over the *in vivo* swine model or the default assumptions. Furthermore, the *in vitro* test can be carried out at Army laboratories. (Anticipated Project Completion - 2005)

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Appendix D

Soil requirements for the different groups

Plants

As	50 to 500
Cd	< 100
Cr	Does not matter
Cu	50 to 500
Pb	200 to 1500
Zn	100 to 500
pH	4 to 8
EC	< 4 dS/m

Earthworms
As: 250 mg/kg
Cd: 100 mg/kg
Cr: 1000 mg/kg
Cu: 200 mg/kg
Ni: 250 mg/kg
Pb: 1000 mg/kg
Zn: 300 mg/kg

The proposed range of pHs for the soil is 4-8.

***In vivo* Swine**

Pb—Minimum concentration 1500 ppm; Preferred concentrations >2500 ppm.
As—Minimum concentration 300 ppm; preferred >500ppm
Cd—Minimum concentration 1000 ppm; preferred > 1500 ppm
Cr—Minimum concentration 1000 ppm. The Cr is something of a guess since it has not been assessed by any group we know of.

Appendix E

References

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