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

ESTCP ER-0517

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14. ABSTRACT

The technical objectives of the investigation were: (1) To provide validation that the relationships between soil properties and *in vitro* bioaccessibility methods can serve 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 in place of or as justification for more-detailed, site-specific bioavailability (e.g., animal dosing), and (3) to promote the use of *in vitro* methods in human health and ecological risk assessments through the upfront involvement of end-users and regulators and the subsequent dissemination of the results of the study in peer-reviewed journals.

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Preface

Most of the technical objectives, methods, results, discussion, conclusions, and recommendations of this study are detailed in Appendices A-F, which were written as stand-alone manuscripts for submission as peer-reviewed publications. Publication in peer-reviewed journals is needed to disseminate and ultimately facilitate the results of this study by site managers. In addition, publication in peer-reviewed literature is crucial to ensuring regulatory and community understanding and acceptance of the scientific results. Appendix A describes the speciation of metals and metalloids in soils that we sampled from U.S. military facilities as measured using synchrotron x-ray absorption techniques. Appendix B describes models for predicting metal toxicity and bioaccumulation in soil invertebrates, with a focus on the validation of laboratory models using metal-contaminated field soils. Appendix C describes a phytoaccumulation study, where comparison of the actual contaminant phytoaccumulation from bioassays with predicted toxicity from *in vitro* models were used to quantify the ability of *in vitro* models to predict actual phytoaccumulation in field DoD soils. Appendix D describes an *in vivo* study of As, Pb, and Cd using the juvenile swine model. Appendix E describes the ability of soil properties and *in vitro* extraction methods to predict bioavailability and bioaccessibility of As, Pb, and Cd in soil. Finally, Appendix F describes the soil properties and metal(loid) concentrations from the soils that we utilized. Liberal citations to these Appendices are made in the report itself, and an effort was made to minimize unnecessary duplication of material between the body of the report and the Appendices themselves. However, some duplication of material was used as needed to allow the report and each of the Appendices to be read as independent documents.

Executive Summary

The Department of Defense (DoD) faces a potentially daunting task of remediating thousands of metal-contaminated sites within the U.S. and its territories that contain unacceptable levels of the toxic metal(loid)s As, Cd, Cr, and Pb. With the exception of Pb contaminated soils, human health and ecological risk drivers have prompted EPA to assume that the total soil metal concentration is 100% bioavailable. Previous SERDP funded research (CU-1166 and CU-1210), however, has shown that the ubiquitous metal-sequestering properties of soil can significantly lower the bioavailability and risk of heavy metals to human and ecological receptors. This investigation brought together regulators, EPA, end-users, and scientists to demonstrate the applicability of these concepts by showing that simple, readily available soil properties can often be used to predict the bioavailability of As, Cd, Cr, and Pb with a reasonable level of confidence. We have shown that *in vitro* methods can often be used for risk assessment of toxic metals in soil by comparing *in vitro* and *in vivo* metal bioavailability studies.

The technical objectives of the investigation were: (1) To provide validation that the relationships between soil properties and *in vitro* bioaccessibility methods can serve 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 in place of or as justification for more-detailed, site-specific bioavailability (e.g., animal dosing), and (3) to promote the use of *in vitro* methods in human health and ecological risk assessments through the upfront involvement of end-users and regulators and the subsequent dissemination of the results of the study in peer-reviewed journals.

Performance Objectives 1 and 2 involved testing the bioavailability screening tools developed in our earlier SERDP studies, which correlate chemical speciation, bioaccessibility, bioavailability, and toxicity of metals (As, Cd, Cr, Pb) 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) Only three sites were considered for the *in vivo* swine dosing studies due to the experimental cost. The use of *in vitro* ecological models were further verified by comparison with *in vivo* ecological bioassay studies of eleven DoD soils (eleven contaminated, eleven control).

Metal Speciation An important first step was characterizing the molecular-level speciation of the metals in the soil with the use of X-ray absorption spectroscopy. Synchrotron X-ray fluorescence microprobe mapping, microbeam X-ray absorption spectroscopy, and bulk sample X-ray absorption spectroscopy were used to determine the oxidation state and molecular coordination environment of As, Cr, and Pb in eleven study soils with variable soil properties. *In vivo* swine dosing trials to determine metal bioavailability, *in vitro* gastrointestinal studies to determine metal bioaccessibility, soil extraction procedures and soil properties used to predict metal bioavailability to plant and soil invertebrates and ecological bioassay studies were also performed on the same set of soils. Findings from synchrotron X-ray studies indicated that Pb is adsorbed as divalent ions or present as organic complexes, rather than in crystalline compounds. Chromium and As are present in their more stable and less toxic inorganic forms, Cr(III) and As(V), except in soil from the Naval Complex at Pearl Harbor, where both As(III) and As(V) are present. Arsenic is bound to iron oxides in the Concord and Pearl samples, and to aluminum oxides in the Hilo soil sample. Arsenic-bearing soils may require more site-specific approaches

to remediation. Lead was not bound in sulfide phases that would be considered stable, meaning that most of the Pb-O in the soils may be liberated under acidic conditions (i.e., in the stomach).

Bioaccumulation and Toxicity Models Metal bioaccumulation and toxicity to soil invertebrates (*E. andrei, En. crypticus, F. candida*) were examined in ESTCP metal-contaminated soils (with paired reference site soils) comprising a wide range of physical and chemical characteristics and metal levels. The predictive ability of a number of different models relating soil properties to oligochaete metal bioaccumulation as a screening tool for estimating metal bioavailability in soils was examined with the intent of validating some of these models for predicting metal bioaccumulation in soil-dwelling oligochaetes.

Key elements for predicting bioaccumulation of metals by soil invertebrates include total metal concentration in the soil, soil physicochemical characteristics, and time. In this study, we examined the application of various models, with varying degrees of success, in predicting the bioaccumulation of metals by earthworms from ESTCP soils. The models can be divided into three categories: 1) Metals for which a large number of models exist in the literature (e.g., Pb, Cd); 2) Metals for which few models exist in the literature (e.g., Cr, Ni); and, 3) Essential metals (e.g., Cu, Zn).

When applying literature-based metal bioaccumulation models to assess Cd and Pb bioaccumulation by earthworms in metal-contaminated field soils, 98% of the variability in earthworm Cd concentrations could be predicted by a model comprising total soil Cd, organic matter content, and soil pH, while 95% of the variability in earthworm Pb concentrations could be predicted by a model including total soil Pb and soil pH. However, both these models over-predicted metal bioaccumulation (Cd Root Mean Square Error (RMSE) - 106%; Pb RMSE - 272%) so their use in predicting bioaccumulation may be limited. A large portion of the variability in the tissue concentrations of As (R^2 - 90%), Cr (R^2 - 77%), and Ni (R^2 - 88%) could be estimated by their concentrations in soil. Even though just a few bioaccumulation models exist for these metals, the models for As (RMSE - 24.2%) and Cr (RMSE - 13.6%) provided acceptable predictions of metal uptake, while the Ni model severely over-predicted uptake (RMSE - 689%). However, for the essential metals Cu and Zn, total soil concentrations combined with soil properties provided a reasonable prediction of tissue concentrations for Cu (RMSE - 24.7%) but not for Zn (RMSE - 590%). A model relating bioaccumulation factor (BAF) of Cd to soil properties provided acceptable predictions of Cd BAFs by *En. crypticus* from ESTCP soils (RMSE - 20%) while no relationship was evident between BAFs and observed metal burdens for Pb and Zn.

Models developed relating 0.5 M $Ca(NO_3)_2$-extractable Cd and Pb to earthworm metal residues did not provide a better prediction of Cd and Pb concentrations in earthworms exposed to ESTCP soils than models selected from the literature that predicted earthworm metal concentrations based upon total metal levels and soil physicochemical characteristics. Models incorporating toxicokinetics of metals were only available for Cd and provided reasonable estimates of Cd concentrations in earthworms (RMSE - 19%). These results indicate that there are no models for a specific metal that would provide good predictions of metal bioaccumulation in all soils and situations.

The capability of soil property/soil extraction models to predict soil invertebrate bioaccumulation of metals from contaminated soils is summarized as follows.

Table ES-1. Summary of the Prediction of Metal Bioaccumulation by Earthworms (*Eisenia fetida*) or Potworm (*Enchytraeus crypticus*) using Soil Property or Soil Extraction Data

Approach	Metal	Model	Summary and ability to predict metal body burdens
Soil properties	As	$\ln As_{ew}=0.9884*\ln As_s - 1.747$ Sample et al. 1998	Based on total As levels; $R^2=0.90$; under predicts 0.8-16-fold, most soils 0.8-3.3 fold; RMSE = 24.2%
	Cd	$\ln Cd_{ew} = 6.018 + 0.787 * \ln Cd_s - 0.106 *OM - 0.402 * pH$ Ma et al. 1983	Based on total Cd, organic matter, pH; $R^2=0.98$; over predicts 3.8-11.3-fold; only eight data points above DL; RMSE = 106%
	Cr	$\log Cr_{ew}=0.69*\log Cr_s -1.05$ Peijnenburg et al. 1999a	Based on total Cr; $R^2=0.73$; under predicts 0.8-7.4-fold; RMSE == 13.6%
	Cu	$\log Cu_{ew}=0.435*\log Cu_s +0.39$ Morgan and Morgan 1988	Based on total Cu; $R^2=0.45$; under predicts 1.3-5.2-fold; RMSE = 24.7%
	Ni	$\log Ni_{ew}=0.98*\log Ni_s +0.67$ Neuhauser et al. 1995	Based on total Ni; $R^2=0.88$; over predicts 11-95-fold; RMSE = 689%
	Pb	$\log Pb_{ew} = 2.65+0.897 *\log Pb_s-3.56*\log pH$ Corp and Morgan 1991	Based on total Pb and pH; $R^2=0.95$; over predicts 0.5-25-fold; RMSE = 272%
	Zn	$\log Zn_{ew}=1.45*\log Zn_s +0.42$ Peijnenburg et al. 1999a	Based on total Zn; $R^2=0.62$; under predicts 1.3-5.2-fold; RMSE = 590%
	Cd	$C_w = 9.32 *e^{-0.008*28} + Cd_s *0.052/0.008*(1-e^{-0.008*28})$ Yu and Lanno 2010	Based on Cherry Point and McLellan soils where total Cd is same as model concentration, one prediction is the same as observed and one is 2-fold higher; with all 8 data points – RMSE = 19%
Calcium Nitrate Extraction	Cd	$\log Cd_{ew} = 0.27*\log Cd_{Ca(NO3)2} + 2.1$ $R^2 = 0.66$,	Only two soils – Cherry Point, McLellan – with total extractable Cd levels; over predicted earthworm Cd 3-6.8-fold; RMSE = 111%
	Pb	$\log Pb_{ew} = 0.32\ Pb_{Ca(NO3)2} + 97$ $R^2 = 0.39$, P=0.008	Only five soils with extractable Pb; over predicted 1.1-3.6-fold; RMSE = 161%
	Zn	$\log Zn_{ew} = 0.02\ Zn_{Ca(NO3)2} + 2.12$, $R^2=0.084$, P=0.21	Only four soils with extractable Zn; under predicted 1.3-2-fold; RMSE = 101%

BAF - Soil properties *En.crypticus*	Cd	log BAF=1.17-0.92*log Clay Peijnenburg et al. 1999b	Only six soils where BAF could be calculated; acceptable under-prediction; RMSE = 21%
	Pb	log BAF=0.35-0.36*pH Peijnenburg et al. 1999b	No relationship
	Zn	log BAF =3.47-0.46 *pH-0.67*log Al_{ox} Peijnenburg et al. 1999b	No relationship

Plant Bioaccumulation Contaminant phytoaccumulation was also determined from plant bioassays for soils from eleven study sites. For ecological risk estimates, metal phytoavailability was estimated from soil-property driven multiple regression models developed using bioaccumulation data from two previous study studies. A separate approach involved the use of soil extraction methods, used to estimate metal(loid) phytoavailability, to predict contaminant phytoaccumulation. Regression models developed using bioaccumulation data from a previous study sponsored by the National Center for Environmental Assessment and SERDP CU-1210 were used to predict contaminant phytoaccumulation in the study soils. Comparison of the actual contaminant phytoaccumulation from bioassays with predicted toxicity from *in vitro* models were used to quantify the ability of *in vitro* models to predict actual phytoaccumulation in field DoD soils. This was the basis for validation of the soil property or soil extraction methods for field DoD soils. The predictive capability required by a soil property/soil extraction models depends on the degree of accuracy of contaminant phytoaccumulation determined by the risk assessor. With some exceptions, both methods were able to predict phytoavailability at RMSE<35% of the measured contaminant tissue value. In general, soil property models were predictive of tissue As, Cd, and Pb. Exceptions were Deseret for As (ryegrass), Hill for Cd (lettuce), and Portsmouth for Pb. In general, the predictive capability of soil extraction methods was adequate to excellent with the exception of Hill for Cd (lettuce) and Portsmouth for Pb.

The predictive capability of soil property / soil extraction models to predict plant phytoaccumulation is summarized as follows.

Table ES-2. Summary of the Prediction of Contaminant Phytoaccumulation using Soil Property or Soil Extraction Soil Data

Approach	Model or Soil Extraction	Ability to Predict Tissue As		Ability to Predict Tissue Cd		Ability to Predict Tissue Pb	
		Lettuce	Ryegrass	Lettuce	Ryegrass	Lettuce	Ryegrass
Properties	MLR	4† Concord Over , 5x‡	4 Deseret Over, 80x	4 Hill Under, 1.7x	4	7 Portsmouth Over, 1.3x ORNL Under, 1.3x	7 Portsmouth Under, 1.2x
	RR	4	4 Deseret Over, 80x	4 Hill Under, 1.7x	4	7 Portsmouth Over, 2x ORNL Over, 2x	7 Portsmouth Over, 1.7x
Soil Extraction	Pore water	3	3 All sites Over, 2x	3	3 Hill Under, 1.6x	4 Portsmouth Under, 4x	4 Portsmouth Under, 3.3x
	Mehlich 3	4	4 all sites Over, 2x to 5x	NA	NA	NA	NA
	Calcium Nitrate	NA	NA	3 Hill Under, 10x	3 Hill Under, 4x	4 Portsmouth Under, 2x	4 Portsmouth Under, 2.5x

† Number of contaminated soils evaluated.

‡ Over prediction of tissue As concentration by a factor of five

In Vitro Testing One of the main objectives of the project was to determine the ability of *in vitro* gastrointestinal methods (i.e., bioaccessibility methods) to predict measured contaminant bioavailability in contaminated soils from study sites. Equations used to predict bioavailability from bioaccessibility methods are available for Pb and As. Relative bioavailable Pb was determined for the Portsmouth soil in our study. The Physiologically Based Extraction Test (PBET) methods (pH 1.5 and 2.5) were able to accurately predict *in vivo* relative bioavailability (RBA) for the Portsmouth soil. The predicted RBA for the PBET method at pH 2.5 was closer to actual *in vivo* RBA than pH 1.5. However both methods predict RBA Pb within the 90% confidence interval. The Ohio State University *In vitro* Gastrointestinal Method (OSU IVG) method i*n vitro* bioaccessible (IVBA Pb) was very close to the *in vivo* RBA Pb. However, information on the ability of the OSU IVG method to predict RBA Pb is very limited whereas in depth validation studies have been conducted for the relative bioaccessibility leaching procedure (RBALP i.e., PBET) method. These results support the use of the PBET method at pH 1.5 and 2.5 to accurately predict *in vivo* RBA Pb. Future validation studies where this approach is expanded from the Portsmouth soil to other DoD soils will increase the confidence of using *in vitro* methods to predict *in vivo* RBA Pb.

Table ES-3. Comparison of Measured and Predicted RBA Pb for the Portsmouth Soil

		Predicted Pb RBA				
Measured Pb RBA, %		PBET pH 1.5		PBET pH 2.5		OSU IVG pH 1.8
Mean	90 % CI†	IVBA, %	RBA, %	IVBA, %	RBA, %	IVBA, %
99	70 - 127	83.3	86.9	80.4	106.2	102.5

† CI = confidence interval

Results from our study show both the OSU IVG and Solubility Bioavailability Research Consortium (SBRC) method were able to predict RBA As in the Deseret soil. The predicted RBA As by all methods ranged from 12.2 % to 16.2%, which is comparable to the *in vivo* RBA As of 14%. Further validation studies of these methods for other contaminated soils from different DoD contaminant sources are warranted. A study investigating the relationship between *in vitro* IVBA Cr and *in vivo* RBA Cr has not been reported. Thus, it was not possible to evaluate the ability of bioaccessible Cr to predict *in vivo* RBA Cr. In our study, a novel immature swine dosing model was used to determine the *in vivo* RBA Cr for the McClellan soil. RBA Cr was 107% with a 90% confidence interval ranging from 76% to 169%. *In vitro* IVBA Cr PBET method, used to measure bioaccessible Cr at pH 1.5 and at pH 2.5, was 10.1% and 19.0%, respectively. The *in vitro* IVBA values were much lower than the *in vivo* RBA Cr. Further research is needed before IVBA can be used to predict *in vivo* RBA Cr.

Table ES-4. Comparison of Measured and Predicted RBA As for the Deseret Soil

		Predicted As RBA					
Measured As RBA, %		OSU IVG gastric		OSU IVG intestinal		SBET gastric	
Mean	90 % CI†	IVBA, %	RBA, %	IVBA,	RBA, %	IVBA, %	RBA, %

				%			
14	13-15	8.45	15.0	8.47	16.2	10.6	12.2

† CI = confidence interval

In general, all of the in vitro methods predicted in vivo RBA As with 90% confidence.

The ability of soil properties to predict As and Cr bioaccessibility (IVBA) was dependent on the contamination source. In general, IVBA As measured by PBET and OSU IVG could be predicted from measured soil Fe properties including Feox or CBD Fe for soils where arsenical pesticide was the contaminant source. However, soil properties of the Deseret soil, where mine tailing was the contaminant source, was not predictive of the measured IVBA As. This finding suggests arsenic may occur as discrete minerals from the mining operation. It is likely the insoluble As minerals in the mining waste did not appreciably dissolve and react with soil components. Therefore, its chemical speciation and IVBA solubility will depend on the mining waste mineral not soil property.

The ability of soil properties (i.e., clay, organic and inorganic carbon) to predict and Cr bioaccessibility (IVBA) was dependent on the contamination source. Good agreement between the measured IVBA Cr and predicted IVBA Cr was found for Hill and McClellan soils. Poor agreement between the measured IVBA Cr and IVBA Cr predicted by soil properties was found for the Cherry Point soil. Differences in Cr chemical speciation in soil may offer an explanation. Water or wastewater treatment was the contaminant source for the Hill and McClellan soils. Incinerator ash was the contaminant source for the Cherry Point soil.

Summary of Soil Properties to Predict Metal Bioavailability

Soil properties, able to predict metal (bio)availability for several contaminated soils in this study, are summarized in the following table. At a minimum, soil property information needed from a site investigation for all contaminants studied are soil pH, clay content, organic C, inorganic C, reactive Fe and Al (FEAL, Feox and/or CBD Fe). Other properties not studied that will affect ecological endpoints include soil salinity and the presence of other toxicants.

Table ES-5. Summary of Soil Properties to Predict Metal Bioavailability

	Contaminant			
	Pb	As	Cr	Cd
Human Soil Ingestion Bioaccessibility	Not evaluated	Feox and FeCBD	Clay content, total organic C, inorganic C	Not evaluated
Plant accumulation Lettuce	pH, OC, FEAL	pH, OC, FEAL	Not evaluated	pH, OC, FEAL
Plant accumulation Ryegrass	pH, OC, FEAL	pH, OC, FEAL	Not evaluated	pH, OC, FEAL
Soil Invertebrates	pH	Total metal	Total metal	pH, OM

These properties will **not** predict metal bioavailability for all soils. A major finding of this study is the contaminant source and likely speciation greatly affects the ability of soil property to predict metal bioavailability. Metal bioavailability was not able to be predicted for several soils where the contaminant source was unweathered mining waste or discrete inorganic mineral forms such as coal ash. Soil properties should NOT be used to predict contaminant bioavailability in these soils. More research on contaminant source and speciation is needed to determine when soil properties can provide an accurate assessment of metal bioavailability. Currently research is in progress, including research funded by SERDP (i.e., ER-1742) to determine the relationship between As speciation and ability to predict As bioavailability to humans.

Summary of Soil Extraction Methods to Predict Metal Bioavailability

Soil exaction methods, able to predict metal (bio)availability for several contaminated soils in this study, are summarized in the following table. Both PBET and OSU IVG were able to very accurately predict RBA As and Pb but for only for 1 soil each. The number of soils evaluated were very limited because of cost constraints associated with in vivo dosing trails required to measure contaminant RBA. More research is needed to evaluate the ability of these methods to predict RBA Pb and RBA As on other contaminated soils.

Soil pore water was able to predict plant tissue concentration of Pb, As, and Cd. Soil extraction with 0.1 M $Ca(NO_3)_2$ was able to predict cationic metal contaminants(i.e. Pb, Cd) but was not evaluated for anionic As contamination. The ability of simply water or dilute calcium nitrate to predict phytoavailable contaminant suggests high solubility of these contaminants in soils. Thus, it is likely that with 0.1 M $Ca(NO_3)_2$ would have also been a good predictor of plant As. However, two cautions should be heeded. The accuracy of these extraction methods to predict plant tissue contamination was limited to ± 35%. Similarly to metal bioaccessibility results, metal bioavailability was not able to be predicted for several soils where the contaminant source was unweathered mining waste (i.e. Deseret) or discrete inorganic mineral forms such as coal ash (i.e. Cherry Point). Soil extraction methods listed in the summary table should NOT be used to predict contaminant bioavailability in these soils. More research on contaminant source and speciation is needed to determine which soil extraction methods can provide an accurate assessment of metal bioavailability.

Table ES-6. Summary of Soil Extraction Methods to Predict Metal Bioavailability

	Contaminant			
	Pb	As	Cr	Cd
Human Soil Ingestion Bioaccessibility	PBET, pH 1.5 PBET, pH 2.5 OSU IVG	OSU IVG SBET	Not evaluated	Not evaluated
Plant accumulation Lettuce	Pore water 0.1 M $Ca(NO_3)_2$	Pore water Mehlich 3	Not evaluated	Pore water 0.1 M $Ca(NO_3)_2$
Plant accumulation Ryegrass	Pore water 0.1 M $Ca(NO_3)_2$	Pore water Mehlich 3	Not evaluated	Pore water 0.1 M $Ca(NO_3)_2$
Soil Invertebrates	Pore water	Not evaluated	Not evaluated	Pore water

	0.5 M $Ca(NO_3)_2$			0.5 M $Ca(NO_3)_2$

As part of Objective 3, immediately upon receiving funding for this endeavor, a two-day workshop was held bringing together state regulators, DoD site end users, EPA officials, and scientists familiar with soil metal bioavailability. The workshop focused on past, current, and future research endeavors investigating soil metal bioavailability methodologies and the possible use of *in vitro* bioaccessibility values in human health risk assessment and policy. At the kickoff workshop, the research strategy was discussed among scientists, regulators, USEPA, and end-users to advance the acceptance of *in vitro* methods in human health and ecological risk assessment and policy. We incorporated the comments of the attendees of the workshop in our research. In addition, also as part of Objective 3, most of the technical objectives, methods, results, discussion, conclusions, and recommendations of this study are detailed in Appendices A-F, which were written as stand-alone manuscripts for submission as peer-reviewed publications. Publication in peer-reviewed journals is needed to disseminate and ultimately facilitate the results of this study to site managers. In addition, publication in peer-reviewed literature is crucial to ensuring regulatory and community understanding and acceptance of the scientific results. The publication of the results of this study are proceeding.

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ACRONYMS AND ABBREVIATIONS

APS	Advanced Photon Source
BAF	Bioaccumulation Factor
BARGE	Bioavailability Research Group of Europe
CEC	Cation Exchange Capacity
DoD	Department of Defense
EcoSSLs	Ecological Soil Screening Levels
EXAFS	Extended X-Ray Absorption Fine Structure
GI	Gastrointestinal
HQ	Hazard Quotient
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectroscopy
IVBA	*In vitro* Bioaccessibility
IVG	*In vitro* Gastointestinal
MLR	Multiple Linear Regression
NAVFAC EXWC	Naval Facilities Engineering and Expeditionary Warfare Center
OSU IVG	Ohio State University *In vitro* Gastrointestinal Method
PBET	Physiologically Based Extraction Test
RBA	Relative Bioaccessibility/Bioavailability
RBALP	Relative Bioaccessibility Leaching Procedure
RMSE	Root Mean Square Error
RR	Ridge Regression
SBRC	*Solubility Bioavailability Research Consortium*
SBET	*Simplified Bioaccessibility Extraction Test*
SOP	Standard Operating Procedure
SSRL	Stanford Synchrotron Radiation Laboratory
TRV	Toxicity Reference Value
UEF	Urinary Excretion Fraction
XANES	X-ray Absorption Near-Edge Structure
XRF	X-ray Fluorescence
XAS	X-ray Absorption Spectroscopy

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1.0 INTRODUCTION

1.1 BACKGROUND

There are thousands of metal-contaminated sites on DoD lands awaiting remediation and closure. Lead, As, Cr, and Cd are toxic (i.e., capable of producing an unwanted, deleterious effect on an organism) metals of particular concern since these metals often control risk-based remedial decisions for soils at DoD sites [1]. Ingestion of contaminated soil by children is the exposure pathway that generally controls remediation goals [2] [3]. With the exception of Pb-contaminated soils, the risk posed by soil ingestion is currently calculated from the total metal (e.g., as measured by USEPA Method 3050B [4]) 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, USEPA's risk assessment guidance implicitly assumes a default relative bioavailability of 100%. For the purposes of this study, "bioavailability" refers to the *in vivo* availability of a contaminant to a biological organism (e.g., a plant, human child, or earthworm), while "bioaccessibility" refers to the amount of a contaminant that can be extracted in an *in vitro* procedure. Ruby et al. [5] provides precise definitions of these and other relevant terms (e.g., relative versus absolute bioavailability, etc.). The toxicity assessment for Pb is unique and is based on a pharmacokinetic model of blood Pb. The default bioavailability assumptions in USEPA'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 [6, 7], mining waste [8, 9] and aggregate [10, 11] 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 [12]. Relatively low Pb bioavailability is a consequence of Pb speciation and the corresponding solubility constraints [13] and of kinetically-controlled dissolution due to limited residence times in the gastrointestinal (GI) tract [14]. Risk assessments based on data from studies using soluble metal salts overestimate the risk posed by these soils [15]. 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 (measured as 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 [16]. Molecular speciation analyses using x-ray absorption spectroscopy (XAS) suggested that Pb(II) was weakly associated with the soil via electrostatic interactions. 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(s). However, not all DoD soils have highly bioaccessible Pb, as molecular speciation (e.g., metallic or precipitated as sparingly soluble species) can significantly reduce Pb bioaccessibility (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 [17]. 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 [18]. Rodriguez et al. [19] 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 [16, 20-22]. Our previous results, for example, have shown that numerous DoD soils throughout the U.S. can effectively sequester As(III/V) and Cr(III/VI), significantly decreasing metal bioavailability. 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. 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 [16, 20]. 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 [21, 22].

Unlike Pb and As, most studies of Zn, Cu, Cd, and Ni bioavailability in soils have focused on ecological bioavailability, primarily plant uptake. It is unlikely that a soil extraction method will replicate the amount of metal absorbed by plants. The plant uptake system is too complex and dynamic to simulate by simple extraction methods in the laboratory. A more reasonable approach may be to use soil extraction methods that are based upon soil chemistry and root physiology and that correlate well with plant uptake of metals. The discipline of Soil Science has used this very concept successfully for the last 50+ years. Chemical extractants cannot extract plant nutrients in the same manner as a living plant under the conditions of the plant root environment. However, good correlations between soil extracts and plant uptake has allowed soil scientists to use that relationship to make reasonable predictions of plant available nutrients in soil and subsequent fertilizer recommendations. Plant uptake studies have shown that these metals are largely immobilized by soils, and only a small fraction is bioavailable. Banjoko et al.

[3] 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 [23] 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 [24-27] is typically bioavailable in soils. In addition, when metal-scavenging Mn [28] or Fe [29] 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 and Cd sometimes remain highly bioaccessible even though they are sequestered by the soil solid phase. 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 [30] 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. [31] reported a 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 [32], soil As [19], and soil Cd [31]. However, none were specifically 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 and an 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 USEPA for screening soil metal levels for wildlife, soil invertebrates, and plants. These values have been developed considering soils in which metals are maximally bioavailable. 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

are currently being employed as an additional method for estimating the bioaccessible fraction of metals in soils.

1.2 OBJECTIVE OF THE DEMONSTRATION

The technical objectives of the investigation are:

(1) To provide validation that the relationships between soil properties and *in vitro* bioaccessibility methods can serve 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 in place of or as justification for more-detailed, site-specific bioavailability (e.g., animal dosing), and

(3) to promote the use of *in vitro* methods in human health and ecological risk assessments through the upfront involvement of end-users and regulators and the subsequent dissemination of the results of the study in peer-reviewed journals.

1.3 REGULATORY DRIVERS

Several recently published studies have summarized the current regulatory climate in regards to these issues. For example, Ehlers and Luthy [33] 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, although individual states have allowed bioavailability adjustments on a case-by-case basis [5]. To help fill this void, the USEPA 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 (e.g., land usage, biogeochemical environment, etc.) are unlikely to change over the relevant timeframe. 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 [34, 35]. Except for Pb, the USEPA'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 or moderately 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.

2.0 TECHNOLOGY

2.1 TECHNOLOGY DESCRIPTION

The purpose of this demonstration was to demonstrate the ability of soil chemical and bioassay methods to predict metal bioavailability for human and ecological risk assessment. The project sought to provide validated evidence that *in vitro* bioaccessibility methods can serve as time- and cost-effective predictive indices of toxic metal bioavailability in DoD soils relative to *in vivo* uptake studies. By quantifying the extent to which soil properties control metal bioavailability, we have shown that the models developed in our previous SERDP projects 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 facilitate 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.

Soil properties, total metal content, speciation, and bioaccessibility and bioavailability (as measured by various *in vitro* and *in vivo* methods, respectively) were determined for metal contaminated soils collected from three DoD sites for the human health models. A similar approach was taken for the *in vitro* ecological model, which was made more robust by considering an additional eight DoD soils (total of eleven contaminated and eleven control soils for the ecological models).

Human Health Metal bioaccessibility and metal bioavailability for three study soils was calculated using soil property-driven models developed from our earlier SERDP studies. Calculated bioaccessibility values were compared with measured bioaccessibility values using *in vitro* gastrointestinal methods for study soils. The physiologically based extraction test (PBET) developed by Ruby et al. [5], was 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. [21, 22], additional soil property-driven models were 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 [20, 36]. In contrast, As(V) bioaccessibility was minimally influenced by changing pH environments. In addition to PBET, the OSU-IVG [37] method was used to measure bioaccessible As. The ability of the OSU-IVG method to predict contaminant bioavailability was determined.

Ecological For ecological risk estimates, metal bioavailability was estimated from multiple regression models developed using bioaccumulation data from 26 soils (CU-1210 and the USEPA-NCEA study [38-43]. Also, the ability of soil extraction methods to predict phytoavailable metals were investigated. Additionally, eight selected DoD sites were tested in addition to the three soils used in the swine study. This was necessary to enhance the robustness of the ecological model [38-43] 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 or DoD soil chemical and physical properties were used to predict metal bioavailability to plants and soil invertebrates. Initially, statistical relationships developed for metal availability from a set of twenty-six soils were used to estimate the chemical availability of

metals in DoD soils, based upon total metal levels and soil physical/chemical characteristics. This was followed by extraction of the DoD soils using several soil extraction methods using pore water, dilute calcium nitrate solution, and Mehlich 3 solution. The ability of soil chemical extractants to predict metal bioavailability to plants was determined. Plant and soil invertebrate bioassays were conducted with DoD soils to determine actual toxicity and bioaccumulation, and these results were compared to the model predictions of toxicity and bioaccumulation.

2.2 TECHNOLOGY DEVELOPMENT

Human Health Within SERDP CU-1166, a predictive model, the Soil BioAccessibility Tool (SBAT) [44] 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 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 [20, 36]. Nevertheless, model validation using *in vivo* studies on actual DoD field samples was 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 an *in vitro* bioaccessibility method 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 [19]. 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 [31, 32]. The Ohio State University IVG (OSU-IVG) method has been shown to be correlated with As [37], Pb [45], and Cd [31]. 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 (United States) data 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, at least at mining sites. However, although site-specific bioavailability adjustments have been made at some sites, regulators in the United States remain uncertain that the *in vitro* methods alone can adequately predict soil metal bioavailability in humans.

Ecological 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 [42, 43, 46, 47], 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 believed that it was timely to apply these techniques to DoD site-specific problems. Such an effort would validate bioaccessibility and bioavailability estimates based on *in vitro* methods and soil properties for DoD sites. Close cooperation with regulators

and end users would 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 USEPA-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) that 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 twenty-two 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 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).

2.3 ADVANTAGES AND LIMITATIONS OF THE TECHNOLOGY

The goal of this initiative was to provide field-validated evidence that *in vitro* bioaccessibility methods can serve as predictive indices of toxic metal bioavailability in DoD soils relative to the more costly and time intensive *in vivo* uptake studies. By quantifying the extent that soil properties control metal bioavailability, we have shown that the predictive models developed in our earlier SERDP studies can be used with a reasonable level of confidence to predict site-specific metal bioavailability for DoD soils throughout the United States. We believe that this upfront investment by ESTCP to compare *in vitro* methods with *in vivo* investigations can potentially save DoD significant remedial cost in the long term.

The lack of wide-spread regulatory acceptance of the *in vitro* methods is the largest potential limitation to widespread application. Another potential limitation with using this technology at DoD sites is that there are different types of metal-contaminated sites within the DoD, e.g., small arms firing ranges, paint residues, past pesticide use, and manufacturing/maintenance activities. The bioavailability of a given metal could vary widely between sites, underscoring the ultimate need for site-specific adjustments.

3.0 PERFORMANCE OBJECTIVES

One of the performance objectives was to test the bioavailability screening tools developed in our earlier SERDP studies, which correlate 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) (Table 3-1). Since ingestion is often the primary human risk driver at contaminated sites [1], human risk by ingestion was evaluated rather than dermal pathways. Only three sites were considered for the *in vivo* swine dosing studies due to the experimental cost. The use of *in vitro* ecological models were further verified by comparison with *in vivo* ecological bioassay studies of eleven DoD soils (eleven contaminated, eleven control). At the kickoff workshop, the research strategy was discussed among scientists, regulators, USEPA, 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 [20-22, 36] and *in vitro* techniques to predict metal bioavailability and risk (i.e., ecological, human). Results obtained from methods developed for assessing metal risk-based endpoints for human our earlier SERDP studies were compared with results from well-established standard methods used to determine human risk (USEPA Risk Assessment Guidance for Superfund) and ecological risk (USEPA Ecological Risk Assessment).

The agreement between the measured and the model-predicted bioavailability was quantified with the root mean square error (RMSE)

$$\mathrm{RMSE} = \left[\frac{1}{n_d - n_p} \sum_{i=1}^{n_d} \left(B_i - \hat{B}_i \right)^2 \right]^{1/2}$$

where n_d is the numbers of data points, n_p is the number of adjustable parameters (zero when used in a purely predictive manner as in this project), i is an index, and B_i and $\hat{B}_i$ are the *i*-th measured and predicted bioavailability, respectively. The RMSE, the square root of the mean squared difference between measured and predicted values, is a measure of the average error between the predicted and measured values. Our goal was for our models to produce RMSE $\leq$ 25%.

Overall performance objectives are shown in Table 3.1. A discussion of these performance objectives as well as supporting performance objectives can be found in Appendices A-F.

Table 3-1. Performance Objectives

Performance Objective	Data Requirements	Success Criteria	Results
Quantitative Performance Objectives			
Ecological Bioassays vs. *in vitro* protocol	Agreement between the measured and empirical model-predicted bioavailability	Significant multiple regression correlation criteria and/or Root Mean Square Error ≤25%	Soil Inverterbrate-Yes[1] Plants-Yes
	Toxicity and bioaccumulation consistent with speciation	Predictive ability of model confirmed	Soil invertebrates – Mixed[2] Plants-Yes
	Estimated risk	Bioassay Hazard Quotients (HQs) and *in vitro* HQs	Soil Inverterbrate-Mixed[3] Plants-Yes
Swine bioassays vs. *in vitro* protocol	Agreement between the measured and empirical model-predicted bioavailability	Root Mean Square Error ≤25%	Pb and As-Yes Cr-No
	Toxicity and bioaccumulation consistent with speciation	Predictive ability of model confirmed	Pb and As-Yes Cr-No
Qualitative Performance Objectives			
Technology Transfer	End-user involvement	Kick-off meeting held and comments of end-users incorporated in research design.	Yes
Ecological bioavailability protocol	Protocol is applicable for evaluating Pb, Cd, Cr, As in soil	Validated statistical model	Soil invertebrates – Mixed Plants-Yes
	End-user acceptance	Results published in peer-reviewed journals.	Pending
Human bioavailability protocol	Protocol is applicable for evaluating Pb, Cr, As in soil	Validated statistical model	Pb and As-Yes Cr-No
	End-user acceptance	Results published in peer-reviewed journals.	Pending

1. Many significant multiple regressions, some acceptable RMSE, not applicable to essential elements, Cu and Zn, that were not at toxic levels and are regulated by the organisms.
2. Speciation did not significantly increase the predictive capacity of bioaccumulation models.
3. Bioaccumulation of metals only, so no HQs; comparison to US EPA EcoSSLs did not reveal trends.

4.0 SITE DESCRIPTION

4.1 SITE LOCATION AND HISTORY

The following three sites were selected for the swine dosing studies:

Portsmouth Naval Shipyard
McClellan Air Force Base
Deseret Chemical Depot

The following sites were used for the ecological bioavailability and *in vitro* bioaccessibility studies:

McClellan Air Force Base
Hill Air Force Base
Marine Corp Air Station Cherry Point
Travis Air Force Base
Portsmouth Naval Shipyard
Naval Support Activity Mechanicsburg
Concord Naval Weapons Site
Naval Complex, Pearl Harbor, HI
Deseret Chemical Depot
Former Sugarcane Fields
Oak Ridge National Laboratory

Hill Air Force Base Hill Air Force Base is located in Ogden, UT. The contaminated area was historically used as sludge drying beds during the treatment of water for potable use.

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.

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.

Naval Support Activity, Mechanicsburg The Naval Support Activity is located in Mechanicsburg, PA. Soil from Site 11, which has functioned as a lead ingot stockpile location from the early 1950s until recent years, is heavily contaminated with lead.

Portsmouth Naval Shipyard The Portsmouth Naval Shipyard is located in Kittery, Maine. Soils from Site 6 are impacted by particulate deposition from historical land use as a temporary storage area of a variety of materials, including lead battery cell plates.

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.

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 were selected.

Concord Naval Weapons Station The Concord Naval Weapons Site is located in Concord, CA. Soils from a site that contains elevated As from pesticide applications were utilized.

Former Sugar Cane Fields Former sugar cane fields located in Hilo on the big island of Hawaii contain high concentrations of As. The use of As-based pesticides during the 1920-1940s is believed to be the source of the contaminant.

Naval Complex, Pearl Harbor Soils located at the Pearl City Fuel Annex contain high levels of As and Pb. The source of As at this site is thought to be historic pesticide or rodenticide use.

Firing Range, Oak Ridge National Laboratory Soils located on the small arms firing range contain elevated concentrations of lead.

4.2 SITE GEOLOGY/HYDROGEOLOGY

The soils types and soil physical and chemical properties are shown in Table 4-1 and 4-2. Please see Appendices A and F for more detailed soil characterization.

Table 4-1 Test Sites and Soil Types

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 Complex, Pearl Harbor	Honolulu, HI	Mollisol
Former Sugar Cane fields	Hilo, HI	Andisol
ORNL Firing Range	Oak Ridge, TN	Ultisol

Table 4-2. Select Soil Properties of contaminated soil (C) and reference (i.e. uncontaminated) soil (R). All soils are < 2 mm fraction.

		Cherry Pt		Concord		Deseret		Hill	Hilo		McCllelan	
	units	C	R	C	R	C	R	C	C	R	C	R
Soil pH, water		5.50	7.43	6.67	6.34	9.28	7.84	7.22	5.88	4.71	4.31	6.66
Soil pH, $CaCl_2$		5.01	6.96	6.15	5.89	7.49	6.91	7.08	5.74	4.73	4.32	6.08
EC	dS/m	0.892	0.353	0.111	0.189	0.544	0.480	0.989	0.820	1.53	0.276	0.119
Alox	mg/kg	6061	909	1522	1672	786	1207	1175	21344	5917	2175	487
Feox	mg/kg	7506	797	3664	4519	863	681	956	25678	7535	4805	804
Mnox	mg/kg	32.2	<25	641	659	313	381	333	484	85.7	<25	125
Org C	%	3.71	0.758	3.13	2.17	0.645	0.792	1.50	7.77	5.69	4.36	0.360
Total C	%	4.54	1.94	3.04	2.13	2.32	1.52	2.66	8.44	5.50	4.66	0.42
CEC	$cmol_c/kg$	9.14	3.94	27.9	27.7	8.37	13.4	11.0	17.1	10.1	13.4	12.0
Sand	%	79.7	80.0	18.4	19.9	36.6	27.5	52.3	61.1	72.3	25.7	59.9
Silt	%	13.5	12.2	40.9	44.3	54.7	53.2	31.3	25.3	17.8	50.2	25.2
Clay	%	6.8	7.8	40.7	35.8	8.7	19.3	16.4	7.8	2.6	24.1	14.9

Soil pH (water): pH measured in 1:1 soil:deionized water suspension
Soil pH ($CaCl_2$): pH measured in 1:2 soil: 0.01 M $CaCl_2$ suspension
EC: electrical conductivity measured in 1:1 soil:deionized water suspension
Alox, Feox, Mnox: reactive oxide fraction measured using acid ammonium oxalate extraction

Table 4-2 (continued).

		Mechanicsburg		ORNL		Pearl City		Portsmouth		Travis	
		C	R	C	R	C	R	C	R	C	**R**
Soil pH, water		8.04	7.46	4.1	3.81	7.34	7.65	6.2	6.2	7.04	6.02
Soil pH, $CaCl_2$		7.04	7.12	3.53	3.14	7.28	7.47	6.04	5.72	6.46	5.63
EC	dS/m	0.209	0.291	0.184	0.152	0.995	0.929	0.089	0.183	0.247	0.261
Alox	mg/kg	1615	2050	388	851	3502	2046	3764	4149	799	885
Feox	mg/kg	1407	2492	507	798	44900	1977	5758	2682	3088	4569
Mnox	mg/kg	290	944	27.4	<25	1014	492	124	70.1	405	547
Org C	%	0.640	1.22	0.326	0.222	2.34	0.29	1.64	1.44	1.09	1.32
Total C	%	4.49	1.43	0.38	0.17	3.33	2.01	2.57	1.72	1.22	1.39
CEC	$cmol_c/kg$	9.74	9.58	2.79	7.90	25.9	39.4	2.73	2.68	17.3	10.8
Sand	%	29.9	9.90	45.7	9.0	48.7	54.7	89.0	86.5	47.6	29.9
Silt	%	36.6	50.0	36.5	33.4	29.2	26.9	8.5	9.6	26.3	44.3
Clay	%	33.5	40.1	17.8	57.6	22.1	18.4	2.5	3.9	26.1	25.8

Soil pH (water): pH measured in 1:1 soil:deionized water suspension
Soil pH ($CaCl_2$): pH measured in 1:2 soil: 0.01 M $CaCl_2$ suspension
EC: electrical conductivity measured in 1:1 soil:deionized water suspension
Alox, Feox, Mnox: reactive oxide fraction measured using acid ammonium oxalate extraction

Table 4-3. Select Properties of ESTCP Contaminated soils (C) and Reference (uncontaminated) soils (R). All soils are < 250 µm fraction.

		Cherry Pt		Concord		Deseret		Hill	Hilo		McCllelan	
	units	**C**	**R**	**C**	**R**	**C**	**R**	**C**	**C**	**R**	**C**	**R**
Alox	mg/kg	10897	988	1746	1765	747	1251	1548	28692	none	3415	650
Feox	mg/kg	13216	821	4207	4752	1037	763	1358	30671	none	6248	1482
Mnox	mg/kg	54.3	<25	634	621	293	224	413	635	none	<25	125
Org C	%	5.94	0.97	2.59	1.79	0.48	0.73	2.02	9.42	none	4.56	0.52
Total C	%	7.71	1.62	3.18	2.11	2.00	1.33	3.31	10.6	none	4.42	0.548
CBD Fe	mg/kg	10824	---	12749	---	6044	---	4530	29606	---	6030	---

Alox, Feox, Mnox: reactive oxide fraction measured using acid ammonium oxalate extraction
CBD Fe: citrate-bicarbonate-dithionite extractable Fe

4.3 CONTAMINANT DISTRIBUTION

The contaminant distributions within the soils are shown in Table 4-3. Please see Appendices A and F for more detailed soil characterization.

Table 4-4 Metal concentrations in contaminated (C) and reference (R) soils (dry weight basis)

Soil		Cd mg/kg	Pb mg/kg	Cr mg/kg	Ni mg/kg	As mg/kg	Zn mg/kg	Cu mg/kg
Mechanicsburg	**R**	<1.0	33	56	36	17	97	19
	C	<1.0	120	39	29	15	98	25
Cherry Point	**R**	<1.0	17	13	3.5	1.7	32	<1.0
	C	19	114	876	78	6.9	486	167
Travis	**R**	<1.0	17	43	23	8.1	70	19
	C	<1.0	2034	42	29	11	225	148
Concord	**R**	<1.0	16	79	98	7.8	101	50
	C	<1.0	22	77	92	220	112	54
McCllelan	**R**	0.7	15	126	60	6.1	32	14
	C	22	193	699	87	9.9	448	241
Point Loma	**R**	<1.0	8.7	23	6.8	3.7	61	11
Portsmouth	**R**	<1.0	48	14	8.4	10	60	12
	C	1.1	3069	11	62	11	500	185
Deseret	**R**	<1.0	20	27	17	11	83	15
	C	<1.0	19	24	16	438	85	13
ORNL	**R**	<1.0	12	48	15	14	85	14
	C	<1.0	966	16	4.2	5.0	30	65
Pearl	**R**	1.4	13	233	182	4.1	133	110
	C	3.6	1466	185	196	619	1804	423
Hilo	**R**	1.3	153	120	561	22	282	69
	C	5.9	2134	140	417	660	1889	224

Point Loma soil was uncontaminated.

5.0 TEST DESIGN

5.1 CONCEPTUAL EXPERIMENTAL DESIGN

Soil collection and characterization A portable field X-ray fluorimeter was used to identify target metal concentrations in the collection areas prior to collecting 10 to 12 buckets of soil, each containing 25 kg. Since the metal concentration in soil can vary greatly between and within the sample buckets, all soil collected from each site was mixed to produce a homogenous composite sample to be used for all investigations. Although the homogenization procedure described below may have impacted the oxidation state of the target metals, it ensures that the characteristics observed using synchrotron X-ray techniques are the same as those used for *in vitro*, ecological bioaccessibility, and swine-dosing bioavailability tests. The disadvantage is that there may be some differences in soil characteristics compared with the soil in its local environment. These differences are expected to be minimal in that the soil samples were collected from the surface, and therefore already exposed to an oxidizing atmosphere; none of the soils were from wetlands or other reducing environments. The homogenization procedure is not expected to affect distribution of target metals on soil particles, so X-ray fluorescence microprobe mapping provides an accurate record of elemental associations that supports interpretation of the metal distribution on soil particles.

Soils were air dried prior to homogenization in a heavy duty electric powered mixer with a 9 ft^3 plastic drum over six hours. A large cement mixer was modified to allow simultaneous homogenization and sieving (<2 mm) of large amounts (250+ kg) of contaminated soil by using a steel cone attachment fitted with a 2-mm sieve. The steel cone attachment, custom built for the cement mixer, allows (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. The mixer is equipped with a dust trap to avoid air dispersion of the material. For soils where clumping is an issue, hardened ceramic balls were placed in the mixer with the soil in order to enhance aggregate breakup without grinding the soil, which could alter its native particle size distribution. Soils were next sieved to <2 mm with a subsample sieved to <270 um. The <2mm samples were used in the *in vitro* and *in vivo* plant and earthworm model studies whereas the <270 um samples were used in the *in vitro* and *in vivo* swine model studies and for synchrotron X-ray interrogation. To verify that soil samples are homogeneous, numerous subsamples (10 or more) were acid digested using USEPA method 3051a followed by Cr, As, Cd, and Pb analysis. Soils are archived at Ohio State University where *in vitro* and *in vivo* plant and earthworm model investigations were performed.

Select, yet the most pertinent (based on our previous SERDP-funded research), soil chemical and physical properties were quantified using established analytical procedures. The soil properties were measured on all soils are total metal analysis, total organic and inorganic carbon, amorphous and crystalline Fe-oxide content, Mn-oxide content, particle size analysis (sand, silt, clay content), cation exchange capacity (CEC) and soil pH. This information was used in the statistical models to 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 were quantified with a variety of high-resolution surface spectroscopy techniques. X-ray absorption spectra on bulk samples of the <270 µm size fraction were collected at the Stanford Synchrotron Radiation Laboratory (SSRL) in May 2007 (beam line 2-3; Pb and As analysis) and January 2008 (beam line 11-2, Cr analysis). In both cases a Si(220) monochrometer was used to control the energy of the incident beam, calibrated by metal foils or known reference compounds. Data were collected in fluorescence geometry using a 13- or 30-element germanium solid-state detector (BL 2-3 and BL 11-2, respectively). Samples were ground to fine powder and mounted in teflon sample holders sealed with Kapton tape. Between three and 25 scans were collected on each sample.

Data files were imported into the Samview module of the X-ray absorption spectroscopy processing program Sixpack [48] where monochrometer energy calibration was verified or corrected, and individual scans were examined to ensure that each solid-state detector channel had successfully recorded data. Noise recorded in malfunctioning channels was eliminated before averaging scans. The averaged data was then imported into the program Athena [49]. The near-edge portions of the X-ray absorption near-edge structure (XANES) were examined and first derivatives calculated to determine the energy position of the absorption edge. Next, spectral backgrounds were subtracted and the extended fine-structure portions of the spectra (EXAFS) were expressed in K-space ($Å^{-1}$), where K represents the momentum wave-vector. The resulting $\chi(K)$ files were imported into the program Artemis [49] for analysis of the EXAFS.

Least squares fitting algorithms of the EXAFS function were applied to determine nearest and second-nearest neighbor atomic identities, coordination numbers, and distances from the target metal(loid), using theoretical phase and amplitude functions generated by the program FEFF[50]. First-shell coordination environments were identified, informed by the oxidation state information obtained from XANES. The energy offset parameter E_0 was constrained to be the same for all atoms included in the fit. Wave amplitudes corresponding to the coordination number around the target metal were allowed to vary, as were the interatomic distances. The Debye Waller factor, a parameter that varies as a function of static and vibrational atomic disorder [51], was held constant and constrained to be the same for all atoms in the first shell.

For samples containing As, theoretical multiple scattering paths within As tetrahedral were generated from the mineral structure of scorodite ($FeAsO_4 \bullet 2H_2O$). Phase and amplitude functions corresponding to 3-leg paths of the form As-O-O-As (12 paths) and 4-leg paths of the form As-O-As-O-As (16 paths) were generated in Artemis using the IFEFFIT module. To test whether including multiple scattering contributions improved the fit for As K edge EXAFS, the multiple scattering paths were applied with distance and degeneracy parameters fixed to their original values, and the Debye Waller factor constrained to 0.001 [52].

Following first-shell fits, second-shell fits were performed if peaks in Fourier transforms of the EXAFS data representing interatomic distances (uncorrected for phase shift) provided evidence of more distal backscatterers. Potential identities of second-shell backscatterers were informed by the soil chemical analyses and, when available, results of the X-ray fluorescence microprobe mapping performed at APS (described below).

Microbeam X-ray techniques were performed at the Advanced Photon Source (Argonne National Laboratories) bending magnet beam line 20-BM, operated by the Pacific Northwest Consortium Collaborative Access Team (PNC-CAT), in February 2008. Microbeam X-ray fluorescence (XRF) spectroscopy was used to assess spatial distributions of the target elements on the soil particle surfaces. Soil grains were dispersed onto Kapton tape, covered with a second layer of tape, and placed at a 45˚ angle to the incident beam. An initial location on the sample with multiple, well-spread out particles was chosen with the aid of a video camera. A constant focal position for all samples was maintained by moving each sample on a motorized rail until it was in focus by a second camera with a viewer outside the hutch. Two-dimensional fluorescence microprobe maps were then acquired to ascertain the distribution of target elements in relation to soil particles.

The images were processed on-site using the PNC-CAT software 2d Scan Plot version 2. Individual element distributions (in relation to the dead-time corrected incident X-ray intensity), and mapped representations of element ratios, were compared visually to detect the areas highest in the target metals to choose locations for collecting microbeam X-ray absorption spectra. In cases where the metal association with other elements was not uniform, more than one spot was chosen. For preparation of X-ray fluorescence map figures, target elements mapped in 2d Scan Plot were saved as jpeg images. These images were imported into the SMAK image processing software package [53], where intensity was re-plotted on a log scale to better visualize the distribution of elements, and converted to greyscale.

X-ray energy at the beamline was controlled using an N_2-cooled Si(111) double-crystal monochrometer. The beam energy was calibrated using an Au foil placed below the beam path and above a caldiode solid-state detector. Part of the beam was deflected downward to excite the foil, and the absorption reading at the caldiode was normalized to the counts in an ion chamber upstream. The beam was focused by means of a 100 mm K-B mirror to approximately 5 μm.

Locations for X-ray absorption spectra (XAS) were chosen from the XRF microbeam maps, described above. At locations where the target metal(loid) appeared elevated on the map, a multichannel analyzer was employed to measure fluorescent X-ray intensity over a range of energies. Elements (atomic number Z>15) present at that location were identified by the energies of the emission peaks. At selected locations, XANES data were collected using a multielement Ge solid-state detector. Each detector element was set up to record the fluorescence intensity within the emission energy range corresponding to a target metal. Twelve detector elements were utilized for each of the contaminants (Cr, As, Pb), and their signals were summed to obtain the relevant XANES spectrum. The summed data was processed using the software Athena, as described above for the spectra collected at SSRL.

These data provided an improved conceptual understanding of the molecular-level speciation of Pb, Cd, Cr, and As in the soils, and how the molecular speciation influenced the resulting bioaccessibility. The metal speciation results were used to confirmed macroscopic observations of metal bioavailability for both the *in vitro* and *in vivo* methods.

More specifically, the geometric relationship between a metal and its nearest neighboring atoms were interpreted to indicate whether it was adsorbed onto a mineral surface or part of the internal

mineral structure. This was accomplished by evaluating the identities, distances, and coordination numbers to atoms closely neighboring the metal by comparison of the EXAFS with theoretical phase and amplitude functions generated from postulated coordination chemistry scenarios.

A metal that is structurally incorporated into the mineral structure likely will not become bioavailable unless the mineral decomposes, whereas a metal that is adsorbed to a particle surface may be mobilized into the dissolved phase if chemical conditions change. For example, introduction of competing ions that can displace the adsorbed metal, a pH change, or a change in redox conditions can destabilize the metal-particle association. An outer-sphere association (electrostatic attraction) is generally less stable than an inner-sphere association (direct chemical bond).

Both As and Cr exhibit multiple potential oxidation states that influence their toxicity. Dissolved As(III) is typically more toxic than As(V) and also has a lower affinity with mineral surfaces. For Cr, it is the oxidized form (Cr(VI)) that is more mobile and toxic than Cr(III). The oxidation states were easily distinguished from the XANES by the energy at which radiation was absorbed by an inner-shell electron. The absorption edge shifts to higher energy for oxidized species, and a characteristic pre-edge peak is associated with Cr(VI) [54]. The edge position and shape was also compared with that of mineral reference standards.

***In vitro* investigations to assess human health risks**

OSU IVG: Ohio State University *In-vitro* Gastrointestinal Method The OSU-IVG (described in more detail in Appendix E) is a rapid, inexpensive and reliable screening tool for determining the potential bioavailability (i.e., bioaccessibility) of soil contaminants including As [37]. 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. Contaminant bioaccessibility is expressed as a percentage of the total contaminant content of the 3test sample. Two bioaccessibility values are determined by the OSU IVG: gastric and insestinal. For gastric bioaccessibility, 150 mL of gastric solution (0.10 M ACS grade NaCl and 1% porcine pepsin, Sigma Aldrich, St. Louis, MO, Cat. No. P7000) is heated in an open extraction vessel, in a 37º C hot water bath. When the solution reaches 37º C, the pH is adjusted to 1.8 ± 0.1 using 6 M trace metal grade HCl followed by addition of the soil (1 g, < 250 µm). The sample is thoroughly mixed with the solution to maintain a homogenous suspension. The pH is continuously monitored and adjusted to 1.8 ± 0.1 for 1 h. After 1 h, 10 mL of gastric solution is removed for analysis. The extract is immediately centrifuged (11,160 g for 15 min) and then filtered (0.45 µm). Filtered extracts are refrigerated (4º C) for preservation prior to analysis. Intestinal bioaccessibility is determined from the gastric sample. The gastric sample is adjusted to 6.5 ± 0.1 using dropwise additions of a saturated NaOH solution followed by the addition of 0.563 g of porcine bile extract (Cat. No. B8631) and 0.563 g of porcine pancreatin (Cat. No. P1750 Sigma Aldrich, St. Louis, MO). The pH is continuously monitored and adjusted to 6.5 ± 0.1. After 2 h of mixing, 10 mL of intestinal solution is collected for analysis. The extract is immediately centrifuged (11,160 g for 15 min) and then filtered (0.45 µm). Filtered extracts are refrigerated (4º C) for preservation prior to analysis. Three replicates analyses of soil test samples are performed to determine bioaccessible contaminants by OSU IVG. Extracts

are analyzed using inductively coupled plasma atomic emission spectroscopy (ICP-AES) or Hg-ICP-AES. Calibration standards, check standards, and dilutions are prepared in 0.1 M ACS grade NaCl, and 0.5 M trace metal grade HCl matrix. A blank and a laboratory control sample are included with each batch of *in vitro* sample extractions for quality control.

PBET – Physiologically Based Extraction Test The physiologically based extraction test (PBET) developed by Ruby et al. [5, 32] was 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. [21, 22] additional soil property-driven models were 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 [20, 36]. 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 and 2.5 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 three study soils was calculated using soil property-driven models developed from our earlier studies. Calculated bioaccessibility values were compared with measured bioaccessibility values using *in vitro* gastrointestinal methods for study soils.

***In vitro* investigations to assess ecological risks**

Soil extraction methods For ecological risk estimates, metal bioavailability was estimated from multiple regression and path analysis models developed using toxicity and bioaccumulation data from 26 soils (CU-1210; previous USEPA-NCEA project). Additionally, 12 selected DoD sites (24 soils) from CU-1166 were tested in addition to the three soils used in the *in vivo* swine test. This was 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 were 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 were used to estimate the chemical availability of metals in DoD soils, based upon total metal levels and soil physical/chemical characteristics. This was followed by extraction of the DoD soils using extraction with several soil chemical extraction methods (e.g., pore water, dilute calcium nitrate and Mehlich 3 solution) [46, 47] to actually measure the chemical availability of metals in DoD soils. These measurements were compared to predicted chemical availability estimated by the models to determine the ability of the models to predict metal availability. The statistical models were used to predict the toxicity of the DoD soils to earthworms and plants, assuming additivity of the toxicity of individual metals. Although the various metals in a potential mixture have different modes of toxic action, it is difficult to make any other assumption than additivity of toxicity. However, we attempted to estimate Toxic Units contributed by each metal to get an estimate of potential toxicity. Bioassays were conducted with DoD soils to determine actual toxicity and these results were compared to the model predictions. Comparison of the actual toxicity from bioassays with predicted toxicity from *in vitro* models was used to quantify the ability of *in vitro* models to predict actual ecotoxicity in

field DoD soils. This served as 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*, were conducted according to Dayton et al. [38-42] with contaminated soils from DoD to provide plant risk-based endpoints of germination, dry matter growth, and tissue metal concentrations. Metal uptake was monitored in both plant species weekly until a steady state was reached, prior to plant bioassays being performed.

Soil Invertebrate Metal bioavailability and ecotoxicity in contaminated soils collected from DoD sites was assessed using soil invertebrate bioassays with earthworms (*Eisenia fetida*), potworms (*Enchytraeus crypticus*), and collembola (*Folsomia candida*) according to standard protocols [55, 56]. Bioassay endpoints included mortality, reproduction, and internal concentration of metals (bioaccumulation).

Swine Metal bioaccessibility calculated by CU-1166 *in vitro* methods using DoD soils were 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.* [57] 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 Pb acetate. The model has also been used successfully to assess the bioavailability of Cd and As.

A study using juvenile swine as test animals was performed to measure the gastrointestinal absorption of Pb from a sample collected from the Portsmouth Naval Shipyard. The test material contained a Pb concentration of 4113 μg/g. The relative bioavailability of Pb in the sample was assessed by comparing the absorption of Pb from the test material to that of a reference material (Pb acetate). Groups of five swine were given oral doses of Pb acetate or test material twice a day for 14 days. The amount of Pb absorbed by each animal was evaluated by measuring the amount of Pb in the blood (measured on days 0, 3, 7, 9, 12, and 15) and the amount of Pb in bone (measured on day 15 at study termination). The amount of Pb present in blood or bone of

animals exposed to test material was compared to that for animals exposed to Pb acetate, and the results were expressed as relative bioavailability (RBA)

A study using juvenile swine as test animals was performed to measure the gastrointestinal absorption of As from a soil sample taken in the vicinity of the Deseret Chemical Depot. The soil sample contained an As concentration of 521 ug/g. The relative bioavailability of As was assessed by comparing the absorption of As from the test material to that of a reference material (sodium arsenate). Groups of five swine were given oral doses of sodium arsenate or the test materials twice a day for 14 days; a group of three non-treated swine served as a control. The amount of As absorbed by each animal was evaluated by measuring the amount of As excreted in the urine (collected over 48-hour periods beginning on days 6, 9, and 12). The urinary excretion fraction (UEF) (the ratio of the amount excreted per 48 hours divided by the dose given per 48 hours) was calculated for both the test soil and sodium arsenate using linear regression analysis. The relative bioavailability (RBA) of As in the test soil compared to that in sodium arsenate was calculated as follows:

$$RBA = \frac{UEF\ (test\ soil)}{UEF(sodium\ arsentae)}$$

A study using juvenile swine as test animals was performed to measure the gastrointestinal absorption of chromium from a soil sample taken in the vicinity of McClellan Air Force Base The soil sample contained a chromium concentration of 593 ug/g. The relative bioavailability of chromium was assessed by comparing the absorption of chromium from the test material to that of a reference material (chromium chloride). Groups of five swine were given oral doses of chromium chloride or the test materials twice a day for 14 days; a group of three non-treated swine served as a control. The amount of chromium absorbed by each animal was evaluated by measuring the amount of chromium excreted in the urine (collected over 48-hour periods beginning on days 6, 9, and 12). The urinary excretion fraction (UEF) (the ratio of the amount excreted per 48 hours divided by the dose given per 48 hours) was calculated for both the test soil and chromium chloride using linear regression analysis. The relative bioavailability (RBA) of chromium in the test soil compared to that in chromium chloride was calculated as follows:

$$RBA = \frac{UEF\ (test\ soil)}{UEF(chromium\ chloride)}$$

Statistics

The ability of bioaccessibility to predict bioavailability. Measured bioaccessible Pb and As for DOD test soils was inserted into previously published regression equations used to predict Pb bioavailability [58] and to predict As bioavailability [19, 37, 59]. Predicted bioavailability was compare with the measured 90% confidence interval for Pb and As bioavailability from swine dosing trials.

The ability of soil properties to predict bioaccessibility. Measured soil properties for DOD test soils was inserted into previously published regression equations used to predict As bioaccessibility [20, 60] and to predict Cr bioavailability [21, 22]. The root square mean error

for predicted-actual bioaccessibility values was used to determine the ability of soil properties to predict As ro Cr bioaccessibility.

The ability of soil properties to predict metal bioavailability to plants. Statistical models were developed using soil property and plant uptake data from a combined NCEA and SERDP ER-1210 database. Both multiple linear regression (MLR) and ridge regression (RR) models were developed. The developed models were evaluated to determine their ability to predict metal bioavailability to plants for the ESTCP study soils. Both types of models were fit to the data using PROC REG in SAS 9.2. For the MLR models, model selection was not performed; we included all five independent variables (pH, OC, FEAL, CEC, and Total) in each model. For the RR models, an extra penalty term is added to the statistical model. This penalty term can be tuned to adjust the parameter estimates, increasing the bias in the parameter estimates while decreasing the influence of multicollinearity on the parameter estimates. These biased estimates produce a model that does not fit the observed data as closely as the MLR. In all cases, the R^2 for the MLR will be superior to the one obtained from the RR. However, the biased estimates produced by the RR often produce a better predictive model, and that was the central goal of our model development.

When using the RR approach, we chose the value of the tuning parameter by selecting the value that minimizes the PRESS statistic. The PRESS statistic is calculated by removing each observation, in turn, from the dataset; fitting the model using the remaining n – 1 observations; using the model fit to obtain a predicted value for the removed observation; and calculating the squared error of prediction for the removed observation. After cycling through each observation in the dataset in this manner, the squared errors of prediction are summed to obtain the final PRESS statistic. The model with the lowest PRESS statistic is declared to have the best predictive ability. PRESS statistics cannot be compared between RR models with different dependent variables, and there isn't a specific value of the PRESS statistic that can be considered adequate for declaring a model to have good predictive ability. However, the PRESS statistic can be used to compare two or more RR models with the same dependent variable.

The ability of soil extraction methods to predict metal bioavailability to plants. Regression models developed using bioaccumulation data from the NCEA study were used to predict contaminant phytoaccumulation in the study soils. Comparison of the actual contaminant phytoaccumulation from bioassays with predicted phytoaccumulation from soil extraction methods were used to quantify the ability of soil extraction models to predict actual phytoaccumulation in field DoD soils.

5.2 BASELINE CHARACTERIZATION

Key observations from the synchrotron X-ray studies are (1) Pb is present as adsorbed divalent ions or as organic complexes, rather than in crystalline compounds, in all of the Pb-rich soil samples; (2) Cr is present as Cr(III), the more stable and less toxic of the two common Cr oxidation states, in all three Cr-rich soil samples; and (3) Arsenic is present in the more stable and less toxic form, As(V), in three of the four As-rich soil samples, but is present as both As(III) and As(V) in the sample from the Naval Complex at Pearl Harbor. Arsenic appears to occur as an adsorbed complex on iron oxides in the Concord and Pearl samples, and as an adsorbed complex on aluminum oxides in the Hilo soil sample. No Pb was found to be bound in

more immobile and less bioaccessible sulfide phases, meaning that most of the Pb-O in the soils can be liberated under acidic conditions (i.e., in the stomach or in the case of percolating acidic soil/groundwater). The finding that Pb is mobilizable in low pH conditions is supported by previous flow-through and leaching experiments performed on the Cherry Point soils [61]. Please see Appendix A for detailed baseline characterization

5.3 TREATABILITY OR LABORATORY STUDY RESULTS

Please see Appendices A through F for detailed study results.

5.4 DESIGN AND LAYOUT OF TECHNOLOGY COMPONENTS

There were no technology components deployed in the field.

5.5 FIELD TESTING

The nominal project schedule is shown in Table 5-1. Investigation-derived wastes (IDW) were disposed of onsite at the individual PI's laboratories. No field equipment was deployed or left in place.

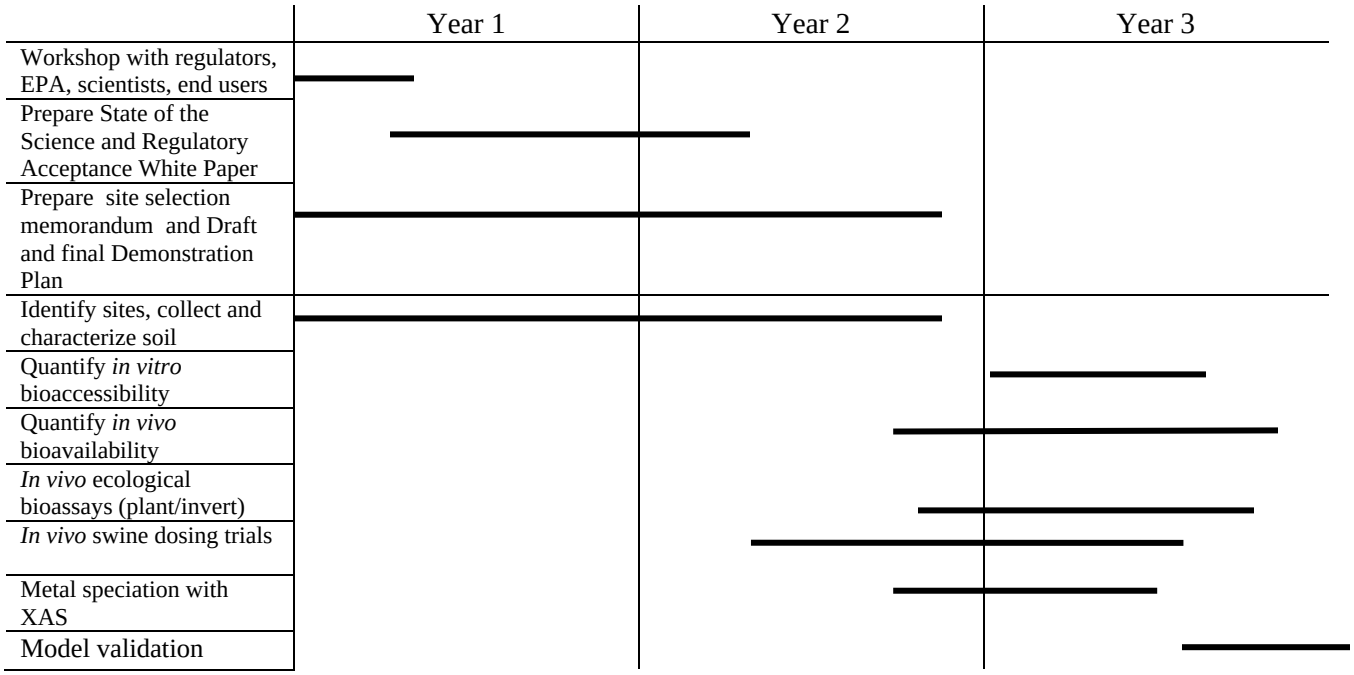
5.6 SAMPLING METHODS

Please see Appendices A through F for detailed sampling methods.

5.7 SAMPLING RESULTS

Please see Appendices A through F for detailed sampling results.

Table 5-1 Project Schedule



The above schedule was based on a nominal project start-date of July 1, 2005. Individual PI start dates varied depending on when funding vehicles were in place.

6.0 PERFORMANCE ASSESSMENT

The technical objectives of the investigation were: (1) To provide validation that the relationships between soil properties and *in vitro* bioaccessibility methods can serve 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 in place of or as justification for more-detailed, site-specific bioavailability (e.g., animal dosing), and (3) to promote the use of *in vitro* methods in human health and ecological risk assessments through the upfront involvement of end-users and regulators and the subsequent dissemination of the results of the study in peer-reviewed journals.

Performance Objectives 1 and 2 involved testing the bioavailability screening tools developed in our earlier SERDP studies, which correlate 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). Only three sites were considered for the *in vivo* swine dosing studies due to the experimental cost. The use of *in vitro* ecological models were further verified by comparison with *in vivo* ecological bioassay studies of eleven DoD soils (eleven contaminated, eleven control).

An important first step was characterizing the molecular-level speciation of the metals in the soil with the use of x-ray absorption spectroscopy. Synchrotron X-ray fluorescence microprobe mapping, microbeam X-ray absorption spectroscopy, and bulk sample X-ray absorption spectroscopy were used to determine the oxidation state and molecular coordination environment of As, Pb, and Cr in eleven study soils with variable soil properties. *In vivo* swine dosing trials to determine metal bioavailability, *in vitro* gastrointestinal studies to determine metal bioaccessibility, soil extraction procedures and soil properties used to predict metal bioavailability to plant and soil invertebrates and ecological bioassay studies were also performed on the same set of soils. Findings from synchrotron X-ray studies indicated that Pb is adsorbed as divalent ions or present as organic complexes, rather than in crystalline compounds. Chromium and As are present in their more stable and less toxic inorganic forms, Cr(III) and As(V), except in soil from the Naval Complex at Pearl Harbor, where both As(III) and As(V) are present. Arsenic is bound to iron oxides in the Concord and Pearl samples, and to aluminum oxides in the Hilo soil sample. Arsenic-bearing soils may require more site-specific approaches to remediation. Lead was not bound in sulfide phases that would be considered stable, meaning that most of the Pb-O in the soils may be liberated under acidic conditions (i.e., in the stomach).

Metal bioaccumulation and toxicity to soil invertebrates (*E. andrei, En. crypticus, F. candida*) were examined in ESTCP metal-contaminated soils (with paired reference site soils) comprising a wide range of physical and chemical characteristics and metal levels. The predictive ability of a number of different models relating soil properties to oligochaete metal bioaccumulation and toxicity as a screening tool for estimating metal bioavailability in soils was examined with the intent of validating some of these models for predicting metal bioaccumulation in soil-dwelling oligochaetes.

Key elements for predicting bioaccumulation of metals by soil invertebrates include metal concentration in the soil, soil physicochemical characteristics, and time. In this study, we examined the application of various models, with varying degrees of success, in predicting the

bioaccumulation of metals by earthworms from ESTCP soils. The models can be divided into three categories: 1) Metals for which a large number of models exist in the literature (e.g., Pb, Cd); 2) Metals for which few models exist in the literature (e.g., Cr, Ni); and, 3) Essential metals (e.g., Cu, Zn).

When applying literature-based metal bioaccumulation models to assess Cd and Pb bioaccumulation by earthworms in metal-contaminated field soils, 98% of the variability in earthworm Cd concentrations could be predicted by a model comprising total soil Cd, organic matter content, and soil pH, while 95% of the variability in earthworm Pb concentrations could be predicted by a model including total soil Pb and soil pH. However, both these models over-predicted metal bioaccumulation (Cd 106%; Pb 272%) so their use in predicting bioaccumulation may be limited. A large portion of the variability in the tissue concentrations of As (90%), Cr (77%), and Ni (88%) could be estimated by their concentrations in soil. Even though just a few bioaccumulation models exist for these metals, the models for As (24.2%) and Cr (13.6%) provided acceptable predictions of metal uptake, while the Ni model severely over-predicted uptake (689%). However, for the essential metals Cu and Zn, total soil concentrations combined with soil properties provided a reasonable prediction of tissue concentrations for Cu (24.7%) but not for Zn (590%). A model relating BAF of Cd to soil properties provided acceptable predictions of Cd BAFs by *En. crypticus* from ESTCP soils (20%) while no relationship was evident between BAFs and observed metal burdens for Pb and Zn.

Models developed relating 0.5 M $Ca(NO_3)_2$-extractable Cd and Pb to earthworm metal residues did not provide a better prediction of Cd and Pb concentrations in earthworms exposed to ESTCP soils than models selected from the literature that predicted earthworm metal concentrations based upon total metal levels and soil physicochemical characteristics. Models incorporating toxicokinetics of metals were only available for Cd and provided reasonable estimates of Cd concentrations in earthworms (19%). These results indicate that there are no models for a specific metal that would provide good predictions of metal bioaccumulation in all soils and situations.

Table 6-1. Summary of the prediction of metal bioaccumulation by earthworms (*Eisenia fetida*) or potworm (*Enchytraeus crypticus*) using soil property or soil extraction data.

Approach	Metal	Model	Summary and ability to predict metal body burdens
Soil properties	As	$\ln As_{ew}=0.9884*\ln As_s - 1.747$ Sample et al. 1998	Based on total As levels; $R^2=0.90$; under predicts 0.8-16-fold, most soils 0.8-3.3 fold; RMSE = 24.2%
	Cd	$\ln Cd_{ew} = 6.018 + 0.787 * \ln Cd_s - 0.106 *OM - 0.402 * pH$ Ma et al. 1983	Based on total Cd, organic matter, pH; $R^2=0.98$; over predicts 3.8-11.3-fold; only eight data points above DL; RMSE = 106%
	Cr	$\log Cr_{ew}=0.69*\log Cr_s -1.05$ Peijnenburg et al. 1999a	Based on total Cr; $R^2=0.73$; under predicts 0.8-7.4-fold; RMSE == 13.6%
	Cu	$\log Cu_{ew}=0.435*\log Cu_s +0.39$ Morgan and Morgan 1988	Based on total Cu; $R^2=0.45$; under predicts 1.3-5.2-fold; RMSE = 24.7%
	Ni	$\log Ni_{ew}=0.98*\log Ni_s +0.67$ Neuhauser et al. 1995	Based on total Ni; $R^2=0.88$; over predicts 11-95-fold; RMSE = 689%
	Pb	$\log Pb_{ew} = 2.65+0.897 *\log Pb_s-3.56*\log pH$ Corp and Morgan 1991	Based on total Pb and pH; $R^2=0.95$; over predicts 0.5-25-fold; RMSE = 272%
	Zn	$\log Zn_{ew}=1.45*\log Zn_s +0.42$ Peijnenburg et al. 1999a	Based on total Zn; $R^2=0.62$; under predicts 1.3-5.2-fold; RMSE = 590%
	Cd	$C_w = 9.32 *e^{-0.008*28} + Cd_s *0.052/0.008*(1-e^{-0.008*28})$ Yu and Lanno 2010	Based on Cherry Point and McLellan soils where total Cd is same as model concentration, one prediction is the same as observed and one is 2-fold higher; with all 8 data points – RMSE = 19%
Calcium Nitrate Extraction	Cd	$\log Cd_{ew} = 0.27*\log Cd_{Ca(NO3)2} + 2.1$ $R^2 = 0.66$,	Only two soils – Cherry Point, McLellan – with total extractable Cd levels; over predicted earthworm Cd 3-6.8-fold; RMSE = 111%
	Pb	$\log Pb_{ew} = 0.32\ Pb_{Ca(NO3)2} + 97$ $R^2 = 0.39$, P=0.008	Only five soils with extractable Pb; over predicted 1.1-3.6-fold; RMSE = 161%
	Zn	$\log Zn_{ew} = 0.02\ Zn_{Ca(NO3)2} + 2.12$, $R^2=0.084$, P=0.21	Only four soils with extractable Zn; under predicted 1.3-2-fold; RMSE = 101%

BAF - Soil properties *En.crypticus*	Cd	log BAF=1.17-0.92*log Clay Peijnenburg et al. 1999b	Only six soils where BAF could be calculated; acceptable under-prediction; RMSE = 21%
	Pb	log BAF=0.35-0.36*pH Peijnenburg et al. 1999b	No relationship
	Zn	log BAF =3.47-0.46 *pH-0.67*log Al_{ox} Peijnenburg et al. 1999b	No relationship

Contaminant phytoaccumulation was also determined from plant bioassays for soils from eleven study sites. For ecological risk estimates, metal phytoavailability was estimated from soil-property driven multiple regression models developed using bioaccumulation data from two previous study studies. A separate approach involved the use of soil extraction methods, used to estimate metal(loid) phytoavailability, to predict contaminant phytoaccumulation. Regression models developed using bioaccumulation data from a previous study sponsored by the National Center for Environmental Assessment were used to predict contaminant phytoaccumulation in the study soils. Comparison of the actual contaminant phytoaccumulation from bioassays with predicted toxicity from *in vitro* models were used to quantify the ability of *in vitro* models to predict actual phytoaccumulation in field DoD soils. This was the basis for validation of the soil property or soil extraction methods for field DoD soils. The predictive capability required by a soil property/soil extraction models depends on the degree of accuracy of contaminant phytoaccumulation determined by the risk assessor. With some exceptions, both methods were able to predict phytoavailability at <35% of the measured contaminant tissue value. In general, soil property models were predictive of tissue As, Cd, and Pb. Exceptions were Deseret for As (ryegrass), Hill for Cd (lettuce), and Portsmouth for Pb. In general, the predictive capability of soil extraction methods was adequate to excellent with the exception of Hill for Cd (lettuce) and Portsmouth for Pb.

The predictive capability of soil property / soil extraction models to predict plant phytoaccumulation is summarized as follows.

Table 6-2. Summary of the Prediction of Contaminant Phytoaccumulation using Soil Property or Soil Extraction Soil Data

Approach	Model or Soil Extraction	Ability to Predict Tissue As		Ability to Predict Tissue Cd		Ability to Predict Tissue Pb	
		Lettuce	Ryegrass	Lettuce	Ryegrass	Lettuce	Ryegrass
Properties	MLR	4† Concord Over , 5x‡	4 Deseret Over, 80x	4 Hill Under, 1.7x	4	7 Portsmouth Over, 1.3x ORNL Under, 1.3x	7 Portsmouth Under, 1.2x
	RR	4	4 Deseret Over, 80x	4 Hill Under, 1.7x	4	7 Portsmouth Over, 2x ORNL Over, 2x	7 Portsmouth Over, 1.7x
Soil Extraction	Pore water	3	3 All sites Over, 2x	3	3 Hill Under, 1.6x	4 Portsmouth Under, 4x	4 Portsmouth Under, 3.3x
	Mehlich 3	4	4 all sites Over, 2x to 5x	NA	NA	NA	NA
	Calcium Nitrate	NA	NA	3 Hill Under, 10x	3 Hill Under, 4x	4 Portsmouth Under, 2x	4 Portsmouth Under, 2.5x

† Number of contaminated soils evaluated.

‡ Over prediction of tissue As concentration by a factor of five

One of the main objectives of the project was to determine the ability of *in vitro* gastrointestinal methods (i.e., bioaccessibility methods) to predict measured contaminant bioavailability in contaminated soils from study sites. Equations used to predict bioavailability from bioaccessibility methods are available for Pb and As.

Relative bioavailable Pb was determined for the Portsmouth soil in our study. The PBET methods (pH 1.5 and 2.5) were able to accurately predict *in vivo* RBA for the Portsmouth soil. The predicted RBA for the PBET method at pH 2.5 was closer to actual *in vivo* RBA than pH 1.5. However both methods predict RBA Pb within the 90% confidence interval. The OSU IVG method IVBA Pb was very close to the *in vivo* RBA Pb. However, information on the ability of the OSU IVG method to predict RBA Pb is very limited whereas in depth validation studies have been conducted for the RBALP (i.e., PBET) method. These results support the use of the PBET method at pH 1.5 and 2.5 to accurately predict *in vivo* RBA Pb. Future validation studies where this approach is expanded from the Portsmouth soil to other DoD soils will increase the confidence of using *in vitro* methods to predict *in vivo* RBA Pb.

Table 6-3. Comparison of measured and predicted RBA Pb for the Portsmouth soil

		Predicted Pb RBA				
Measured Pb RBA, %		PBET pH 1.5		PBET pH 2.5		OSU IVG pH 1.8
Mean	90 % CI†	IVBA, %	RBA, %	IVBA, %	RBA, %	IVBA, %
99	70 - 127	83.3	86.9	80.4	106.2	102.5

† CI = confidence interval

Results from our study show both the OSU IVG and SBRC method were able to predict RBA As in the Desert soil. The predicted RBA As by all methods ranged from 12.2 % to 16.2%, which is comparable to the *in vivo* RBA As of 14%. Further validation studies of these methods for other contaminated soils from different DoD contaminant sources are warranted. A study investigating the relationship between *in vitro* IVBA Cr and *in vivo* RBA Cr has not been reported. Thus, it was not possible to evaluate the ability of bioaccessible Cr to predict *in vivo* RBA Cr. In our study, a novel immature swine dosing model was used to determine the *in vivo* RBA Cr for the McClellan soil. RBA Cr was 107% with a 90% confidence interval ranging from 76% to 169%. *In vitro* IVBA Cr PBET method, used to measure bioaccessible Cr at pH 1.5 and at pH 2.5, was 10.1% and 19.0%, respectively. The *in vitro* IVBA values were much lower than the *in vivo* RBA Cr. Further research is needed before IVBA can be used to predict *in vivo* RBA Cr.

Table ES-4. Comparison of measured and predicted RBA As for the Deseret soil

	Predicted As RBA		
Measured As RBA, %	OSU IVG gastric	OSU IVG intestinal	SBET gastric

Mean	90 % CI†	IVBA, %	RBA, %	IVBA, %	RBA, %	IVBA, %	RBA, %
14	13-15	8.45	15.0	8.47	16.2	10.6	12.2

† CI = confidence interval

In general, all of the in vitro methods predicted in vivo RBA As with 90% confidence.

Studies of the determination of soil properties on *in vivo* bioavailability or *in vitro* bioaccessibility are very limited. To our knowledge, these relationships have not been reported for Pb and limited studies exist for As and Cr. Key soil physical and chemical properties (e.g. particle size, CEC, Fe-oxides, TOC/TIC, pH) were identified as controlling the extent of toxic metals bioaccessibility as measured using the PBET that simulated the digestive system of humans. The bioaccessibility results (*in vitro*) were found to be in excellent agreement with molecular-level metal speciation studies, which confirmed that key soil properties control metal bioavailability.

The ability of soil properties to predict As and Cr bioaccessibility (IVBA) was dependent on the contamination source. In general, IVBA As measured by PBET and OSU IVG could be predicted from measured soil Fe properties including Feox or CBD Fe for soils where arsenical pesticide was the contaminant source. However, soil properties of the Deseret soil, where mine tailing was the contaminant source, was not predictive of the measured IVBA As. This finding suggests arsenic may occur as discrete minerals from the mining operation. It is likely the insoluble As minerals in the mining waste did not appreciably dissolve and react with soil components. Therefore, its chemical speciation and IVBA solubility will depend on the mining waste mineral not soil property.

The ability of soil properties (i.e., clay, organic and inorganic carbon) to predict and Cr bioaccessibility (IVBA) was dependent on the contamination source. Good agreement between the measured IVBA Cr and predicted IVBA Cr was found for Hill and McClellan soils. Poor agreement between the measured IVBA Cr and IVBA Cr predicted by soil properties was found for the Cherry Point soil. Differences in Cr chemical speciation in soil may offer an explanation. Water or wastewater treatment was the contaminant source for the Hill and McClellan soils. Incinerator ash was the contaminant source for the Cherry Point soil.

<u>Summary of Soil Properties to Predict Metal Bioavailability</u>

Soil properties, able to predict metal (bio)availability for several contaminated soils in this study, are summarized in the following table. At a minimum, soil property information needed from a site investigation for all contaminants studied are soil pH, clay content, organic C, inorganic C, reactive Fe and Al (FEAL, Feox and/or CBD Fe). Other properties not studied that will affect ecological endpoints include soil salinity and the presence of other toxicants.

Table 6-5. Summary of Soil Properties to Predict Metal Bioavailability

	Contaminant			

	Pb	As	Cr	Cd
Human Soil Ingestion Bioaccessibility	Not evaluated	Feox and FeCBD	Clay content, total organic C, inorganic C	Not evaluated
Plant accumulation Lettuce	pH, OC, FEAL	pH, OC, FEAL	Not evaluated	pH, OC, FEAL
Plant accumulation Ryegrass	pH, OC, FEAL	pH, OC, FEAL	Not evaluated	pH, OC, FEAL
Soil Invertebrates	pH	Total metal	Total metal	pH, OM

These properties will **not** predict metal bioavailability for all soils. A major finding of this study is the contaminant source and likely speciation greatly affects the ability of soil property to predict metal bioavailability. Metal bioavailability was not able to be predicted for several soils where the contaminant source was unweathered mining waste or discrete inorganic mineral forms such as coal ash. Soil properties should NOT be used to predict contaminant bioavailability in these soils. More research on contaminant source and speciation is needed to determine when soil properties can provide an accurate assessment of metal bioavailability. Currently research is in progress, including research funded by SERDP (i.e., ER-1742) to determine the relationship between As speciation and ability to predict As bioavailability to humans.

Summary of Soil Extraction Methods to Predict Metal Bioavailability

Soil exaction methods, able to predict metal (bio)availability for several contaminated soils in this study, are summarized in the following table. Both PBET and OSU IVG were able to very accurately predict RBA As and Pb but for only for 1 soil each. The number of soils evaluated were very limited because of cost constraints associated with in vivo dosing trails required to measure contaminant RBA. More research is needed to evaluate the ability of these methods to predict RBA Pb and RBA As on other contaminated soils.

Soil pore water was able to predict plant tissue concentration of Pb, As, and Cd. Soil extraction with 0.1 M $Ca(NO_3)_2$ was able to predict cationic metal contaminants(i.e. Pb, Cd) but was not evaluated for anionic As contamination. The ability of simply water or dilute calcium nitrate to predict phytoavailable contaminant suggests high solubility of these contaminants in soils. Thus, it is likely that with 0.1 M $Ca(NO_3)_2$ would have also been a good predictor of plant As. However, two cautions should be heeded. The accuracy of these extraction methods to predict plant tissue contamination was limited to ± 35%. Similarly to metal bioaccessibility results, metal bioavailability was not able to be predicted for several soils where the contaminant source was unweathered mining waste (i.e. Deseret) or discrete inorganic mineral forms such as coal ash (i.e. Cherry Point). Soil extraction methods listed in the summary table should NOT be used to predict contaminant bioavailability in these soils. More research on contaminant source and speciation is needed to determine which soil extraction methods can provide an accurate assessment of metal bioavailability.

Table 6-6. Summary of Soil Extraction Methods to Predict Metal Bioavailability

	Contaminant			
	Pb	As	Cr	Cd
Human Soil Ingestion Bioaccessibility	PBET, pH 1.5 PBET, pH 2.5 OSU IVG	OSU IVG SBET	Not evaluated	Not evaluated
Plant accumulation Lettuce	Pore water 0.1 M $Ca(NO_3)_2$	Pore water Mehlich 3	Not evaluated	Pore water 0.1 M $Ca(NO_3)_2$
Plant accumulation Ryegrass	Pore water 0.1 M $Ca(NO_3)_2$	Pore water Mehlich 3	Not evaluated	Pore water 0.1 M $Ca(NO_3)_2$
Soil Invertebrates	Pore water 0.5 M $Ca(NO_3)_2$	Not evaluated	Not evaluated	Pore water 0.5 M $Ca(NO_3)_2$

As part of Objective 3, immediately upon receiving funding for this endeavor, a two-day workshop was held bringing together state regulators, DoD site end users, EPA officials, and scientists familiar with soil metal bioavailability. The workshop focused on past, current, and future research endeavors investigating soil metal bioavailability methodologies and the possible use of *in vitro* bioaccessibility values in human health risk assessment and policy. At the kickoff workshop, the research strategy was discussed among scientists, regulators, USEPA, and end-users to advance the acceptance of *in vitro* methods in human health and ecological risk assessment and policy. We incorporated the comments of the attendees of the workshop in our research. In addition, also as part of Objective 3, most of the technical objectives, methods, results, discussion, conclusions, and recommendations of this study are detailed in Appendices A-F, which were written as stand-alone manuscripts for submission as peer-reviewed publications. Publication in peer-reviewed journals is needed to disseminate and ultimately facilitate the results of this study by site managers. In addition, publication in peer-reviewed literature is crucial to ensuring regulatory and community understanding and acceptance of the scientific results. The publication of the results of this study are proceeding.

Please see Appendices A through F for a detailed performance assessment.

7.0 COST ASSESSMENT

Cost is an important part of the decision making process when doing bioavailability assessments and making risk management decisions. Questions a project manager must ask themselves include:

- How can I balance the cost of in vivo studies with the desire for reduced uncertainty when making risk assessment conclusions?

- What is the potential return on investment of a bioavailability study? Would adjustments to the RBA at the site lead to higher remedial goals? Would higher remedial goals allow for a reduced remedial footprint and reduced costs?

- Is there existing data that indicates reduced bioavailability of metals contaminants at the site?

- Does the project schedule allow for the time required to complete a bioavailability assessment?

The following sections provide cost information to help remediation professionals begin to answer these questions.

7.1 COST MODEL

The following tables provide simple cost model information. Site-specific bioavailability assessment will require a sampling and analysis plan, sample collection and reporting. These costs are estimated in Table 7-1. *In vitro* study costs are presented next in table 7-2, followed by costs for the in vivo studies demonstrated in this study.

Table 7-1. Cost Model for Bioavailability Assessment: Sample Collection and Reporting

Sample Collection and Reporting

Cost Element	Data Tracked During the Demonstration	Unit type: Number	Unit Cost	Estimated Costs
Sampling and Analysis Plan	• Personnel required and associated labor • Materials	Sampling and Analysis Plan Document: 1	$8,000	$8,000
Sample collection and preparation	• Costs associated with labor and materials tracked	XRF: 1/sample Sample collection: 1/sample Grinding and sieving: 1/sample	$450/sample	$1,350
Reporting	• Costs associated with labor tracked	Report documenting results of entire project: 1	$20,000	$20,000

Assumptions: Approximately 1 acre site with 3 samples. Sample collection and preparation includes necessary grinding and sieving for bioavailability studies.

Table 7-2. Cost Model for Bioavailability Assessment: *In vitro* Bioaccessibility

In Vitro Bioaccessibility

Cost Element	Data Tracked During the Demonstration	Unit type: Number	Unit Cost	Estimated Costs
In Vitro Tests	• Personnel required and associated labor • Analytical laboratory costs • Reporting	Set of three tests	$600 $110	$1,800 $330
Total				$2,130

Assumptions: Each soil sample includes the following three replicate laboratory tests: reference, contaminated, and lab reference. Approximately 1 acre site with 3 samples. Sample collection and preparation includes necessary grinding and sieving for bioavailability studies.

Table 7-3. Cost Model for Bioavailability Assessment: Plant Toxicity Tests

Plant Toxicity Tests

Cost Element	Data Tracked During the Demonstration	Unit type: Number	Unit Cost	Estimated Costs
Plant Toxicity Tests –	• Personnel required and associated labor • Analytical laboratory costs	Lab technician, per unit cost (set of three tests/sample)	$3,000	$9,000
		Metals analysis and soil parameters	$500	$1,500
Waste disposal	Hazardous waste or standard soil disposal			$200
Reporting			$55/hr	$275
Total				$10,975

Assumption: Each soil sample includes the following three toxicity tests: reference, contaminated, and lab reference. Approximately 1 acre site with 3 samples. Sample collection and preparation includes necessary grinding and sieving for bioavailability studies.

Table 7-4. Cost Model for Bioavailability Assessment: Soil Invertebrate Toxicity Tests

Soil Invertebrate Toxicity Tests

Cost Element	Data Tracked During the Demonstration	Unit type: Number	Unit Cost	Estimated Costs
Soil Invertebrate Toxicity Tests – Earthworm, Potworm, and Collembola	• Personnel required and associated labor • Analytical laboratory costs	Lab technician, per unit cost (set of three tests)	$4,000 (Earthworm - $1,200 Enchytraeid - $1,200 Collembola - $1,600)	$12,000
		Metals analysis and soil parameters	$500	$1,500
Waste disposal	Hazardous waste or standard soil disposal			$200
Total				$4,700

Assumption: Each soil sample includes the following three toxicity tests: reference, contaminated, and lab reference. Approximately 1 acre site with 3 samples. Sample collection and preparation includes necessary grinding and sieving for bioavailability studies.

Table 7-5. Cost Model for Bioavailability Assessment: In Vivo Swine Study

In Vivo Swine Study

Cost Element	Data Tracked During the Demonstration	Unit type: Number	Unit Cost	Estimated Costs
Soil In Vivo Swine Study	• Personnel required and associated labor • Analytical laboratory costs	Lab technician, per unit cost	$20,500	$61,500
		Animals/Supplies	$7,500	$22,500
		Laboratory Analysis	$8,500	$25,500
Waste disposal	Hazardous waste or standard soil disposal			$200
Total				$109,700

All cost elements are provided on a per unit basis in the above tables. It is assumed that for the lower cost options such as an *in vitro* study, more samples could be analyzed leading to a broader understanding of RBA at the site.

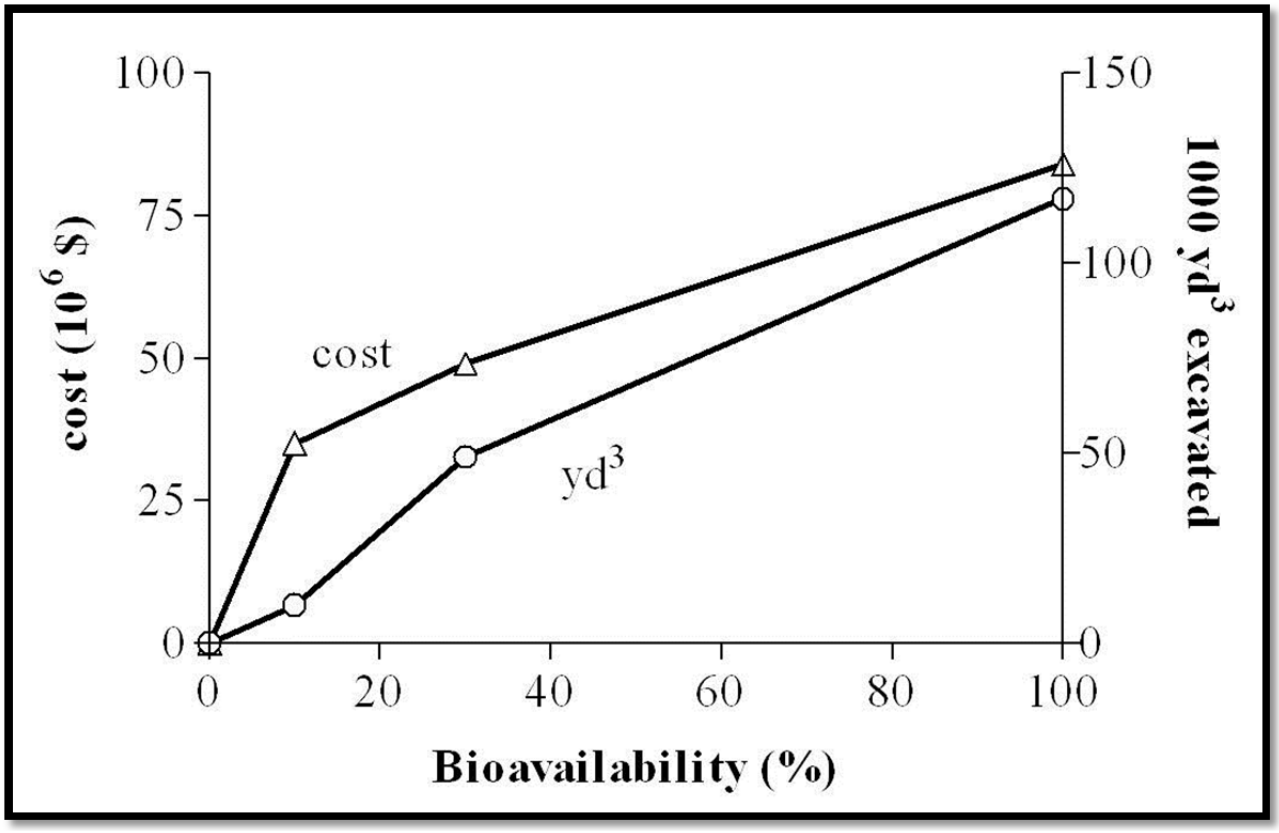
7.2 COST DRIVERS

A site specific bioavailability analysis will vary in cost according to site specific factors that drive how many and what type of analysis is required. These variations in cost are apparent in the tables shown in Section 7.1. A significant driver in the determination of whether or not to pursue an adjustment of RBA is the potential cost avoidance.

Removal is the primary remedial technology available for soils contaminated with the metals studied. Soil removal, transportation and disposal costs for metal-contaminated soils can exceed $1,000 per cubic yard. A significant reduction in remedial footprint can easily justify the expense of in vivo studies at some sites. An example is provided in Table 7-6 and Figure 7-1. This example shows an Hg-contaminated site where the initial remedial goal of 50 mg/kg was based on the assumption that the Hg at the site was the soluble form $HgCl_2$ and was 100% bioavailable. Speciation and bioavailability studies were done and the risk assessment was revised based on the adjusted RBA of 10%. The final remedial goal for the site was 400 mg/kg reflecting an RBA of 10%, significantly reducing the footprint of the remediation area. The reduced footprint correlated with a more than 100,000 yd^3 reduction in soil volume to be removed and avoided almost $50 million in unnecessary remediation costs.

Table 7-6. Example Bioavailability Adjustment Cost/Benefit Analysis

Bioavailability (%)	Remediation Goal (mg Hg/kg soil)	1000 yd^3 excavated	Cost (10^6 1995 $)
100	50	120	81
30	180	54	49
10	400	10	34



Courtesy of Auburn University

Figure 7-1. Example Bioavailability Adjustment Cost/Benefit Analysis

7.3 COST ANALYSIS

Consideration of cost should be part of the decision making process when determining whether bioavailability analyses are appropriate for a given site. Figure 7-2 provides a logical process to control costs related to bioavailability analysis. If metals concentrations in site soils indicate unacceptable risk using the hazard quotient (HQ) approach, a review of soil properties and

current bioavailability assumptions should be done. If soil properties indicate that metals may be less bioavailable than assumed in the risk assessment, the next step towards adjusting the RBA is *in vitro* analysis. Before undertaking *in vitro* analysis consideration should be given to the site specific factors impacting the cost/benefit equation for the site. 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; and c) whether or not existing data support the likelihood of reduced bioavailability.

If *in vitro* studies are completed and do indicate reduced RBA, the degree of certainty related to those adjustments should be documented for the project team. Understanding the results of the *in vitro* study in context can help the project team make the decision to use the results of the *in vitro* study in site risk assessment decisions. The team will also have the information necessary to determine if in vivo studies are required for making RBA adjustment decisions at the site and what the potential benefits of such studies are for the site.

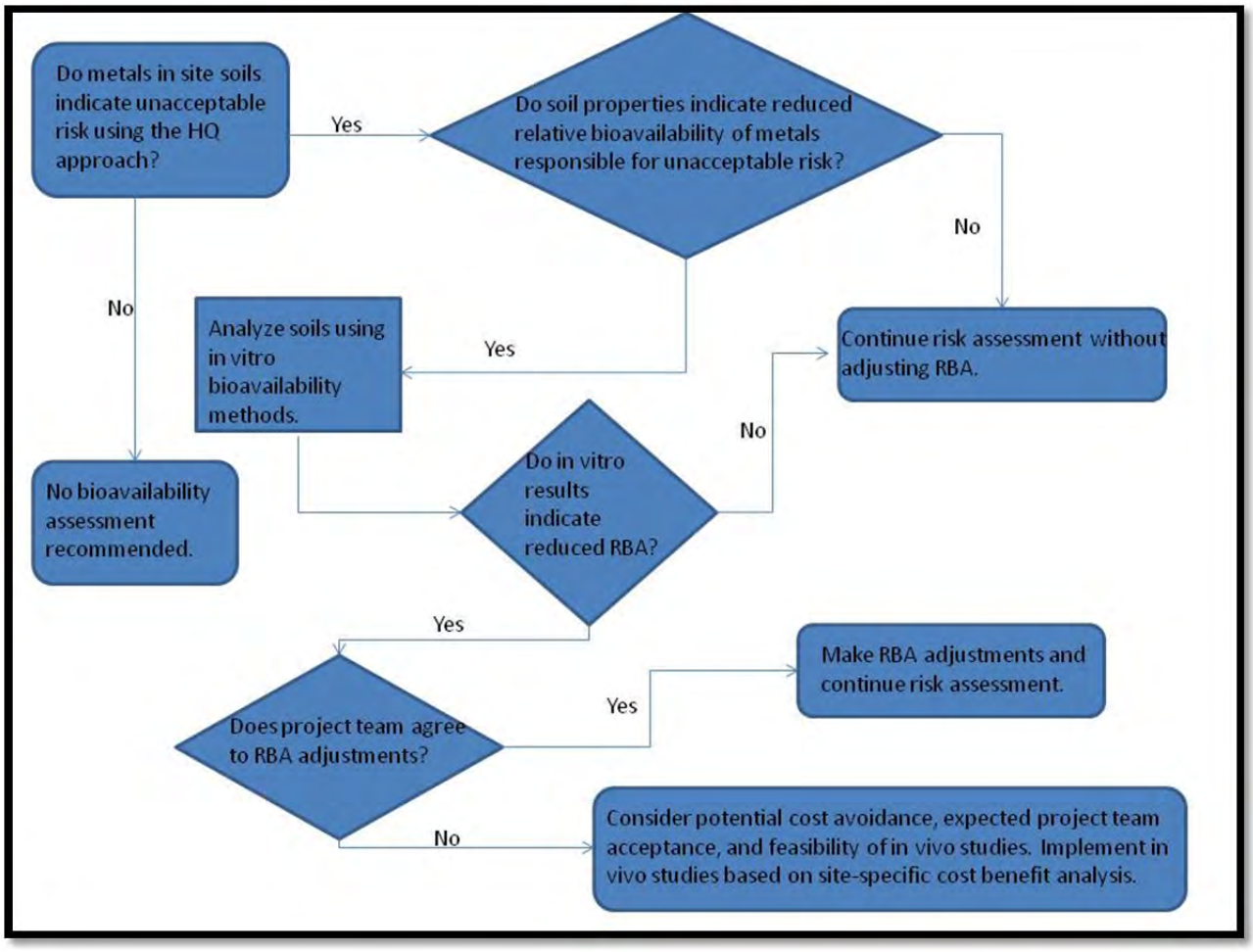


Figure 7-2. Process to Control Costs Related to Bioavailability Analysis

8.0 IMPLEMENTATION ISSUES

Results from this study show *in vitro* gastrointestinal methods can be used to predict bioavailable Pb and As via soil ingestion human exposure pathway. However the number of soils / sites were limited due to project costs. Further validation studies of these methods for other contaminated soils from different contaminant sources are warranted to increase acceptance of these methods in human health risk assessment by regulatory bodies. The ability of soil properties to predict bioavailability was inconsistent and contaminant source dependent. Soil properties were accurate predictors for some soil/contaminant source combinations but not others. Further studies are needed before a more detailed contaminant speciation model can be used to determine which soils may be suitable for estimating metal bioavailability using soil properties.

The predictive capacity afforded by soil property / soil extraction models depends to a large degree on the degree of accuracy of contaminant phytoaccumulation determined by the risk assessor. With some exceptions, both methods were able to predict phytoavailability at < 35% of the measured contaminant tissue value. In general, soil property models were predictive of tissue As, Cd, and Pb. Exceptions were Deseret for As (ryegrass), Hill for Cd (lettuce), and Portsmouth for Pb. In general, the predictive capability of soil extraction methods was adequate to excellent with the exception of Hill for Cd (lettuce) and Portsmouth for Pb.

In assessing the bioavailability and toxicity of metals in the soils of this study, it was apparent that soil invertebrates, particularly oligochaetes, exhibited reduced reproduction relative to the laboratory reference soil, in site reference soils. This was most extreme for earthworms, where reproduction in site reference soils was significantly lower in all but one site reference soil. Enchytraeid reproduction was lower in about half the site reference soils, while there was no effect of site reference soil on reproduction in Collembola. This suggests, that of the three soil invertebrates tests, earthworms are the least relevant since the soil types tested were unsuitable for earthworm reproduction regardless of whether elevated levels of metals were present. The reliance on earthworm testing of soils is widespread but may not be correct for certain soils, since *E. andrei* prefer soils rich in organic matter and reproduce poorly in soils with elevated sand or silt content. Enchytraeids are naturally found in a wider array of soils and can thrive in soils with a higher sand or silt content. Arthropods, such as Collembola, are affected even less by soil properties. The evaluation of metal bioavailability in soils with properties not conducive to testing with earthworms should incorporate tests using other soil invertebrates that are either indigenous to the soils being tested or which reproduce adequately in the test soils (e.g., enchytraeids, collembola, mites). In addition, soils found on DoD sites may be composites of soils that have been manually moved from a number of areas and deposited at sites distant from their origin. Additionally, many of these soils may not be suitable for earthworm inhabitation due to physical compaction, low moisture and organic matter content, and the presence of unmeasured chemicals. In short, the soils may be considered test substrates with unique properties, rather than actual soils, and warrant site-specific testing for chemical bioavailability and toxicity rather than assessment using standard extraction and chemical analysis.

Ecological Soil Screening Levels (EcoSSLs) are conservative screening levels for contaminants in soil that are preferentially based upon toxicity data from soils where soil physical and chemical characteristics provide conditions of maximum chemical bioavailability. At least for

soil invertebrates and plants, there does not appear to be any clear relationship between toxicity and EcoSSL levels for metals in the DoD soils tested, as toxicity was observed in site reference soils as well as those where metal levels did not exceed EcoSSLs. Both field and laboratory research on evaluating the utility of EcoSSL in site specific investigations is warranted.

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. Translating soil properties into field-scale risk assessment adjustments will also require consideration of future site uses that may alter soil characteristics and the subsurface environment and hence, bioavailability. 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.

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. 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. 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. The ultimate publication of the results of this study will significantly help bridge this data gap. Publications and abstracts related to this study are described below in Table 8-1.

PUBLICATIONS

Yu, S., Lanno, R.P. 2010. Uptake kinetics and subcellular compartmentalization of cadmium in acclimated and unacclimated earthworms (Eisenia andrei). Environ. Toxicol. Chem. 29:1568-1574.

ABSTRACTS

Hawkins, Amy, Nick Basta, Elizabeth Dayton, Roman Lanno, Mark Barnett, Phil Jardine, Stan Casteel, and Kaye Savage. 2009. Soil Properties, Metal Bioavailability and Risk Assessment. Partners in Environmental Technology Technical Symposium & Workshop sponsored by Strategic Environmental Research and Development Program (SERDP) and Environmental Security Technology Certification Program (ESTCP), Washington, DC. Dec 1-3, 2009.

Basta, N.T., S. D. Whitacre, E. A. Dayton, P. M. Jardine, J. S. Richey, S.W. Casteel, and A.L. Hawkins. 2011. Predicting Arsenic Bioavailability in Contaminated Soils Using Bioaccessibility or Soil Properties. 11th International Conference for Trace Element Biogeochemistry (ICOBTE), Florence, Italy. July 3-7, 2011.

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Lanno, R.P., Yu, S. 2010. Validation of laboratory models to predict metal bioaccumulation in earthworms using metal-contaminated field soils. SETAC 30th Annual Meeting, Portland, Oregon, Nov. 7-11.

Yu, S., Lanno, RP. 2009. Uptake kinetics and subcellular compartmentalization of cadmium in acclimated and unacclimated earthworms (Eisenia andrei). SETAC 29th Annual Meeting, New Orleans, Louisiana, Nov. 19-23.

Yu, S., Lanno, R.P. 2009. The effect of soil properties on metal bioavailability to earthworms: Validation of laboratory models using metal-contaminated field soils. SETAC 29th Annual Meeting, New Orleans, Louisiana, Nov. 19-23.

Yu, S., Anderson, H., Basta, N, Lanno, R.P. 2008. The effect of soil properties on metal bioavailability to earthworms: Validation of laboratory models using metal-contaminated field soils. SETAC 29th Annual Meeting, Tampa Bay, FL, Nov. 16-20.

Table 8-1. Publications and Abstracts

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APPENDIX A. METAL AND METALLOID SPECIATION IN CONTAMINATED SOILS FROM U.S. MILITARY FACILITIES USING SYNCHROTRON X-RAY TECHNIQUES

Savage, K.S.[1], Covey, A.K.[2], Weinman, B.[3], Lanno, R.P.[4], Casteel, S.W.[5], Basta, N.T.[6], Dayton, E.A.[6], Barnett, M.O.[7], and Hawkins, A[8].

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ABSTRACT

Cleanup strategies at numerous Department of Defense (DoD) facilities awaiting remediation require a better understanding of how the contaminants of interest are bound in the soils to address their long-term fate and toxicity. Synchrotron X-ray fluorescence microprobe mapping, microbeam X-ray absorption spectroscopy, and bulk sample X-ray absorption spectroscopy were used to determine the oxidation state and molecular coordination environment of arsenic, lead, and chromium in ten soils with variable soil properties, selected from contaminated DoD lands for assessment of health and ecological hazards. *In vivo* bioaccessability studies, swine dosing trials, ecological bioassay studies were also performed on the same set of soils. Findings from synchrotron X-ray studies indicate that Pb is adsorbed as divalent ions or present as organic complexes, rather than in crystalline compounds. Cr and As are present in their more stable and less toxic inorganic forms, Cr(III) and As(V), except in soil from the Naval Complex at Pearl Harbor, where both As(III) and As(V) are present. As is bound to iron oxides in the Concord and Pearl samples, and to aluminum oxides in the Hilo soil sample. Arsenic-bearing soils may require more site-specific approaches to remediation. Pb was not bound in sulfide phases that would be considered stable, meaning that most of the Pb-O in the soils may be liberated under acidic conditions (i.e., in the stomach or in the case of percolating acidic soil/groundwater).

INTRODUCTION

Numerous sites on Department of Defense (DoD) lands will require remediation. These sites currently rank somewhere on the order of 8000, with most locations owing their contamination to industrial, commercial, training, and weapons testing activities (Figure A-1). With a majority (~70%) of these sites involving soils contaminated with elevated concentrations of various metalloids (As) and metals (Cr, Cd, Cu), and with the DoD charged with the task of remediating these properties for revitalization and development (Salatas et al., 2004), characterizing the extent to which the elevated concentrations pose a human health and/or ecological problem is an important part of determining the best remediation strategies. Successful measures in the management and restoration ~100,000 tons of contaminated soils from landfill and naval shipyard sites impacting the Chesapeake Bay watershed (Lane et al., 2007) set a good precedent for the DoD's ability to effectively deal with the task of cleaning

these properties, but questions still remain about the extent to which the contaminations actually pose a problem.

Currently, models used for risk assessment by the DoD and EPA are predicated on the assumption that the entire pool of contaminants are bioavailable (Stewart et al., 2003a). While elevated concentrations of metals such as arsenic (used as a former pesticide), chromium (used in plating), and lead (used in former firing ranges) are contaminants in DoD soils, little is known about how the contaminants are actually sequestered, as well as the extent to which they actually pose a health problem: contaminants stably sorbed and/or precipitated in crystalline compounds are typically less available, and hence less toxic, than contaminants sequestered in more labile pools of the soils. Since the soils at each of 8000 sites have different bulk geochemical (e.g. pH, organic matter content, sesquioxides potentially providing sorption sites) and physical properties (i.e., specific surface area, surface charge, porosity), it is unclear how toxic and mobile the contaminants are in the different soil materials (Stewart et al., 2003b). For instance, soils in the arsenic-contaminated Concord, Hilo, and Pearl City locations range from matrices comprised of aridisols, andisols, and mollisols, respectively (ESTCP-ER-0517 2007). The different minerals and organic components comprising the soils in each of these locations (i.e., aridisols < mollisol < andisol % organic carbon) could mean that the geochemical and biological availability of As, Cr, and Pb is site-specific, requiring different remediation strategies (Stewart et al., 2003; Chorover et al., 2004; Salatas et al., 2004). The occurrence of Blackfoot's disease in Taiwan is an example of how differences in bulk material can impact toxicity, with naturally occurring organic matter in the Taiwanese sediments resulting in more toxic forms of arsenic poisoning versus other Asian aquifers with high arsenic and low organic content (Reza et al., 2007). Hence, to evaluate the true context within which a contaminant is toxic, it is not only important to quantify how much of a contaminant is present in the soil, it is equally important to characterize the material with which it is associated, and how it is sequestered in that material.

The redox sensitivity of As and Cr adds another level of complexity to determining the level of toxicity. For instance, As(III) is more toxic than As(V) (Cullen and Reimer, 1989), and Cr(VI) is anionic and more toxic than the cationic Cr(III) (Stewart et al., 2003). While Pb is also redox sensitive, it almost always speciates as Pb(II) at Earth surface conditions (Lollar et al., 2004). Despite the high Pb levels associated with some mine sites, Pb is typically not a problem when high levels of sulfide are also present. Under the vadose type of conditions presumably

present in most of the DoD soils, sulfides are not likely to precipitate at high enough levels to effectively sequester all of the Pb, Cr, and As added to the soil. To determine how each of these contaminants is predominantly situated in each of these settings, X-ray absorption spectroscopy can be used on bulk soil samples to see how the As, Cr, and Pb is bound in the soils. Currently, there is no consensus on how these contaminants are interacting with their host soils, making it difficult to determine how available or how immobile they are in their present conditions.

In this study, synchrotron X-ray techniques including X-ray fluorescence microprobe mapping, microbeam X-ray absorption spectroscopy, and bulk X-ray absorption spectroscopy (XAS) were employed to assess the mechanisms of metal sequestration. The XAS near-edge structure, XANES, was utilized to determine the oxidation state of the target metals and the extended fine structure, EXAFS, was employed to assess their atomic coordination environment in the soil samples. Comparison with theoretical models and with spectra from relevant model compounds enable distinction between adsorption and substitution/coprecipitation modes of metal sequestration. The approach was to first identify particular soil grains within the samples that are elevated in the target elements using X-ray fluorescence, followed by microbeam XAS on the targeted regions. Microbeam techniques provide a complementary tool to bulk EXAFS analysis by providing direct information about heterogeneity within the sample.

The geometric relationship between a metal and its nearest neighboring atoms may be interpreted to indicate whether it is adsorbed onto a mineral surface or part of the internal mineral structure. A metal that is structurally incorporated into the mineral structure likely will not become bioavailable unless the mineral decomposes, whereas a metal that is adsorbed to a particle surface may be mobilized into the dissolved phase if chemical conditions change. For example, introduction of competing ions that can displace the adsorbed metal, a pH change, or a change in redox conditions can destabilize the metal-particle association. An outer-sphere association (electrostatic attraction) is generally less stable than an inner-sphere association (direct chemical bond). The type of association can be evaluated geometrically according to the distances between the metal and second-neighbor heavy atoms at the mineral surface.

MATERIALS AND METHODS

SOIL SELECTION

DoD facilities with different soil properties, but all contaminated with Cr, As, and/or Pb were selected for in vivo bioaccessability studies, swine dosing trials, ecological bioassay studies, and X-ray interrogation. Soil types hypothesized to strongly sequester metals as well as soil types thought to have poor metal sequestering potential were desired. For example, sandy, high pH aridisols at Hill Air Force Base and Deseret Chemical Depot, with limited capacity to sequester metals, were expected to have high metal bioaccessibility. Silty, neutral pH soils from Travis Air Force Base, with good to excellent metal sequestering properties, were expected to have low metal bioaccessibility. Acidic, Fe-oxide rich soils such as have excellent capacity to sequester anions such as As, and potentially poor capacity to sequester cations such as Cd and Pb. Characteristics of soils chosen for bioaccessibility and X-ray studies are shown in Table A-1.

SOIL COLLECTION

A portable field X-ray fluorimeter was used to identify target metal concentrations in the collection areas prior to collecting 10 to 12 buckets of soil, each containing 25 kg. Since the metal concentration in soil can vary greatly between and within the sample buckets, all soil collected from each site was mixed to produce a homogenous composite sample to be used for all investigations. Although the homogenization procedure described below may have impacted the oxidation state of the target metals, it ensures that the characteristics observed using synchrotron X-ray techniques are the same as those used for in vitro, ecological bioaccessibility, and swine-dosing bioavailability tests. The disadvantage is that there may be some differences in soil characteristics compared with the soil in its local environment. These differences are expected to be minimal in that the soil samples were collected from the surface, and therefore already exposed to an oxidizing atmosphere; none of the soils were from wetlands or other reducing environments. The homogenization procedure is not expected to affect distribution of target metals on soil particles, so X-ray fluorescence microprobe mapping provides an accurate record of elemental associations that supports interpretation of the metal distribution on soil particles.

Soils were air dried prior to homogenization in a heavy duty electric powered mixer with a 9 cu ft. plastic drum over six hours. A large cement mixer was modified to allow simultaneous homogenization and sieving (<2 mm) of large amounts (250+ kg) of contaminated soil by using a steel cone attachment fitted with a 2-mm sieve. The steel cone attachment, custom built for the

Table A-1. Soils selected for studies

Soil	Soil Type	Contaminant	Source of Contamination	pH	Organic Carbon (%)	Oxalate Extractable Fe (g/kg)	Aqua Regia Digestible Contaminant (mg/kg) < 250 um soil sample	Study Use[A]
Cherry Point	Entisol	Cr, Pb	Incinerator Site	6.39	-	5.33	639, 91	w, iv, s
Concord	Vertisol	As	Pesticides	6.67	3.58	4.09	220[B]	w, iv
Deseret	Aridisol	As	Flooding of Mine Tailings	9.28	1.13	1.01	544	s
Hill	Entisol	Cr	Sludge Drying Beds	7.22	2.4	1.32	369	w, iv
Hilo	Andisol	As	Arsenic Pesticides	-	-	-	-	s
McClellan	Alfisol	Cr, Pb	Wastewater Treatment Lagoon	4.31	6.08	6.09	574, 164	w, iv, s
Mechanicburg	Ultisol	Pb	Lead Ingot Stockpile	8.04	2.12	1.95	223	w, iv
ORNL	Ultisol	Pb	Small Arms Firing Range	-	-	-	-	
Pearl	Mollisol	As	Pesticides/Rodenticides	-	-	-	-	w, iv
Portsmouth	Inceptisol	Pb	Temporary Storage Area (e.g. Lead Battery Cell Plates)	6.2	3.24	8.93	4113	w, iv
Travis	Alfisol	Pb	Small Arms Firing Range	7.04	2.12	3.53	2416	w, iv

[A] w: ecological bioavailability (worm toxicity tests). iv: in vitro bioaccessibility. s: swine dosing
[B] <2mm soil sample used for aqua regia digestion

cement mixer, allows (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. The mixer is equipped with a dust trap to avoid air dispersion of the material. For soils where clumping is an issue, hardened ceramic balls were placed in the mixer with the soil in order to enhance aggregate breakup without grinding the soil, which could alter its native particle size distribution. Soils were next sieved to < 2 mm with a subsample sieved to < 270 um. The < 2mm samples were used in the in vitro and in vivo plant and earthworm model studies whereas the < 270 um samples were used in the in vitro and in vivo swine model studies, and for synchrotron X-ray interrogation. To verify that soil samples are homogeneous, numerous subsamples (10 or more) were acid digested using USEPA method 3051a followed by Cr, As, Cd, and Pb analysis. Soils are archived at Ohio State University where in vitro and in vivo plant and earthworm model investigations were performed.

X-RAY ABSORPTION SPECTROSCOPY

X-ray absorption spectra on bulk samples of the <270 μm size fraction were collected at the Stanford Synchrotron Radiation Laboratory (SSRL) in May 2007 (beam line 2-3; Pb and As analysis) and January 2008 (beam line 11-2, Cr analysis). In both cases a Si(220) monochrometer was used to control the energy of the incident beam, calibrated by metal foils or known reference compounds. Data were collected in fluorescence geometry using a 13- or 30-element germanium solid-state detector (BL 2-3 and BL 11-2, respectively). Samples were ground to fine powder and mounted in teflon sample holders sealed with Kapton tape. Between three and 25 scans were collected on each sample.

Data files were imported into the Samview module of the X-ray absorption spectroscopy processing program Sixpack (Webb, 2004) where monochrometer energy calibration was verified or corrected, and individual scans were examined to ensure that each solid-state detector channel had successfully recorded data. Noise recorded in malfunctioning channels was eliminated before averaging scans. The averaged data was then imported into the program Athena (Ravel and Newville, 2005). The near-edge portions of the spectra (XANES) were examined and first derivatives calculated to determine the energy position of the absorption edge. Next, spectral backgrounds were subtracted and the extended fine-structure portions of the spectra (EXAFS) were expressed in K-space ($Å^{-1}$), where K represents the momentum wave-

vector. The resulting χ(K) files were imported into the program Artemis (Ravel and Newville, 2005) for analysis of the EXAFS.

Least squares fitting algorithms of the EXAFS function were applied to determine nearest and second-nearest neighbor atomic identities, coordination numbers, and distances from the target metal(loid), using theoretical phase and amplitude functions generated by the program FEFF (Ankudinov, 2002). First-shell coordination environments were identified, informed by the oxidation state information obtained from XANES. The energy offset parameter E_0 was constrained to be the same for all atoms included in the fit. Wave amplitudes corresponding to the coordination number around the target metal were allowed to vary, as were the interatomic distances. The Debye Waller factor, a parameter that varies as a function of static and vibrational atomic disorder (O'Day et al., 1994), was held constant and constrained to be the same for all atoms in the first shell.

For samples containing arsenic, theoretical multiple scattering (MS) paths within As tetrahedral were generated from the mineral structure of scorodite (FeAsO4•2H2O). Phase and amplitude functions corresponding to 3-leg paths of the form As-O-O-As (12 paths) and 4-leg paths of the form As-O-As-O-As (16 paths) were generated in Artemis using the IFEFFIT module. To test whether including multiple scattering contributions improved the fit for As K edge EXAFS, the multiple scattering paths were applied with distance and degeneracy parameters fixed to their original values, and the Debye Waller factor constrained to 0.001 (Beaulieu and Savage, 2005).

Following first-shell fits, second-shell fits were performed if peaks in Fourier transforms of the EXAFS data representing interatomic distances (uncorrected for phase shift) provided evidence of more distal backscatterers. Potential identities of second-shell backscatterers were informed by the soil chemical analyses and, when available, results of the X-ray fluorescence microprobe mapping performed at APS (described below).

X-RAY FLUORESCENCE MICROPROBE

Microbeam X-ray techniques were performed at the Advanced Photon Source (Argonne National Laboratories) bending magnet beam line 20-BM, operated by the Pacific Northwest Consortium Collaborative Access Team (PNC-CAT), in February 2008. Microbeam X-ray fluorescence (XRF) spectroscopy was used to assess spatial distributions of the target elements

on the soil particle surfaces. Soil grains were dispersed onto Kapton tape, covered with a second layer of tape, and placed at a 45˚ angle to the incident beam. An initial location on the sample with multiple, well-spread out particles was chosen with the aid of a video camera. A constant focal position for all samples was maintained by moving each sample on a motorized rail until it was in focus by a second camera with a viewer outside the hutch. Two-dimensional fluorescence microprobe maps were then acquired to ascertain the distribution of target elements in relation to soil particles.

The images were processed on-site using the PNC-CAT software 2d Scan Plot version 2. Individual element distributions (in relation to the dead-time corrected incident X-ray intensity), and mapped representations of element ratios, were compared visually to detect the areas highest in the target metals to choose locations for collecting microbeam X-ray absorption spectra. In cases where the metal association with other elements was not uniform, more than one spot was chosen. For preparation of X-ray fluorescence map figures, target elements mapped in 2d Scan Plot were saved as jpeg images. These images were imported into the SMAK image processing software package (Webb, 2006), where intensity was replotted on a log scale to better visualize the distribution of elements, and converted to greyscale.

MICROBEAM X-RAY ABSORPTION SPECTROSCOPY

X-ray energy at the beamline was controlled using an N_2-cooled Si(111) double-crystal monochrometer. The beam energy was calibrated using an Au foil placed below the beam path and above a caldiode solid-state detector. Part of the beam was deflected downward to excite the foil, and the absorption reading at the caldiode was normalized to the counts in an ion chamber upstream. The beam was focused by means of a 100 mm K-B mirror to approximately 5 μm (Antonette et al., 2001).

Locations for X-ray absorption spectra (XAS) were chosen from the XRF microbeam maps, described above. At locations where the target metal(loid) appeared elevated on the map, a multichannel analyzer (MCA) was employed to measure fluorescent X-ray intensity over a range of energies. Elements (atomic number $Z > 15$) present at that location were identified by the energies of the emission peaks. At selected locations, XANES data were collected using a multielement Ge solid-state detector. Each detector element was set up to record the fluorescence intensity within the emission energy range corresponding to a target metal. Twelve detector

elements were utilized for each of the contaminants (Cr, As, Pb) and their signals were summed to obtain the relevant XANES spectrum. The summed data was processed using the software Athena, as described above for the spectra collected at SSRL.

RESULTS AND DISCUSSION

PB- AND CR-RICH SOILS

Lead displays a wide range of coordination environments in soils. Nearest-neighbor oxygen atoms in common Pb minerals can be as close as 2.16 Å in plattnerite [$Pb(IV)O_2$] and up to 2.62 Å in anglesite ($PbSO_4$), and the number of coordinating oxygen atoms can vary from 4 to 12. Furthermore, the coordination environment can be highly symmetrical, with identical first-shell Pb-O distances, or distorted, with three or more Pb-O distances. In some minerals, such as pyromorphite, there are two Pb sites, with different coordination environments. Pb can also be directly coordinated with sulfur, as in galena (Pb-S: 2.97 Å). Sorbed onto iron or aluminum oxides, Bargar et al (1997a, 1997b) found Pb-O distances in the range 2.3 to 2.4 Å. In organic matter complexes, Pb-O distances have been reported as 2.3 Å in lignins (Marmiroli, 2005), 2.41 Å in cellulose (Marmiroli, 2005), 2.32 and 2.46 Å in humates at pH 6 and pH 4, respectively (Xia et al., 1997). Lead can be also be coordinated with carbon, as in trimethyl or tetramethyl lead (Pb-C: 2.14 – 2.18 Å, Glidewell, 1990). Direct Pb-Pb associations occur in organic dilead compounds, in the 2.77 – 2.98 Å range (Glidewell, 1990).

Chromium is generally present in contaminated soils as either tetrahedrally coordinated Cr(VI) or octahedrally coordinated Cr(III). Cr(VI) is the more mobile and toxic species, while Cr(III) is typically present in stable minerals such as chromite and can also substitute for Fe(III) in minerals such as magnetite. The presence of Cr(VI) is indicated in Cr K edge XANES spectra by the presence of a significant pre-edge feature associated with 1s to 3d electron transitions in tetrahedrally coordinated Cr(VI) (Peterson et al., 1997). Because backscattering phase and amplitude paths of Cr and Fe at the same distance are not readily distinguishable, it can be difficult to ascertain the identity of second-shell backscatterers that could represent either Cr or Fe.

Results of X-ray investigation for Pb- and Cr-rich samples are presented in Figure A-2 (X-ray fluorescence microprobe maps) and Figure A-3 (X-ray absorption spectra). In all cases, Pb is divalent, coordinated with oxygen, and does not appear to be associated with crystalline

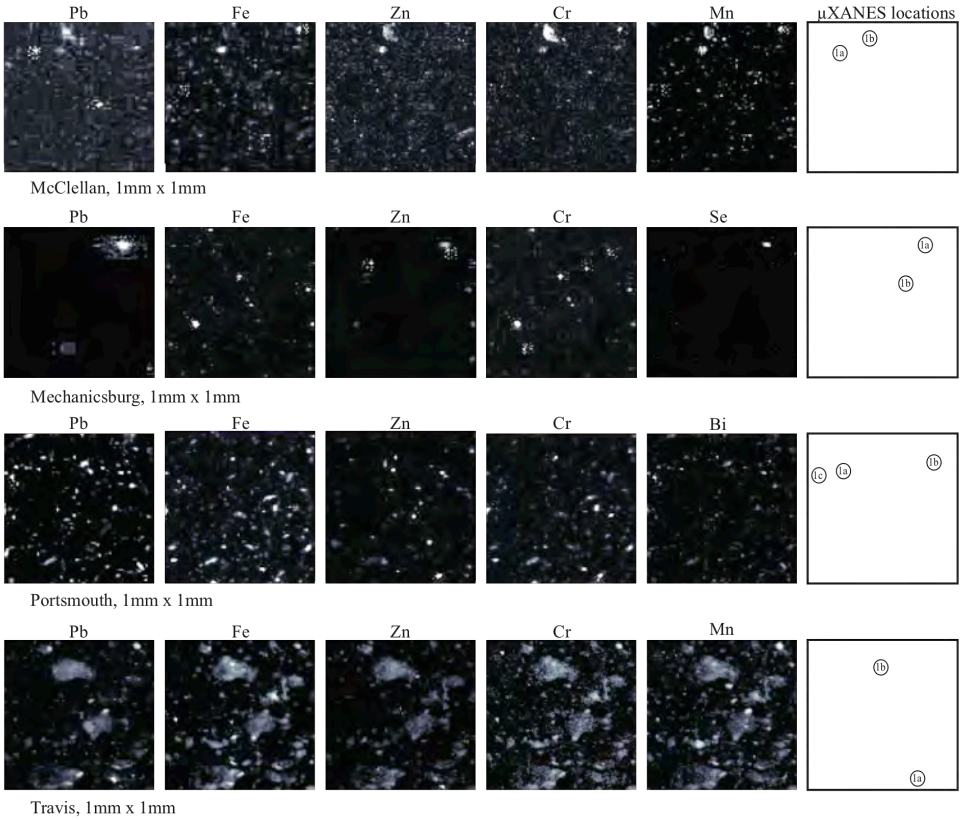

Figure A-2. X-ray fluorescence microprobe maps showing distributions of Pb, Fe, Zn, Cr, and Se, Bi, or Mn in contaminated soil samples collected from the McClellan air force base (Sacramento, CA), Naval Support Activity site (Mechanicsburg, PA), Portsmouth Naval Shipyard (Kittery, ME) and Travis Air Force Base (Fairfield, CA). Locations where microbeam XANES and/or X-ray fluorescence spectra were collected are shown at right.

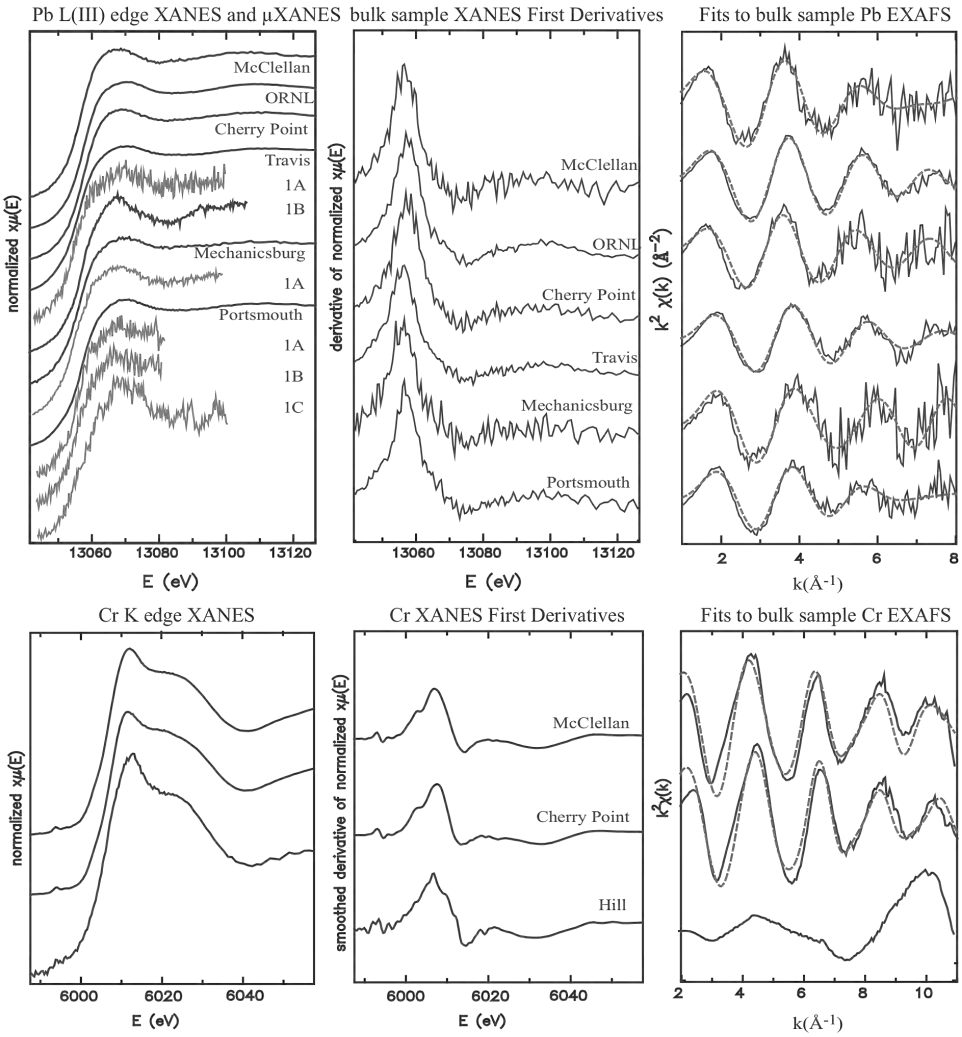

Figure A-3. Lead L(III) edge X-ray absorption spectra and Cr K edge X-ray absorption spectra of contaminated soil samples showing near-edge features for bulk samples and selected microbeam analyses (XANES), first derivatives of XANES spectra for bulk soil samples, and fits to Extended X-ray Absorption Fine Structure spectra (EXAFS) for bulk soil samples. Fit parameters are shown in Table 2.

Table A-2. Parameters used to generate fits to EXAFS data shown in Figures A-3 and A-5.

Sample	shell	R(Å)	N	s
McClellan-Pb	Pb-O	2.27	0.5	0.005
	Pb-O	2.34	1.3	0.005
	Pb-O	2.55	1.7	0.005
McClellan-Cr	Cr-O	1.99	4.5	0.002
	Cr-Cr	3.04	1.0	0.0053
	Cr-Fe	3.39	0.4	0.0053
ORNL	Pb-O	2.25	0.6	0.005
	Pb-O	2.36	0.8	0.005
	Pb-O	2.48	1.6	0.005
Cherry Point-Pb	Pb-O	2.34	1.6	0.005
	Pb-O	2.57	0.9	0.005
Cherry Point-Cr	Cr-O	1.99	4.5	0.003
	Cr-Cr/Fe	2.55	0.5	0.006
	Cr-Cr/Fe	3.45	1.3	0.006
Travis	Pb-O	2.16	0.2	0.005
	Pb-O	2.28	1.4	0.005
	Pb-O	2.48	0.8	0.005
Mechanicsburg	Pb-O	2.28	1.9	0.005
	Pb-O	2.56	1.4	0.005
Portsmouth	Pb-O	2.26	1.4	0.01
	Pb-O	2.47	1.3	0.01
Concord	As-O	1.68	4	0.0018
	As-MS	*	*	0.0010
	As-Fe	2.93	0.63	0.0046
	As-As	3.88	1	0.0018
Deseret	As-O	1.68	4	0.0017
	As-MS	*	*	0.0010
Hilo	As-O	1.69	4	0.0020
	As-MS	*	*	0.0010
	As-Al	3.21	1.4	0.0070
Pearl	As-O	1.75	3	0.0041
	As-Fe	2.92	0.5	0.01
	As-Fe	3.32	0.25	0.01

* Multiple scattering paths described in text

compounds, but rather associated with organic matter or as a poorly crystalline lead oxide. There is scant EXAFS evidence for association with iron oxides but X-ray fluorescence microprobe maps show a fairly consistent Pb correlation with iron. Cr is present exclusively as Cr(III), and is coordinated by oxygen in two of the Cr-rich samples, with the third sample (Hill AFB) undetermined.

McClellan Air Force Base, Sacramento, CA

Soils at McClellan Air Force Base are fine-grained alfisols with slight acidity and significant organic matter, contaminated with Pb, Cr, and Cd from a former wastewater treatment lagoon. As shown on Figure A-2, results of X-ray fluorescence microprobe show that Pb is distributed heterogeneously on soil particles. On some soil particles Pb is present with Mn, Zn, and Cr, while on others it coexists with Fe. A low signal to noise ratio and an interference with bismuth at 13419 eV permits limited interpretation of the X-ray absorption spectra. Only the first shell was fit (Table A-1). The local oxygen coordination environment is similar to that in β-PbO (massicot) in that there are contributions from oxygen backscatterers at three distances within the range 2.27 – 2.55 Å. This distal range is slightly expanded relative to massicot (2.22 – 2.48 Å), and the lack of strong second-shell backscattering atoms indicates that Pb is not part of an ordered crystalline structure. The oxygen coordination shell is more complex than what is observed for Pb adsorbed to Fe or Al sesquioxides, where oxygen backscatterers are typically within 2.3 – 2.4 Å of Pb. Based on the high organic content and low pH of the contaminated soil (4.3, compared to 6.1 – 6.6 in the uncontaminated soil), it is possible that Pb is associated with organic matter. The relatively long Pb-O distance (2.46 Å) is consistent with Pb humate at pH 4 (Xia et al., 1997) or Pb acetate (Manceau et al., 1996).

Chromium K-edge EXAFS of the McClellan soil show a local coordination environment similar to that found in chromite, with comparable distances for O, Cr, and Fe backscatterers. However, calculated coordination numbers for Fe and Cr are lower and the XANES spectrum lacks features characteristic of crystalline chromite, suggesting a disordered long-range coordination environment. A heterogeneous chromium distribution is also suggested by the X-ray fluorescence microprobe maps, which show a non-uniform correlation with Fe. The Cr XANES indicates only Cr(III) is present, indicated by the absence of the significant pre-edge feature associated with Cr(VI) in the Cr K edge XANES spectrum.

Firing range at Oak Ridge National Laboratory, Tennessee

These soils are highly weathered acidic ultisols, abundant in silt and clay. The bulk X-ray absorption spectrum displays a simple oscillation with no significant second-shell backscattering atoms evident in the Fourier transform (Figure A-3). The local coordination environment is similar to that in the McClellan sample. Based on sample mineralogy, Pb could be adsorbed onto clay minerals or onto sesquioxides, with substantial disorder indicated by the lack of an observed second-shell backscattering contribution.

Marine Air Corps Station, Cherry Point, NC

This poorly developed entisol, thought to be contaminated from incineration debris, is high in organic matter and very high in iron (about 11 wt.%). The <250 μm size fraction has lower metal concentrations than the <2 mm size fraction, indicating that metals may reside primarily in solid incinerator waste particles. Both Cr and Pb EXAFS were impacted by the presence of other elements (Mn and Bi, respectively), limiting interpretation of these spectra. Two Pb-O distances, 2.34 Å and a smaller contribution at 2.57 Å provided the best fit to the lead L(III) edge EXAFS. Cr is present as Cr(III), as indicated by the absence of the significant pre-edge feature associated with tetrahedrally coordinated Cr(VI) in the Cr K edge XANES spectrum (Peterson et al., 1997). in addition to oxygen, chromium and/or iron contribute to the fit to Cr K edge EXAFS data. Oxygen and the further backscatterer (Cr or Fe) distances are consistent with chromite, but the closer of these backscatterer distances (Cr or Fe) is shorter (2.55 Å, compared to 2.96 Å in chromite), and, like the McClellan sample, the XANES lacks features characteristic of chromite.

Travis Air Force Base, Fairfield, CA

The silt and clay loam from this site, a former small arms range, is elevated in Pb and other metals, as well as Sb. The X-ray fluorescence microprobe map shows a common distribution pattern for Pb and Zn. On some grains, Pb and Zn are also associated with Fe, Cr, and Mn. In one location, Pb is not associated with any of these other elements. Microbeam XANES analyses on two high-Pb spots are different from one another, despite similarities in elemental associations, suggesting a variety of coordination environments for Pb in this sample.

Three Pb-O distances provided the best fit to the bulk EXAFS data, 2.16, 2.28 and 2.48 Å. The soil environment is interpreted to be highly heterogeneous, leading to a distribution of sorption geometries for Pb. The association with Fe observed on the X-ray fluorescence microprobe map suggests that some of the Pb is likely to be associated with iron oxides, but poorly crystalline lead oxides derived from bullet fragments may predominate.

Naval Support Activity Site, Mechanicsburg, PA

This silty clay ultisol collected from a lead ingot stockpile location is relatively low in metals other than Pb. On the X-ray fluorescence microprobe map, Pb is observed in the same locations as Zn and Se, and not with Fe or Cr. The microbeam XANES spectrum is similar to the bulk spectrum. Although Pb is not in a crystalline compound, its local coordination environment may be similar to that in β-PbO (massicot), similar to the Cherry Point sample. The Fourier transform (not shown) indicates the possibility of a second-neighbor backscattering contributor relatively distant from Pb, at approximately 3.8 Å; however, this spectrum has a low signal:noise ratio and therefore a fit to this second shell could not be constrained. Both the microprobe map and the soil data suggest Pb is not associated with sesquioxides. A mixture of Pb metal or alloy, and poorly crystalline Pb oxides, is a reasonable interpretation in the context of the contamination scenario. Sorption on clay minerals is also a possibility.

Portsmouth Naval Shipyard, Kittery, Maine

Pb in this organic-rich, iron-poor sand/silt inceptisol was introduced from storage of battery cell plates and other materials. In X-ray fluorescence microprobe images, areas of elevated Pb are associated with Cr and/or Bi, and less consistently with Fe. Microbeam XANES support multiple coordination environments for Pb, and no fit was confidently achieved for the bulk EXAFS spectrum beyond the local Pb-O shell, which is similar to the other samples with the best fit by two oxygen distances, 2.26 Å and 2.47 Å.

Hill Air Force Base, Ogden, UT

Chromium contamination at this site, together with cadmium and lead, was left in a sludge drying bed following drinking water treatment. This sandy entisol has high organic matter content (8% TOC). The X-ray absorption near edge structure and first derivative indicate all Cr

is present as Cr(III). The EXAFS spectrum is unusual (Fig. 3) and no fit to this data was performed.

AS-RICH SOILS

The geochemical controls on As retention in soils are important to health risk assessments and remediation strategies because As toxicity, mobility, and bioavailability are functions of its oxidation state and local chemical environment (Foster et al., 1998). Arsenic in soil environments is typically found as arsenate, As(V) tetrahedrally coordinated by oxygen at 1.68 Å, or arsenite, As(III) coordinated by three oxygen atoms at 1.75 Å. Organic forms of arsenic can also be present and are usually an indicator of biological activity involving arsenic, or application of organic arsenic compounds such as when poultry litter containing roxarsone (from a feed additive) is spread as a fertilizer. Different forms of arsenic can be distinguished in X-ray absorption spectra by the energy position of the absorption edge, which increases with increasing oxidation state, by the shape of the XANES spectrum, and by the coordination environment assessed from EXAFS data. Results of the synchrotron X-ray studies are presented in Figures A-4 and A-5.

Concord Naval Weapons Site, CA

The silty clay vertisol at the Concord site is impacted by arsenic that was introduced from pesticide application. The X-ray fluorescence microprobe map indicates heterogeneity in As distribution. Most arsenic is associated with Fe, Mn, and Zn, but there are a few grains where As is present without other elements that were included in the mapping scheme. XANES spectra, both in bulk and on a high-As grain, indicate only As(V). The bulk EXAFS is consistent with arsenate adsorbed on iron oxides at a high density, with As-As as well as As-Fe contributions to the fit (Table A-2). Both As-Fe and As-As distances are significantly shorter than in scorodite. The spectra and microprobe maps are not consistent with other minerals that have been found in pesticide-impacted soils, such as schultenite (Cances et al., 2005), arseniosiderite (Cances et al., 2008) or chromated copper arsenate (Bull et al., 2000).

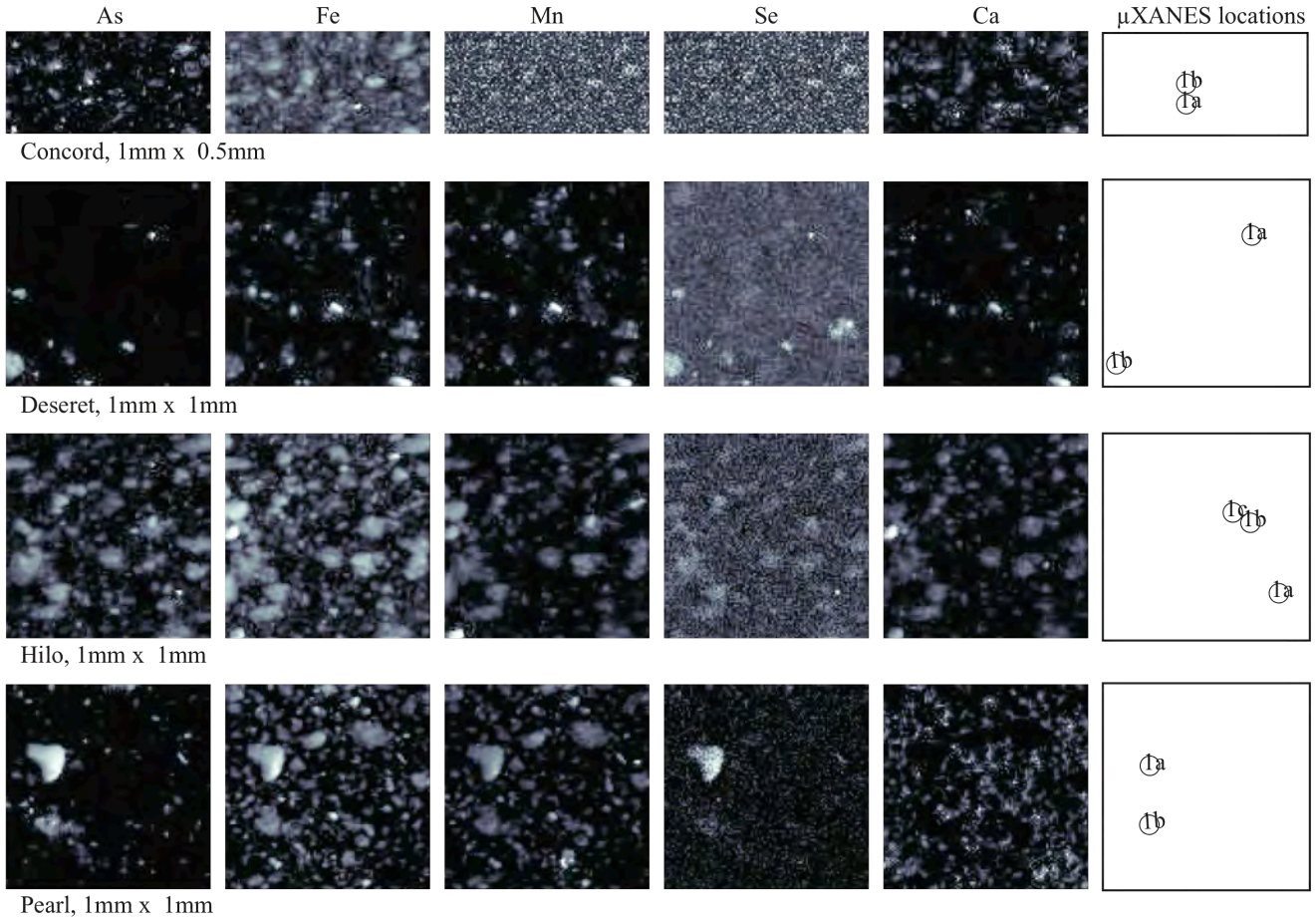

Figure A-4. X-ray fluorescence microprobe maps showing distributions of As, Fe, Mn, Se, and Ca for contaminated soil samples collected from the Concord Naval Weapons site (CA), Deseret Chemical Depot (Tooele, UT), former sugar cane fields near Hilo (HI) and the Pearl Harbor Naval Complex (HI). Locations where microbeam XANES and/or X-ray fluorescence spectra were collected are shown at right.

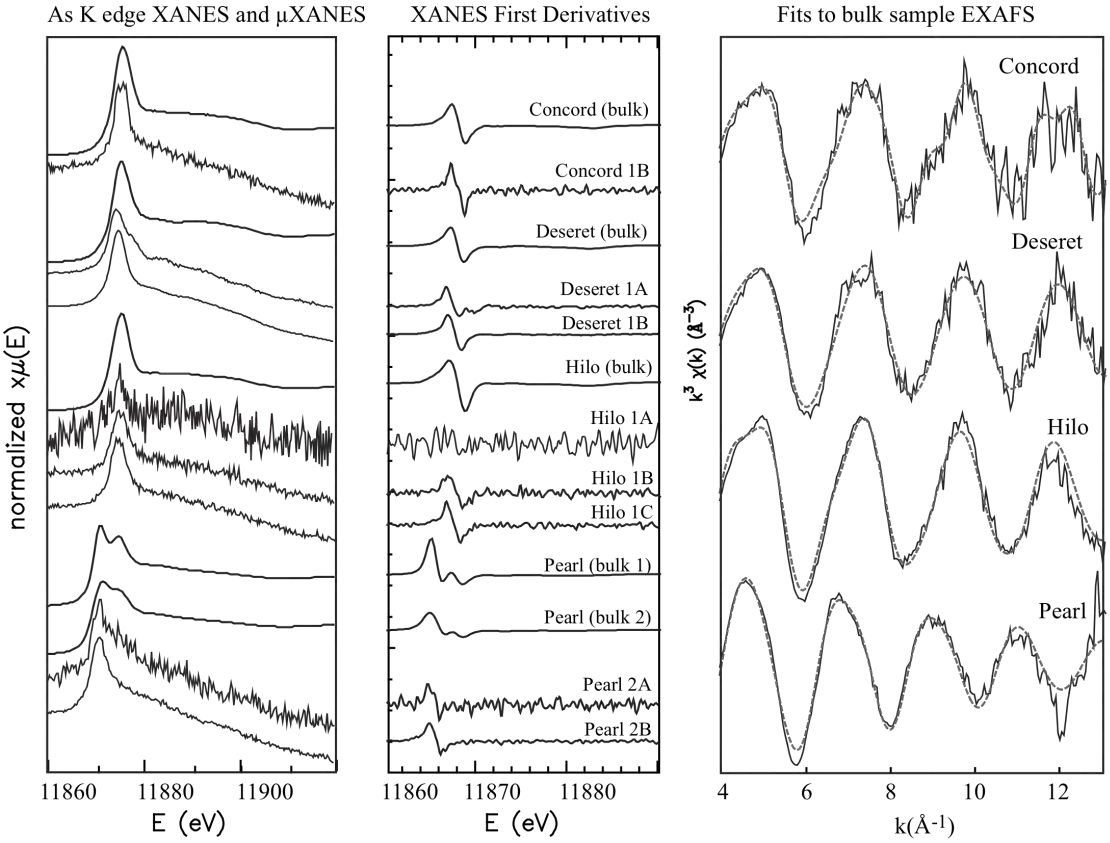

Figure A-5. Arsenic K edge X-ray absorption spectra of contaminated soil samples showing XANES for bulk samples and selected microbeam analyses, first derivatives of XANES spectra, and fits to EXAFS for bulk soil samples. Fit parameters are shown in Table A-2.

Deseret Chemical Depot, Tooele, UT

Arsenic in this silty sand aridisol originates from mine tailings that flooded the site in the 1930s. The pH of this soil is unusually high, 9.3. Arsenic is well correlated with selenium on the microprobe map. Its relationship to iron is variable; some high-arsenic grains are free of iron whereas others are well matched. Including a contribution from multiple scattering in the arsenate tetrahedron considerably improves the fit to the first oscillation in the EXAFS spectrum. Neither iron nor aluminum backscatterers contribute significantly to the pattern.

Former sugar cane fields, Hilo, HI

Similar to the Concord site, arsenic in the Hilo andisol is thought to have originated from pesticide applications in the 1920s - 1940s. The X-ray fluorescence microprobe map shows that arsenic is primarily associated with iron, but there are two locations elevated in arsenic that do not contain iron. One of these is high in selenium. The bulk EXAFS includes a multiple scattering component associated with the arsenate tetrahedron. A good fit is achieved with Al at 3.16 Å as a second-shell backscatterer, consistent with the presence of colloidal allophanes and imogolite in the sample. Ferrihydrite is also ubiquitous and a small second-neighbor contribution from Fe can be fit as well.

Naval Complex, Pearl Harbor, HI

Of the As-bearing soil samples this is the only one in which As is observed as As(III) on the XANES spectra. The bulk sample includes contributions from both As(III) and As(V), but the two microbeam XANES spectra show only As(III). The bulk EXAFS is best fit with 3 oxygen atoms at a distance of 1.75 Å, consistent with arsenite predominance. Iron backscattering at 2.9 Å is consistent with arsenite adsorption on ferrihydrite (Ona-Nguema 2005; Manning et al., 1998). The reducing environment preserving As(III) is consistent with the high organic content of the mollisol.

SUMMARY

Key observations from the synchrotron X-ray studies are (1) Pb is present as adsorbed divalent ions or as organic complexes, rather than in crystalline compounds, in all of the Pb-rich soil samples; (2) Cr is present as Cr(III), the more stable and less toxic of the two common Cr

oxidation states, in all three Cr-rich soil samples; and (3) Arsenic is present in the more stable and less toxic form, As(V), in three of the four As-rich soil samples, but is present as both As(III) and As(V) in the sample from the Naval Complex at Pearl Harbor. Arsenic appears to occur as an adsorbed complex on iron oxides in the Concord and Pearl samples, and as an adsorbed complex on aluminum oxides in the Hilo soil sample.

In terms of remediation, As strategies will likely require more site-specific approaches versus Pb, which despite soil differences, is more ubiquitously speciated less toxically and with less mobility. No Pb was found to be bound in more immobile and less bioaccessible sulfide phases, meaning that most of the Pb-O in the soils can be liberated under acidic conditions (i.e., in the stomach or in the case of percolating acidic soil/groundwater). The finding that Pb is mobilizable in low pH conditions supported by previous flow-through and leaching experiments performed on the Cherry Point soils (Bang 2004).

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APPENDIX B

Models for predicting metal toxicity and bioaccumulation in soil invertebrates: Validation of laboratory models using metal-contaminated field soils

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Models for predicting metal toxicity and bioaccumulation in soil invertebrates: Validation of laboratory models using metal-contaminated field soils

Introduction

There are thousands of metal-contaminated sites on DoD lands awaiting remediation and closure. The toxic metals lead (Pb), arsenic (As), chromium (Cr), and cadmium (Cd) are of particular concern since they often control risk-based remedial decisions for soils at DoD sites (Exponent, 2001). The ecological risk of metals is directly related to their bioavailability which is the fraction of metal that is absorbed across external membranes (e.g., gut, cuticle, dermis) and enters the body of an organism. The same total metal concentration may result in soil invertebrate responses from complete mortality to 100% survival depending upon the physical/chemical characteristics of the soil (Bradham et al. 2006; Lanno et al. 2004). As such total metal levels in soil may not be the best predictors of toxicity and bioaccumulation. Metal bioavailability can be altered by several soil physical and chemical properties such as Fe-oxide content, organic matter content, and pH. For example, when metal-scavenging manganese (Mn) (Boularbah et al., 1996) or iron (Fe) (Chlopecka and Adriano, 1996) oxyhydroxides are added to soil, metal bioavailability decreased. Certain soil conditions were also found to enhance metal bioavailability. For example, when the soil Fe-oxide content was below 0.5% on a mass basis, the bioavailability of As increased dramatically, particularly in alkaline soils (Yang et al., 2002, 2003). Similarly, for DoD soils low in organic and inorganic carbon, the bioavailability of Cr (III) and Cr (VI) was significantly higher relative to soils that contained higher levels of organic and inorganic carbon (Stewart et al., 2003a, b; Jardine et al., 1999).

Although the concept of metal bioavailability is acknowledged by risk assessors, regulators,

and in guidance documents, there are no consistent, standardized approaches for the application of metal bioavailability considerations in site cleanup actions. EPA's risk assessment guidance implicitly assumes a default relative bioavailability of 100%, or exposure dose is usually measured as total metal concentration, since most bioavailability models are based upon laboratory tests conducted with soils spiked with soluble metal salts. However, this is a conservative assumption, and may overstate the risk posed by less soluble metals in field soils (Davis et al., 1992). Several studies have shown that metals are largely immobilized by soils, and only a small fraction is bioavailable. For example, Banjoko and McGrath (1991) found that most of the zinc (Zn) (78%) present in soil existed in the recalcitrant residual fraction and was not available to maize grown in the soils. Recent research has indicated that reference dose criteria used for soil As and Cr are often highly conservative because the indigenous metal-sequestering properties of many soils can significantly lower the metal bioavailability relative to commonly used default values (Yang et al., 2002, 2003; Stewart et al., 2003a, b). Also, numerous studies 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 that are commonly used in toxicological studies.

A range of tools is available to study metal bioavailability, from microscopy, to chemical extractions, to bioassays. Studies have also focused on the application of these techniques specifically to DoD sites (Battelle and Exponent, 2000; Kelley et al., 2002). Based on previous scientific and technical advances in the area of *in vitro* and *in vivo* metal bioavailability in soils, this Environmental Security Technology Certification Program (ESTCP) project was proposed to validate the ability of these techniques to predict metal bioavailability for human and ecological

risk assessment on DoD sites, and to investigate the role of soil properties in controlling metal bioavailability.

This section of the report examines the results of earthworm (*Eisenia andrei*), potworm (*Enchytraeus crypticus, En. albida*), and collembola (*Folsomia candida*) bioassays conducted in soils from metal-contaminated DOD sites with paired reference sites. The goals of this study were: 1) To assess metal bioaccumulation and toxicity to soil invertebrates in a wide range of DOD soils varying in physical and chemical characteristics and metal levels, and 2) To attempt to validate various models relating soil properties or metal extracts to oligochaete metal bioaccumulation and toxicity as a screening tool for predicting metal bioavailability in soils.

Methods

Soil collection

Eleven DOD sites, differing in geographical location, physical/chemical characteristics, source, and level of metal contamination were selected for soil invertebrate bioassays. For each site, approximately 25 kg of both contaminated soil and site reference soil (the same soil series but uncontaminated, i.e., natural background levels of Cd, Pb, As) were collected. A portable X-Ray Fluorescence meter was used to evaluate approximate metal levels of the soil in the collection area. All soil samples collected from one site were thoroughly mixed to produce one homogenous sample. Prior to soil invertebrate and plant bioassays, soil properties and total metal content were determined.

Soil preparation and physicochemical characteristics

Prior to soil invertebrate bioassays, properties of ESTCP soils were determined according

to standard methods of soil analysis (Sparks et al. 1996). Soil pH was measured in both 1:1 soil:deionized water suspension and 1:2 soil: 0.01 M $CaCl_2$ suspension. Electrical conductivity (EC) was measured in 1:1 soil:deionized water suspension. Reactive Al-oxide, Fe-oxide, and Mn-oxide fractions were measured using acid ammonium oxalate extraction.

Webster soil (Ames, IA; 2.4% OC, 35.6% clay, pH 5.5) was used as a lab standard reference soil to monitor test organism performance. Before the test, ESTCP soils (11 contaminated soils paired with 11 reference soils) and Webster soil were sieved to 2.0 mm, air-dried, hydrated with deionized water to achieve a moisture content of 50% of their water-holding capacity (Environment Canada 2004), and left to equilibrate overnight.

Test organism culture and maintenance

Sexually mature adult earthworms (*Eisenia andrei*), each with a developed clitellum, weighing approximately 0.3 to 0.5 g wet weight, were obtained from a lab culture maintained in separated dairy solids (SDS; Ohio Agricultural Research and Development Center, Wooster, OH) (Environment Canada 2004). Sexually mature *Enchytraeus albidus* were obtained from a lab culture which was maintained in a mixture of 1:1 Webster soil and potting soil, and was watered and fed with ground oatmeal twice per week. Sexually mature *Enchytraeus crypticus* were obtained from a lab culture which was maintained in Sassafras soil, and was watered and fed with ground oatmeal twice a week (OECD 2003). Adult Collembola (*Folsomia candida*) were obtained from a lab culture which was maintained in a mixture of 9:1 plaster of Paris and powdered charcoal, and was watered and fed with Baker's yeast once per week (ISO 1999).

Soil invertebrate bioassays

E. andrei reproduction bioassays were conducted according to standard procedures for earthworm reproduction tests (Environment Canada 2004). Four replicates were prepared for each soil with ten worms placed on the surface of the soil (200 g dry weight) in each test chamber (glass mason jars; 500 ml; Ball, Muncie, IN). Test chambers were sealed with perforated metal lids (one hole, ~2.0 mm, to allow gas exchange) and screw collars. During tests, all the test chambers were maintained under continuous fluorescent lighting at 20±2 ºC. The moisture content of the soil was checked by comparison with initial complete test chamber weights. If weight differed by >5%, deionized water was applied using a spray applicator. The worms were fed with 25 g of SDS once a week. The duration of reproduction test was 56 days. On day 28, adult worms were removed from the soils, counted, placed on moistened filter paper for 24 h to void their gut contents, and then stored in Nalgene bottles (Fisher Scientific, Pittsburgh, PA) and frozen (–70 ºC). Test chambers were returned to the environmental chamber and on day 56, earthworm hatchlings were counted.

Enchytraeus (potworm) bioassays were conducted according to the OECD Guideline 220 (2003). Four replicates were prepared for each soil with ten worms placed on the surface of the soil (20 g dry weight) in each test chamber (glass mason jars; 100 ml; Ball, Muncie, IN). Test chambers were sealed with perforated metal lids (one hole, ~2.0 mm, to allow gas exchange) and screw collars. During the tests, all the test chambers were maintained under continuous fluorescent lighting at 20±2 ºC. The moisture content of the soil was checked by comparison with initial complete test chamber weights. If weight differed by >5%, deionized water was applied using a spray applicator. Potworms were fed with 25 mg of ground oatmeal twice a week. The durations of *En.crypticus* reproduction and *En.albidus* bioaccumulation tests were 28 and 21

days, respectively. For *En.crypticus* reproduction test, on day 14, adult worms were removed from the soils, counted, and then stored in vials and frozen (–70ºC). Test chambers were returned to the environmental chamber and on day 28, hatchlings were counted. For *En.albidus* bioaccumulation tests, adult worms were removed from the soils on day 21, counted, and then stored in vials and frozen (–70ºC).

Collembola bioassays were conducted according to ISO 11267 (1999). Four replicates were prepared for each soil with ten Collembola placed on the surface of the soil (25 g dry weight) in each test chamber (glass mason jars; 100 ml; Ball, Muncie, IN). Test chambers were sealed with a perforated metal lids (one hole, ~2.0 mm, to allow gas exchange) and screw collars. During the tests, all the test chambers were maintained under continuous fluorescent lighting at 20±2ºC. The moisture content of the soil was checked by weight differential and sprayed with deionized water if moisture content decreased more than 5%. Organisms were fed with three granules of Baker's yeast once a week. The duration of Collembola reproduction test was 42 days. On day 28, adult Collembola were removed from the soils, counted, and test chambers returned to the environmental chamber, and on day 42, hatchlings were counted. Adults were pooled and stored in vials at -70ºC until metals analysis.

Total metal and 0.5 M $Ca(NO_3)_2$-extractable metal analysis

Total metal content of soil was determined using acid digestion (USEPA 3051a, 2007) followed by analysis using high-resolution inductively coupled plasma mass spectrometry (ICP-MS). Soil samples (0.5 g) were oven-dried at 105ºC for 2 h, weighed, mixed with 10 ml of concentrated trace metal-grade HNO_3 (Fisher Scientific, Pittsburgh, PA), and digested in a closed Teflon bottle in a microwave oven (Ethos 320; Milestone Inc., Monroe, CT) at 180ºC for

10 min. After cooling at ambient temperature, the solution was diluted to 50 ml with deionized water, and then any residual soil was removed by filtration (0.22 μm). Metal concentrations in the digests were determined with ICP-MS (Elan 6000; Perkin Elmer Sciex., Woodbridge, ON Canada), and reported based upon the dry weight of soil. QA/QC measures included duplicate analyses, metal spikes, blanks, and analyses of standard reference soil ‘‘sandy soil B’’ CRM-SA-B, Environmental Express, Mt. Pleasant, SC). Measured metal concentrations of the standard reference soil were within performance acceptance limits.

To determine $Ca(NO_3)_2$-extractable metals, soil samples (1.0 g, dry weight) were placed in 50-ml centrifuge tubes and extracted with 20.0 ml of 0.5 M $Ca(NO_3)_2$ solution. The samples were shaken end-to-end on a reciprocal shaker for 16 h. The soil extracts were centrifuged at 10,000 rpm for 15 min and supernatants decanted and filtered through a 0.45 μm membrane filter. Supernatants were acidified with 1.0 mL of trace metal concentrated HCl and stored in acid-rinsed Nalgene bottles at 4 °C until analysis of metal by ICP-MS.

Earthworm metal analysis

For earthworm bioassays, two worms from each replicate were thawed and pooled, oven-dried at 105 ºC to a constant weight, and weighed before the metal analysis. Each earthworm sample was mixed with 10 ml of 25% (v/v) concentrated trace metal-grade HNO_3 (Fisher Scientific, Pittsburgh, PA), and digested in a closed Teflon bottle in a microwave oven (Ethos 320; Milestone Inc., Monroe, CT) at 180ºC for 10 min. After cooling at ambient temperature, the solution was made up to 50 ml with deionized water in a volumetric flask. Concentrations of Cd, Cu, Pb, Cr, Ni, Zn, and As in the digests were determined with inductively coupled plasma mass spectroscopy (ICP-MS; Elan 6000; Perkin Elmer Sciex., Woodbridge, ON Canada).

For *Enchytraeus* bioassays, all the worms from two replicates were thawed and pooled together, oven-dried at 105 ºC to a constant weight, and weighed before the metal analysis. Each sample was mixed with 10 ml 12.5% (v/v) concentrated trace metal-grade HNO_3 (Fisher Scientific, Pittsburgh, PA), and digested in a closed Teflon bottle in a microwave oven (Ethos 320; Milestone Inc., Monroe, CT) at 180ºC for 10 min. Concentrations of Cd, Cu, Pb, Cr, Ni, Zn, and As in the digests were determined with ICP-MS (Elan 6000; Perkin Elmer Sciex., Woodbridge, ON Canada). All adult collembola from all replicates were pooled and digested for metals analysis as above, but metal levels were below detection limits.

Quality assurance/quality control (QA/QC) procedures for total metal analysis in organism tissues included metal analysis of procedural blanks, spikes, and certified reference material (lobster hepatopancreas, TORT-2, National Research Council, Canada). Measured metal concentrations of the standard reference tissue were within performance acceptance limits.

Data analysis

Mortality, reproduction, and tissue metal concentration data were tabulated in a Microsoft Excel spreadsheet (Microsoft 2000, 9.0.2812). Student t-tests were used to compare data from Webster soil and from ESTCP soils to compare parameters between ESTCP soils and the laboratory reference soil. Student t-tests were also used to compare mean response parameters betweeen contaminated soils and their corresponding reference soils.

A literature review was performed to assemble empirical models relating earthworm toxicity endpoints or tissue metal concentrations to total metal soil concentrations and soil properties. Empirical models developed from previous studies (Lanno and Basta 2003; Yu and Lanno, 2010) were also applied to predict earthworm metal bioaccumulation. Tissue metal concentrations were then predicted using these models, and the correlations between predicted values and actual

measured values were tested to validate the application of these models to metal-contaminated ESTCP field soils. The agreement between the measured and the model-predicted bioavailability was quantified with the root mean square error (RMSE):

$$\mathrm{RMSE} = \left[\frac{1}{n_d - n_p} \sum_{i=1}^{n_d} \left(B_i - \hat{B}_i \right)^2 \right]^{1/2}$$

where n_d is the numbers of data points, n_p is the number of adjustable parameters (zero when used in a purely predictive manner as in this project), i is an index, and B_i and $\hat{B}_i$ are the *i*-th measured and predicted bioavailability, respectively. The RMSE, the square root of the mean squared difference between measured and predicted values, is a measure of the average error between the predicted and measured values. The goal for models was to produce RMSE ≤ 25%.

Results

Soil properties and total metal content of the contaminated and reference soils varied widely (Tables B-1 and B-2). Comparing soil properties over all soils, soil pH ranged from 3.1 to 7.5, Al-oxide content from 345 to 21,344 mg/kg, Fe oxide content from 507 to 25,678 mg/kg, organic carbon content from 0.2 to 7.8%, cation exchange capacity (CEC) from 2.7 to 39 cmol$_c$/kg, and clay content from 2.5 to 58%. Total metal concentrations ranged from 0.7 to 22 mg/kg for Cd, 12 to 3,069 mg/kg for Pb, 11 to 876 mg/kg for Cr, 3.5 to 561 mg/kg for Ni, 1.7 to 660 mg/kg for As, 30 to 1,889 mg/kg for Zn, and 1.0 to 423 mg/kg for Cu. Such a wide range in metal concentrations and soil properties provided a reasonable challenge for validating models predicting metal bioaccumulation by soil invertebrates as related to soil physicochemical

Table B-1. Selected properties of ESTCP metal-contaminated soils (C) and reference soils (R).

Soil		Soil pH water	Soil pH $CaCl_2$	EC dS/M	Al_{ox} mg/kg	Fe_{ox} mg/kg	Mn_{ox} mg/kg	Org C %	Total C %	CEC cmolc/kg	Sand %	Silt %	Clay %	WHC %
Mechanicsburg	**R**	7.5	7.1	0.3	2050	2492	944	1.2	1.4	9.6	9.9	50	40	47
	C	8.0	7.0	0.2	1615	1407	290	0.6	4.5	9.7	30	37	34	36
Cherry Point	**R**	7.4	7.0	0.4	909	797	<25	0.8	1.9	3.9	80	12	7.8	32
	C	5.5	5.0	0. 9	6061	7506	32	3.7	4.5	9.1	80	14	6.8	40
Travis	**R**	6.0	5.6	0.3	885	4569	547	1.3	1.4	11	30	44	26	41
	C	7.0	6.5	0.3	799	3088	405	1.1	1.2	17	48	26	26	36
Concord	**R**	6.3	5.9	0.2	1672	4519	659	2.2	2.1	28	20	44	36	48
	C	6. 7	6.2	0.1	1522	3664	641	3.1	3.0	28	18	41	41	47
McCllelan	**R**	6.7	6.1	0.1	487	804	125	0.4	0.4	12	60	25	15	43
	C	4.3	4.3	0.3	2175	4805	<25	4.4	4.7	13	26	50	24	52
Portsmouth	**R**	6.2	5.7	0.2	4149	2682	70	1.4	1.7	2.7	87	9.6	3.9	32
	C	6.2	6.0	0.1	3764	5758	124	1.6	2.6	2.7	89	8.5	2.5	28
Deseret	**R**	7.8	6.9	0.5	1207	681	381	0.8	1.5	13	28	53	19	31
	C	9.3	7.5	0.5	786	863	313	0.7	2.3	8.4	37	55	8.7	35
ORNL	**R**	3.8	3.1	0.2	851	798	<25	0.2	0.2	7.9	9.0	33	58	44
	C	4.1	3.5	0.2	388	507	27	0.3	0.4	2.8	46	37	18	41
Pearl	**R**	7.7	7.5	0.9	2046	1977	492	0.3	2.0	39	54.7	26.9	18.4	52
	C	7.3	7.3	1.0	3502	44900	1014	2.3	3.3	26	48.7	29.2	22.1	52
Hilo	**R**	4.7	4.7	1.5	5917	7535	86	5.7	5.5	10	72.3	17.8	2.6	40
	C	5.9	5.7	0.8	21344	25678	484	7.8	8.4	17	61.1	25.3	7.8	45
Webster	**R**	6.1	5.5	----	1320	2350	395	2.4	----	28	26	32	42	36

properties.

Survival, reproduction, and metal concentrations in the tissues of earthworms (*E. andrei*) from bioassays conducted in contaminated and reference soils are shown in Table B-3. Reduced survival was only observed in Deseret contaminated soil in which the number of live adult worms on day 28 was significantly lower than both Webster soil and the Deseret reference soil. Earthworm survival in all soils tested, except Deseret contaminated soil, was above the validation limit (90% survival) for earthworm bioassays (Environment Canada 2004).

Significantly lower reproduction was observed in all ESTCP soils (contaminated and reference) except for the Concord reference soil in which the number of hatchlings was not significantly different from Webster soil. For about half of the ESTCP soils, reproduction was below the validation limit (30 juveniles per 10 adult worms) for earthworm reproduction bioassays (Environment Canada 2004). *E. andrei* reproduction was significantly lower than in its corresponding reference soil only in Travis soil, suggesting that the decrease in reproduction in most ESTCP soils was due to the effects of the soil matrix, and only in Travis were effects of metal contamination on reproduction observed. Whole-body metal concentrations of *E. andrei* suggest a general trend of increased metal bioaccumulation from contaminated soils. In many cases, metal concentrations in worms exposed to contaminated soils were significantly higher than metal concentrations in worms exposed to either corresponding reference soils or Webster soil, or both. In two cases, Cr for Pearl and As for Cherry Point, metal concentrations in earthworms exposed to contaminated soils were significantly higher than in worms exposed to Webster soil, but significantly lower than in earthworms exposed to corresponding site reference soils. Although these concentrations are statistically significant, the actual differences in concentrations are only about 2.5-fold. For As, whole-body concentrations in *E. andrei* exposed

Table B-2. Total metal content of ESTCP contaminated soils (C) and reference soils (R) along with US EPA Ecological Screening Levels (EcoSSLs) and background soil levels. Cells highlighted in yellow are above US EPA EcoSSLs for the respective metals. Values for metal background levels in soil are take from US EPA EcoSSL documents.

Soil		Cd mg/kg	Pb mg/kg	Cr mg/kg	Ni mg/kg	As mg/kg	Zn mg/kg	Cu mg/kg
Mechanicsburg	**R**	<1.0	33	56	36	17	97	19
	C	<1.0	120	39	29	15	98	25
Cherry Point	**R**	<1.0	17	13	3.5	1.7	32	<1.0
	C	19	114	876	78	6.9	486	167
Travis	**R**	<1.0	17	43	23	8.1	70	19
	C	<1.0	2034	42	29	11	225	148
Concord	**R**	<1.0	16	79	98	7.8	101	50
	C	<1.0	22	77	92	220	112	54
McCllelan	**R**	0.7	15	126	60	6.1	32	14
	C	22	193	699	87	9.9	448	241
Portsmouth	**R**	<1.0	48	14	8.4	10	60	12
	C	1.1	3069	11	62	11	500	185
Deseret	**R**	<1.0	20	27	17	11	83	15
	C	<1.0	19	24	16	438	85	13
ORNL	**R**	<1.0	12	48	15	14	85	14
	C	<1.0	966	16	4.2	5.0	30	65
Pearl	**R**	1.4	13	233	182	4.1	133	110
	C	3.6	1466	185	196	619	1804	423
Hilo	**R**	1.3	153	120	561	22	282	69
	C	5.9	2134	140	417	660	1889	224
Webster	**R**	<1.0	0.06	0.07	0.8	4.5	3.0	41
Invertebrate Eco SSL		140	1700	NA	280	NA	120	80
Background in U.S. soils		0.3-0.5	20	40-50	15-20	5-7	18-25	55

in all reference and contaminated ESTCP soils were significantly higher than in *E. andrei* exposed to Webster soil. *E. andrei* survival and reproduction in Webster reference soil were well above validitry criteria, suggesting that observed results were related to test soils and not test organism health or standard test conditions.

Reduced survival was observed in about half of the ESTCP soils in which Enchytraeid survival was also below the validation limit (80% survival) for *En. crypticus* bioassays (OECD Guideline 220, 2003) (Table B-4). However, in only two contaminated soils (Mechanicsburg and Portsmouth), was the number of live adult *En. crypticus* on day 14 significantly lower than in

corresponding site reference soils, suggesting that only in these soils was an effect of metal contamination on survival observed. ORNL soil was intolerable by *En. crypticus* with complete mortality in both reference and contaminated soil. Significantly lower reproduction was observed in about half of the ESTCP soils in which reproduction was also below the validation limit (250 juveniles/10 adult worms) for *En. crypticus* reproduction bioassays (OECD Guideline 220, 2003). However, only in three contaminated soils (Mechanicsburg, Portsmouth, and Pearl) was *En. crypticus* reproduction significantly lower than in corresponding site reference soils, which means only in those soils was an effect of metal contamination on reproduction observed. Whole-body metal concentrations in *En. crypticus* suggest a general trend of increased metal bioaccumulation from the most contaminated soils where whole-body metal concentrations in potworms exposed to contaminated soils were significantly higher than potworms exposed to corresponding site reference soils or Webster soil, or both. In soils where metal concentrations were only marginally higher than in site reference soils, no increase in metal concentrations was observed. *En.crypticus* survival and reproduction in Webster reference soil were well above validitry criteria, suggesting that observed results were related to test soils and not test organism health or standard test conditions.

Collembola (*F. candida*) survival was reduced in seven ESTCP soils in six of which survival was also below the validation limit (80% survival) for Collembola bioassays (Table B-5) (ISO 11267 1999). However, in only three contaminated soils (Travis, Concord, ORNL) was the number of live adult Collembola on day 28 significantly lower than in corresponding reference soils, suggesting possible effects of metal contamination. Significantly lower reproduction was observed in about half of the ESTCP soils, but only in Cherry Point contaminated soil was reproduction below the validation limit (100 juveniles reproduced by 10 adult Collembola) for

Table B-3. *E. andrei* survival, reproduction, and tissue metal concentrations of Webster soil, ESTCP contaminated soils (C) and reference soils (R).

Soil		Survival on Day 28	Hatchlings on Day 56	Tissue concentration on Day 28 (mg/kg)						
				Cd	Cu	Pb	Zn	Cr	Ni	As
Webster		10	77	3.9	35	1.1	179	2.5	3.4	0.7
Mechanicsburg	R	9.8	28[24]	2.0	35	1.8	174	3.6	2.7	3.6*
	C	9.8	31[2]	2.0	33	6.6**	156	3.7	2.7	8.0**
Cherry Point	R	9.5	11[24]	1.3	22	0.9	102	1.9	0.9	5.0*
	C	10	36[2]	40**	64**	9.5**	187**	65**	15**	1.7[1]
Travis	R	10	27[24]	2.0	31	1.8	155	3.2	4.2	8.8*
	C	10	20[234]	1.8	508**	143**	170**	3.5	3.2	17**
Concord	R	9.5	76	2.4	37	1.6	147	5.2*	11*	1.7
	C	9.3	65[2]	2.0	27	0.4	135	2.0	4.1	110**
McCllelan	R	9.8	57[2]	3.3	34	0.7	153	2.1	2.8	2.0
	C	9.5	42[2]	86**	63**	5.3**	197**	18**	21**	4.1**
Portsmouth	R	9.8	53[2]	3.5	38	9.6*	154	3.6	2.9	4.0*
	C	9.5	44[2]	3.3	62**	295**	214**	3.1	4.8	4.5
Deseret	R	9.5	17[24]	3.5	19	1.5	137	2.6	2.2	5.5*
	C	7[234]	11[24]	8.7**	38**	4.1**	257**	4.8**	6.2**	138**
ORNL	R	9.8	10[24]	2.2	26	1.5	176	1.5	1.9	1.9
	C	9.3	7.0[24]	1.5	36**	490**	210**	1.6	1.5	2.5*
Pearl	R	9.8	11[24]	2.5	30	1.8	169	17*	16*	1.6
	C	10	31[2]	5.0	44	85**	251**	8.8[1]	21*	126**
Hilo	R	10	3.5[24]	2.0	21	22*	153	15*	40*	5.8*
	C	10	19[24]	2.9	51**	384**	372**	20	37	143**

For tissue metal concentration:

* Significantly higher (α =0.05) than Webster soil

** Significantly higher (α =0.05) than corresponding reference soil and Webster soil - blue

[1] Significantly lower (α =0.05) than corresponding reference soil, but significantly higher (α =0.05) than Webster soil

For survival and reproduction:

[2] Significantly lower (α =0.05) than Webster soil – highlighted in purple

[3] Significantly lower (α =0.05) than corresponding reference soil - highlighted in green

[4] Below the validation limit for bioassay set by Environment Canada (2004)

Table B-4. *En. crypticus* survival, reproduction, and whole-body metal concentrations in worms exposed to Webster reference soil, ESTCP contaminated soils (C) and reference soils (R).

Soil		Survival on Day 14	Hatchlings on Day 28	Whole-body concentration on Day 14 (mg/kg)						
				Cd	Cu	Pb	Zn	Cr	Ni	As
Webster		9.7	406	3.1	20	5.0	369	8.6	11	1.9
Mechanicsburg	R	6.5[24]	172[24]	5.4	14	4.3	447	3.1	4.7	0.9
	C	1.8[234]	80[234]	9.0**	19	6.6	498	7.9***	12***	7.2**
Cherry Point	R	4.8[24]	136[24]	2.5	21	5.3	301	7.1	6.1	7.8*
	C	8.3	322	34**	45**	52**	338	295**	30**	2.7
Travis	R	8.5	224[24]	8.1*	21	11*	334	17*	8.5	1.4
	C	5[24]	226[24]	2.7	24	124**	382	3.9	11	1.7
Concord	R	10	298	4.3	19	4.4	266	7.8	13	2.4
	C	7.5[24]	203[24]	2.9	14	5.4	351	6.5	14	17**
McCllelan	R	10	337	1.3	12	1.6	166	3.3	3.7	1.7
	C	8.5	306	46**	53**	24**	518**	66**	60**	13**
Portsmouth	R	7.8[24]	245[24]	2.8	13	20	285	3.7	2.3	1.8
	C	1.8[234]	0[234]	2.7	14	169**	827**	5.8	3.9	1.9
Deseret	R	10	331	2.2	9.4	12*	183	1.6	2.5	1.8
	C	10	299	4.4***	6.1	4.7	193	10***	12***	63**
ORNL	R	0[24]	0[24]	-----	-----	-----	-----	-----	-----	-----
	C	0[24]	0[24]	-----	-----	-----	-----	-----	-----	-----
Pearl	R	10	393	0.3	11	6.1	289	18*	13	1.3
	C	10	231[234]	4.0***	142**	421**	801**	58**	55**	213**
Hilo	R	5.5[24]	249[24]	2.1	13	78*	158	72*	118*	25*
	C	10	520	6.0**	38**	1458**	687**	68*	118*	510**

For tissue metal concentration:

* Significantly higher (α =0.05) than Webster soil

** Significantly higher (α =0.05) than corresponding reference soil and Webster soil – highlighted in blue

*** Significantly higher (α =0.05) than corresponding reference soil

For survival and reproduction:

[2] Significantly lower (α =0.05) than Webster soil – highlighted in purple

[3] Significantly lower (α =0.05) than corresponding reference soil – highlighted in green

[4] Below the validation limit for bioassay set by the OECD Guideline 220 (2003)

Table B-6. *Folsomia candida* survival and reproduction of Webster soil, ESTCP contaminated soils (C) and reference soils (R).

Soil		Survival on Day 28	Hatchlings on Day 42
Webster		9.9	529
Mechanicsburg	R	10	435
	C	9.5	251[23]
Cherry Point	R	10	331[2]
	C	10	97[234]
Travis	R	10	514
	C	6.3[234]	365[23]
Concord	R	10	482
	C	5.5[234]	372[23]
McCllelan	R	9.5	513
	C	9.8	367[23]
Portsmouth	R	9.3	308[2]
	C	8.8	365[2]
Deseret	R	5.5[24]	408
	C	10	425
ORNL	R	10	243[2]
	C	7.0[234]	279[2]
Pearl	R	10	452
	C	9.0	582
Hilo	R	7.0[24]	400
	C	8.5[2]	249[2]

For survival and reproduction:

[2] Significantly lower (α =0.05) than Webster soil.

[3] Significantly lower (α =0.05) than corresponding reference soil – highlighted in green

[4] Below the validation limit for bioassay set by ISO 11267 (1999).

Collembola reproduction bioassays (ISO 11267, 1999). In five contaminated soils (Mechanicsburg, Cherry Point, Travis, Concord, McCllelan), Collembola reproduction was significantly lower than in their corresponding reference soils, suggesting effects of metal contamination in these soils. *F. candida* survival and reproduction in Webster reference soil were well above validitry criteria, suggesting that observed results were related to test soils and not test organism health or standard test conditions.

In order to quantitatively assess the factors that affect metal bioaccumulation and toxicity to *E. andrei*, a literature review was conducted to assemble empirical models relating earthworm toxicity endpoints or whole-organism metal concentrations to total metal concentrations in soils and soil properties. One hundred and thirty-four models from 18 studies were collected with two models for As, 31 for Cd, one for Cr, 30 for Cu, three for Ni, 36 for Pb, and 31 for Zn. No models relating toxicity to metal concentrations and soil physicochemical characteristics were found, and all the models were bioaccumulation models using either bioaccumulation factor (BAF) or whole-organism metal concentration as a dependent variable. Each model had its own specific earthworm species and ranges of soil parameters that it described. All the models are listed in Appendix B-1 with a summary of the studies from which these models were collected.

Data on soil properties and total metal content of ESTCP soils were applied to these models, and predicted values of BAF or whole-organism metal concentrations were obtained. Then, the correlation between predicted values and values actually measured by bioassay was examined, and the correlation coefficients (R^2) were listed in Appendix B-1.

There were only two models for As, but one of them provided a good fit to the ESTCP data ($R^2 = 0.91$, RMSE = 24.2%, Figure B-1) with total soil As concentration accounting for about 91% of the variability observed in earthworm As concentrations. This correlation should be interpreted with caution since there are two groups of points, one including very low levels of As in soil while the other group of data points represent As contaminated sites. The grouping of data points was similar for Cr (Figure B-2) and Ni (FigureB- 3), both of which had just a few models in the literature, especially models relating bioaccumulation to soil property parameters. However, the total soil concentrations of these metals accounted for a large portion of variability in earthworm tissue concentrations of these metals, 73% for Cr and 88% for Ni. Although the

regression model fit was good, predicted Ni concentrations in earthworms were much higher than measured values (RMSE = 689%), resulting in poor predictive ability for this model. This may be a result of extrapolation since the range of Ni concentrations in soils from which the model was developed was very narrow (1.3-2.4 mg/kg) while ESTCP soils had a much wider range of Ni concentrations (3.5 to 561 mg/kg). However, for Cr, the model under-predicted Cr bioaccumulation but, with a RMSE = 13.6%, this model provided good predictive agreement.

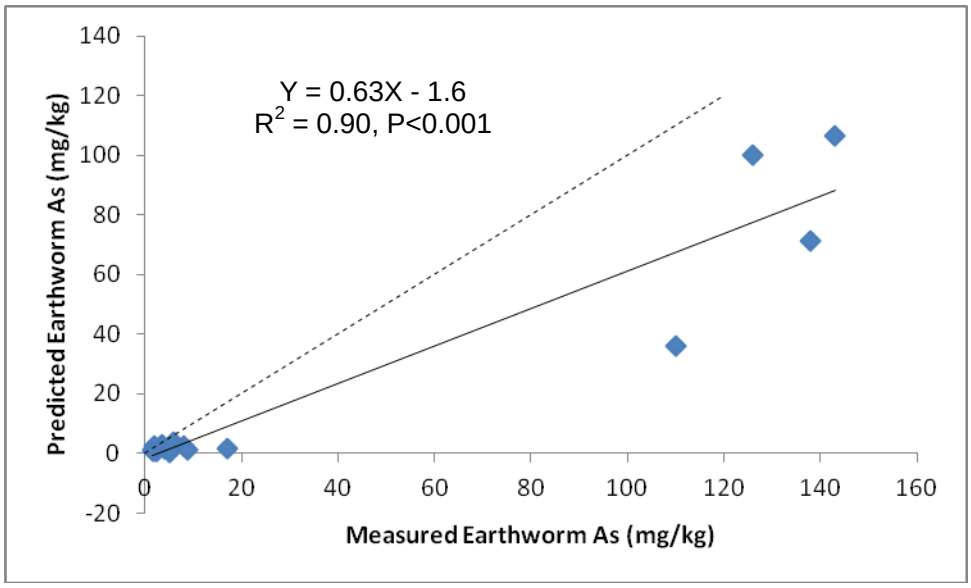

Figure B-1. Relationship between predicted and measured As concentrations in earthworms (*Eisenia andrei*) exposed to ESTCP soils as predicted by the model: ln As_{ew}=0.9884*ln As_s-1.747 (Sample et al. 1998). Dotted line is 1:1 predicted:measured As.

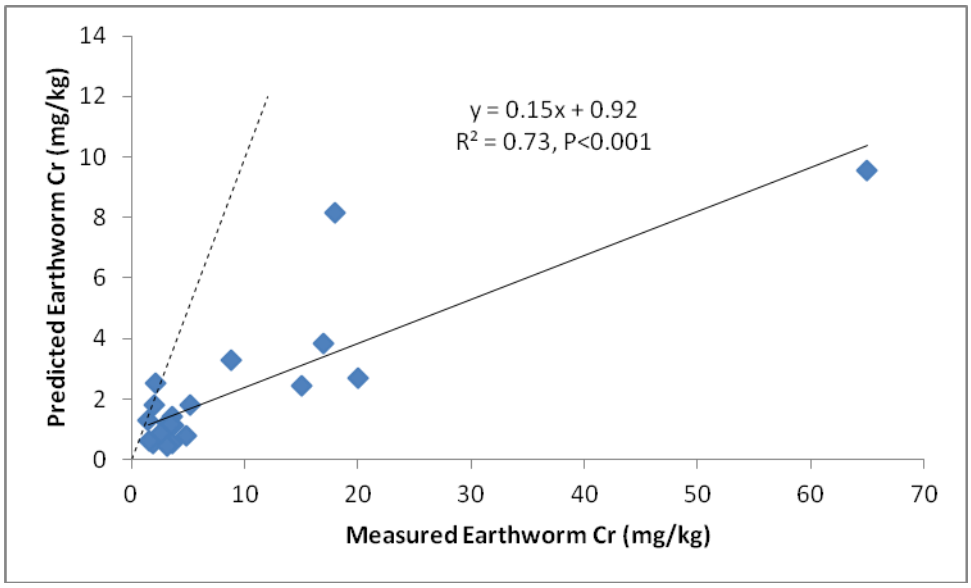

Figure B- 2. Relationship between predicted and measured Cr concentrations in earthworms (*Eisenia andrei*) exposed to ESTCP soils as predicted by the model: log Cr_{ew}=0.69*log Cr_s-1.05 (Peijnenburg et al. 1999a). Dotted line is 1:1 predicted:measured Cr.

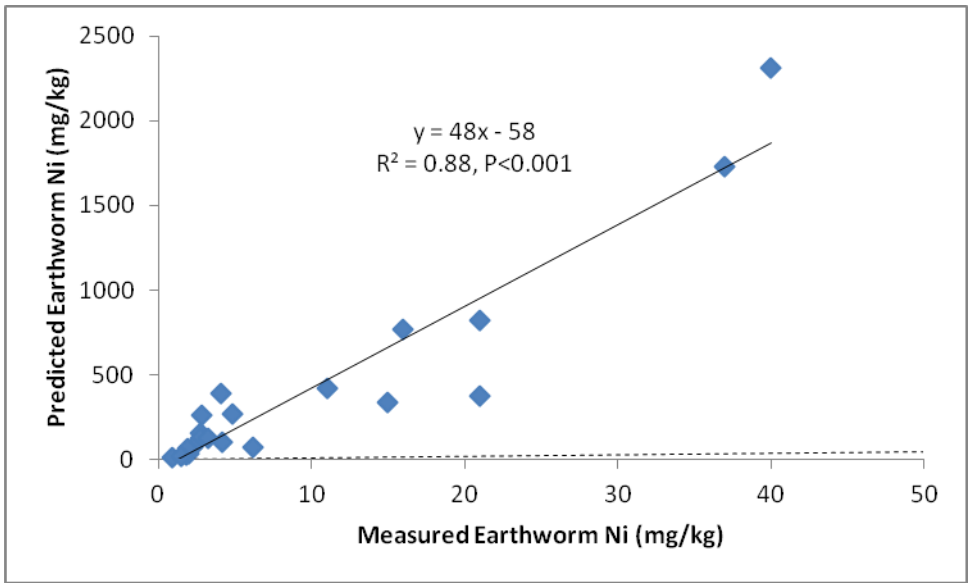

Figure B-3. Relationship between predicted and measured Ni concentrations in earthworms (*Eisenia andrei*) exposed to ESTCP soils as predicted by the model: log Ni_{ew}=0.98*log Ni_s+0.67 (Neuhauser et al. 1995). Dotted line is 1:1 predicted:measured Ni.

There were 31 models for Cd, and 16 of them used total soil Cd concentration as the only parameter in the model. Among these models, the largest R^2 value was 0.82, suggesting that total soil Cd concentration could account for about 82% of the variability in earthworm Cd concentrations. The best fitting model was a model using total soil Cd concentration, soil pH, and organic matter content (OM) as parameters, and these three parameters could account for about 98% of the variability in earthworm Cd concentrations (Figure B-4). Although such a high R^2 value was partly due to the nature of our data (only eight data points were included because total soil Cd concentrations in some ESTCP soils were below detection limits, and of the eight data points, most were in the low concentration range), that model was still the best compared to the other 30 models. Although this model fit the ESTCP data very well, it had a poor predictive capacity with a RMSE of 106% .

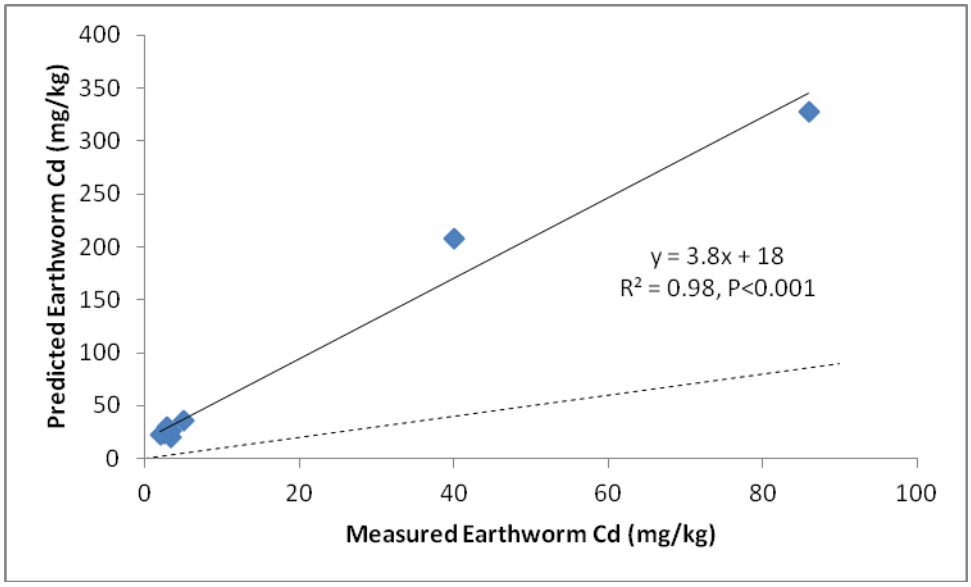

Figure B-4. Relationship between predicted and measured Cd concentrations in earthworms (*Eisenia andrei*) exposed to ESTCP soils as predicted by the model: ln Cd_{ew}=6.018+0.787*ln Cd_s-0.106*OM-0.402*pH (Ma et al. 1983). Dotted line is 1:1 predicted:measured Cd.

One issue related to these empirical, multiple-regression type models is that they do not consider metal uptake kinetics in predicting bioaccumulation. Cd uptake kinetics have been shown to be linear over short time periods (as in these ESTCP tests) and not reach steady state during experimental exposure (Spurgeon and Hopkin 1999; Sheppard et al. 1997; Neuhauser et al. 1995; van Gestel et al. 1993), but recent models have been developed that describe Cd uptake kinetics and estimate steady-state concentrations over longer time periods (Yu and Lanno, 2010). In this recent study, one of the exposure concentrations was 20 mg Cd/kg in Webster soil, resulting in the following model:

$$C_w = 9.32*e^{-0.008*28} + Cd_s*0.052/0.008*(1- e^{-0.008*28})$$

where C_w is the total Cd concentration in the earthworm and C_s is the total Cd concentration in the soil. Application of this model to predicting steady-state Cd bioaccumulation by *E.andrei* in two ESTCP soils (Cherry Point and McLellan) that had total Cd concentrations similar to that used in model development (19 and 22 mg Cd/kg, respectively) resulted in a reasonable approximation for Cd uptake by *E. andrei* exposed to Cherry Point soil (40 mg Cd/kg measured vs 32 mg Cd/kg predicted), but under-predicted Cd bioaccumulation in McLellan soil (86 mg Cd/kg measured vs 36 mg Cd/kg predicted). When the remainder of the ESTCP soils were included in the model estimation, the kinetics model provided accepatable predictive capacity with a RMSE of 19%, based upon eight data points.

There were 36 models for Pb, and 20 of them used total soil Pb concentration as the only parameter in the model. Among these models, the largest R^2 value was 0.62 suggesting that total soil Pb concentration could account for about 62% of the variability in earthworm Pb concentrations at most. Including soil pH as another parameter in the model significantly

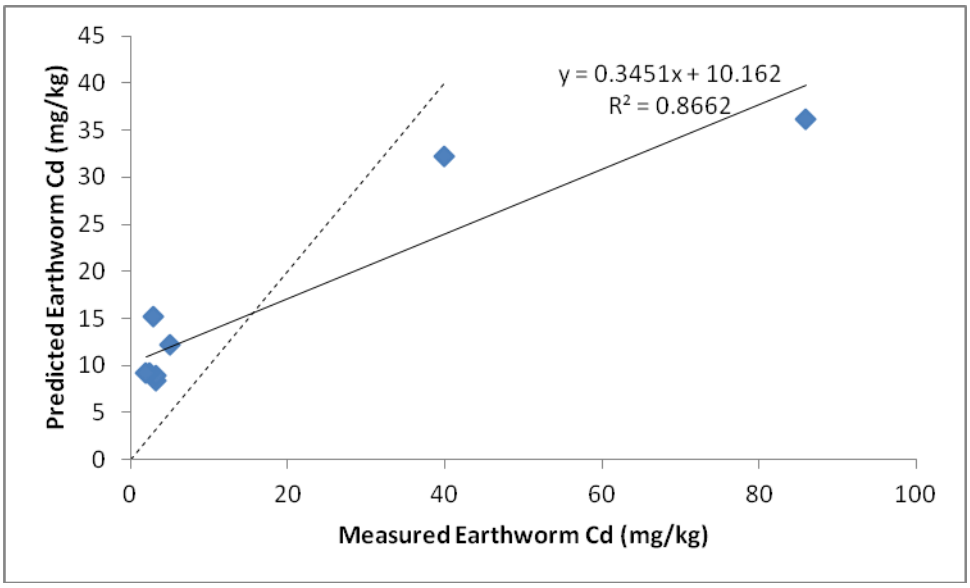

Figure B-5. Relationship between predicted and measured Cd concentrations in earthworms (*Eisenia andrei*) exposed to ESTCP soils as predicted by the model: $C_w = 9.32*e^{-0.008*28} + Cd_s*0.052/0.008*(1- e^{-0.008*28})$ (Yu and Lanno 2010). Dotted line is 1:1 predicted:measured Pb.

improved the explanatory ability of the model, with R^2 values increasing to around 0.9, but adding OM or CEC to the model did not improve the relationship significantly. The best fitting model included total soil Pb concentration and soil pH as parameters and accounted for about 95% of the variability in earthworm Pb concentrations (Figure B-6). However, the model consistently over-predicted Pb bioaccumulation, with RMSE of 272%.

There were 30 models for Cu, and 23 of them incorporated total soil Cu concentration as the only parameter in the model. Among these models, the best accounted for about 45% of the variability in earthworm Cu concentrations (Figure B-7), under-predicted Cu bioaccumulation (RMSE = 24.7%). Adding soil property parameters such as soil pH or OM to the model did not improve the model significantly. The situation was the same for Zn. There were 31 models for Zn, and 22 of them used total soil Zn concentration as the only parameter in the model. Total soil Zn

concentration could account for about 62% of the variability in earthworm Zn concentrations, but

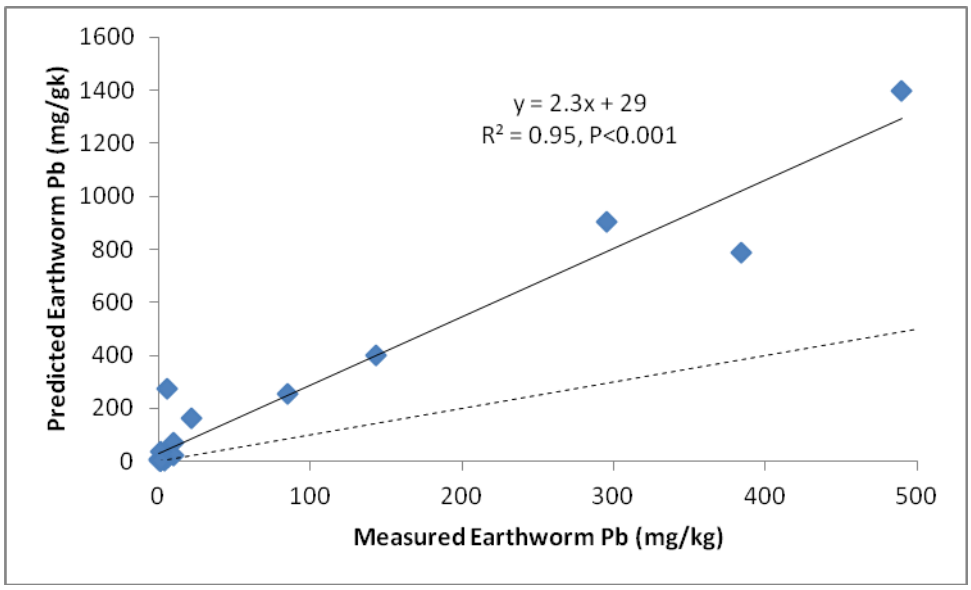

Figure B-6. Relationship between predicted and measured Pb concentrations in earthworms (*Eisenia andrei*) exposed to ESTCP soils as predicted by the model: log Pb_{ew}=2.65+0.897*log Pb_s-3.56*log pH (Corp and Morgan 1991). Dotted line is 1:1 predicted:measured Pb.

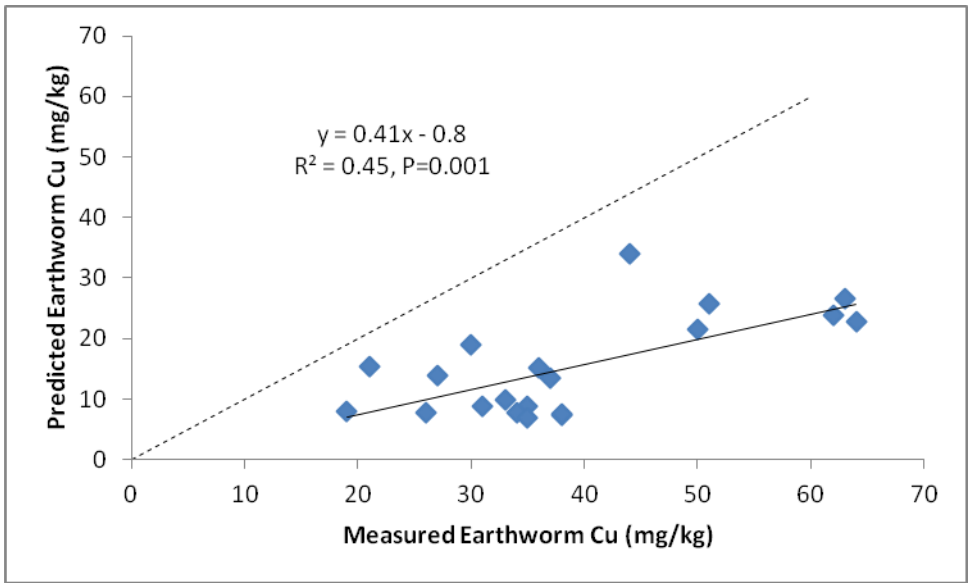

Figure B-7. Relationship between predicted and measured Cu concentrations in earthworms (*Eisenia andrei*) exposed to ESTCP soils as predicted by the model: log Cu_{ew}=0.435*log Cu_s+0.39 (Morgan and Morgan 1988). Dotted line is 1:1 predicted:measured Cu.

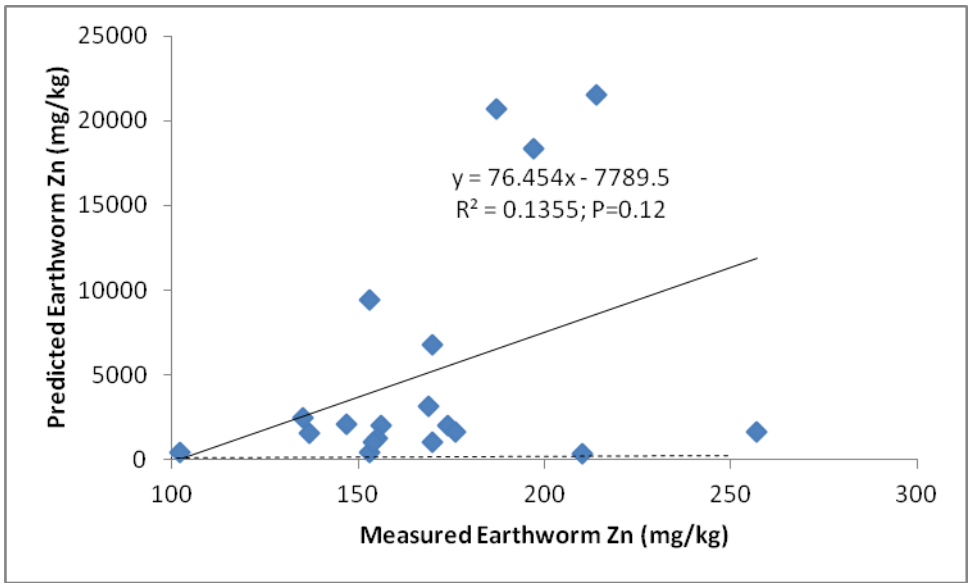

Figure B-8. Relationship between predicted and measured Zn concentrations in earthworms (*Eisenia andrei*) exposed to ESTCP soils as predicted by the model: log Zn_{ew}=1.45*log Zn_s+0.42 **(**Peijnenburg et al. 1999a). Dotted line is 1:1 predicted:measured Zn.

this model over-predicted extremely high Zn concentrations in earthworms (RMSE = 590%) (Figure B-8). Adding soil property parameters such as soil pH or OM to the model did not significantly improve the model.

In order to quantitatively assess the factors that affect metal accumulation and toxicity to *En. crypticus*, a literature review was performed to assemble empirical models relating Enchytraeid toxicity endpoints or tissue metal concentrations to total metal concentrations in soils and soil properties. No toxicity models were found, and three bioaccumulation models using

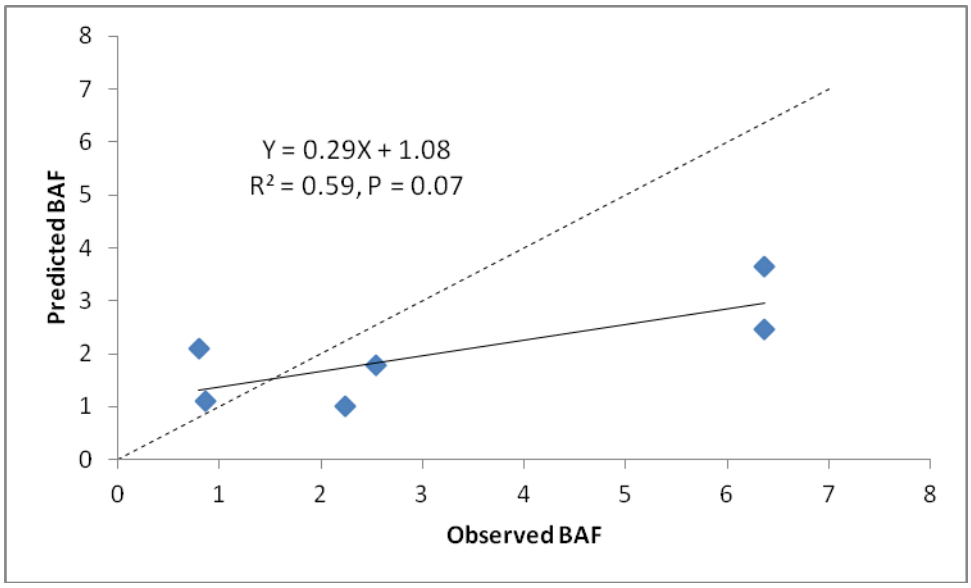

Figure B-9. Relationship between Cd BAF predicted by model: log BAF=1.17-0.92*log Clay (Peijnenburg et al. 1999b) and Cd BAF measured in *En. crypticus* exposed to ESTCP soils. Dotted line represents 1:1 predicted:observed.

bioaccumulation factor (BAF) as a dependent variable from Peijnenburg et al. (1999) were assembled with for Cd, Pb, and Zn. Data on soil properties and total metal content of ESTCP soils were applied to these models, and predicted values of BAF were obtained. The relationship between predicted BAFs and measured BAFs for enchytraeids exposed to ESTCP soils (Figures B-9-B-11) was very weak for Pb and Zn, but provided a good fit for Cd (RMSE = 21%), based upon four data points.

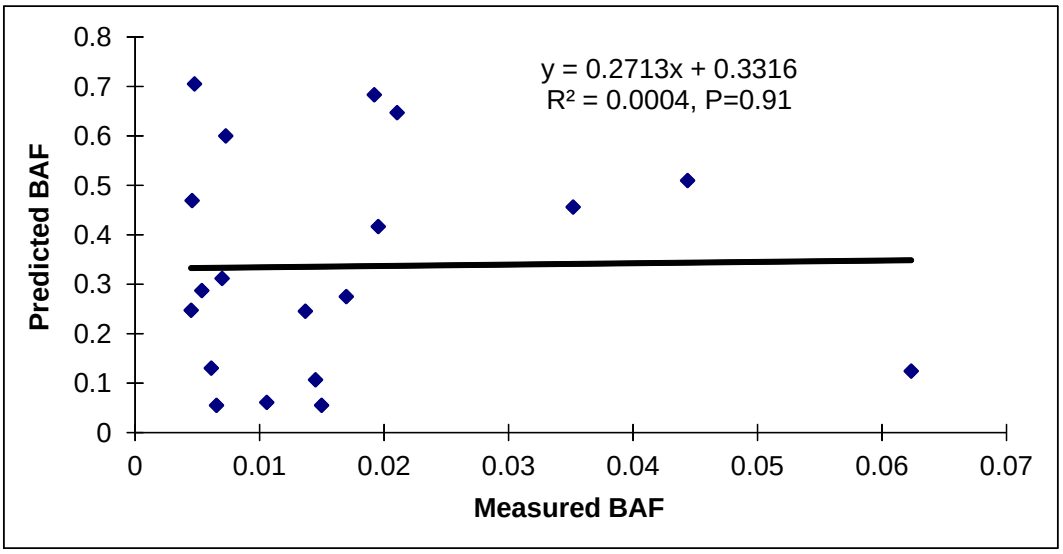

Figure B-10. Relationship between Pb BAF predicted by model: log BAF=0.35-0.36*pH (Peijnenburg et al. 1999b) and Pb BAF measured in *En. crypticus* exposed to ESTCP soils.

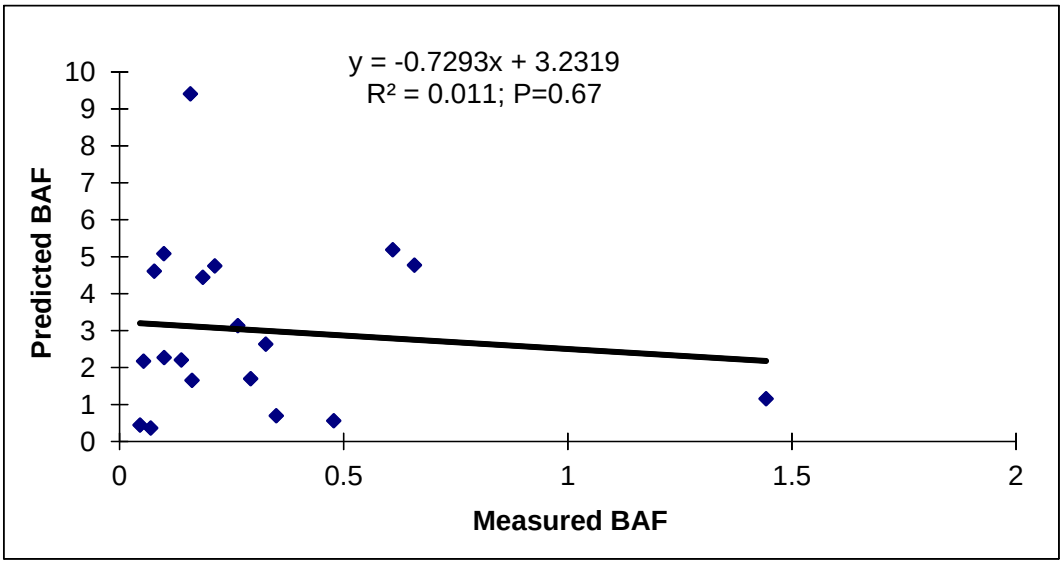

Figure B-11. Relationship between Zn BAF predicted by model: log BAF=3.47-0.46*pH-0.67*log Al_{ox} (Peijnenburg et al. 1999b) and Zn BAF measured in *En. crypticus* exposed to ESTCP soils.

A literature search was conducted for models relating metal toxicity or bioaccumulation in Collembola to total metal concentrations and soil physical/chemical properties, but none was found.

For many of the ESTCP contaminated soils, 0.5 M $Ca(NO_3)_2$-extractable metal levels were below limits of quantitation (Table B-6). No As or Cr was detected in 0.5 M $Ca(NO_3)_2$ extracts from any of the soils and Cd was only detected in Cherry Point and McCllelan soils. Pb was detected in 0.5 M $Ca(NO_3)_2$ extracts from five soils (Hilo, McCllelan, ORNL, Portsmouth, Travis) and extractable Pb ranged from 2-62% of measured total metal levels. Zn was detected in 0.5 M $Ca(NO_3)_2$ extracts from four soils (Cherry Point, Hilo, McCllelan, Portsmouth) and extractable Zn ranged from 10-38% of measured total metal levels.

Table B-6. Extractable (0.5 M $Ca(NO_3)_2$) metal content (mg/kg) of ESTCP contaminated soils. Values in parentheses are the percent of total metal that is extractable with a 0.5 M $Ca(NO_3)_2$ solution.

	As	Cd	Cr	Pb	Zn
Concord	<20	<2	<2	<2	<20
Cherry Point	<20	16.6(88%)	<2	<2	109 (22%)
Deseret	<20	<2	<2	<2	<20
Hilo	<20	<2	<2	33 (1.5%)	535 (28%)
McCllelan	<20	15.5(71%)	<2	27(14%)	171 (38%)
Mechanicsburg	<20	<2	<2	<2	<20
ORNL	<20	<2	<2	603(62%)	<20
Pearl	<20	<2	<2	<2	<20
Portsmouth	<20	<2	<2	507(17%)	52 (10%)
Travis	<20	<2	<2	48 (2.4%)	<20

Models developed in previous studies (Lanno and Basta 2003) that established correlations between 0.5 M $Ca(NO_3)_2$-extractable metals and bioaccumulation by earthworms were also used

to predict metal bioaccumulation by earthworms exposed to ESTCP soils. Lanno and Basta (2003) models were based upon 22 soils differing in physicochemical properties that were amended with one concentration of either Pb or Zn, or three concentrations of Cd, allowing equilibration with the soils to determine the bioavailable fraction of metals. The model in Figure B-12 relating log Pb concentration in earthworms to log 0.5 M $Ca(NO_3)_2$-extractable Pb (R^2 – 0.39) was based upon soils amended with 2,000 mg/kg Pb. Except for McCllelan, this model tended to under-predict Pb bioaccumulation by *E. andrei* (Figure B-13; R^2 – 0.43; RMSE – 161%).

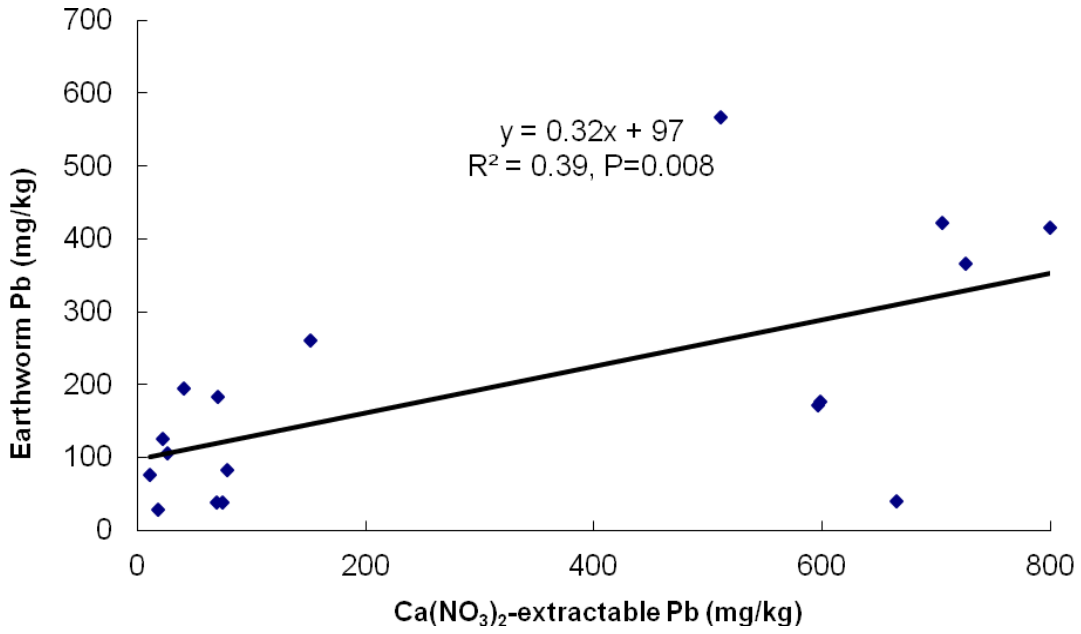

Figure B-12. Relationship between Pb concentrations in earthworms and 0.5 M $Ca(NO_3)_2$-extractable Pb. Model was developed using 22 soils differing in physicochemical characteristics amended with 2,000 mg/kg Pb.

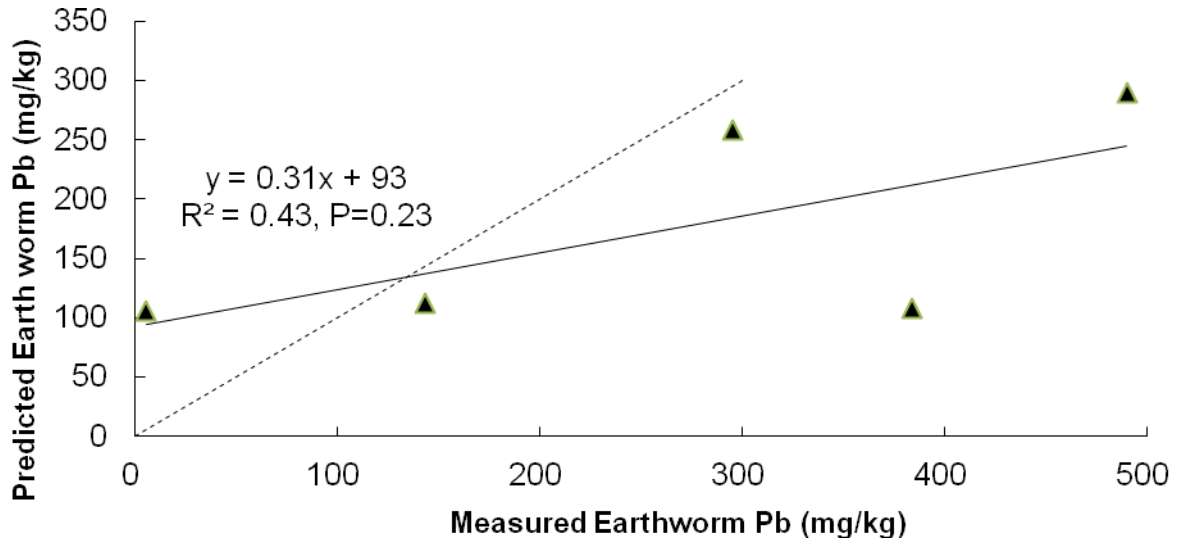

Figure B-13. Relationship between predicted and measured Pb concentrations in earthworms (*Eisenia andrei*) exposed to ESTCP soils as predicted by the model: $\log Pb_{worm} = 0.343 \log Pb_{0.5\ M\ Ca(NO3)2\text{-}extractable} + 1.4$. Dotted line represents 1:1 predicted:measured.

A regression model applied to the data in Figure B-14 relating log Zn concentrations in earthworms to log 0.5 M $Ca(NO_3)_2$-extractable Zn (R^2 – 0.08) was not significant (P = 0.21). These soils were all amended with the same concentration of Zn, 300 mg/kg, and the mean (±95% CL) Zn concentration in earthworms was 145 (±7.1) mg/kg. The mean (±95% CL) Zn concentration in earthworms exposed to ESTCP soil (Table B-3) was 183 (±23.2) mg/kg. Although these means are statistically different, they are not expected to cause biological effects as the mean Zn concentration in earthworms exposed to Webster reference soil was 179 mg/kg.

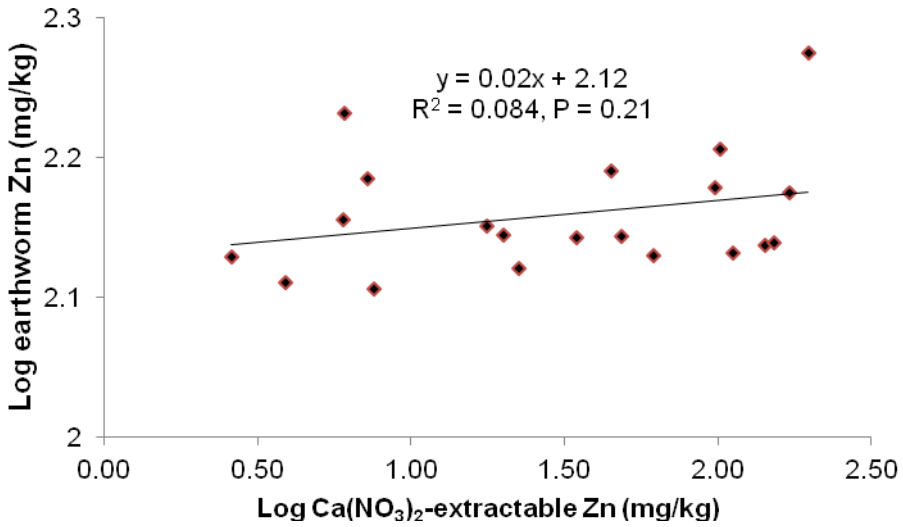

Figure B-14. Relationship between log Zn concentrations in earthworms and log 0.5 M $Ca(NO_3)_2$-extractable Zn. Model was developed using 22 soils differing in physicochemical characteristics amended with 300 mg/kg Zn.

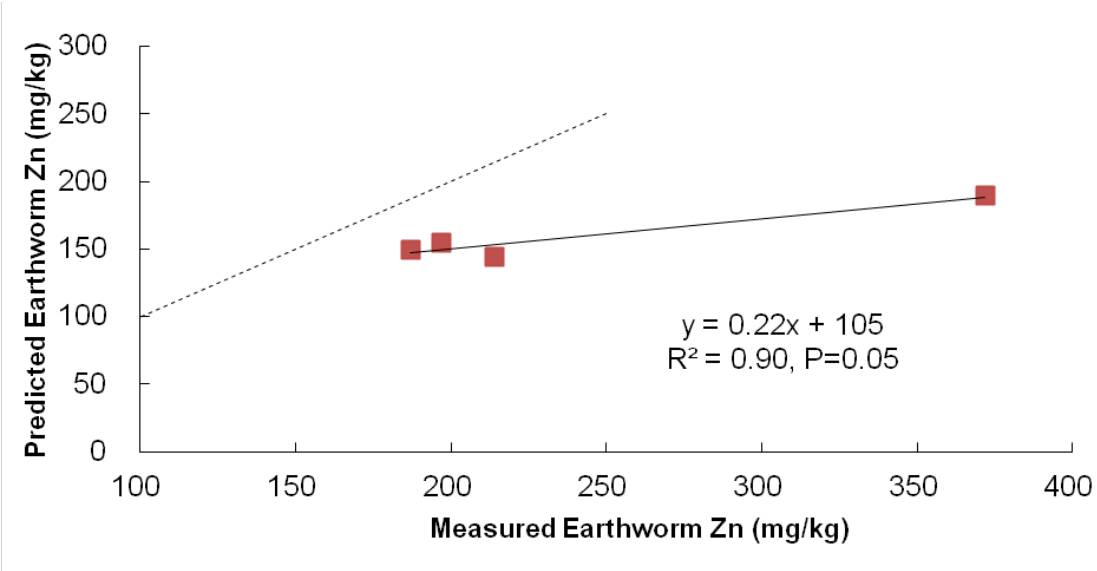

Figure B-15. Relationship between predicted and measured Pb concentrations in earthworms (*Eisenia andrei*) exposed to ESTCP soils as predicted by the model: $\log Zn_{worm} = 0.02 \log Zn_{0.5\ M\ Ca(NO3)2\text{-}extractable} + 2.1$. Dotted line represents 1:1 predicted:measured.

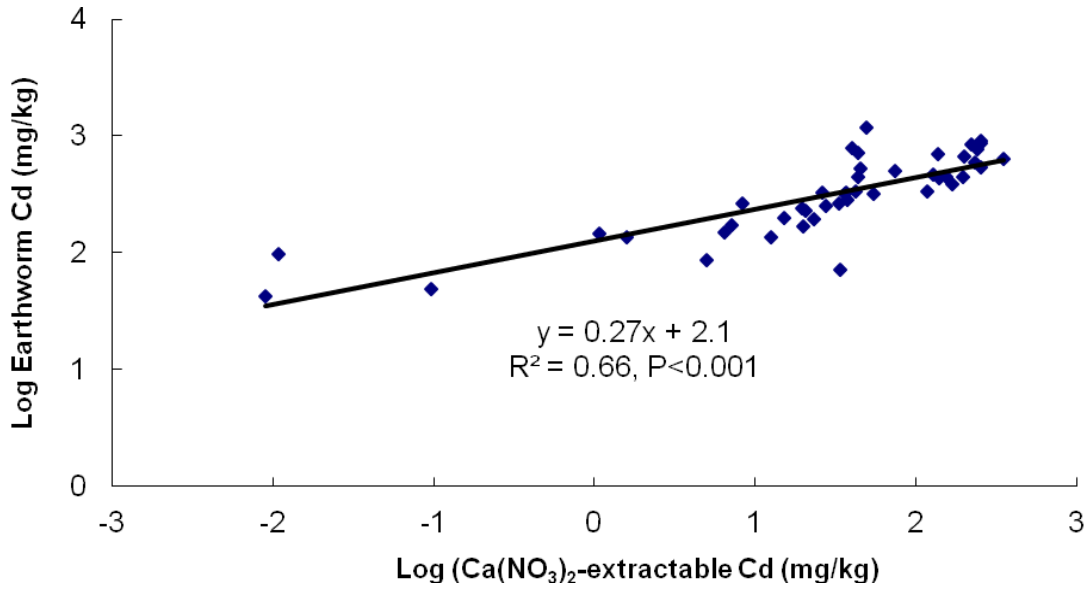

Figure B-16. Relationship between log Cd concentrations in earthworms and log 0.5 M $Ca(NO_3)_2$-extractable Cd. Model was developed using 27 soils differing in physicochemical characteristics and Cd amendment levels (nominal amended levels: 10, 50, or 300 mg/kg Cd).

The model in Figure B-16 relating log Cd concentrations in earthworms to log 0.5 M $Ca(NO_3)_2$-extractable Cd (R^2 – 0.66) was based upon 27 soils differing in physicochemical characteristics and amended with three different levels of Cd, either 10, 50, or 300 mg/kg Cd (nominal). Since there were only two ESTCP soils for which $Ca(NO_3)_2$-extractable Cd was detectable (Cherry Point and McCllelan) a correlation analysis could not be conducted. Measured Cd concentrations in worms exposed to Cherry Point and McCllelan soils were 40 and 86 mg/kg, respectively and the model over-predicted Cd bioaccumulation. The model predicted Cd concentrations in earthworms of 271 and 266 mg Cd/kg for worms exposed to Cherry Point and McCllelan soils, respectively.

Discussion

Soil composition was the dominant factor affecting reproduction in *E. andrei*, with hatchling production significantly lower in 95% of the ESTCP soils (reference and contaminated) compared to the Webster reference soil, but did not markedly affect adult earthworm survival. Reproduction tests conducted with *E. crypticus* and *F. candida* were less affected by soil composition with 57% and 33% of the tests, respectively, showing significantly lower reproduction than test organisms exposed to the Webster reference soil. All reproduction and survival tests conducted with Webster reference soil met validation criteria for the respective tests, suggesting healthy test organisms were used in all tests. Since ESTCP test soil composition was a variable influencing the outcome of the tests, it would be reasonable to compare test organism responses to their respective site reference soils if responses in both soils were lower than in the Webster lab reference soil. For tests conducted with *E. andrei*, in only two cases, survival in Deseret soil and reproduction in Travis soil, were earthworm responses significantly lower in contaminated compared to site reference soils (Table B-3). Arsenic appeared to be the only element in Deseret soil that was elevated relative to its site reference soil, but As levels were still lower than in Hilo (660 mg As/kg) and Pearl (619 mg As/kg) soils, where no significant mortality was observed. Whole-body residues of As were similar in worms exposed to contaminated Deseret, Hilo, and Pearl soils and were in a range that was not associated with mortality in previous studies (Lanno and Basta 2003). It would appear that increased mortality observed in worms exposed to contaminated Deseret soil was not due directly to the effects of As levels in the soil. Similarly, in Travis soil, Pb appeared to be the only metal that was elevated to a range where toxicity might occur, and decreased reproduction in earthworms exposed to contaminated Travis soil would not seem to be caused by elevated Pb concentrations alone. In

tests with *En. crypticus*, survival and reproduction were lower in contaminated Mechanicsburg and Portsmouth soils relative to their site reference soils, and reproduction was reduced in Pearl soil relative to its reference soil. Metal levels in soil do not explain potworm responses in contaminated Mechanicsburg soil as only Pb levels are significantly higher than the site reference (Table B-2), but do not fall in a range that would be toxic to potworms. Potworms exposed to contaminated Mechanicsburg soil accumulated significantly higher levels of As relative to potworms exposed to the site reference (Table B-4), but these levels were much lower than in potworms exposed to other ESTCP soils where no effects on survival or reproduction were observed, so As is not a likely cause of the observed effects (Karjalainen et al. 2009). Contaminated Portsmouth soil was particularly toxic to *En. crypticus*, with Pb and Zn levels significantly higher than in site reference soil. Together with potworms exposed to Pearl soil, Zn concentrations in potworms exposed to Portsmouth were the highest of all the ESTCP soils tested. Reproduction in potworms exposed to Pearl soil was also reduced significantly relative to the site reference soil (Table B-4). In contrast to Portsmouth soil, contaminated Pearl soil not only had elevated levels of Pb and Zn, but also Cu and As relative to the site reference soil. However, the toxicity was much less than observed in Portsmouth. Responses of collembola (*F. candida*) were affected much less by the physicochemical properties of ESTCP soils (Table B-5), as would be expected since collembolans are in contact much less with soil than oligochaetes and dermal absorption of metals is greatly reduced. General trends suggest that for collembola exposed to Mechanicsburg, Travis, and ORNL soils, Pb was the only element with elevated concentrations relative to site reference soils and may be responsible for the observed effects. In addition to elevated Pb concentrations, As and Zn were also elevated in Hilo soil relative to site reference soil. In collembola exposed to Cherry Point and McCllelan soils, Cr levels were

elevated relative to site reference soils and may be responsible for the observed effects. Similarly, As appears to be responsible for observed effects in Concord soil.

Comparing total metal levels in all the ESTCP soils reveals that some exceeded their respective US EPA EcoSSLs (see Table B-2 for EcoSSL values). EcoSSLs are total contaminant concentrations in soil that are protective of ecological receptors that commonly come into contact with soil or ingest biota that live in or on soil. These are very conservative values that consider bioavailability and can be used to identify those contaminants of potential concern in soils requiring further evaluation in a baseline ecological risk assessment and are not soil quality standards. Although there was observed toxicity in at least one test species in all soils in which total metal levels exceeded EcoSSLs, toxicity was also observed in soils (Mechanicsburg, Deseret, Concord, ORNL) where no metal levels exceeded EcoSSLs. This may be attributable to toxicity resulting from metals with no available EcoSSL (e.g., As in Deseret), extremes in soil physical or chemical parameters (e.g., pH in ORNL), or other physical or chemical factors in the soils. EcoSSLs don't appear to provide much insight for assessing the potential toxicity of the DoD soils.

A survey of the literature did not reveal any regression-type models relating earthworm toxicity endpoints, such as mortality and reproduction, to total soil metal concentrations and soil properties. In the ESTCP studies, little mortality was observed, but decreased reproduction was observed in almost all the ESTCP soils and could be related to either the effects of soil matrix or the effects of metal contamination. Soil properties, particularly reduced organic carbon and pH, as well as texture extremes, have been shown to negatively impact the reproduction of oligochaetes during soil toxicity tests (Kuperman et al. 2006; Chelinho et al. 2011). ESTCP soils with low organic carbon content (<1.5%) and extreme textures (>50% sand) were

apparently not suitable for *E. andrei* reproduction even though no metal contamination existed and organisms were fed during bioassays, resulting in poor reproduction even in many uncontaminated reference soils. Compared to the corresponding reference soils, reproduction was significantly lower in only two contaminated soils, which could possibly be related to the metal toxicity. However, for the cases where reproduction rates in site reference soils were below validation limits (Environment Canada 2004), comparisons with responses in matched metal-contaminated soil would still be necessary and useful for the interpretation of potential toxic effects of metals. Feral *E. andrei* do not naturally inhabit soils low in organic matter and the relevance of data generated from *E. andrei* bioassays for ecological risk assessment should be used with caution, but few other standardized tests for soil-dwelling organisms exist as alternatives. The relevance of *E. andrei* as a model organism may be better understood if tests on the same soils with other indigenous earthworm species provided similar results. Other soil invertebrates that could live and reproduce in the specific soil texture may also be an alternative. For soils where no other species can be tested, earthworms may still be a possible choice since robust methods and standard protocols exists for earthworm bioassays, but additional information is needed on the effects of soil physicochemical parameters on reproduction.

The Terrestrial Biotic Ligand Model (TBLM) predicts the toxicity of metals in soils based upon estimating the free metal ion species in soil using a speciation model (WHAM VI; Tipping 1998) and relating that to binding of the metal to a biotic ligand resulting in observed toxicity (Thakali et al. 2006). To date, TBLM models exist only for Cu and Ni, limiting any application to the ESTCP data set since the TBLM is not designed to exam the toxicity of metal mixtures. Additionally, in soils where Cu and Ni levels were above their respective EcoSSL values, no decrease in *Eisenia* reproduction was observed relative to the reference soils.

Earthworm metal bioaccumulation models are usually developed from laboratory bioassays conducted with a specific earthworm species and a set of soils with a limited range of soil parameters. In order to be widely applied in ecological risk assessment, these models need to be validated with different data sets, especially if the model is to be applicable to field-contaminated soils. In this report, we examined the application of literature-based earthworm metal bioaccumulation models using data from bioassays conducted with metal-contaminated ESTCP field soils.

The predictive capacity of earthworm bioaccumulation models in the literature relating tissue metal concentrations to total soil metal concentrations and soil properties differs among metals. Few studies have examined the bioaccumulation of As, Cr, and Ni in earthworms and there are few models predicting tissue concentrations of these metals in relation to soil properties. However, among these few models, good relationships between measured metal levels in earthworms exposed to ESTCP soils and metal levels predicted by the models were found (R^2 range – 0.73-0.90), all using total soil concentration as the only independent variable (Figures B-1-B-3). Acceptable relationships between predicted and measured As (RMSE = 24.2%) and Cr (RMSE = 13.6%) were observed (Table B-7). The correlation observed between measured and predicted Cr levels in earthworms was contrary to that of Sample et al. (1998) which indicated that Cr concentrations in earthworms were poorly predicted by total soil Cr concentrations. Chromium concentrations predicted by the best fitting model (R^2 – 0.73) (Peijnenburg et al. 1999a) under-predicted Cr concentrations measured in earthworms exposed to ESTCP soils (Figure B-2, Table B-7). The bioaccumulation of Cr is highly dependent on chemical species, with Cr(VI) being more bioavailable than Cr(III) (Eisler 1986). Therefore, the difference between predicted and measured earthworm Cr concentrations may be related to the differences

in Cr speciation between soils used to develop the model and ESTCP soils. The available data for Ni in the literature were contradictory, indicating either positive, negative, or no correlation between total soil Ni and worm Ni concentrations (Neuhauser et al. 1995; Abdul Rada and Bouché 1995; Beyer et al. 1982; Sample et al. 1998). Also, the best fitting model for Ni in our study (R^2 – 0.88; Neuhauser et al. 1995; RMSE = 689%) grossly over-predicted earthworm Ni concentrations (Figure B-3; Table B-7), likely due to the narrow range of soil Ni from which the model was developed and extrapolation beyond Ni concentrations used in model development for the ESTCP soils.

Several studies focused on the bioaccumulation of Cd and Pb in earthworms, which provided a substantial number of models for comparison. For Cd, tissue concentration was best predicted by a model comprising total soil Cd, OM, and soil pH as the independent variables (Figure B-4), while for Pb, tissue concentrations were best predicted by a model using total soil Pb and soil pH as the independent variables (Figure B-6). Both Cd (RMSE = 106%) and Pb (RMSE = 106%) over-predicted metal levels in earthworms. Negative coefficients associated with these soil parameters indicated that increases in these parameters would decrease metal bioaccumulation in earthworms. This was consistent with the findings of several studies (Beyer et al. 1987; Janssen et al. 1997; Ma 1982; Morgan and Morgan 1988; Peijnenburg et al. 1999a; Peramaki et al. 1992). Cadmium and Pb are non-essential metals with little regulation of uptake by earthworms (Dallinger 1993; Van Gestel et al. 1993). Total soil metal concentration combined with soil properties accounted for greater than 90% of the variability in earthworm tissue burdens of these metals.

Although several bioaccumulation models existed for Cu and Zn, predictive capabilities were poor. The best model (Morgan and Morgan 1988) for Cu bioaccumulation explained about

45% of the variability in earthworm tissue burdens using total soil Cu as the independent variable (Figure B-7), while for Zn, about 62% of the variability in earthworm tissue burdens could be explained by total soil Zn concentrations (Peijnenburg et al. 1999a). However, this model over-predicted worm Zn concentrations by two to three orders of magnitude (RMSE = 590%; Figure B-8). Adding soil property parameters to the bioaccumulation models did not significantly improve the prediction. Therefore, other factors besides total soil metal concentration and soil properties affected the bioaccumulation of these metals. Both Cu and Zn are essential metals, and their uptake can be regulated by earthworms (Morgan and Morgan 1988; Van Gestel et al. 1993). Evidence of the regulation of Cu and Zn uptake was observed in earthworms exposed to the ESTCP soil since over a broad range of soil concentrations (30 to 1,889 mg/kg for Zn (63-fold); 1.0 to 423 mg/kg for Cu (423-fold)), tissue concentrations increased only slightly and remained within a range of 3.3 to 3.6-fold (102 to 372 mg/kg for Zn; 19 to 64 mg/kg for Cu), for Zn and Cu, respectively. The ability to regulate tissue levels of essential metals such as Cu and Zn explains why total soil metal concentrations combined with soil properties could not provide a reasonable prediction of earthworm tissue concentrations of Cu and Zn, especially if no toxicity was observed. Models predicting Cu and Zn levels in earthworms over the range of bioavailable soil metal concentrations that are not toxic to earthworms provide little useful information for risk assessment. The development of models that consider toxicity above the threshold of bioavailable essential metals (e.g., TBLM for Cu; Thakali et al. 2006) are required for assessing the risks of these metals to invertebrates in soils.

Another parameter often used to describe metal bioaccumulation is the bioaccumulation factor (BAF) which is the ratio of the concentration of metal in an organism to the concentration in the soil, at steady state with respect to the accumulation kinetics of the metal. Peijnenburg et al.

(1999b) developed models relating BAFs of Cd, Pb, and Zn in *En. crypticus* to soil physicochemical properties, and the application of these models to predicting BAF in *En. crypticus* exposed to ESTCP soils provided no correlations for Pb and Zn, but provided an acceptable prediction for Cd, based upon six soils (RMSE = 21%) (Figures B-9-B-11). Poor correlations for Pb and Zn may be due to the fact that assumptions regarding the independence of BAF with exposure concentration do not hold true for metals as they do with organic compounds that bioaccumulate and an inverse relationship had been observed between BAF and exposure concentration for both essential metals and non-essential metals (McGeer et al. 2003).

Models for predicting metal bioaccumulation in earthworms that incorporate total metal concentrations attempt to account for differences in the bioavailability of metals by using varying physicochemical characteristics as additional explanatory variables (Bradham et al. 2006). Another method of estimating the bioavailable fraction of metals in soils is to measure metal concentrations in aqueous extracts. Solutions ranging from distilled water to salt extracts of varying molarities have been used to estimate bioavailable metal concentrations (Lanno and Basta 2003). In an attempt to incorporate measures of the bioavailable fraction of metals in soils into a predictive model, models were developed relating 0.5 M $Ca(NO_3)_2$-extractable Cd, Pb, and Zn to earthworm metal residues (Lanno and Basta 2003; Figures B-12, B-14, B-16). However, for Cd and Pb, these models did not provide a better relationship between predicted and measured earthworm concentrations of Cd (RMSE – 111%) and Pb (RMSE – 161%) (Table B-7) than models selected from the literature that predicted earthworm metal concentrations based upon total metal levels and soil physicochemical characteristics (Figures B-4, B-6). The model relating 0.5 M $Ca(NO_3)_2$-extractable Zn to Zn concentrations in earthworms was not significant (P=0.21). These soils were all amended with the same concentration of Zn, 300

mg/kg, and the mean (±95% CL) Zn concentration in earthworms was 145 (±7.1) mg/kg. The mean (±95% CL) Zn concentration in earthworms exposed to ESTCP soil (Table B-3) was 183 (±23.2) mg/kg. Although these means are statistically different, they are not expected to cause biological effects as the mean Zn concentration in earthworms exposed to Webster reference soil was 179 mg/kg. In this case, the 0.5 M $Ca(NO_3)_2$-extractable Zn model provide better results and under-predicted (RMSE – 101%) literature models based upon total Zn concentrations and soil physicochemical characteristics. This is not unexpected since Zn concentrations are maintained at relatively constant internal concentrations in earthworms since Zn is an essential element.

Other variables that should be included in models predicting metal bioaccumulation in earthworms is exposure time and metal uptake kinetics (Yu and Lanno, 2010). For many non-essential elements (e.g., As, Cd, Pb), bioaccumulation is time-dependent with kinetics best described using a linear regression model over short-term exposures (e.g., up to 56 days). For this reason, bioaccumulation models relating metal concentrations in organisms to total soil metal concentrations and soil physicochemical characteristics may predict bioaccumulation over a specified, short duration of exposure but their relevance to predicting life-time metal bioaccumulation in soil invertebrates is unclear. In order to develop models for Cd bioaccumulation by earthworms, Yu and Lanno (2010) examined long-term uptake of Cd by *E. andrei* over a 224-day period and were able describe uptake using a one-compartment first order kinetics (1CFOK) model. Application of this model to predicting steady-state Cd bioaccumulation by *E.andrei* in two ESTCP soils (Cherry Point and McLellan) that had total Cd concentrations similar to that used in model development (19 and 22 mg Cd/kg, respectively) resulted in a very close approximation for Cd uptake by *E. andrei* exposed to Cherry Point soil

(40 mg Cd/kg measured vs 32 mg Cd/kg predicted), but under-predicted two-fold Cd bioaccumulation in McLellan soil (86 mg Cd/kg measured vs 36 mg Cd/kg predicted). When the entire set of ESTCP soils was included (Figure B-5), Cd bioaccumulation in earthworms was slightly over-predicted (RMSE – 19%). While kinetics-based models appear to hold some promise in predicting metal bioaccumulation, very few long-term kinetics studies are currently available for model development.

ESTCP soils were often contaminated with multiple metals and it was assumed that the effects of individual metals were additive, meaning there was no interaction between different metals with respect to toxicity or bioaccumulation. Application of bioaccumulation models to ESTCP data addressed each element independently without considering metal interactions on bioaccumulation. However, such assumptions may not always be valid since metal interactions exist and may affect metal bioaccumulation. For example, Bey et al. (1982) observed that although total soil Cd accounted for 87% of the variability of in Cd uptake by earthworms (82% in this study), inclusion of Zn in the model significantly improved the model fit and accounted for an additional 5% of variability. Increased concentrations of Zn in soil were negatively correlated with Cd in worms, theoretically due to the competition between Zn and Cd at uptake sites.

Species is also an important factor affecting metal bioaccumulation by earthworms, with several studies suggesting that endogeic species feeding on soil often accumulate higher metal concentrations than epigeic species feeding on surface organic litter (Langdon et al. 2005; Spurgeon and Hopkin 1996; Morgan and Morgan 1992, 1993, 1999; Dai et al. 2004; Beyer et al. 1987). Some studies have developed different sets of models for different species (Laszczyca et al. 2004; Morgan and Morgan 1988; van Vliet et al. 2005; Wright and Stringer 1980). Corp and

Morgan (1991) even differentiated models between native and introduced *Lumbricus rubellus*. In ESTCP tests, *E. andrei*, a robust species that can easily be cultured in large quantities in the laboratory, was used as the test organism. *E. andrei* has a higher reproductive rate and a shorter generation time than other species, and is responsive to a wide range of contaminants. However, the choice of *E. andrei* in toxicity and accumulation studies has been a source of criticism, principally because it is not naturally a soil-dwelling species but inhabits environments rich in organic matter such as compost piles. Additionally, since it is an epigeic species (Bouché 1972), some studies have found that *E. andrei* was less sensitive to contaminants than other species (Langdon et al. 2005; Spurgeon and Weeks 1998). For some metals, the results of our study were consistent with such findings. For example, Figures B-4 and B-6 showed that earthworm Cd and Pb concentrations in *E. andrei* were much lower than worm Cd and Pb concentrations predicted by models developed from *Lumbricus rubellus* (Ma et al. 1983; Corp and Morgan 1991). Therefore, in order to apply models developed from a specific species to another species, interspecies transfer coefficients may need to be developed.

Table B-7. Summary of the prediction of metal boaccumulation by earthworms (*Eisenia fetida*) using soil property or soil extraction data.

Approach	Metal	Model	Summary and ability to predict metal body burdens
Soil properties	As	$\ln As_{ew}=0.9884*\ln As_s - 1.747$ Sample et al. 1998	Based on total As levels; $R^2=0.90$; under predicts 0.8-16-fold, most soils 0.8-3.3 fold; RMSE = 24.2%
	Cd	$\ln Cd_{ew} = 6.018 + 0.787 * \ln Cd_s - 0.106 *OM - 0.402 * pH$ Ma et al. 1983	Based on total Cd, organic matter, pH; $R^2=0.98$; over predicts 3.8-11.3-fold; only eight data points above DL; RMSE = 106%
	Cr	$\log Cr_{ew}=0.69*\log Cr_s -1.05$ Peijnenburg et al. 1999a	Based on total Cr; $R^2=0.73$; under predicts 0.8-7.4-fold; RMSE == 13.6%
	Cu	$\log Cu_{ew}=0.435*\log Cu_s +0.39$ Morgan and Morgan 1988	Based on total Cu; $R^2=0.45$; under predicts 1.3-5.2-fold; RMSE = 24.7%
	Ni	$\log Ni_{ew}=0.98*\log Ni_s +0.67$ Neuhauser et al. 1995	Based on total Ni; $R^2=0.88$; over predicts 11-95-fold; RMSE = 689%
	Pb	$\log Pb_{ew} = 2.65+0.897 *\log Pb_s-3.56*\log pH$ Corp and Morgan 1991	Based on total Pb and pH; $R^2=0.95$; over predicts 0.5-25-fold; RMSE = 272%
	Zn	$\log Zn_{ew}=1.45*\log Zn_s +0.42$ Peijnenburg et al. 1999a	Based on total Zn; $R^2=0.62$; under predicts 1.3-5.2-fold; RMSE = 590%
	Cd	$C_w = 9.32 *e^{-0.008*28} + Cd_s *0.052/0.008*(1-e^{-0.008*28})$ Yu and Lanno 2010	Based on Cherry Point and McLellan soils where total Cd is same as model concentration, one prediction is the same as observed and one is 2-fold higher; with all 8 data points –RMSE = 19%
Calcium Nitrate Extraction	Cd	$\log Cd_{ew} = 0.27*\log Cd_{Ca(NO3)2} + 2.1$ $R^2 = 0.66$,	Only two soils – Cherry Point, McLellan – with total extractable Cd levels; over predicted earthworm Cd 3-6.8-fold; RMSE = 111%
	Pb	$\log Pb_{ew} = 0.32 Pb_{Ca(NO3)2} + 97$ $R^2 = 0.39$, P=0.008	Only five soils with extractable Pb; over predicted 1.1-3.6-fold; RMSE = 161%
	Zn	$\log Zn_{ew} = 0.02 Zn_{Ca(NO3)2} + 2.12$, $R^2=0.084$, P=0.21	Only four soils with extractable Zn; under predicted 1.3-2-fold; RMSE = 101%
BAF - Soil properties *En.crypticus*	Cd	log BAF=1.17-0.92*log Clay Peijnenburg et al. 1999b	Only six soils where BAF could be calculated; acceptable under-prediction; RMSE = 21%
	Pb	log BAF=0.35-0.36*pH Peijnenburg et al. 1999b	No relationship
	Zn	log BAF =3.47-0.46 *pH-0.67*log Al_{ox} Peijnenburg et al. 1999b	No relationship

Conclusions

Metal bioaccumulation and toxicity to soil invertebrates (*E. andrei, En. crypticus, F. candida*) were examined in ESTCP metal-contaminated soils (with paired reference site soils) comprising a wide range of physical and chemical characteristics and metal levels. The predictive ability of a number of different models relating soil properties to oligochaete metal bioaccumulation and toxicity as a screening tool for estimating metal bioavailability in soils was examined with the intent of validating some of these models for predicting metal bioaccumulation in soil-dwelling oligochaetes.

Key elements for predicting bioaccumulation of metals by soil invertebrates include metal concentration in the soil, soil physicochemical characteristics, and time. A review of the literature revealed many models that used total soil metal concentration as the lone predictor variable of metal bioaccumulation by soil invertebrates, and in this research some of the better models for predicting metal bioaccumulation for a few of the metals (e.g., Cr, Ni) were still those based solely upon total metal concentrations, perhaps due to the lack of many models. More recent models have attempted to include metal bioavailability in the prediction of bioaccumulation by relating soil physicochemical characteristics to metal uptake in the models. An alternative approach uses the concentration of metals in an aqueous extract of the soil as a measure of bioavailability and a predictor variable. All of the previous models do not take into consideration a basic paradigm of bioaccumulation, the kinetics of metal uptake. Very recent models have attempted to incorporate metal toxicokinetics into predictions of Cd bioaccumulation in

earthworms. In this study, we have examined the application of all these models, with varying degrees of success, to predicting the bioaccumulation of metals by earthworms from ESTCP soils. Three models (Cd, Pb, Zn) relating BAF in *En. crypticus* to soil properties were available for enchytraeids and none was found for collembola. The models can be divided into three categories: 1) Metals for which a large number of models exist in the literature (e.g., Pb, Cd); 2) Metals for which few models exist in the literature (e.g., Cr, Ni); and, 3) Essential metals (e.g., Cu, Zn).

When applying literature-based metal bioaccumulation models to assess Cd and Pb bioaccumulation by earthworms in metal-contaminated field soils, 98% of the variability in earthworm Cd concentrations could be predicted by a model comprising total soil Cd, organic matter content, and soil pH (Ma et al. 1983), while 95% of the variability in earthworm Pb concentrations could be predicted by a model including total soil Pb and soil pH **(**Corp and Morgan 1991). However, both these models over-predicted metal bioaccumulation (RMSE Cd – 106%; Pb – 272%) so their use in predicting bioaccumulation may be limited. A large portion of the variability in the tissue concentrations of As (90% - Sample et al. 1998), Cr (77% - Peijnenburg et al. 1999a), and Ni (88% - Neuhauser et al. 1995) could be estimated by their concentrations in soil. Even though just a few bioaccumulation models exist for these metals, the models for As (RMSE – 24.2%) and Cr (RMSE – 13.6%) provided acceptable predictions of metal uptake, while the Ni model severely over-predicted uptake (RMSE – 689%). However, for the essential metals Cu and Zn, total soil concentrations combined with soil

properties provide a reasonable prediction of tissue concentrations for Cu (RMSE – 24.7%) but not for Zn (RMSE – 590%). These results should be viewed cautiously, since the outcome of model prediction has little relevance since exposure concentrations in ESTCP soils were all in the range where metal body burdens could be regulated by worms. A model relating BAF of Cd to soil properties (Peijnenburg et al. 1999b) provided acceptable predictions of Cd BAFs by *En. crypticus* from ESTCP soils (RMSE – 20%) while no relationship was evident between BAFs and observed metal burdens for Pb and Zn.

Models developed relating 0.5 M $Ca(NO_3)_2$-extractable Cd and Pb to earthworm metal residues (Lanno and Basta 2003) did not provide a better prediction of Cd and Pb concentrations in earthworms exposed to ESTCP soils than models selected from the literature that predicted earthworm metal concentrations based upon total metal levels and soil physicochemical characteristics. Models incorporating toxicokinetics of metals were only available for Cd (Yu and Lanno, 2010) and provided reasonable estimates of Cd concentrations in earthworms (RMSE – 19%). Suffice to say, there are no models for a specific metal that would provide good predictions of metal bioaccumulation in all soils and situations. For Cd and Pb, since there were many models to choose from in the literature, it was possible to find one that provided reasonable predictions for Cd and Pb bioaccumulation. Models that used extractable metals did not account for as much of the observed variability but may have more general applicability to soils differing in

physicochemical characteristics, while toxicokinetic models for Cd show some promise but have only been applied in very few situations.

Metal bioaccumulation itself is only one line of evidence in an ecological risk assessment and other endpoints, such as reproduction and survival, provide important evidence of the effects of metals in soils. Reproduction and survival bioassays conducted in ESTCP soils provided ample evidence of the confounding effects of soil matrix composition on responses by the standard test organism *E. andrei*. It was also evident that the effects of soil composition were less confounding in bioassays conducted with enchytraeids or collembolan, suggesting that earthworms may not be the most suitable test organism for certain endpoints in soils of poor composition, especially with respect to organic carbon and soil texture extremes. While earthworms may be suitable for assessing metal bioaccumulation from some soils, other soil invertebrates may be more suitable for reproduction and survival endpoints. Toxicity tests exist or are being developed for soil invertebrates such as oribatid mites (Princz et al. 2010), isopods (Loureiro et al. 2005), and other invertebrates (Løkke and van Gestel, 1998) which may provide data on the toxicity of metals in soils to which earthworms are not suited.

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Appendix B-1: Summary of earthworm metal bioaccumulation models

Metal	Model	R^2	Reference
As	log BAF=-1.03*log Alox+1.29	0.0369	Janssen et al. 1997
	ln As_{ew}=0.9884*ln As_s-1.747	0.9045	Sample et al. 1998
Cd	log BAF=-0.43*pH+1.36*log Clay-1.39*log OM+3.19	0.8806	Janssen et al. 1997
	log Cd_{ew}=0.27*log Cd_s+1.4	0.7254	Heikens et al. 2001
	log Cd_{ew}=0.3*log Cd_s-0.3	0.7337	Wright and Stringer 1980
	log Cd_{ew}=0.3*log Cd_s+1.1	0.7337	Corp and Morgan 1991
	log Cd_{ew}=0.32*log Cd_s-0.09	0.7391	Van Vliet et al. 2005
	log Cd_{ew}=0.32*log Cd_s+0.33	0.7391	Wright and Stringer 1980
	log Cd_{ew}=0.39*log Cd_s+1.1	0.7572	Heikens et al. 2001
	log Cd_{ew}=0.45*log Cd_s+1.45	0.7718	Spurgeon and Hopkin 1996
	log Cd_{ew}=0.47*log Cd_s+1.2	0.7764	Morgan and Morgan 1988
	log Cd_{ew}=0.5*log Cd_s+0.7	0.7832	Wright and Stringer 1980
	log Cd_{ew}=0.51*log Cd_s+1.5	0.7854	Neuhauser et al. 1995
	log Cd_{ew}=0.56*log Cd_s+0.67	0.7961	Wright and Stringer 1980
	log Cd_{ew}=0.61*log Cd_s+1.3	0.8062	Morgan and Morgan 1988
	log Cd_{ew}=0.66*log Cd_s+1.21	0.8157	Neuhauser et al. 1995
	log Cd_{ew}=0.69*log Cd_s+0.4	0.8211	Van Vliet et al. 2005
	ln Cd_{ew}=0.486*ln Cd_s+3.740	0.78	Ma et al. 1983
	ln Cd_{ew}=0.5512*ln Cd_s+2.8216	0.7943	Sample et al. 1998
	Cd_{ew}=52.15*log Cd_s+84.37	0.6426	Laszczyca et al. 2004
	Cd_{ew}=84.91*log Cd_s+22.596	0.6426)	Laszczyca et al. 2004
	log Cd_{ew}=1.28+0.324*log Cd_s-0.23*log pH	0.7906	Corp and Morgan 1991
	log Cd_{ew}=1.34+0.566*log Cd_s-0.171*log pH	0.8234	Corp and Morgan 1991
	ln Cd_{ew}=5.538+0.664*ln Cd_s-0.404*pH	0.9643	Ma et al. 1983
	log Cd_{ew}=1.14-0.079*pH	0.4456	Beyer et al. 1987
	log Cd_{ew}=1.207+0.618*log Cd_s-0.194*log OM	0.8183	Morgan and Morgan 1988
	log Cd_{ew}=1.417+0.492*log Cd_s-0.181*log OM	0.7957	Morgan and Morgan 1988
	log Cd_{ew}=1.93+0.480*log Cd_s-0.548*log OM	0.2571	Corp and Morgan 1991
	log Cd_{ew}=2.04+0.209*log Cd_s-0.709*log OM	0.0471	Corp and Morgan 1991
	ln Cd_{ew}=4.233+0.612*ln Cd_s-0.107*OM	0.723	Ma et al. 1983

	$\log Cd_{ew}=2.37+0.519*\log Cd_{s}-0.57*\log pH-0.585*\log OM$	0.5376	Corp and Morgan 1991
	$\ln Cd_{ew}=6.018+0.787*\ln Cd_{s}-0.106*OM-0.402*pH$	0.9801	Ma et al. 1983
	$Cd_{ew}=713*(0.98*Cd_{soil\ pH\text{-}extactable}+0.02*Cd_{gut\ pH\text{-}extactable})-11.2$	---	Saxe et al. 2001
Cr	$\log Cr_{ew}=0.69*\log Cr_{s}-1.05$	0.7324	Peijnenburg et al. 1999
Cu	$\log BAF=-0.65*\log Feox-0.38*\log Clay+1.38$	0.2177	Janssen et al. 1997
	$\log Cu_{ew}=0.01*\log Cu_{s}+0.23$	0.4198	Wright and Stringer 1980
	$\log Cu_{ew}=0.1*\log Cu_{s}+2.5$	0.4314	Wright and Stringer 1980
	$\log Cu_{ew}=0.14*\log Cu_{s}+1.07$	0.4359	Grelle and Descamps 1998
	$\log Cu_{ew}=0.15*\log Cu_{s}+1.2$	0.4369	Heikens et al. 2001
	$\log Cu_{ew}=0.229*\log Cu_{s}+0.726$	0.444	Morgan and Morgan 1988
	$\log Cu_{ew}=0.25*\log Cu_{s}-0.54$	0.4455	Peijnenburg et al. 1999
	$\log Cu_{ew}=0.291*\log Cu_{s}+0.944$	0.4481	Corp and Morgan 1991
	$\log Cu_{ew}=0.326*\log Cu_{s}+0.798$	0.4497	Corp and Morgan 1991
	$\log Cu_{ew}=0.41*\log Cu_{s}-0.47$	0.4518	Van Vliet et al. 2005
	$\log Cu_{ew}=0.435*\log Cu_{s}+0.39$	0.4519	Morgan and Morgan 1988
	$\log Cu_{ew}=0.45*\log Cu_{s}+0.81$	0.4518	Spurgeon and Hopkin 1996
	$\log Cu_{ew}=0.46*\log Cu_{s}+0.6$	0.4517	Heikens et al. 2001
	$\log Cu_{ew}=0.487*\log Cu_{s}+0.327$	0.4512	Morgan and Morgan 1988
	$\log Cu_{ew}=0.5*\log Cu_{s}+1.2$	0.4509	Wright and Stringer 1980
	$\log Cu_{ew}=0.5*\log Cu_{s}+1.8$	0.4509	Wright and Stringer 1980
	$\log Cu_{ew}=0.51*\log Cu_{s}-0.47$	0.4506	Van Vliet et al. 2005
	$\log Cu_{ew}=0.53*\log Cu_{s}-0.34$	0.4499	Van Vliet et al. 2005
	$\log Cu_{ew}=0.57*\log Cu_{s}+0.39$	0.448	Neuhauser et al. 1995
	$\log Cu_{ew}=0.67*\log Cu_{s}+0.35$	0.4404	Neuhauser et al. 1995
	$\ln Cu_{ew}=0.2414*\ln Cu_{s}+1.8059$	0.4449	Sample et al. 1998
	$Cu_{ew}=1.52*\log Cu_{s}+5.35$	0.4148	Laszczyca et al. 2004
	$Cu_{ew}=34.8*\log Cu_{s}-44.6$	0.4442	Kennette et al. 2002
	$Cu_{ew}=14.88+0.344*Cu_{s}$	0.392	Ma et al. 1983
	$Cu_{ew}=18.43+0.340*Cu_{s}-0.738*pH$	0.3949	Ma et al. 1983
	$\log Cu_{ew}=0.895+0.308*\log Cu_{s}-0.0561*\log OM$	0.4134	Corp and Morgan 1991
	$\log Cu_{ew}=1.48+0.194*\log Cu_{s}-0.310*\log OM$	0.0001	Corp and Morgan 1991
	$Cu_{ew}=21.56+0.349*Cu_{s}-1.272*OM$	0.3778	Ma et al. 1983
	$Cu_{ew}=20.57+0.350*Cu_{s}-1.307*OM+0.238*pH$	0.3759	Ma et al. 1983
	$Cu_{ew}=24.9*(1.25*Cu_{soil\ pH\text{-}extactable}-0.25*Cu_{gut\ pH\text{-}extactable})+11.9$	---	Saxe et al. 2001
Ni	$\log BAF=-0.70*\log Alox+0.47$	0.203	Janssen et al. 1997
	$\log Ni_{ew}=0.98*\log Ni_{s}+0.67$	0.8827	Neuhauser et al. 1995
	$\ln Ni_{ew}=2.862+0.2074*pH$	0.0942	Sample et al. 1998
Pb	$\log BAF=-0.61-0.74*\log clay$	0.0142	Peijnenburg et al. 1999
	$\log BAF=-0.78*\log clay-0.45*\log Feox+0.46$	0.1199	Janssen et al. 1997
	$\log Pb_{ew}=0.22*\log Pb_{s}+0.64$	0.6107	Spurgeon and Hopkin 1996

	$\log Pb_{ew}=0.5*\log Pb_s-0.1$	0.6156	Wright and Stringer 1980
	$\log Pb_{ew}=0.5*\log Pb_s-1.1$	0.6156	Wright and Stringer 1980
	$\log Pb_{ew}=0.6*\log Pb_s-0.3$	0.6079	Heikens et al. 2001
	$\log Pb_{ew}=0.6*\log Pb_s+0.8$	0.6079	Corp and Morgan 1991
	$\log Pb_{ew}=0.61*\log Pb_s+0.02$	0.6069	Neuhauser et al. 1995
	$\log Pb_{ew}=0.69*\log Pb_s+0.096$	0.5982	Corp and Morgan 1991
	$\log Pb_{ew}=0.74*\log Pb_s+0.05$	0.592	Neuhauser et al. 1995
	$\log Pb_{ew}=0.8*\log Pb_s-0.5$	0.584	Grelle and Descamps 1998
	$\log Pb_{ew}=0.9*\log Pb_s-0.23$	0.5696	Morgan and Morgan 1988
	$\log Pb_{ew}=0.9*\log Pb_s-0.8$	0.5696	Wright and Stringer 1980
	$\log Pb_{ew}=0.9*\log Pb_s-1.1$	0.5696	Wright and Stringer 1980
	$\log Pb_{ew}=1.04*\log Pb_s-1.073$	0.5483	Morgan and Morgan 1988
	$\log Pb_{ew}=1.47*\log Pb_s-1.66$	0.4828	Van Vliet et al. 2005
	$\log Pb_{ew}=1.50*\log Pb_s-0.83$	0.4785	Van Vliet et al. 2005
	$\ln Pb_{ew}=0.7612*\ln Pb_s+0.0752$	0.5892	Sample et al. 1998
	$\ln Pb_{ew}=0.999*\ln Pb_s+0.525$	0.5547	Ma et al. 1983
	$Pb_{ew}=18.4*\log Pb_s-27$	0.529	Kennette et al. 2002
	$Pb_{ew}=31.008*\log Pb_s-69.097$	0.1958	Laszczyca et al. 2004
	$Pb_{ew}=74.43*\log Pb_s-167.9$	0.2139	Laszczyca et al. 2004
	$\log Pb_{ew}=1.24+0.830*\log Pb_s-2.12*\log pH$	0.8897	Corp and Morgan 1991
	$\log Pb_{ew}=2.140+1.720*\log Pb_s-7.097*\log pH$	0.8412	Morgan and Morgan 1988
	$\log Pb_{ew}=2.173+1.518*\log Pb_s-5.678*\log pH$	0.923	Morgan and Morgan 1988
	$\log Pb_{ew}=2.65+0.897*\log Pb_s-3.56*\log pH$	0.9474	Corp and Morgan 1991
	$\ln Pb_{ew}=4.355+1.056*\ln Pb_s-0.925*pH$	0.9113	Ma et al. 1983
	$\ln Pb_{ew}=5.233+0.7253*\ln Pb_s-0.82195*pH$	0.8432	Sample et al. 1998
	$\ln Pb_{ew}=1.261+1.146*\ln Pb_s-0.297*OM$	0.4005	Ma et al. 1983
	$\log Pb_{ew}=3.690+1.660*\log Pb_s-6.878*\log pH-1.010*\log CEC$	0.6538	Morgan and Morgan 1988
	$\log Pb_{ew}=4.154+1.530*\log Pb_s-6.657*\log pH-0.898*\log CEC$	0.6582	Morgan and Morgan 1988
	$\ln Pb_{ew}=4.157+1.113*\ln Pb_s-0.167*OM-0.746*pH$	0.6941	Ma et al. 1983
	$Pb_{ew}^{1/2}=-0.502*pH-0.064*OC-0.429*FEAL-0.177*CEC$	---	Bradham et al. 2006
	$Pb_{ew}=112*(0.97*Pb_{\text{soil pH-extactable}}+0.03*Pb_{\text{gut pH-extactable}})+10.4$	---	Saxe et al. 2001
	$Mortality^{1/2}=-0.75*pH+0.073*OC-0.40*FEAL+0.067*CEC$	---	Bradham et al. 2006
	$(\text{Relative Reproduction})^{1/2}=0.273*pH+0.176*OC+0.402*FEAL+0.414*CEC$	---	Bradham et al. 2006
Zn	$\log BAF=-0.39*pH+2.1$	0.0483	Posthuma et al. 1998
	$\log BAF=-0.39*pH-1.06*\log Alox+0.73*\log Clay+3.04$	0.1914	Janssen et al. 1997
	$\log Zn_{ew}=0.01*\log Zn_s+0.23$	0.447	Wright and Stringer 1980
	$\log Zn_{ew}=0.1*\log Zn_s+2.5$	0.476	Wright and Stringer 1980
	$\log Zn_{ew}=0.14*\log Zn_s+1.07$	0.4883	Grelle and Descamps 1998
	$\log Zn_{ew}=0.16*\log Zn_s+2.5$	0.4942	Heikens et al. 2001
	$\log Zn_{ew}=0.17*\log Zn_s+1.8$	0.4972	Heikens et al. 2001

	$\log Zn_{ew}=0.18*\log Zn_s+2.27$	0.5001	Corp and Morgan 1991
	$\log Zn_{ew}=0.2*\log Zn_s+2.1$	0.5058	Morgan and Morgan 1988
	$\log Zn_{ew}=0.27*\log Zn_s+2.09$	0.5249	Neuhauser et al. 1995
	$\log Zn_{ew}=0.29*\log Zn_s+2.2$	0.53	Corp and Morgan 1991
	$\log Zn_{ew}=0.36*\log Zn_s+0.74$	0.5468	Van Vliet et al. 2005
	$\log Zn_{ew}=0.38*\log Zn_s+1.81$	0.5512	Neuhauser et al. 1995
	$\log Zn_{ew}=0.49*\log Zn_s+0.52$	0.5729	Van Vliet et al. 2005
	$\log Zn_{ew}=0.5*\log Zn_s+1.2$	0.5746	Wright and Stringer 1980
	$\log Zn_{ew}=0.5*\log Zn_s+1.8$	0.5746	Wright and Stringer 1980
	$\log Zn_{ew}=0.55*\log Zn_s+0.30$	0.5827	Van Vliet et al. 2005
	$\log Zn_{ew}=0.69*\log Zn_s+0.41$	0.6004	Van Vliet et al. 2005
	$\log Zn_{ew}=1.45*\log Zn_s+0.42$	0.6191	Peijnenburg et al. 1999
	$\ln Zn_{ew}=0.2373*\ln Zn_s+5.0981$	0.5162	Sample et al. 1998
	$\ln Zn_{ew}=0.241*\ln Zn_s+6.047$	0.5172	Ma et al. 1983
	$Zn_{ew}=-188.9*\log Zn_s+1466.7$	0.4437	Laszczyca et al. 2004
	$Zn_{ew}=151*\log Zn_s-19$	0.4437	Kennette et al. 2002
	$Zn_{ew}=612.19*\log Zn_s-675$	0.4437	Laszczyca et al. 2004
	$\log Zn_{ew}=1.86+0.250*\log Zn_s-0.643*\log pH$	0.4359	Corp and Morgan 1991
	$\log Zn_{ew}=2.42+0.202*\log Zn_s-0.281*\log pH$	0.4762	Corp and Morgan 1991
	$\ln Zn_{ew}=4.453+0.234*\ln Zn_s+0.12845*pH$	0.4234	Sample et al. 1998
	$\ln Zn_{ew}=6.791+0.343*\ln Zn_s-0.270*pH$	0.3505	Ma et al. 1983
	$\ln Zn_{ew}=6.056+0.313*\ln Zn_s-0.073*OM$	0.1313	Ma et al. 1983
	$\ln Zn_{ew}=6.878+0.439*\ln Zn_s-0.088*OM-0.298*pH$	0.1407	Ma et al. 1983
	$Zn_{ew}=7.67*(0.82*Zn_{soil\ pH\text{-}extactable}+0.18*Zn_{gut\ pH\text{-}extactable})+102$	---	Saxe et al. 2001

M_{ew}: concentration of metal M in the earthworm (mg/kg).

M_s: total concentration of metal M in the soil (mg/kg).

OM: organic matter content (%).

BAF: Bioaccumulation Factor= M_{ew} / M_s.

Clay: clay content (%).

Feox: Fe oxyhydroxide concentration (mmol/kg).

Alox: Al oxyhydroxide concentration (mmol/kg).

CEC: cation exchange capacity (cmol/kg).

FEAL: amorphous iron and aluminum oxides (mol/kg)

$M_{soil\ pH\text{-}extactable}$: soluble metal concentration determined after 24-h batch extraction in unbuffered DI water (μg/kg).

$M_{gut\ pH\text{-}extactable}$: soluble metal concentration predicted at pH 7.0 (μg/kg).

Reference	Metal	Species	Experiment Type	Parameter Range
Beyer et al. 1987	Cd	*Aporrectodea tuberculata*	field	
Bradham et al. 2006	Pb	*Eisenia andrei*	lab	pH: 3.8-7.8 CEC: 3.01-32.4 cmol/kg OM: 5-30% FEAL: 0.009-0.195 mol/kg Soil Pb: 2000 mg/kg
Corp and Morgan 1991	Cd, Cu, Pb, Zn	*Lumbricus rubellus* (native and introduced)	lab	pH: 3.5-8.1 OM: 4-35% Soil Cd: 0.33-266 mg/kg Soil Cu: 22-816 mg/kg Soil Pb: 91-37700 mg/kg Soil Zn: 416-96800 mg/kg
Grelle and Descamps 1998	Cu, Pb, Zn	*Eisenia fetida*	lab	Soil Cu: 0.5-13 mg/kg Soil Pb: 5-798 mg/kg Soil Zn: 0.4-973 mg/kg
Heikens et al. 2001	Cd, Cu, Pb, Zn	mix	field	Soil Cd: 0.1-100 mg/kg Soil Cu: 10-10000 mg/kg Soil Pb: 100-10000 mg/kg Soil Zn: 10-10000 mg/kg
Janssen et al. 1997	As, Cd, Cu, Ni, Pb, Zn	*Eisenia andrei*	lab	pH: 3.0-7.2 OM: 2-21.8% Clay: 0.8-33.8% Fe_{ox}: 201-16569 mg/kg Al_{ox}: 94.4-3462 mg/kg CEC: 1.7-41.8 cmol/kg Soil As: 0.75-71.2 mg/kg Soil Cd: 0.1-49.5 mg/kg Soil Cu: 1.3-109.9 mg/kg Soil Ni: 0.6-47.5 mg/kg Soil Pb: 70.4-847.4 mg/kg Soil Zn: 5.2-3109 mg/kg
Kennette et al. 2002	Cu, Pb, Zn	*Lumbricus terrestris*	lab	Soil Cu: 23.5-2890 mg/kg Soil Pb: 33.6-7110 mg/kg Soil Zn: 40.1-14600 mg/kg
Laszczyca et al. 2004	Cd, Cu, Pb, Zn	*Eisenia fetida* *Lumbricus terrestris*	field	Soil Cd: 0.84-82 mg/kg Soil Cu: 10.7-47 mg/kg Soil Pb: 136-2635 mg/kg Soil Zn: 151-10154 mg/kg
Ma et al. 1983	Cd, Cu, Pb, Zn	*Lumbricus rubellus*	field	pH: 3.5-6.1

				OM: 2.2-8.6% Soil Cd: 0.1-5.7 mg/kg Soil Cu: 1-130 mg/kg Soil Pb: 14-430 mg/kg Soil Zn: 10-1220 mg/kg
Morgan and Morgan 1988	Cd, Cu, Pb, Zn	*Lumbricus rubellus* *Dendrobaena veneta*	field	Soil Cd: 0.1-350 mg/kg Soil Cu: 26-2740 mg/kg Soil Pb: 170-24600 mg/kg Soil Zn: 160-45000 mg/kg
Neuhauser et al. 1995	Cd, Cu, Ni, Pb, Zn	mix	field	Soil Cd: 0.01-1000 mg/kg Soil Cu: 0.1-1000 mg/kg Soil Ni: 1.25-2.4 mg/kg Soil Pb: 0.01-100000 mg/kg Soil Zn: 10-100000 mg/kg
Peijnenburg et al. 1999	Cr, Cu, Pb, Zn	*Eisenia andrei*	lab	Soil Cr: 3.2-987.9 mg/kg Soil Cu: 1.1-108 mg/kg Soil Pb: 3.5-849.5 mg/kg Soil Zn: 5.3-3138.7 mg/kg
Posthuma et al. 1998	Zn	*Eisenia fetida*	lab	Soil Zn: 52.3-3112.6 mg/kg
Sample et al. 1998	As, Cd, Cu, Ni, Pb, Zn	mix	field	pH: 2.8-9.96 Soil As: 0.77-79.2 mg/kg Soil Cd: 0.06-467 mg/kg Soil Cu: 3.43-1000 mg/kg Soil Ni: 11.4-57 mg/kg Soil Pb: 0.79-24550 mg/kg Soil Zn: 12.5-183000 mg/kg
Saxe et al. 2001	Cd, Cu, Pb, Zn	*Eisenia andrei*	lab	pH: 3.52-7.9
Spurgeon and Hopkin 1996	Cd, Cu, Pb, Zn	mix	field	Soil Cd: 0.5-312 mg/kg Soil Cu: 6.3-2610 mg/kg Soil Pb: 56.2-15600 mg/kg Soil Zn: 31.6-32900 mg/kg
Van Vliet et al. 2005	Cd, Cu, Pb, Zn	*Lumbricus rubellus* *Aporrectodea caliginosa* *Allolobophora chlorotica*	field	Soil Cd: 2.81-4.5 mg/kg Soil Cu: 69.9-95.3 mg/kg Soil Pb: 186.5-269.4 mg/kg Soil Zn: 555.8-784.7 mg/kg
Wright and Stringer 1980	Cd, Cu, Pb, Zn	*Lumbricus terrestris* *Aporrectodea caliginosa* *Allolobophora chlorotica* *Aporrectodea longa* *Aporrectodea rosea*	field	Soil Cd: 1-10 mg/kg Soil Pb: 92-147 mg/kg Soil Zn: 89-617 mg/kg

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APPENDIX C
PLANT PHYTOACCUMULATION STUDY

PREDICTION OF CONTAMINANT PHYTOACCULUMATION USING SOIL PROPERTY OR SOIL EXTRACTION SOIL DATA

Contaminant phytoaccumulation was determined from plant bioassays for soils from 12 study sites. For ecological risk estimates, metal phytoavailability was estimated from soil-property driven multiple regression models developed using bioaccumulation data from two previous study studies; SERDP ER-1210 (Dayton et al., 2006) and a study sponsored by the National Center for Environmental Assessement (NCEA, Lanno et al., 2003). A separate approach involved the use of soil extraction methods, used to estimate metal(loid) phytoavailability, to predict contaminant phytoaccumulation. Regression models developed using bioaccumulation data from the NCEA study were used to predict contaminant phytoaccumulation in the study soils. Comparison of the actual contaminant phytoaccumulation from bioassays with predicted toxicity from in vitro models were used to quantify the ability of in vitro models to predict actual phytoaccumulation in field DoD soils. This was the basis for validation of the soil property or soil extraction methods for field DoD soils.

MATERIALS AND METHODS

Soil Contaminant Spiking and Ageing for NCEA and SERDP soils

Uncontaminated soils were spiked with metal salts and aged to minimize the "salt effect" (Basta et al., 2005). Soils were spiked with only one metal to avoid competitive adsorption effects. Soils from the NCEA study (Table C-1) were spiked with one contaminant and to achieve one contaminant soil concentration. NCEA soils were spike with reagent grade $Na_2HAsO_4 \cdot 7H_2O$ at 250mgAs/kg, $Cd(NO_3)_2 \cdot 4\ H_2O$, at 50 mg/kg, or $Pb(NO_3)_2$ at 2000 mg Pb/kg. Soils from the SERDP ER-1210 study (Table C-2) were spiked with one contaminant at multiple contaminant concentrations. ER-1210 soils were spiked with reagent grade $Na_2HAsO_4 \cdot 7H_2O$ at 10, 50, 100, 200 and 300 mgAs/kg, $Cd(NO_3)_2 \cdot 4\ H_2O$, at 10, 50, 100, 200 and 300 mg/kg, or $Pb(NO_3)_2$ at 250, 500, 1000, 3000 and 5000 mg Pb/kg. One liter of spiking solution was mixed with 5.0 kg of soil. Additional deionized water was added to form a saturated paste and was thoroughly mixed. The spiked soils underwent 3 wet-dry cycles at 60 °C for 24 h. Heavy metals added as a salt can result in a "salt effect" where metal availability is greater in spiked soil than non-spiked contaminated soil. The 3 wet-dry cycles reduces the salt effect by increasing the reaction between the soil matrix and metal contaminants. Soil salinity, as measured by the electrical conductivity (EC) of a water-saturated soil paste, was measured in spiked soils to ensure that metal salt spiking had not increased salinity enough to inhibit germination. The EC of spiked soils was determined after the second wet-dry cycle. Soils that had EC > 1.5 dS/m were leached with deionized water until the soil EC < 1.5 dS/m. Spiked soils that had EC < 1.5 dS/m were not leached. The final metal/loid concentration of spiked soils after leaching was confirmed, by microwave assisted acid digestion according to U.S. EPA Method 3051 to be within 10% of the expected spike level. Spiked soils were then aged by wetting drying the soils for 2 years to ensure complete reaction of the contaminant with the soil thereby minimizing the salt effect.

Table C-1. Soil properties and summary statistics of NCEA soils

		Soil Properties†					
Soil	Soil Horizon	FEAL	CEC	OC	AspH‡	CdpH‡	PbpH‡
		mol/kg	cmol/kg	%			
Canisteo	A	0.0570	30.5	3.00	7.55	7.80	7.60
Dennis	A	0.0830	9.77	1.90	4.90	4.80	4.75
Dennis	B	0.0660	14.6	0.80	5.60	5.65	5.20
Norge	A	0.0450	4.57	1.20	4.00	4.00	3.80
Hanlon	A	0.0440	16.3	1.60	7.00	6.50	6.65
Taloka	A	0.0550	4.85	1.20	4.65	5.10	4.15
Kirkland	A	0.0610	14.0	1.45	5.10	4.80	4.80
Luton	A	0.0690	32.4	2.00	7.15	7.05	6.60
Mansic	A	0.0260	16.5	1.50	7.95	7.30	7.70
Mansic	B	0.0110	11.7	0.53	8.00	7.75	7.80
Osage	A	0.1280	28.3	2.30	6.00	6.50	6.0
Osage	B	0.1950	27.5	2.00	6.15	6.10	5.90
Pond Creek	A	0.0580	10.7	1.90	4.65	4.60	4.10
Pond Creek	B	0.0490	12.5	0.80	5.95	5.75	5.15
Teller	A	0.0300	3.01	0.85	4.30	3.90	4.30
Pratt	A	0.0100	4.40	0.90	6.30	5.50	4.60
Pratt	B	0.0090	3.40	0.50	6.00	5.45	5.15
Richfield	B	0.0330	22.4	1.10	7.55	6.70	6.35
Summit	A	0.0890	29.4	2.40	7.25	6.95	6.95
Summit	B	0.0360	27.6	1.25	6.65	6.85	6.45
SERDP							
Kirkland	A	0.0619	14.2	1.43	6.27	6.27	6.27
Richfield	B	0.0470	27.9	0.657	7.76	7.76	7.76
Teller	A	0.0690	4.13	0.406	4.78	4.78	4.78
Sassafras	A	0.0530	4.15	0.721	5.49	5.49	5.49
Webster	A	0.0910	25.7	2.39	6.06	6.06	6.06
Minimum		0.009	3.01	0.406	4.00	3.90	3.80
Maximum		0.195	32.4	3.00	8.00	7.80	7.80
Mean		0.059	16.0	1.39	6.12	5.98	5.77

† FEAL is reaction Fe and Al oxides, CEC is soil cation exchange capacity, OC is organic carbon content.

‡ The pH measured in soil spiked with As, or Cd, or Pb.

Table C-2. Taxonomic classifications of soils from the NCEA and SERDP projects.

Soil Taxonomic Classification	Soil Series	Horizon
Fine-loamy, mixed, superactive, calcareous, mesic Typic Endoaquolls	Canisteo	A
Fine, mixed, thermic Aquic Argiudolls	Dennis	A
		B
Loamy, mixed, active, thermic Arenic Haplustalfs	Dougherty	A
Coarse-loamy, mixed, superactive, mesic Cumulic Hapludolls	Hanlon	A
Fine, mixed, superactive, thermic Udertic Paleustolls	Kirkland	A
Fine, smectitic, mesic Typic Endoaquerts	Luton	A
Fine-loamy, mixed, superactive, thermic Aridic Calciustolls	Mansic	A
		B
Fine-silty, mixed, active, thermic Udic Paleustolls	Norge	A
Fine, smectitic, thermic, Typic Epiaqerts	Osage	A
		B
Fine-silty, mixed, superactive, thermic Pachic Argiustolls	Pond Creek	A
		B
Sandy, mixed, mesic Lamellic Haplustalfs	Pratt	A
		B
Fine, smectitic, mesic Aridic Argiustolls	Richfield	B
Fine-loamy, siliceous, semiactive, mesic Typic Hapludults	Sassafras	A
Fine, smectitic, thermic oxyaquic Vertic Argiudolls	Summit	A
		B
Fine, mixed, thermic Mollic Albaqualfs	Taloka	A
Fine-loamy, mixed, active, thermic Udic Argiustolls	Teller	A
Fine-loamy, mixed, superactive, mesic Typic Endoaquolls	Webster	A

Determination of Soil Properties

All analyses were performed on duplicate samples of air-dried soil (< 2 mm). Soil pH was determined in 1:1 soil: deionized water suspension using a combination pH electrode (Thomas, 1996). Because metal salt addition can cause acidification due to metal hydrolysis (Basta and Tabatabai (1992), soil pH was measured on control (unspiked) and on metal-spiked soils. Soil pH measured on metal-spiked soils was used for all statistical analyses using soil pH. Soil organic carbon (OC) was determined by oxidation of organic C by acid dichromate reduction (Heanes, 1984). Amorphous Fe and Al oxide content was determined by acid ammonium oxalate extraction (McKeague and Day, 1996) and CEC was determined using the unbuffered salt ($BaCl_2$) extraction method (Sumner and Miller, 1996). Blanks, spikes and a certified reference soil (CRM020-050, RTC Corporation, Laramie, WY, USA) were used for quality assurance and quality control in the determination of Pb levels.

Soil Extraction Methods

Three soil extraction methods were used. Calcium nitrate solution was used to extract cationic Cd and Pb from study soils. Soil extraction with 0.1 M $Ca(NO_3)_2$ solution at 1:20 soil:solution ratio. Soil was extracted for 16 h followed by filtration of the supernatant using 0.45 µ membrane filtration. Deionized water was used to estimate pore water As, Cd, and Pb in soils. Pore water was determined by extraction of soil with deionized water (1:1 w/w) for 4 h followed by filtration of the supernatant using 0.45 µ membrane filtration. Mehlich 3 soil extraction, commonly used to measure phytoavailable phosphate, was used to extract phytoavailable As in soil. Soil was extracted according to Mehlich (1984). In this procedure an acidic solution containing fluoride extracts soil at 1:10 soil:solution ratio for 5 min followed by filtration of the supernatant using 0.45 µ membrane filtration. All extracted metal(loids) were quantified using ICP AES.

Plant Bioassay

Spiked soil (800 g) was mixed with 50%, by volume, vermiculite in 1 L pots. To prevent Pb from leaching, the pots were not allowed to drain. Twenty lettuce (*Lactuca sativa var. Paris Island Cos)* seeds were planted per pot. Three replicates of each soil (Pb-spiked and control) were grown in a completely randomized design. Plants were grown 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 that all soils had adequate nutrition, macro nutrients were tested and adjusted. Plant available phosphorus (P) and potassium (K) were determined using the Mehlich 3 (Mehlich, 1984) extraction with subsequent analysis by ICP-AES. Plant available nitrogen (NO_3-N and NH_4-N) was determined by a 1*M* KCl extraction followed by automated flow injection analysis (Mulvaney, 1996). All soils had adequate levels of plant nutrients after fertilizer addition of Miracle Gro™ (15% N + 30% P_2O_5 + 15% K_2O). To balance nitrogen due to NO_3 addition as the Pb salt with soil spiking, an additional 200 mg/kg of N was applied to the control pots as NH_4NO_3. Percent germination was determined at 7 days. Pots were thinned to 5 plants per pot at 14 days. Lettuce was harvested after 40 days, rinsed in deionized water, and dried at 70°C for 48 h and crushed by hand. The dried material was weighed to determine dry matter growth (DMG). Dry lettuce tissue (0.25 g) was predigested for 4 h in 10 mL of nitric acid. Predigested samples were digested at 140°C for 4h, or until clear (Zarcinas et al., 1987).

Filtered (0.45 μm) solutions were analyzed for Pb by ICP-AES. To account for differences in lettuce biological endpoints due to differences in soil quality (i.e., acidity, texture), dry matter growth (DMG) and germination (G) are presented relative to their controls.

Statistical Analyses

Statistical modeling was performed using two multiple regression models: multiple linear regression (MLR) and ridge regression (RR). Both types of models were fit to the data using PROC REG in SAS 9.2. For the MLR models, model selection was not performed; we included all five independent variables (pH, OC, FEAL, CEC, and Total) in each model. For the RR models, an extra penalty term is added to the statistical model. This penalty term can be tuned to adjust the parameter estimates, increasing the bias in the parameter estimates while decreasing the influence of multicollinearity on the parameter estimates. These biased estimates produce a model that does not fit the observed data as closely as the MLR. In all cases, the R^2 for the MLR will be superior to the one obtained from the RR. However, the biased estimates produced by the RR often produce a better predictive model, and that was the central goal of our model development.

When using the RR approach, we chose the value of the tuning parameter by selecting the value that minimizes the PRESS statistic. The PRESS statistic is calculated by removing each observation, in turn, from the dataset; fitting the model using the remaining n – 1 observations; using the model fit to obtain a predicted value for the removed observation; and calculating the squared error of prediction for the removed observation. After cycling through each observation in the dataset in this manner, the squared errors of prediction are summed to obtain the final PRESS statistic. The model with the lowest PRESS statistic is declared to have the best predictive ability. PRESS statistics cannot be compared between RR models with different dependent variables, and there isn't a specific value of the PRESS statistic that can be considered adequate for declaring a model to have good predictive ability. However, the PRESS statistic can be used to compare two or more RR models with the same dependent variable.

Statistical models were developed using soil property and plant uptake data from a combined NCEA and SERDP ER-1210 database. Both MLR and RR models were developed. The developed models were evaluated to determine their ability to predict contaminant uptake for the ESTCP study soils.

RESULTS AND DISCUSSION

Soil Properties

The range in soil chemical and physical properties for the NCEA and SERDP soils is shown in Table C-1. The amorphous Fe plus Al oxide (FEAL) content ranged from 0.009 to 0.195 mol/kg, with a mean of 0.059 mol/kg . The coefficient of variation (CV) for amorphous Al ranged from 0.1 to 5.8% with a mean CV value of 2.8%. The coefficient of variation (CV) for amorphous Fe ranged from 0.5 to 7.7% with a mean CV value of 3.9%. There was a wide range in soil CEC from 3.01 to 32.4 $cmol_c$/kg with a mean of 16.0 $cmol_c$/kg. The coefficient of variation (CV) for soil CEC ranged from 0.0 to 1.5% with a mean CV value of 0.5%. Soil OC

ranged from 0.406 to 3.00 % with a mean of 1.39%. The coefficient of variation (CV) for soil OC ranged from 0.0 to 12.5% with a mean CV value of 2.7%.

Spiking of soils with cationic metal salts can decrease the soil pH. Therefore, the soil pH after spiking and ageing was used for statistical analysis using soil pH. The pH for As-spiked soils ranged from 3.80 to 7.80 with a mean of 5.60. The pH for Cd-spiked soils ranged from 3.90 to 7.80 with a mean of 5.98. The pH for Pb-spiked soils ranged from 3.80 to 7.80 with a mean of 5.77 (Table C-1). The coefficient of variation (CV) for soil pH ranged from 0.0 to 1.1% with a mean CV value of 0.4%. Comparison of soil pH of the same soil spiked with As, Cd, or Pb shows the Pb lowered pH significantly for several soils. This consistent with acidity from hydrolysis of metal salts (i.e., Pb, Cd) because Pb was added in the greatest molar amount. Lead decreased soil pH up to 0.6 unit on unbuffered sandy soils such as Pond Creek (Table P1) but had lesser effect on highly buffered clay (i.e., Osage) or calcareous soil (i.e., Mansic). Many studies do not measure soil pH after spiking. This could be a significant source of error if ignored.

Plant Phytoaccumulation of As, Cd, and Pb from study soils

NCEA Plant Bioassay

Both lettuce and ryegrass were grown in NCEA soils. Tissue contaminant concentration is summarized in Table C-3. The wide range in contaminant tissue concentrations (Table C-3) resulting from a single soil spike level indicates that phytoavailability is being modified by soil properties. Plants grown in NCEA soils spiked at 250 mg As/kg had wide range in lettuce tissue As ranging from 0.76 to 31.1 mg/kg with a mean of 11.1 mg/kg and a wide range in ryegrass tissue As ranging from 4.39 to 109 mg/kg with a mean of 26.1 mg/kg. Plants grown in NCEA soils spiked at 50 mg Cd/kg had a wide range in lettuce tissue Cd ranging from 23.8 to 128 mg/kg with a mean of 66.0 mg/kg and wide range in ryegrass tissue Cd ranging from 3.00 to 66.6 mg/kg with a mean of 27.0 mg/kg. Plants grown in NCEA soils spiked at 2000 mg Pb/kg had lettuce tissue Pb ranging from 3.22 to 114 mg/kg with a mean of 41.6 mg/kg and ryegrass tissue Pb ranging from 16 to 236 mg/kg with a mean of 90.3 mg/kg.

SERDP Plant Bioassay

Ryegrass tissue contaminant concentration is summarized in Table C-4. Ryegrass tissue As concentration, ranged from 0.7 to 3.7 mg As/kg for soils spiked with 10 mg As/kg, from 4.20 to 13.3 mg As/kg for soils spiked with 50 mg As/kg, from 8.89 to 17.5 mg As/kg for soils spiked with 100 mg As/kg and from 16.7 to 21.9 mg As/kg for soils spiked with 200 mg As/kg. Ryegrass tissue Cd widely ranged from 3.40 to 32 mg Cd/kg for soils spiked with 10 mg Cd/kg, and from 11.4 to 91.7 mg Cd/kg for soils spiked with 50 mg Cd/kg. Ryegrass tissue Pb widely ranged from 3.88 to 110 mg Pb/kg for soils spiked with 250 mg Pb/kg, from 6.32 to 91.2 mg Pb/kg for soils spiked with 500 mg Pb/kg, from 16.8 to 143 mg Pb/kg for soils spiked with 1000 mgPb/kg and from 114 to 167 mgPb/kg for soils spiked with 3000 mg Pb/kg. Missing data points are due to plant death and missing soil for Richfield As (Table C-4).

ESTCP Plant Bioassay

Both lettuce and ryegrass were grown in ESTCP soils. Data is shown for all dry matter growth (DMG) and metal(loid) tissue concentrations (Table C-5). Metal(loid) plant uptake occurred on

uncontaminated and contaminated soils. Only plant tissue data for contaminated soils (Table C-5 bolded and underlined values) were used for statistical analyses.

Lettuce DMG ranged from 0.110 to 5.60g with a mean of 3.33g, while ryegrass tissue ranged from 1.02g to 7.75 g with a mean of 3.58g. In As contaminated soils, tissue As had a narrow range from 2.88 to 5.16 mg/kg for lettuce and < 2 to 4.64 mg/kg for ryegrass. Mean tissue As was lower for ESTCP soils (Table C-5) than mean lettuce and ryegrass As grown on NCEA (Table C-3) and SERDP soils (Table C-4). Total soil As alone cannot explain this difference because several ESTCP soils had As contents much greater than the ≤ 250 mg/kg As of the

Table C-3. Contaminant phytoaccumulation in plant bioassay lettuce and ryegrass tissue for NCEA soils.

		Tissue Contaminant†					
		Tissue As		Tissue Cd		Tissue Pb	
Soil Series	Horizon	Lettuce	Ryegrass	Lettuce	Ryegrass	Lettuce	Ryegrass
		mg/kg					
Canisteo	A	na	16	na	10.6	8.72	36.6
Dennis	A	2.43	15	60.4	21.5	na	93.7
Dennis	B	0.763	4.39		3.0	na	84.4
Norge	A	26.5	26.1	82.9	47.5	61.3	236
Hanlon	A	na	15.9	23.8	16.9	9.2	45
Taloka	A	29.7	26.1	77	29.1	37.7	150
Kirkland	A	14.8	14.8	52.3	27.9	50.4	121
Luton	A	4.58	19.5	na	18.5	16.5	31
Mansic	A	na	35.7	na	na	60	58.0
Mansic	B	na	109	na	na	na	64.0
Osage	A	5.16	.	28.1	13.2	3.22	16.0
Osage	B	5.6	4.78	28.9	15.5	15.3	25
Pond Creek	A	31.1	16.8	57.4	26.2	43.1	144
Pond Creek	B	9.42	16.1	75.7	26.2	na	80.3
Teller	A	na	25	128	66.6	108	186
Pratt	A	na	na	na	na	na	122
Pratt	B	na	88.9	na	64.8	na	148
Richfield	B	na	17.3	na	na	na	75
Summit	A	1.61	8.28	na	11.1	na	17
Summit	B	1.12	9.54	na	17.8	13.4	38.0
min		0.763	4.39	23.8	3	3.22	16.0
max		31.1	109	128	66.6	114	236
mean		11.1	26.1	66.0	27	41.6	90.3

† na is data not available because of phytotoxicity

Table C-4. Contaminant phytoaccumulation in plant bioassay lettuce and ryegrass tissue for SERDP ER-1210 soils.

	Tissue contaminant concentration, mg/kg, for each soil type				
Soil Contaminant concentration mg/kg	Kirkland	Richfield	Teller	Sassafras	Webster
As					
10	0.7		3.7	0.96	1.13
50	5.34		13.3	7.12	4.2
100	15.7		17.5	16.3	8.89
200	21.9		na	na	16.7
Cd					
10	5.2	5.6	32	18.4	3.4
50	19.4	16.2	91.7	91.4	11.4
Pb					
250	9.5	3.88	42.9	110	4.56
500	20.1	14.4	91.2	179	6.32
1000	68.2	49.7	143	na	16.8
3000	167	114	na	na	na

† na is data not available because of phytotoxicity

Table C-5. Dry matter growth and contaminant phytoaccumulation in plant bioassay lettuce and ryegrass tissue for ESTCP study soils.

	Lettuce Tissue				Ryegrass Tissue			
			Tissue					
Soil	DMG†	As	Cd	Pb	DMG	As	Cd	Pb
	g	mg/kg			g	mg/kg		
Concord	4.63	**4.83‡**	1.0	<2	7.75	4.64	<0.2	<2
Cherry Point	0.110	<2	**29.3**	<2	4.49	<2	**5.55**	<2
Deseret	0.65	**5.16**	1.38	<2	1.02	**2.87**	0.27	<2
Hill	3.20	<2	**78.0**	<2	1.27	<2	**20.3**	<2
Hilo	2.58	**3.69**	2.17	**7.63**	2.84	**2.36**	0.27	**6.76**
McCllelan	5.05	<2	**29.7**	**2.00**	4.91	<2	**8.65**	**<2**
Mechanichsburg	2.96	<2	0.26	**8.96**	2.59	<2	<0.2	**2.10**
ORNL	2.99	<2	0.69	**47.3**	2.71	<2	<0.2	**51.6**
Pearl City	5.60	**2.88**	1.21	**5.73**	6.81	**<2**	<0.2	**<2**
Port	4.39	<2	1.49	**93.8**	1.51	<2	0.99	**262**
Travis	4.45	<2	0.57	**35.6**	3.51	<2	0.38	**76.6**

† DMG is dry matter growth.

‡ Bolded and underlined values represent values from contaminated soils and were used for statistical analyses.

NCEA and SERDP soils. Thus, several ESTCP soils significantly reduced phytoavailable As.

In Cd contaminated ESTCP soils, tissue Cd concentrations ranged from 29.3 to 78.0 mg/kg for lettuce and 5.55 to 20.3 mg/kg for ryegrass. These values are comparable to tissue Cd found in NCEA and SERDP soils. In Pb contaminated soils, tissue Pb concentrations ranged from 2.00 to 93.8 mg/kg for lettuce and < 2 to 262 mg/kg for ryegrass. Where tissue concentrations was below detection limit (<2 mg/kg), half of the detection limit (1 mg/kg) was used in statistical analysis.

Soil Extractions

Extractable contaminant data for NCEA and ESTCP soils is shown in (Table C-6). Data was included only if there is corresponding plant tissue data. For NCEA soils pore water extractable As ranged from 0.020 to 20.3 mg/kg with a mean of 5.56 mg/kg, while Cd ranged from 0.030 to 17.2 mg/kg with a mean of 2.72 mg/kg and Pb ranged from 0.150 to 124 mg/kg with a mean of 21.8 mg/kg. Calcium nitrate extractable Cd ranged from 5.00 to 49.3 with a mean of 26.3, and Pb ranged from 5.11 to 1085 mg/kg with a mean of 417. Mehlich 3 extractable As ranged from 8.64 to 164 mg/kg with a mean of 70.3 (Table C-6). For ESTCP soils pore water extractable As ranged from < 0.1 to 1.19 mg/kg with a mean of 0.518 mg/kg, while Cd ranged from < 0.1 to 0.869 mg/kg with a mean of 0.417 mg/kg and Pb ranged from 0.160 to 33.3 mg/kg with a mean of 9.88 mg/kg. Calcium nitrate extractable Cd ranged from < 2 to 16.6 with a mean of 12.0, and Pb ranged from < 2 to 603 mg/kg with a mean of 244. Mehlich 3 extractable As ranged from 7.35 to 15.5 mg/kg with a mean of 11.1 (Table C-6).

Prediction of Contaminant Phytoacculumation using Soil Property Data

Statistical Regression Prediction Models

Multiple regression and Ridge regression models developed from the NCEA soil database for lettuce and NCEA plus SERDP soil databases for ryegrass tissue concentration are shown in Table C-7. The PRESS statistic is reported for each ridge regression (RR) model. Because PRESS is only used for RR and not the multiple regression models without ridge regression, the PRESS is 0 for MLR models in Table C-7.

For the models of toxins in lettuce, the PRESS statistic was significantly smaller in the ridge regression models than the MLR models indicating that reducing the influence of multicollinearity and allowing some bias in parameter estimates was beneficial for predictive ability. For the models of toxins in grass, the ridge regression and MLR models produced similar PRESS statistics indicating that the ridge adjustment did not lead to much improvement in predictive ability.

Results of both MLR and RR equations are shown in Table C-7. The MLR relationship for lettuce As vs. soil properties was not significant ($R^2 = 0.60$, $P < 0.247$), while it was strongly significant for ryegrass As ($R^2 = 0.66$, $P < 0.0001$). There was a weak relationship for lettuce Cd ($R^2 = 0.864$, $P < 0.32$) while it was strongly significant for ryegrass Cd ($R^2 = 0.86$, $P < 0.001$). The relationship for lettuce Pb was weak ($R^2 = 0.73$, $P < 0.059$) yet strong for ryegrass Pb ($R^2 =$

Table C-6. Pore water (1:1), calcium nitrate, and Mehlich 3 extractable

contaminants from NCEA and contaminated ESTCP soils. Data was included only if there is corresponding plant tissue data.

Soil	Pore Water			Calcium Nitrate		Mehlich 3
	As	Cd	Pb	Cd	Pb	As
	mg/kg					
			NCEA			
CAN	8.44	0.040	0.740	7.20	10.7	107
DEN A	0.300	1.44	4.48	36.4	598	16.1
DEN B	0.020	.	2.73	27.4	596	8.64
DOUG	.	4.70	31.93	36.8	800	.
HAN	13.9	0.450	1.32	15.2	70.3	107
KIRK	2.01	1.29	3.16	37.6	665	55.9
LUT	1.49	0.080	0.530	12.6	17.6	68.2
MAN A	16.9	.	0.750	6.50	26.1	145
MANB	20.3	.	0.150	5.00	40.5	.
Norge	.	9.19	56.07	32.9	990	57.4
OSA	0.230	0.160	1.70	19.9	69.1	.
OSB	0.120	0.270	0.640	23.3	74.4	17.4
PCA	7.49	1.82	13.21	33.1	5.11	73.1
PCB	0.49	0.430	7.21	43.8	705	59.6
PRATA	.	.	90.83	40.2	1085	97.5
PRATB	.	3.93	124.3	49.3	1047	114
RICH	8.65	0.200	4.44	19.6	151	164
SUMA	0.320	0.090	0.470	8.30	21.7	24.4
SUMB	0.020	0.030	0.170	20.8	78.3	23.3
TALOKA	3.81	4.86	21.47	42.4	726	59.2
TELLER	10.1	17.21	92.07	33.7	977	67.6
min	0.020	0.030	0.150	5.00	5.11	8.64
max	20.3	17.2	124	49.3	1085	164
mean	5.56	2.72	21.8	26.3	417	70.3
			ESTCP			
Con	0.199	.	.	.	.	12.8
CP	.	0.350	.	16.6	.	.
Des	1.19	.	.	.	.	7.35
Hill	.	0.033	.	3.84	.	.
Hilo	0.164	<0.1	0.534	<2	33.2	15.5
MC	.	0.869	.	15.5	26.7	.
Mech	.	.	.	.	<2	.
ORNL	.	.	33.3	.	603	.
Port	.	.	5.53	.	507	.
PC	<0.1	.	.	.	<2	8.70

Table C-6 (continued). Pore water (1:1), calcium nitrate, and Mehlich 3

extractable contaminants from NCEA and contaminated ESTCP soils. Data was included only if there is corresponding plant tissue data.

Soil	Pore Water			Calcium Nitrate		Mehlich 3
	As	Cd	Pb	Cd	Pb	As
	---------- mg/kg ----------					
Travis	.	.	0.16	.	48.1	.
min	<0.1	<0.1	0.160	<2	<2	7.35
max	1.19	0.869	33.3	16.6	603	15.5
mean	0.518	0.417	9.88	12.0	244	11.1

Table C-7. Predictive equations to determine contaminant phytoacculumation from soil properties and total contaminant content. All equations have the form log plant tissue = intercept + a pH + b OC + c FEAL + d CEC + e Total. Values listed under pH, OC, FEAL, CEC, and Total are regression coefficients.

	Model	Press	Intercept	pH	OC	FEAL	CEC	Total†	R^2	P value
Lettuce As	MLR	0	6.51	-0.278	0.471	-2.49	0.0138	-0.021	0.60	<0.247
	Ridge	0.75	2.7	-0.113	0.0924	-0.244	-0.0073	0.005		
Ryegrass As	MLR	0	0.297	0.125	0.084	-2.416	-0.027	0.0031	0.66	<0.0001
	Ridge	0.05	0.547	0.087	0.0293	-2.95	-0.184	0.0029		
Lettuce Cd	MLR	0	2.44	-0.119	-0.145	0.432	-0.0089	0.005	0.864	<0.0318
	Ridge	0.65	2.42	-0.0753	-0.094	-0.426	-0.0065	-0.0002		
Ryegrass Cd	MR	0	1.55	-0.043	-0.167	0.241	-0.0133	0.01189	0.856	<0.0001
	Ridge	0.1	1.68	-0.063	-0.152	-0.17	-0.0104	0.0105		
Lettuce Pb	MLR	0	1.79	0.0026	-0.2	-0.86	-0.022	0.00022	0.725	<0.0588
	Ridge	1.65	1.39	-0.04	-0.124	-1.08	-0.0077	0.00038		
Ryegrass Pb	MLR	0	2.02	-0.032	-0.129	-0.202	-0.025	0.00033	0.817	<0.0001
	Ridge	0.06	2.17	-0.056	-0.132	-0.738	-0.02	0.00031		

† Total is the total contaminant soil content (mg/kg) for As, Cd, or Pb.

Table C-7. Summary of the Prediction of Contaminant Phytoaccumulation using Soil Property or Soil Extraction Soil Data

Approach	Model or Soil Extraction	Ability to Predict Tissue As		Ability to Predict Tissue Cd		Ability to Predict Tissue Pb	
		Lettuce	Ryegrass	Lettuce	Ryegrass	Lettuce	Ryegrass
Properties	MLR	4 Concord Over , 5x	4 Deseret Over, 80x	4 Hill Under, 1.7x	4	7 Portsmouth Over, 1.3x ORNL Under, 1.3x	7 Portsmouth Under, 1.2x
	RR	4	4 Deseret Over, 80x	4 Hill Under, 1.7x	4	7 Portsmouth Over, 2x ORNL Over, 2x	7 Portsmouth Over, 1.7x
Soil Extraction	Pore water	3	3 All sites Over, 2x	3	3 Hill Under, 1.6x	4 Portsmouth Under, 4x	4 Portsmouth Under, 3.3x
	Mehlich 3	4	4 all sites Over, 2x to 5x	NA	NA	NA	NA
	Calcium Nitrate	NA	NA	3 Hill Under, 10x	3 Hill Under, 4x	4 Portsmouth Under, 2x	4 Portsmouth Under, 2.5x

0.82, P <0.001). In part, the stronger predictive equations for ryegrass vs. lettuce As, Cd, and Pb are due to differences in the size of their respective databases. Lettuce multiple linear regression models were developed using only the NCEA soil properties whereas ryegrass models were developed from NCEA and SERDP spiked soils. Therefore, there are more data for ryegrass than lettuce. This resulted in generally weaker lettuce regression relationships than ryegrass. For lettuce As n = 12, while for ryegrass As n = 32. For lettuce Cd n = 11, while for ryegrass Cd n = 26. Finally for lettuce Pb n = 13, while for ryegrass Pb n = 37.

These models were developed to determine if they could predict ESTCP tissue contaminant content from ESTCP soil properties and contaminant soil content (i.e., total in Table C-7). To illustrate the predictive ability of the developed models ESTCP tissue content was plotted against a 1:1 line generated from ESTCP tissue data. The RR model closely predicted the tissue As for lettuce grown on ESTCP soils (Fig. C-1A). With the exception of the Concord soil, the MLR model closely predicted the tissue As for lettuce grown on ESTCP soils (Fig. C-1A). Both RR and MLR model closely predicted ryegrass tissue As with the exception of the Deseret soil (Fig C-1B. The prediction models grossly over predicted tissue As content for Deseret.

Both RR and MLR models were able to predict lettuce Cd grown on ESTCP soils except for the Hill soil (Fig. C-2A). The models under predicted tissue Cd by approximately 1.7x for lettuce grown on the Hill soil. Both RR and MLR models were able to predict ryegrass Cd content grown on ESTCP for all soils (Fig. C-2B). For both lettuce and ryegrass Cd, ridge predictions were generally slightly better than MLR predictions.

Both RR and MLR models were able to predict lettuce Pb grown on ESTCP soils except for the Portsmouth and ORNL soils (Fig. C-3A). The MLR under predicted lettuce tissue Pb about 1.3x and RR over predicted tissue Pb by 2x for the ORNL soil. Both RR and MLR over predicted lettuce tissue Pb for the Portsmouth soil by about 2x and 1.3x, respectively. Both RR and MLR models were able to predict ryegrass Pb content grown on ESTCP for all soils (Fig. C-3B). However, RR over predicted tissue Pb about 1.7x while MLR slightly under predicted ryegrass tissue Pb 1.2x for the Portsmouth soil.

Prediction of Contaminant Phytoacculumation using Soil Extraction Data

Simple regression models, using the NCEA soil extraction and lettuce tissue data (Figs. C-4 to C-9) were developed to determine if soil extraction methods could be predictive of contaminant bioavailability. Pore water extractable As was significantly related between lettuce tissue As (r^2 = 0.837, P < 0.01) (Fig. C-4A) and ryegrass tissue As (r^2 = 0.474, P < 0.01) (Fig. C-4B). Soil test Mehlich 3 extractable As was a significantly related between lettuce tissue As (r^2 = 0.515, P < 0.05) (Fig. C-5A) and ryegrass tissue (r^2 = 0.416, P <0.05) (Fig. C-5B).

Pore water extractable Cd was significantly related between lettuce tissue Cd (r^2 = 0.658, P < 0.01) (Fig. C-6A) and ryegrass tissue Cd (r^2 = 0.678, P < 0.01) uptake (Fig. C-6B). Similarly, calcium nitrate extractable Cd was significantly related between lettuce tissue Cd (r^2 = 0.411, P < 0.05) (Fig. C-7A) and ryegrass tissue Cd (r^2 = 0.459, P < 0.01) (Fig. C-7B).

Pore water extractable Pb there was significantly related between lettuce tissue Pb ($r^2 = 0.693$, $P < 0.01$) (Fig. C-8A) and ryegrass tissue Pb ($r^2 = 0.454$, $P < 0.05$) (Fig. C-8B). Similarly, calcium nitrate extractable Pb was significantly related between lettuce tissue Pb ($r^2 = 0.538$, $P < 0.01$) (Fig. C-9A) and ryegrass tissue Pb ($r^2 = 0.622$, $P < 0.01$) (Fig. C-9B).

The ESTCP soil extractable and plant tissue data were plotted on the simple regressions (Figs. C-4 to C-9) developed using the NCEA data to illustrate how well those models could predict contaminant uptake in plants grown on ESTCP soils. Pore water extractable As accurately predicted lettuce tissue As (Fig. P4A), but over estimated ryegrass tissue As by 2x (Fig. C-4B). Mehlich 3 extractable As slight under estimated ESTCP lettuce tissue As (Fig. C-5A) and over estimated ESTCP ryegrass As 2x to 5x (Fig. C-5B).

Pore water extractable Cd was able to predict ESTCP lettuce Cd (Fig. C-6A), but underestimated lettuce tissue uptake for the Hill soil by 1.6x. Pore water extractable Cd was a fairly good predictor of ryegrass tissue Cd (Fig. C-6B). Calcium nitrate extractable Cd was an accurate predictor of ESTCP lettuce tissue Cd except for the Hill site which was greatly underestimated (ca. 10x, Fig. C-7A). Calcium nitrate extractable Cd was an accurate predictor of ESTCP lettuce tissue Cd except for the Hill site (Fig. C-7B) which was underestimated by 4x.

Pore water extractable Pb was able to predict ESTCP lettuce Pb (Fig. C-8A),but underestimated tissue Pb for the Port soil by a factor of 4x. Similarly, pore water extractable Pb was able to predict ESTCP ryegrass Pb (Fig. C-8B), but underestimated tissue Pb for the Port soil by a factor of 3.3x. Calcium nitrate extractable Pb was able to predict ESTCP lettuce tissue Pb, except for the Port soil which was underestimated by a factor of 2x (Fig. C-9A). Similarly, calcium nitrate extractable Pb was able to predict ESTCP ryegrass tissue Pb, except for the Port soil which was underestimated by a factor of 2.5x (Fig. C-9B).

Summary of the Predictive Capability of Soil Property and Soil Extraction Models

The predictive capability required by a soil property / soil extraction models depends on the degree of accuracy of contaminant phytoaccumulation determined by the risk assessor. With some exceptions, both soil property and soil extraction models were able to predict phytoavailability at < 35% of the measured contaminant tissue value. In general, soil property models were predictive of tissue As, Cd, and Pb (Table C-7). Exception were Deseret for As (ryegrass), Hill for Cd (lettuce), and Portsmouth for Pb. Similar findings were found for soil extraction models. In general, soil extraction models were predictive of tissue As, Cd, and Pb. Exceptions were the same as found for soil property models but soil extractions over predicted ryegrass tissue As for all soils.

The predictive capability by soil property / soil extraction models by soil is summarized in Table C-8. Comparison of MLR with RR shows the predictive capability was similar. Predictive capability was improved by RR vs. MLR for Concord (As) and ORNL (Pb). In general predictive capability of soil extraction methods was adequate to excellent with the exception of Hill for Cd (lettuce) and Portsmouth for Pb. Similar predictive capabilities were found for pore water vs. calcium nitrate extraction for Pb and Cd (Table C-8).

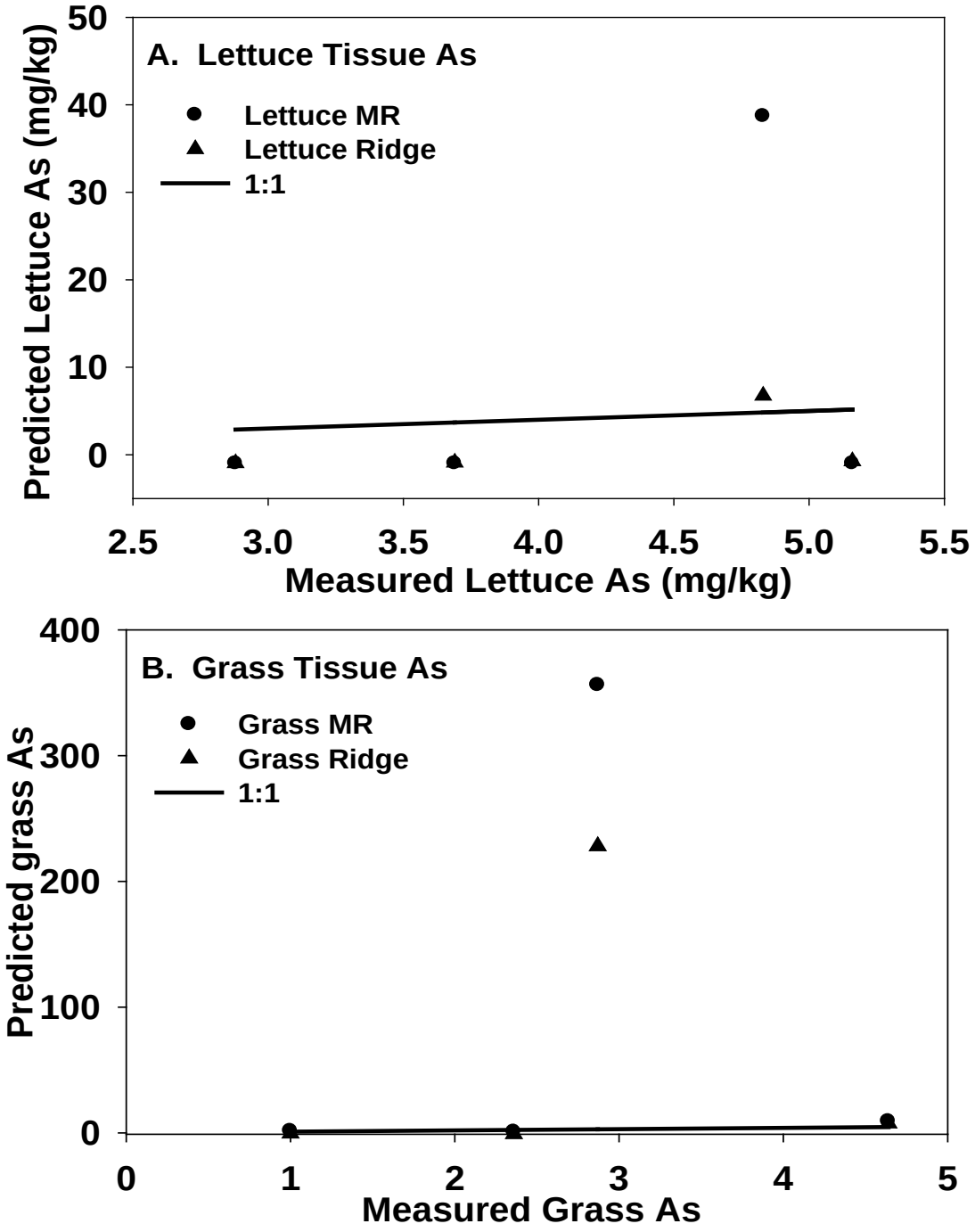

Figure C-1. Comparison of Predicted Lettuce (A) and Ryegrass (B) Arsenic Concentration from Multiple Regression and Ridge Regression with Measured Plant Concentrations

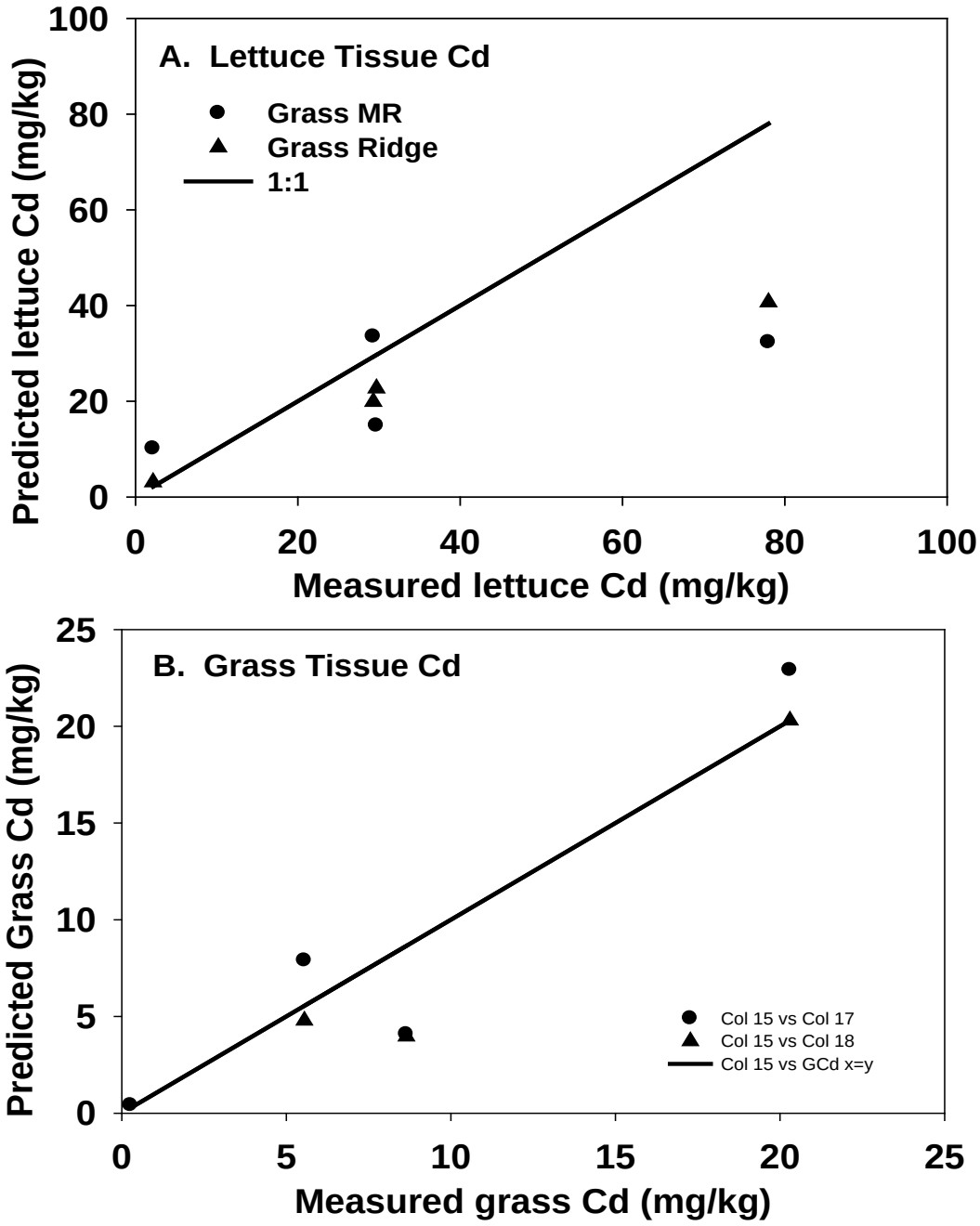

Figure C-2. Comparison of Predicted Lettuce (A) and Ryegrass (B) Cadmium Concentration from Multiple Regression and Ridge Regression with Measured Plant Concentrations

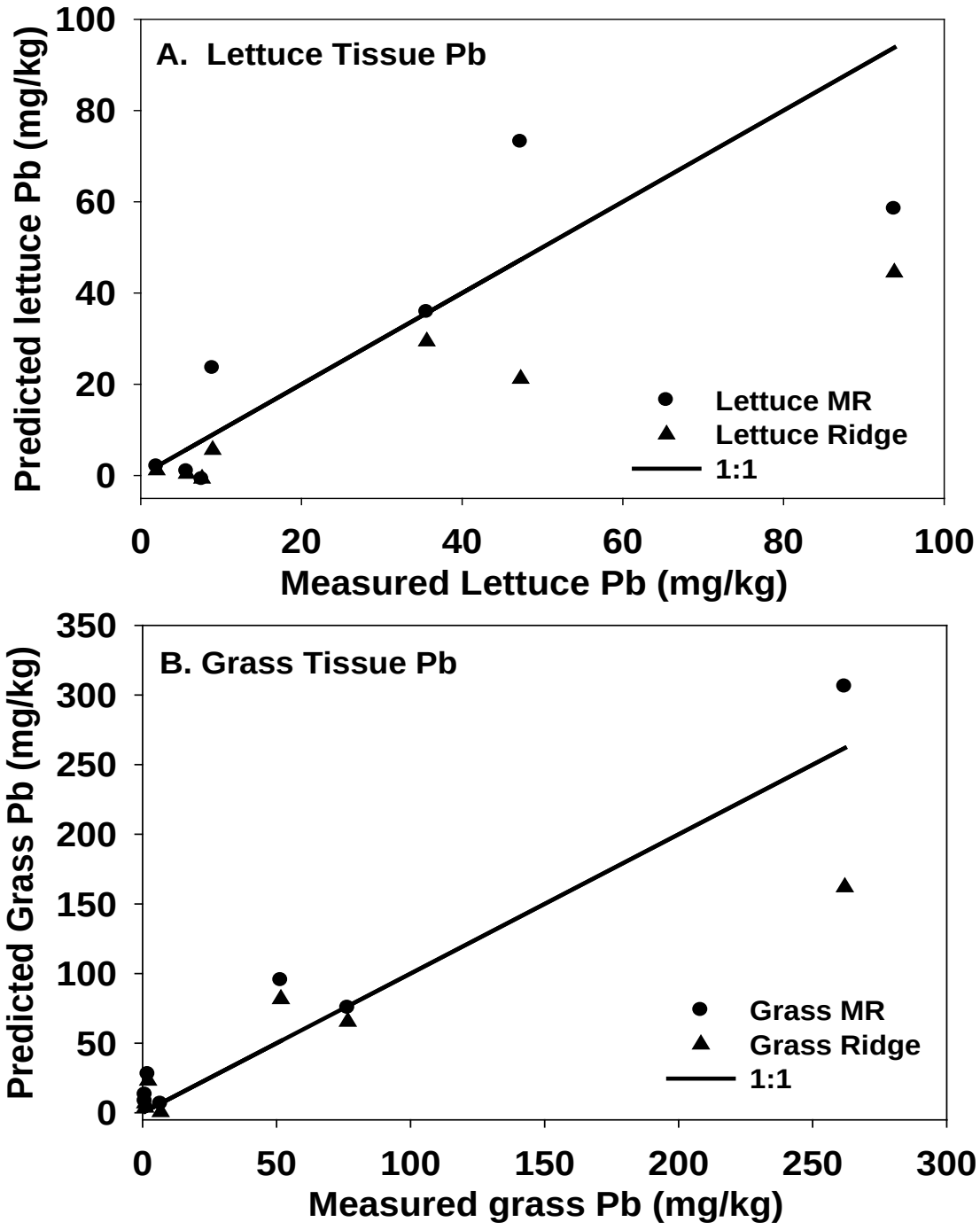

Figure C-3. Comparison of Predicted Lettuce (A) and Ryegrass (B) Lead Concentration from Multiple Regression and Ridge Regression with Measured Plant Concentrations

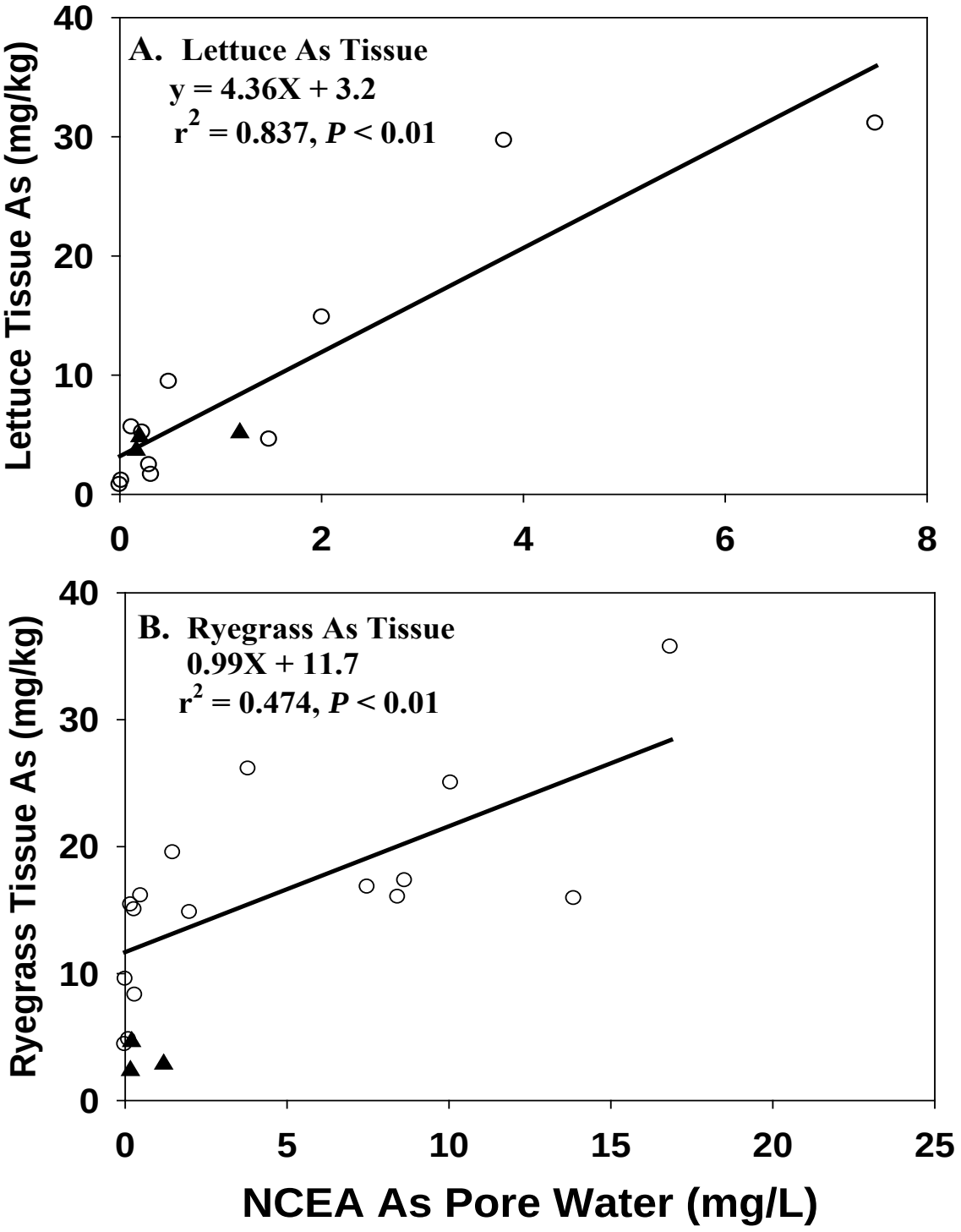

Figure C-4. Relationship between Lettuce (A) and Ryegrass (B) As concentration and Soil Pore Water As for NCEA soils.

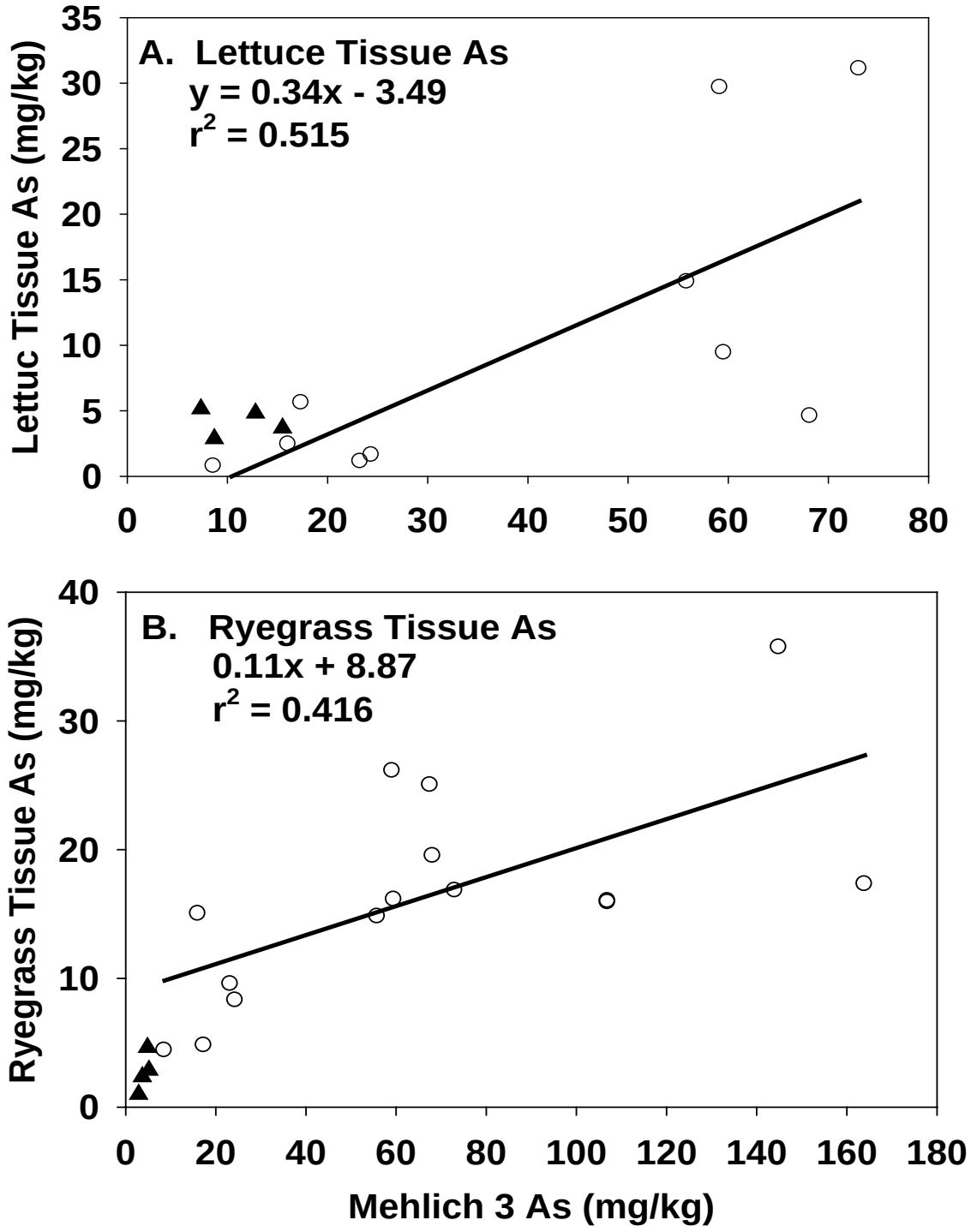

Figure C-5. Relationship between Lettuce (A) and Ryegrass (B) As concentration and Soil Mehlich 3 Extractable As for NCEA soils.

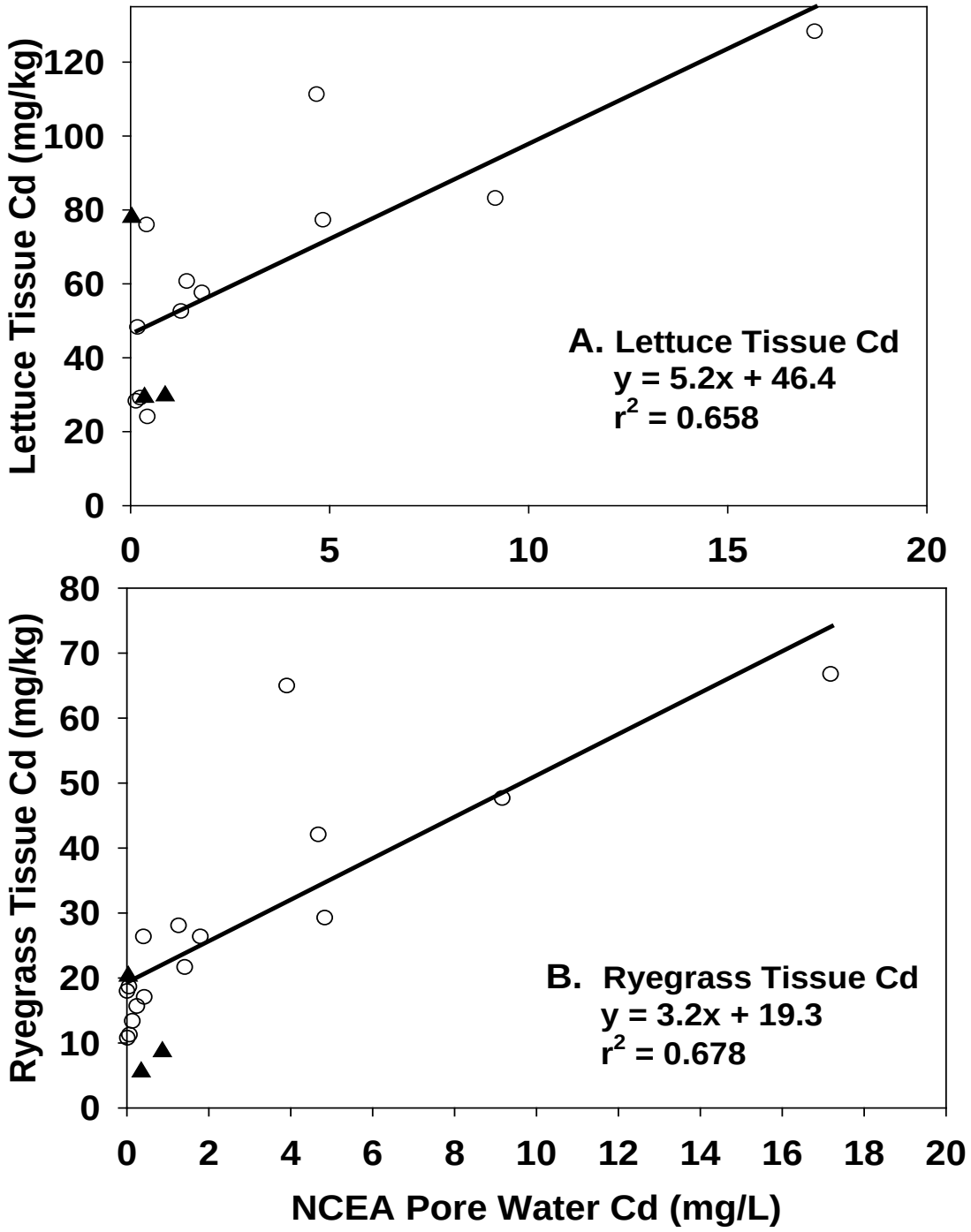

Figure C-6. Relationship between Lettuce (A) and Ryegrass (B) As concentration and Soil Pore Water Cd for NCEA soils.

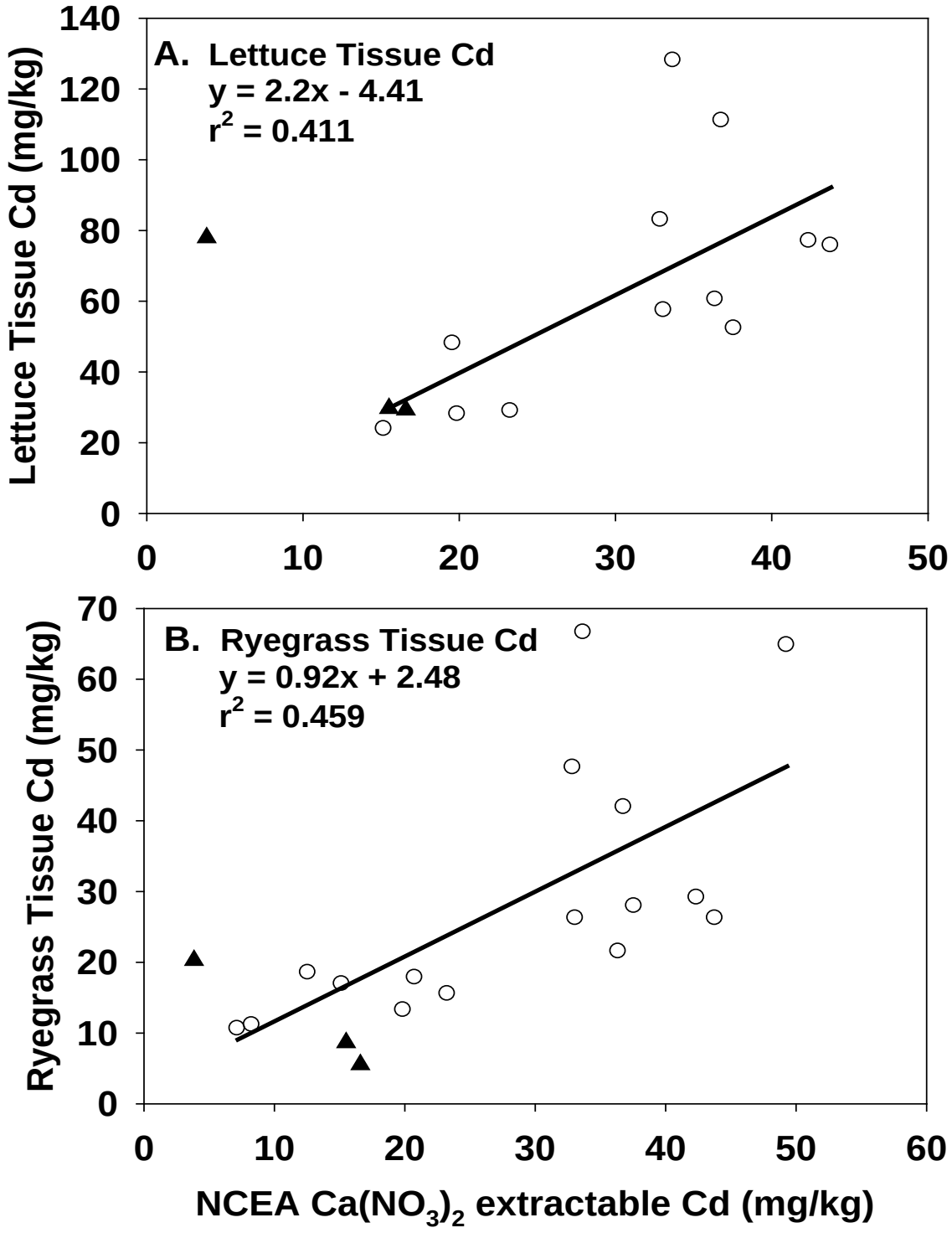

Figure C-7. Relationship between Lettuce (A) and Ryegrass (B) As Concentration and Soil 0.1 M Calcium Nitrate Extractable Cd for NCEA soils.

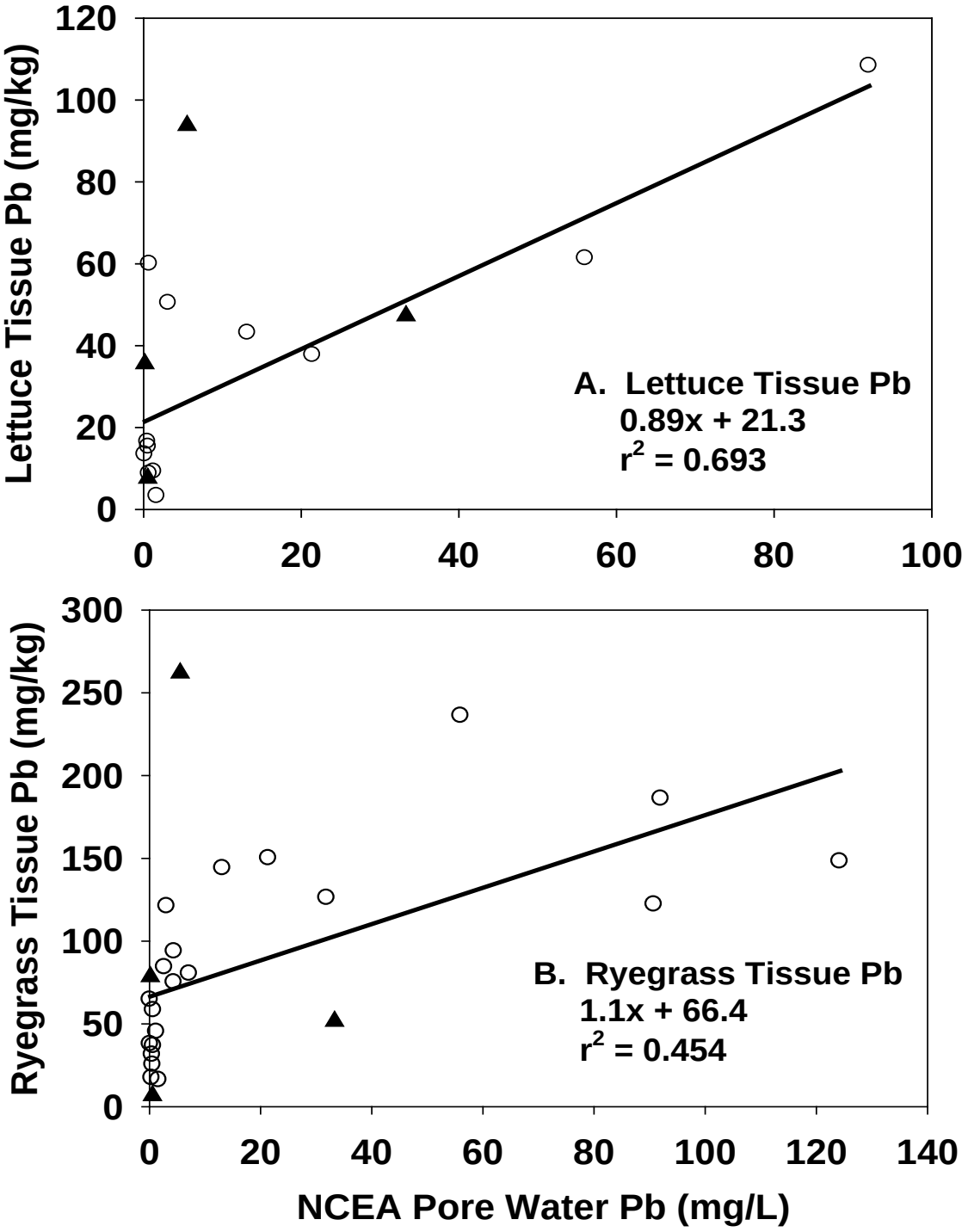

Figure C-8. Relationship between Lettuce (A) and Ryegrass (B) As concentration and Soil Pore Water Pb for NCEA soils.

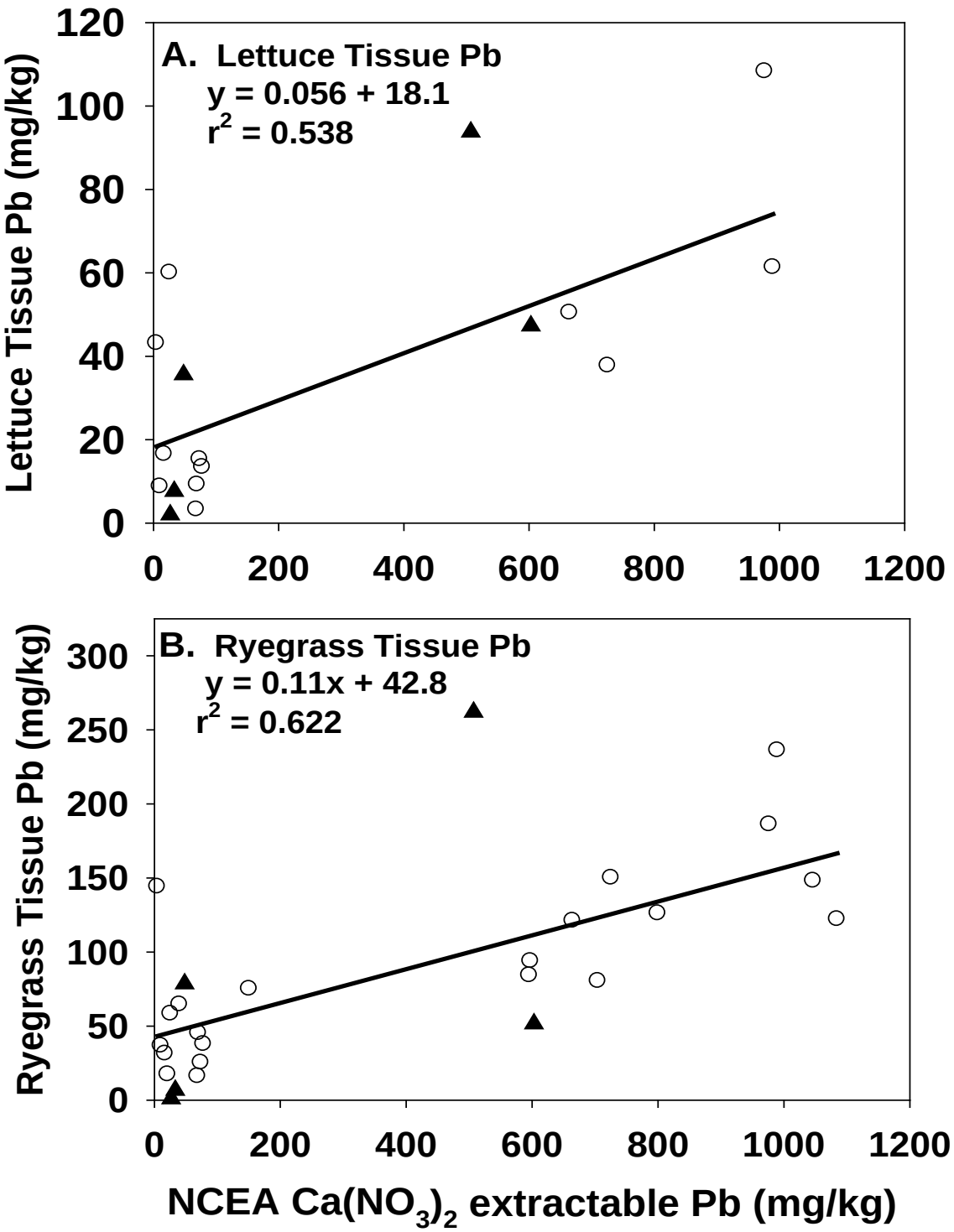

Figure C-9. Relationship between Lettuce (A) and Ryegrass (B) As Concentration and Soil 0.1 M Calcium Nitrate Extractable Pb for NCEA soils.

Table C-8. **Summary of the Prediction Capability†of Contaminant Phytoacculumation using Soil Property or Soil Extraction Soil Data, by soil**

Soil		Soil Property Model		Soil Extraction Model		
		MLR	RR	Pore Water	Mehlich 3	Ca(NO3)2
Con	As	P	E	E (lettuce) A (rye)	E (lettuce) A (rye)	
CP	As	A	A			
	Cd			A		A
Des	As	P	P	E (lettuce) A (rye)	E (lettuce) A (rye)	
Hill	Cd	P	A	P (lettuce) E (rye)		P (lettuce) A (rye)
Hilo	As	A	A	E (lettuce) A (rye)	E (lettuce) A (rye)	
	Pb	A	A			
MC	Cd	E	E	A		A
	Pb	E	E			A
Mech	Pb	E	E			
ORNL	Pb	P (lettuce) ‡ A (rye)	E	A		A
PC	As	E	E		E (lettuce) A (rye)	
	Pb	E	E			
Port	Pb	P (lettuce) A (rye)	P (lettuce) A (rye)	P		P
Travis	Pb	E	E	A		A

† Predictive capability, E = excellent, A=adequate, P=poor.

‡ Poor for lettuce, adequate for ryegrass

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APPENDIX D

RELATIVE BIOAVAILABILITY OF AS, PB, AND /OR CR IN SOIL

Relative bioavailability of Pb or As or Cr were determined in 3 study soils from juvenile swine dosing trials. Relative bioavailable Pb was determined for soil collected from the Portsmouth Naval Shipyard. Relative bioavailable As was determined for soil collected from the Deseret Chemical Depot. Relative bioavailable Cr was determined for soil collected from the McClellan Air Force Base.

Relative bioavailable Pb of soil from the Portsmouth Naval Shipyard

A study using juvenile swine as test animals was performed to measure the gastrointestinal absorption of lead from a sample collected from the Portsmouth Naval Shipyard. The test material contained a lead concentration of 4113 μg/g. The relative bioavailability of lead in the sample was assessed by comparing the absorption of lead from the test material to that of a reference material (lead acetate). Groups of five swine were given oral doses of lead acetate or test material twice a day for 14 days. The amount of lead absorbed by each animal was evaluated by measuring the amount of lead in the blood (measured on days 0, 3, 7, 9, 12, and 15) and the amount of lead in bone (measured on day 15 at study termination). The amount of lead present in blood or bone of animals exposed to test material was compared to that for animals exposed to lead acetate, and the results were expressed as relative bioavailability (RBA). The RBA results for the sample in this study are summarized in Table D-1. The lead RBA estimates are approximately 99% for the test material. This value is higher than the default value for lead in soil that is usually employed when reliable site-specific data are lacking. This indicates that the lead in this material is about as well absorbed as soluble lead. This relative bioavailability estimate may be used to improve accuracy and decrease uncertainty in estimating human health risks from exposure to this site-specific test material. Supporting detailed information of experimental data used to derive the RBA Pb value for the Portsmouth soil follows Table D-1.

Relative bioavailable As of soil the Deseret Chemical Depot

A study using juvenile swine as test animals was performed to measure the gastrointestinal absorption of arsenic from a soil sample taken in the vicinity of the Deseret Chemical Depot The soil sample contained an arsenic concentration of 521 ug/g. The relative bioavailability of arsenic was assessed by comparing the absorption of arsenic from the test material to that of a reference material (sodium arsenate). Groups of five swine were given oral doses of sodium arsenate or the test materials twice a day for 14 days; a group of three non-treated swine served as a control. The amount of arsenic absorbed by each animal was evaluated by measuring the amount of arsenic excreted in the urine (collected over 48-hour periods beginning on days 6, 9, and 12). The urinary excretion fraction (UEF) (the ratio of the amount excreted per 48 hours divided by the dose given per 48 hours) was calculated for both the test soil and sodium arsenate using linear regression analysis.

Table D-1. Relative Bioavailable Pb of the Portsmouth Soil

NAVY3 Lead RBA Estimates (All Data)

Measurement Endpoint	Estimated Soil RBA (90% Confidence Interval)
Blood Lead AUC	1.02 (0.63 - 1.37)
Femur Lead	0.95 (0.71 - 1.30)
Point Estimate	0.99 (0.70 - 1.27)

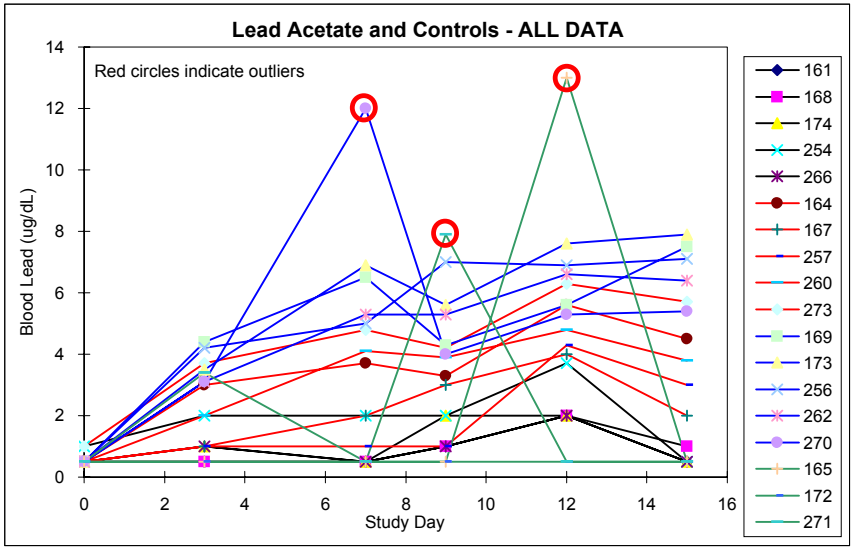
TABLE D-~~2~~ IDENTIFICATION OF POTENTIAL BLOOD LEAD OUTLIERS

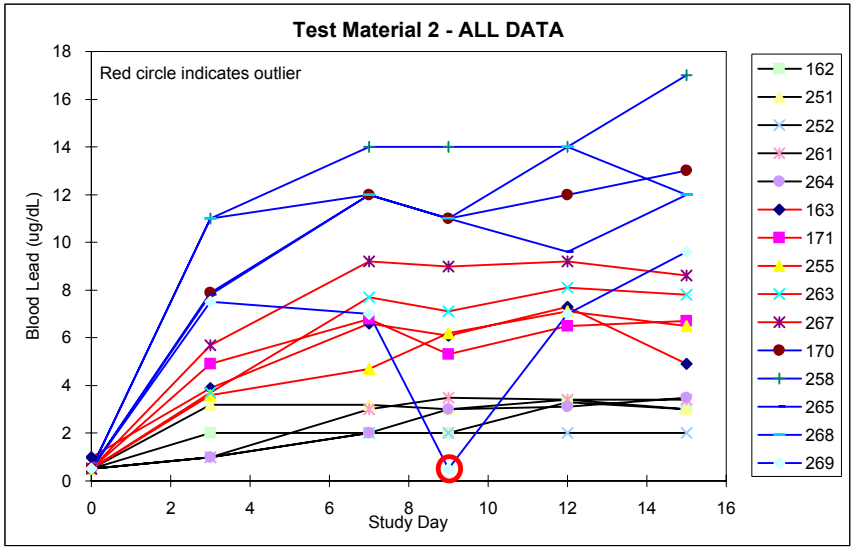
Material Administered	Group	Pig Number	Target Dose	Blood Lead (µg/dL) by Day					
				0	3	7	9	12	15
Lead Acetate	1	161	25	0.5	0.5	0.5	1.0	2.0	0.5
Lead Acetate	1	168	25	0.5	0.5	0.5	1.0	2.0	1.0
Lead Acetate	1	174	25	0.5	1.0	0.5	2.0	2.0	0.5
Lead Acetate	1	254	25	1.0	2.0	2.0	2.0	3.7	0.5
Lead Acetate	1	266	25	0.5	1.0	0.5	1.0	2.0	0.5
Lead Acetate	2	164	75	0.5	3.0	3.7	3.3	5.6	4.5
Lead Acetate	2	167	75	0.5	1.0	2.0	3.0	4.0	2.0
Lead Acetate	2	257	75	0.5	1.0	1.0	1.0	4.3	3.0
Lead Acetate	2	260	75	0.5	2.0	4.1	3.9	4.8	3.8
Lead Acetate	2	273	75	1.0	3.7	4.8	4.2	6.3	5.7
Lead Acetate	3	169	225	0.5	4.4	6.5	4.3	5.6	7.5
Lead Acetate	3	173	225	0.5	3.5	6.9	5.6	7.6	7.9
Lead Acetate	3	256	225	0.5	4.2	5.0	7.0	6.9	7.1
Lead Acetate	3	262	225	0.5	3.1	5.3	5.3	6.6	6.4
Lead Acetate	3	270	225	0.5	3.1	12.0	4.0	5.3	5.4
Test Material 3	4	162	75	0.5	2.0	2.0	2.0	3.3	3.0
Test Material 3	4	251	75	0.5	3.2	3.2	3.0	3.4	3.0
Test Material 3	4	252	75	0.5	1.0	2.0	2.0	2.0	2.0
Test Material 3	4	261	75	0.5	1.0	3.0	3.5	3.4	3.4
Test Material 3	4	264	75	0.5	1.0	2.0	3.0	3.1	3.5
Test Material 3	5	163	225	1.0	3.9	6.6	6.1	7.3	4.9
Test Material 3	5	171	225	0.5	4.9	6.8	5.3	6.5	6.7
Test Material 3	5	255	225	0.5	3.6	4.7	6.2	7.1	6.5
Test Material 3	5	263	225	0.5	3.7	7.7	7.1	8.1	7.8
Test Material 3	5	267	225	0.5	5.7	9.2	9.0	9.2	8.6
Test Material 3	6	170	675	0.5	7.9	12.0	11.0	12.0	13.0
Test Material 3	6	258	675	0.5	11.0	14.0	14.0	14.0	17.0
Test Material 3	6	265	675	0.5	7.8	12.0	11.0	9.6	12.0
Test Material 3	6	268	675	0.5	11.0	12.0	11.0	14.0	12.0
Test Material 3	6	269	675	0.5	7.5	7.0	0.5	7.0	9.6
Control	7	165	0	0.5	0.5	0.5	0.5	13.0	0.5
Control	7	172	0	0.5	0.5	0.5	0.5	0.5	0.5
Control	7	271	0	0.5	3.4	0.5	7.9	0.5	0.5

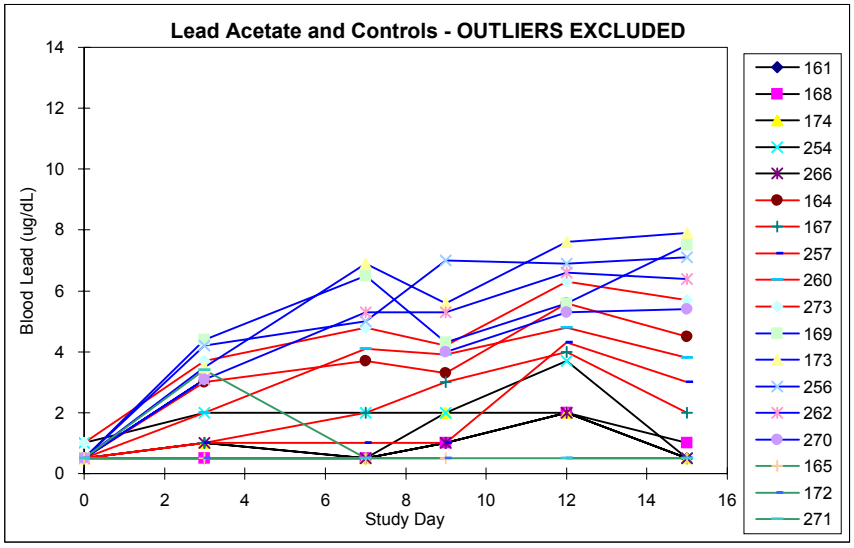
Dose units: µg/kg-d

Data point judged to be outlier; excluded from further analyses

FIGURE D-1 BLOOD LEAD DATA BY DAY







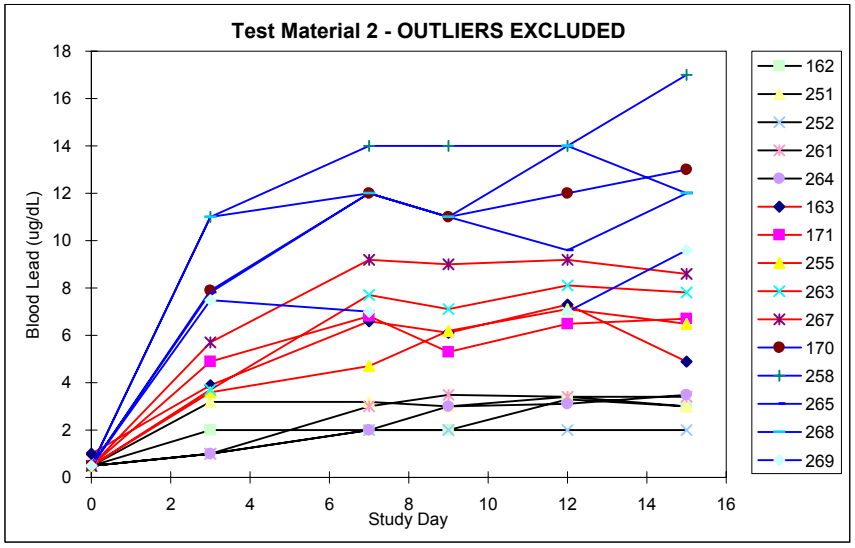
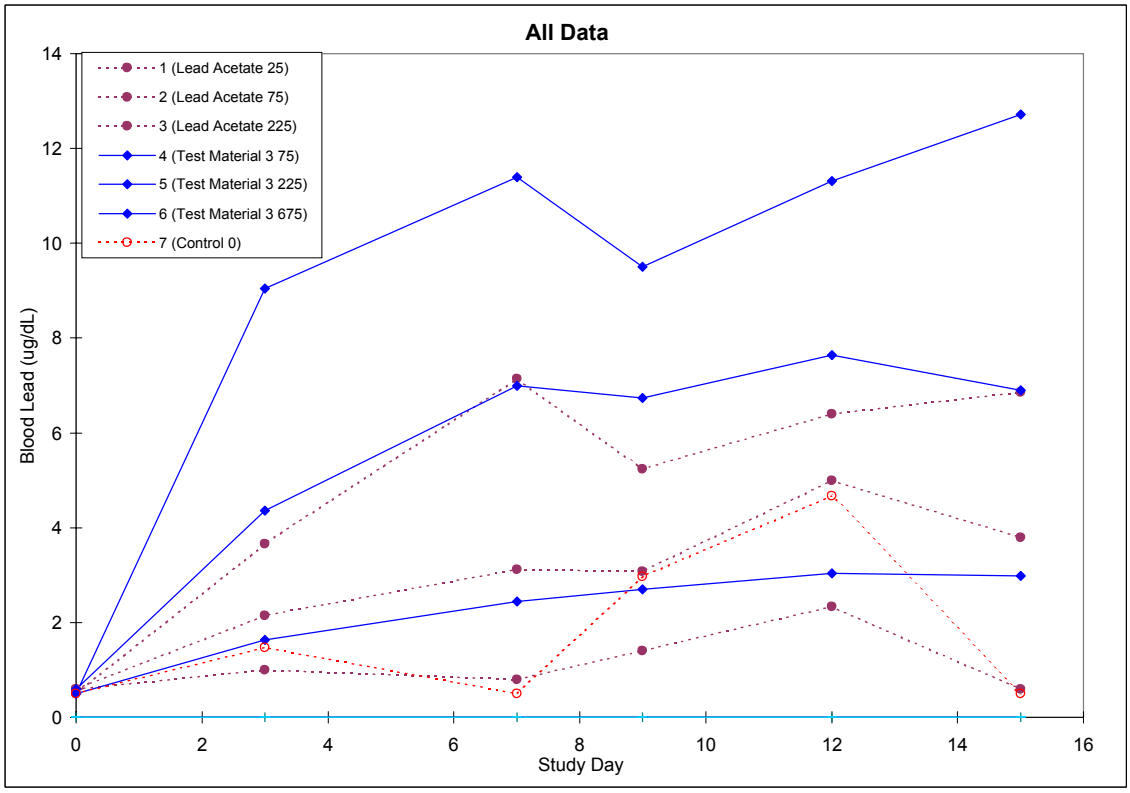


FIGURE D-2 GROUP MEAN BLOOD LEAD BY DAY



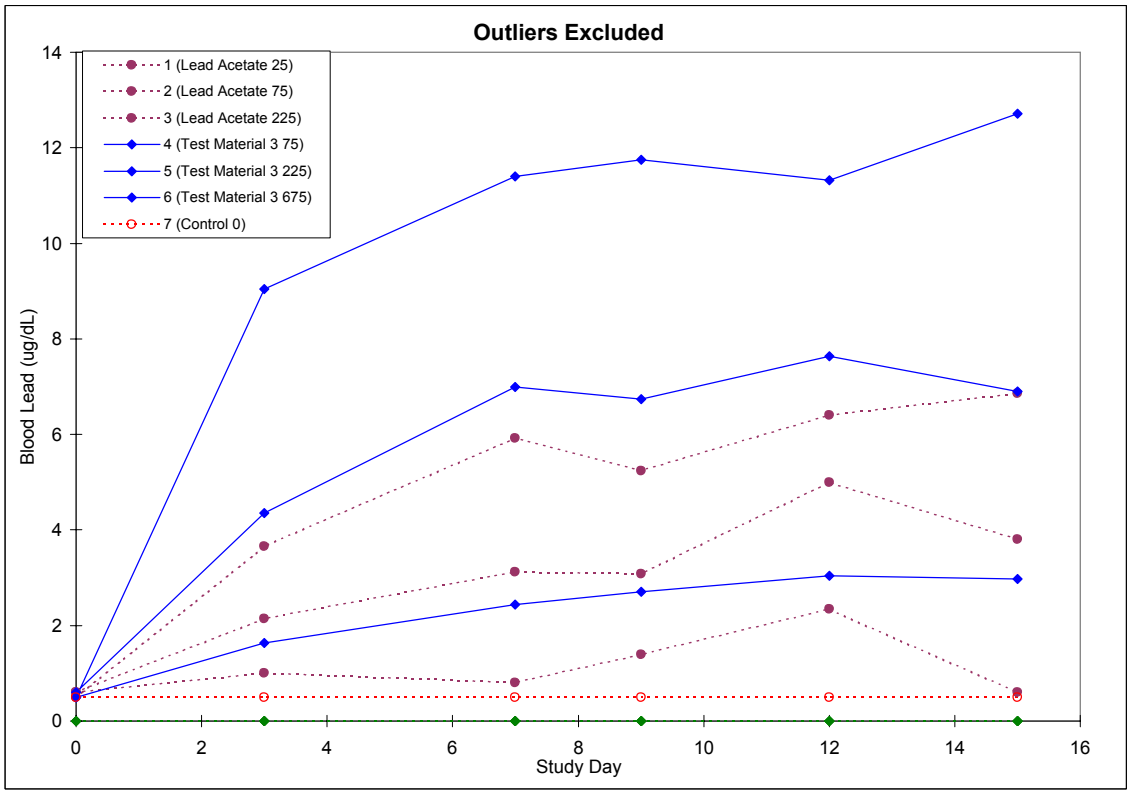
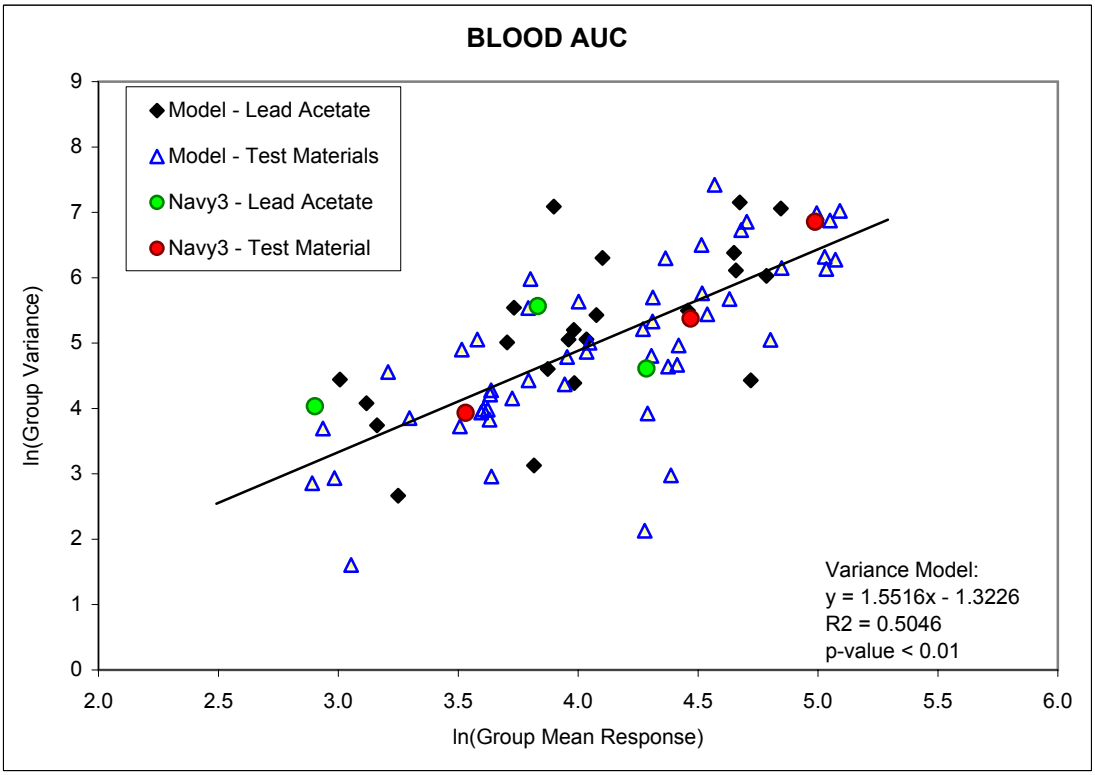
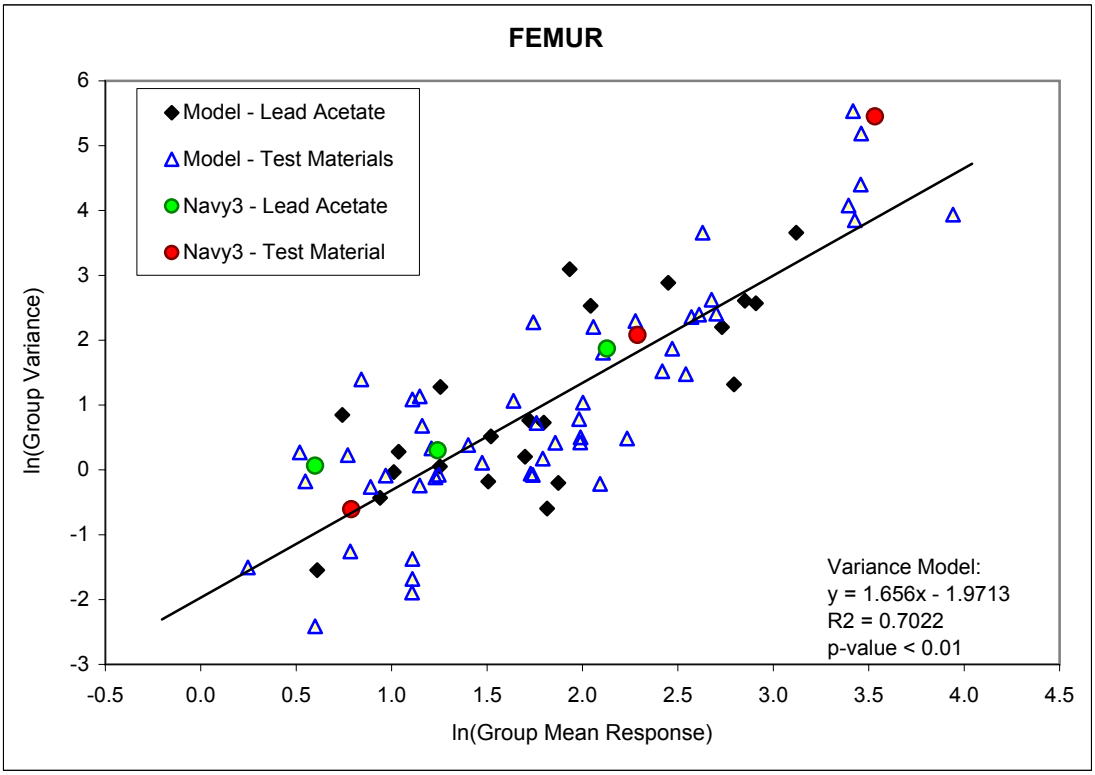


TABLE D-3 BLOOD LEAD AREA UNDER CURVE DETERMINATIONS

Group	Pig Number	AUC (µg/dL-days) for Time Interval Shown					AUC Total (µg/dL-days)
		0-3	3-7	7-9	9-12	12-15	
1	161	1.50	2.00	1.50	4.50	3.75	13.25
1	168	1.50	2.00	1.50	4.50	4.50	14.00
1	174	2.25	3.00	2.50	6.00	3.75	17.50
1	254	4.50	8.00	4.00	8.55	6.30	31.35
1	266	2.25	3.00	1.50	4.50	3.75	15.00
2	164	5.25	13.40	7.00	13.35	15.15	54.15
2	167	2.25	6.00	5.00	10.50	9.00	32.75
2	257	2.25	4.00	2.00	7.95	10.95	27.15
2	260	3.75	12.20	8.00	13.05	12.90	49.90
2	273	7.05	17.00	9.00	15.75	18.00	66.80
3	169	7.35	21.80	10.80	14.85	19.65	74.45
3	173	6.00	20.80	12.50	19.80	23.25	82.35
3	256	7.05	18.40	12.00	20.85	21.00	79.30
3	262	5.40	16.80	10.60	17.85	19.50	70.15
3	270	5.40	13.60	7.70	13.95	16.05	56.70
4	162	3.75	8.00	4.00	7.95	9.45	33.15
4	251	5.55	12.80	6.20	9.60	9.60	43.75
4	252	2.25	6.00	4.00	6.00	6.00	24.25
4	261	2.25	8.00	6.50	10.35	10.20	37.30
4	264	2.25	6.00	5.00	9.15	9.90	32.30
5	163	7.35	21.00	12.70	20.10	18.30	79.45
5	171	8.10	23.40	12.10	17.70	19.80	81.10
5	255	6.15	16.60	10.90	19.95	20.40	74.00
5	263	6.30	22.80	14.80	22.80	23.85	90.55
5	267	9.30	29.80	18.20	27.30	26.70	111.30
6	170	12.60	39.80	23.00	34.50	37.50	147.40
6	258	17.25	50.00	28.00	42.00	46.50	183.75
6	265	12.45	39.60	23.00	30.90	32.40	138.35
6	268	17.25	46.00	23.00	37.50	39.00	162.75
6	269	12.00	29.00	14.00	21.00	24.90	100.90
7	165	1.50	2.00	1.00	1.50	1.50	7.50
7	172	1.50	2.00	1.00	1.50	1.50	7.50
7	271	5.85	7.80	1.00	1.50	1.50	17.65

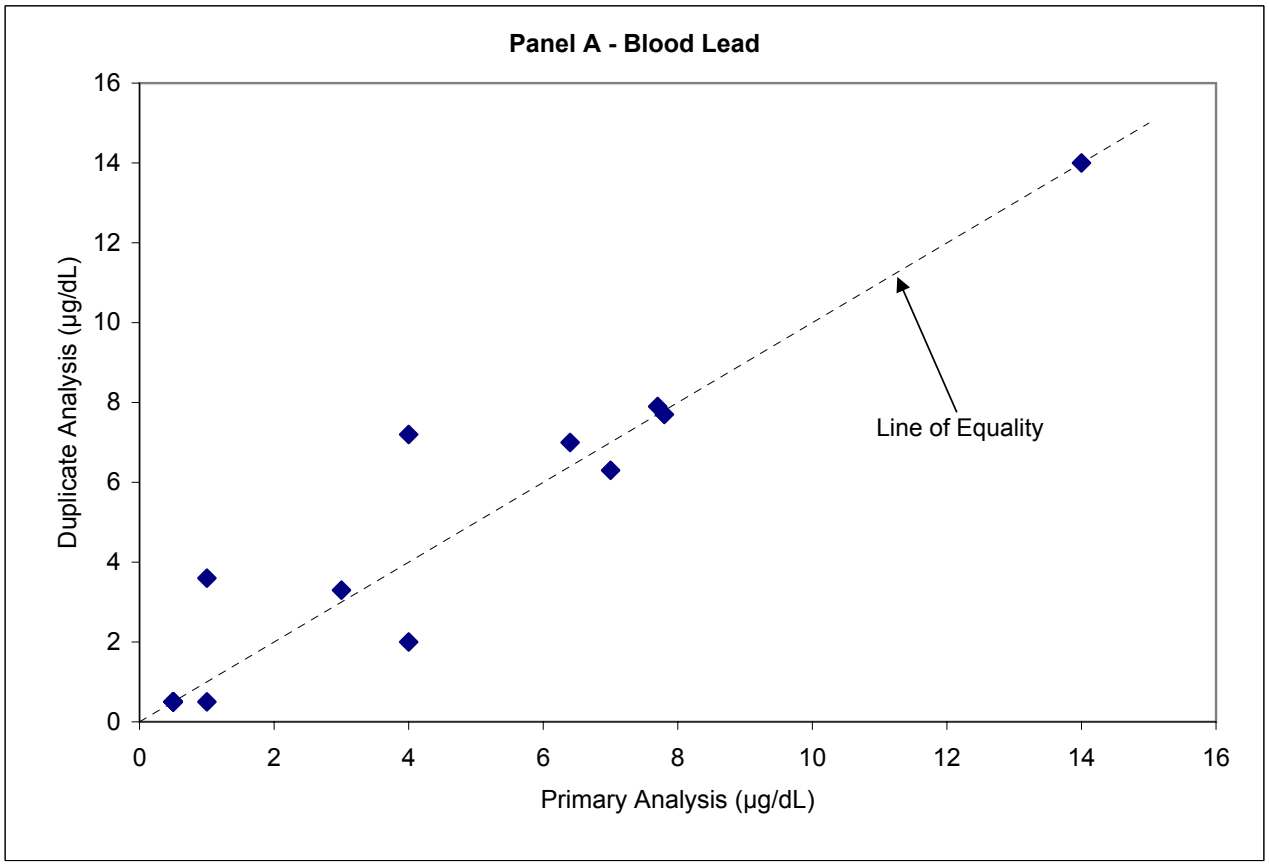
FIGURE D-3 VARIANCE MODELS





4_MSE3 Variance.xls (Fig4-2_All Endpoints)

FIGURE D-4 SAMPLE PREPARATION REPLICATES



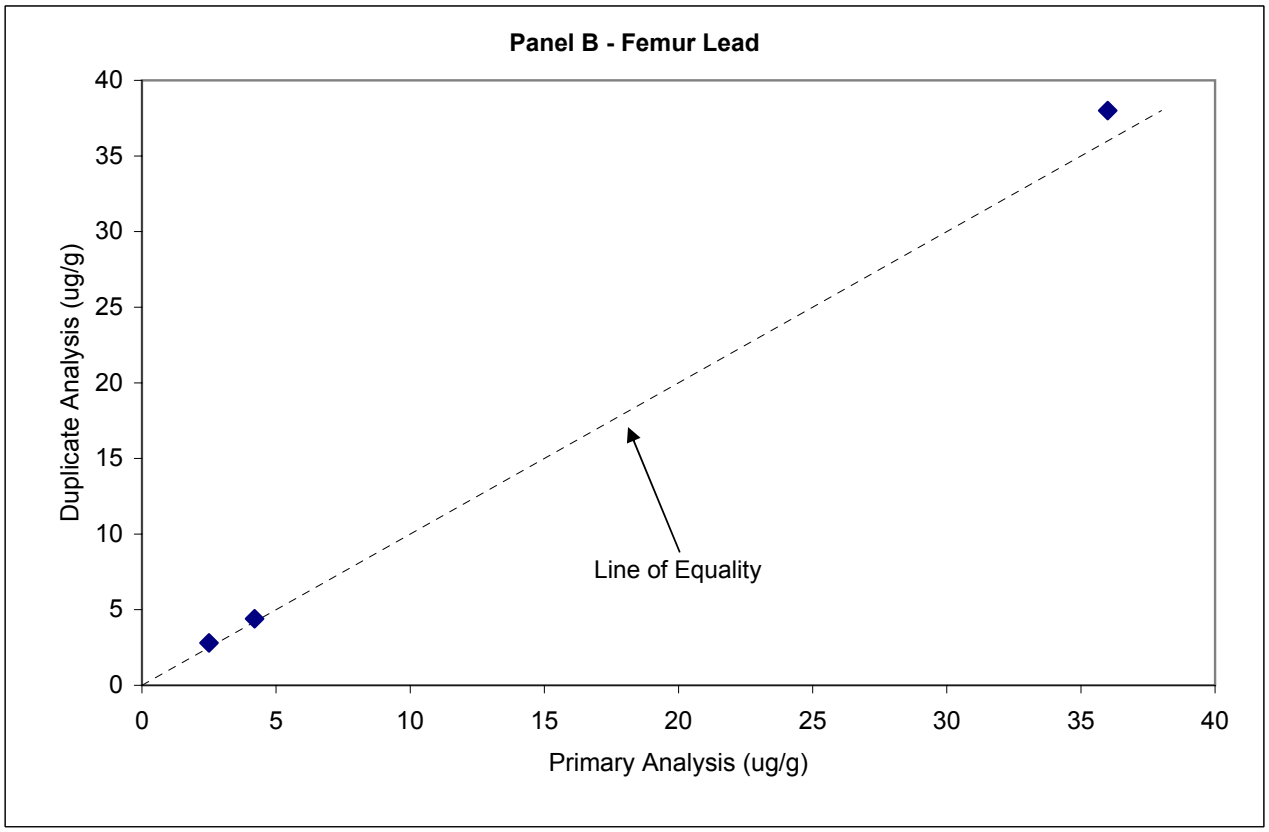
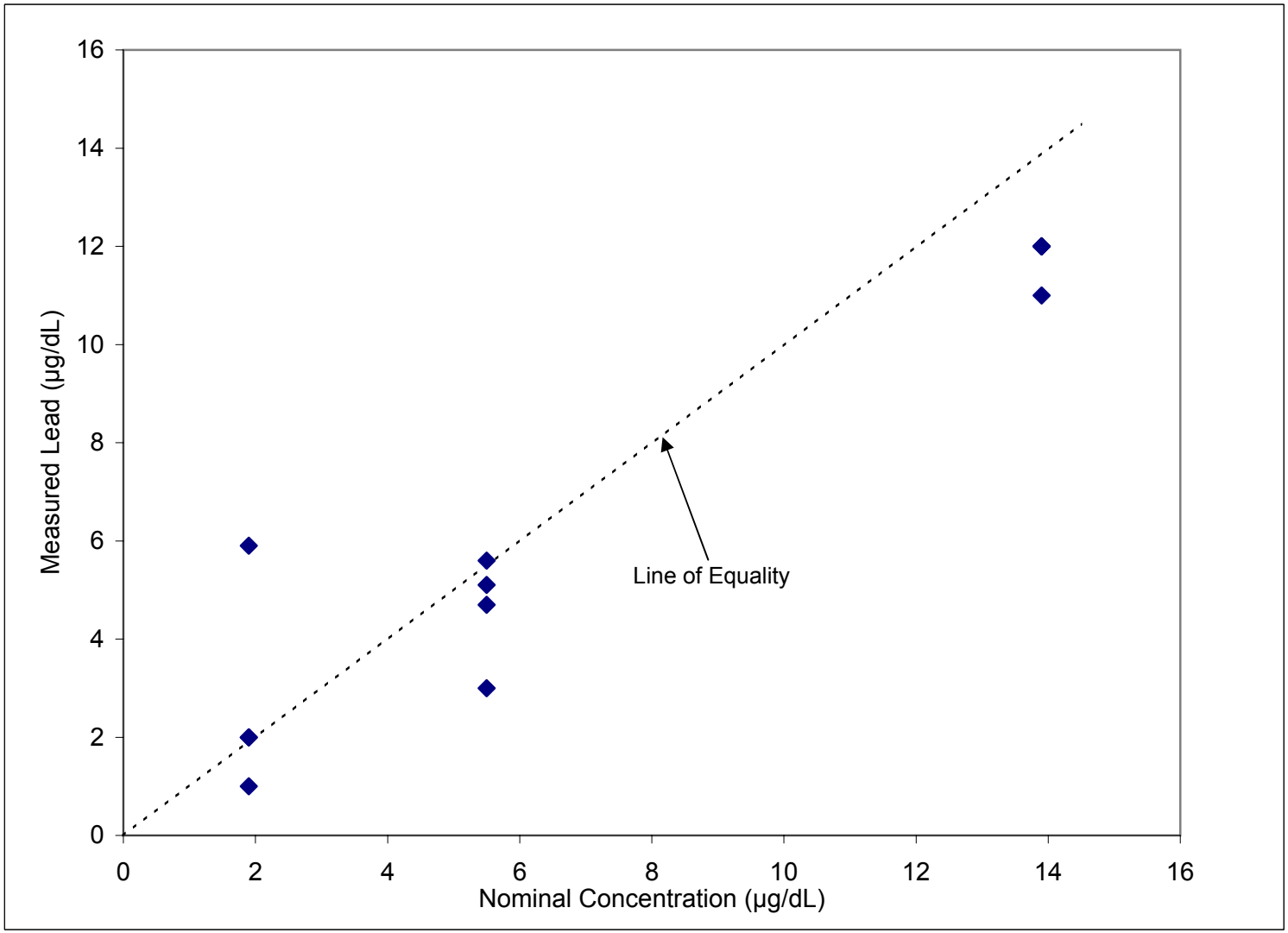


FIGURE D-5 CDC BLOOD LEAD CHECK SAMPLES



5_Navy3 Lab QC analysis.xls (Fig2-3_CDC)

The relative bioavailability (RBA) of arsenic in the test soil compared to that in sodium arsenate was calculated as follows:

$$RBA = \frac{UEF(test\ soil)}{UEF(sodium\ arsenate)}$$

Results are summarized in Table D-3. The arsenic RBA estimates are approximately 14% for the test material. These values are much lower than the default value range of 80%-100% for arsenic in soil that is usually employed when reliable site-specific data are lacking. This indicates that the arsenic in this material is not as well absorbed as soluble arsenic. This relative bioavailability estimate may be used to improve accuracy and decrease uncertainty in estimating human health risks from exposure to this site-specific test material. Supporting detailed information of experimental data used to derive the RBA As for the Deseret soil follows Table D-3.

Table D-3. Relative Bioavailable As of the Deseret Soil

Navy1 Arsenic - Deseret Chemical Depot
RBA Estimates, Outliers Excluded

Measurement Endpoint	Estimated RBA (90% Confidence Interval)
Days 6/7	0.13 (0.11 - 0.15)
Days 9/10	0.13 (0.12 - 0.14)
Days 12/13	0.14 (0.13 - 0.16)
All Days	0.14 (0.13 - 0.15)

Table D-4 Arsenic - Deseret Chemical Depot
RBA Estimates, Outliers Excluded

Measurement Endpoint	Estimated RBA (90% Confidence Interval)
Days 6/7	0.13 (0.11 - 0.15)
Days 9/10	0.13 (0.12 - 0.14)
Days 12/13	0.14 (0.13 - 0.16)
All Days	0.14 (0.13 - 0.15)

TABLE D-5 DOSING PROTOCOL

Group	Number of Animals	Dose Material Administered	Arsenic Dose (µg/kg-day)	
			Target	Actual [a]
1	5	Sodium Arsenate	25	27.6
2	5	Sodium Arsenate	50	55.8
3	5	Sodium Arsenate	100	109.8
4	5	Test Material 1	40	44.8
5	5	Test Material 1	80	87.6
6	5	Test Material 1	160	172.9
7	3	Control	0	0.0

[a] Calculated as the administered daily dose divided by the measured or extrapolated daily body weight, averaged over days 0-14 for each animal and each group. Actual dose for Group 5 excludes the doses administered to pig number 127, which was euthanized on day 11.

Doses were administered in two equal portions given at 9:00 AM and 3:00 PM each day. Doses were held constant based on a body weight of 13.9 kg, the expected mean weight during the exposure interval (14 days). Actual mean body weight across all animals during the exposure interval was 12.9 kg.

TABLE D-6 TYPICAL FEED COMPOSITION

TestDiet 5TXP: Porcine Grower Purified Diet with Low Lead[1]

INGREDIENTS

Corn Starch, %	25.2	Potassium Phosphate, %	0.87
Sucrose, %	20.9648	Calcium Carbonate, %	0.7487
Glucose, %	16	Salt, %	0.501
Soy Protein Isolate, %	14.9899	Magnesium Sulfate, %	0.1245
Casein - Vitamin Free, %	8.5	DL-Methionine, %	0.0762
Powdered Cellulose, %	6.7208	Choline Chloride, %	0.0586
Corn Oil, %	3.4046	Vitamin/Mineral Premix, %	0.0577
Dicalcium Phosphate, %	1.7399	Sodium Selenite, %	0.0433

NUTRITIONAL PROFILE[2]

Protein, %	**21**	**Fat, %**		**3.5**
Arginine, %	1.42	Cholesterol, ppm		0
Histidine, %	0.61	Linoleic Acid, %		1.95
Isoleucine, %	1.14	Linolenic Acid, %		0.03
Leucine, %	1.95	Arachidonic Acid, %		0
Lysine, %	1.56	Omega-3 Fatty Acids, %		0.03
Methionine, %	0.49	Total Saturated Fatty Acids, %		0.43
Cystine, %	0.23	Total Monounsaturated Fatty Acids, %		0.82
Phenylalanine, %	1.22	Polyunsaturated Fatty Acids, %		1.98
Tyrosine, %	1.03			
Threonine, %	0.88			
Tryptophan, %	0.32	**Fiber (max), %**		**6.8**
Valine, %	1.16			
Alanine, %	0.95	**Carbohydrates, %**		**62.2**
Aspartic Acid, %	2.33			
Glutamic Acid, %	4.96	**Energy (kcal/g)** [3]		**3.62**
Glycine, %	0.79	*From:*	*kcal*	*%*
Proline, %	1.83	Protein	0.84	23.1
Serine, %	1.25	Fat (ether extract)	0.315	8.7
Taurine, %	0	Carbohydrates	2.487	68.3
Minerals		**Vitamins**		
Calcium, %	0.8	Vitamin A, IU/g		1.7
Phosphorus, %	0.72	Vitamin 0-3 (added), IU/g		0.2
Phosphorus (available), %	0.4	Vitamin E, IU/kg		11
Potassium, %	0.27	Vitamin K (as menadione), ppm		0.52
Magnesium, %	0.04	Thiamin Hydrochloride, ppm		1
Sodium, %	0.3	Ribonavin, ppm		3.1
Chlorine, %	0.31	Niacin, ppm		13
Fluorine, ppm	0	Pantothenic Acid, ppm		9
Iron, ppm	82	Folic Acid, ppm		0.3
Zinc, ppm	84	Pyridoxine, ppm		1.7
Manganese, ppm	3	Biotin, ppm		0.1
Copper, ppm	4.9	Vitamin B-12, mcg/kg		15
Cobalt, ppm	0.1	Choline Chloride, ppm		410
Iodine, ppm	0.15	Ascorbic Acid, ppm		0
Chromium, ppm	0			
Molybdenum, ppm	0.01			
Selenium, ppm	0.26			

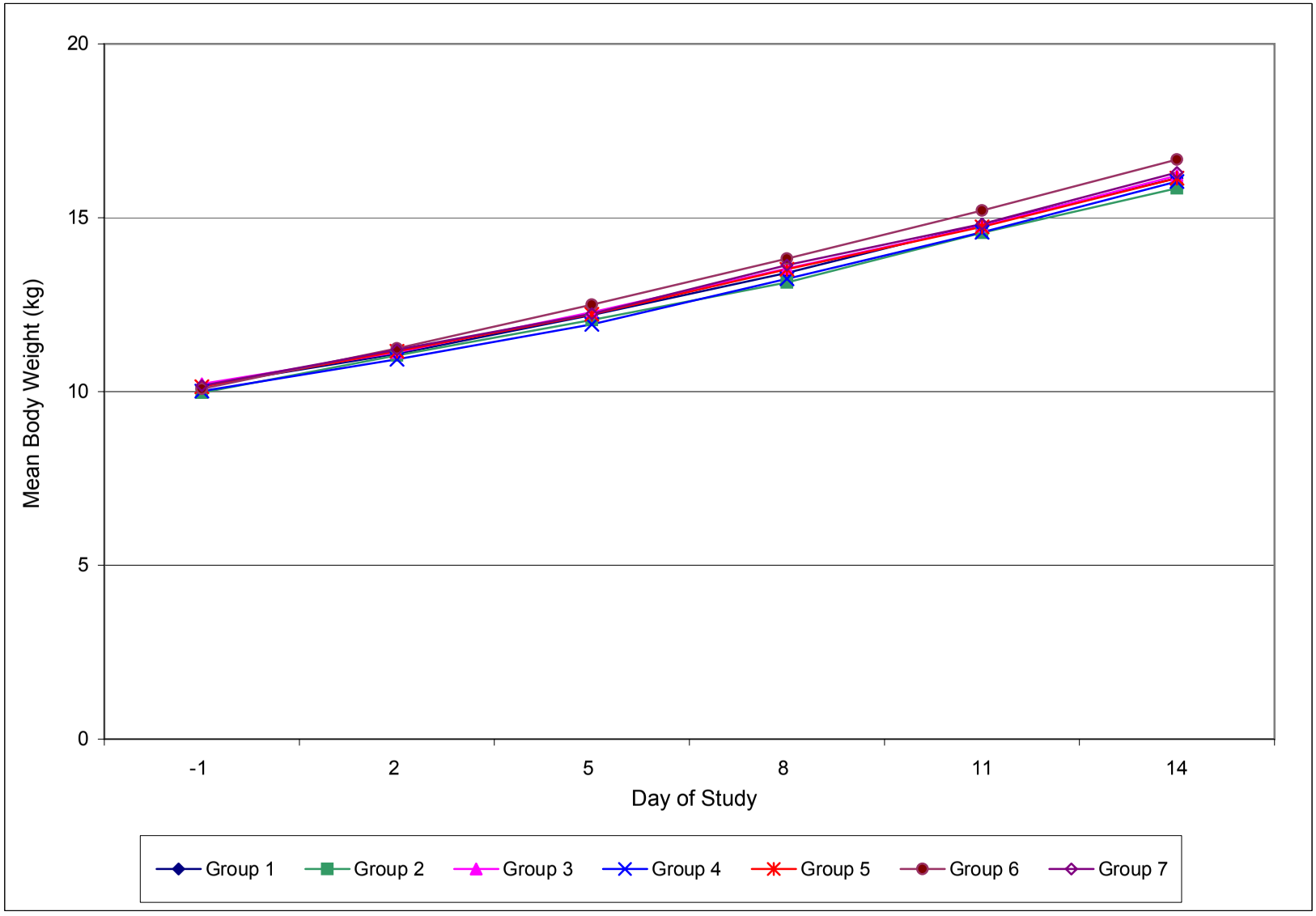
FOOTNOTES

[1] This special purified diet was originally developed for lead RBA studies.

[2] Based on the latest ingredient analysis information. Since nutrient composition of natural ingredients varies, analysis will differ accordingly. Nutrients expressed as percent of ration on an As Fed basis except where otherwise indicated.

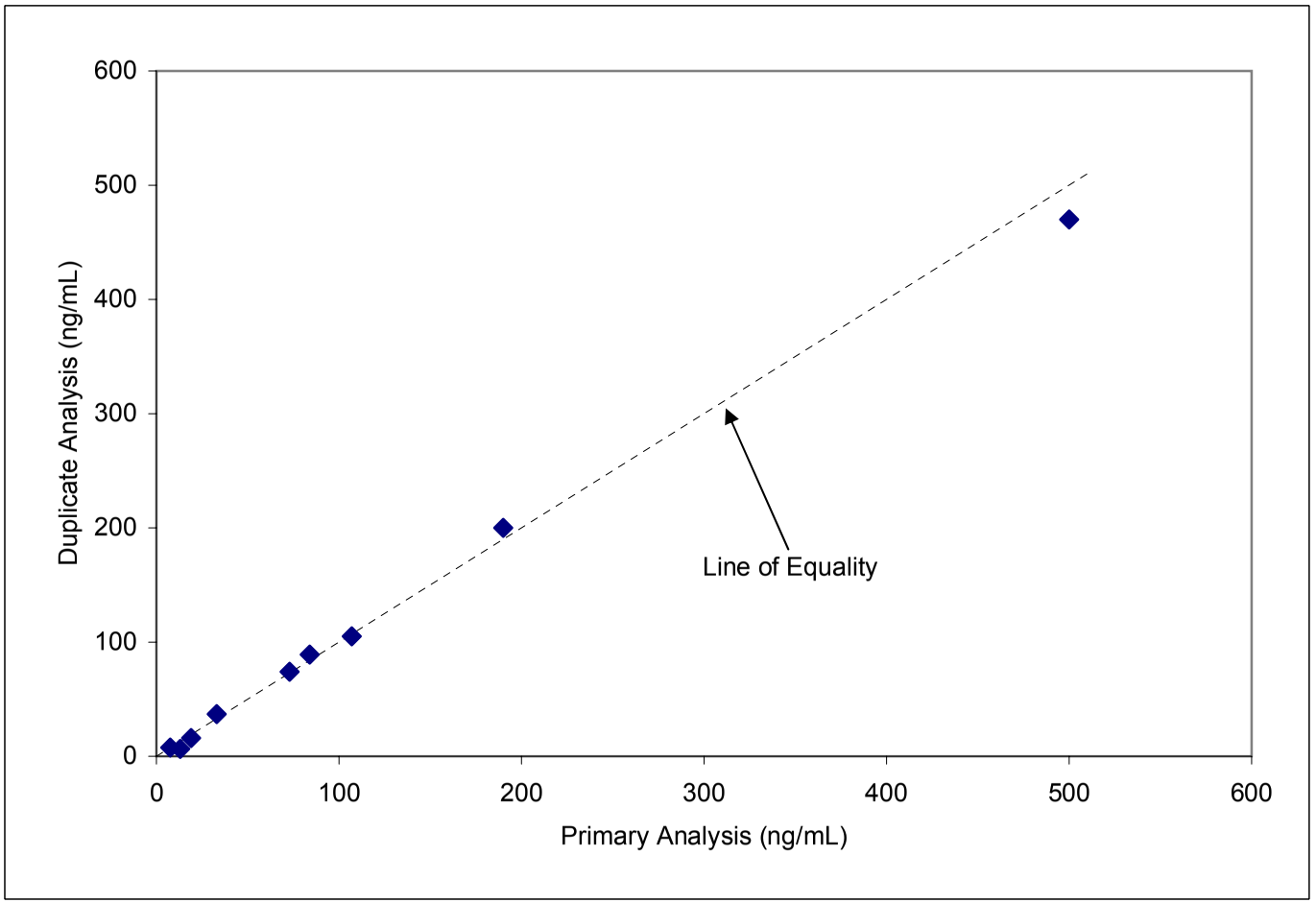
[3] Energy (kcal/gm) - Sum of decimal fractions of protein, fat and carbohydrate x 4,9,4 kcal/gm respectively.

FIGURE D-5 BODY WEIGHT GAIN



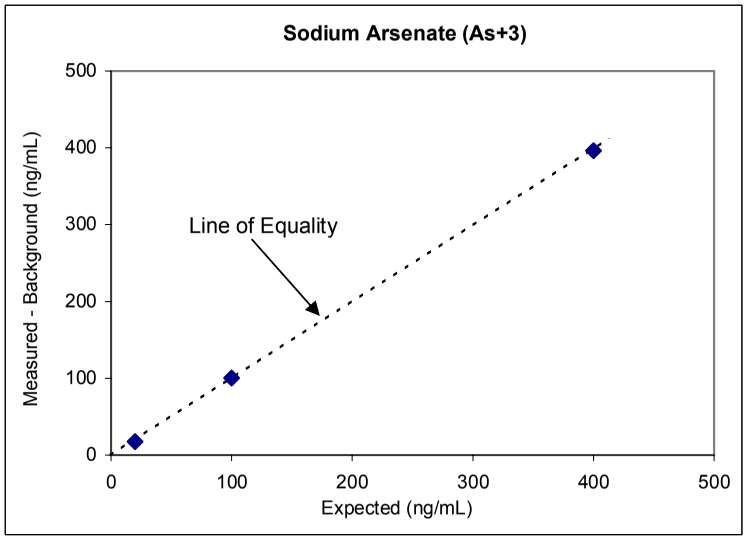
1_Navy1 Weights.xls (Fig2-1_group weights)

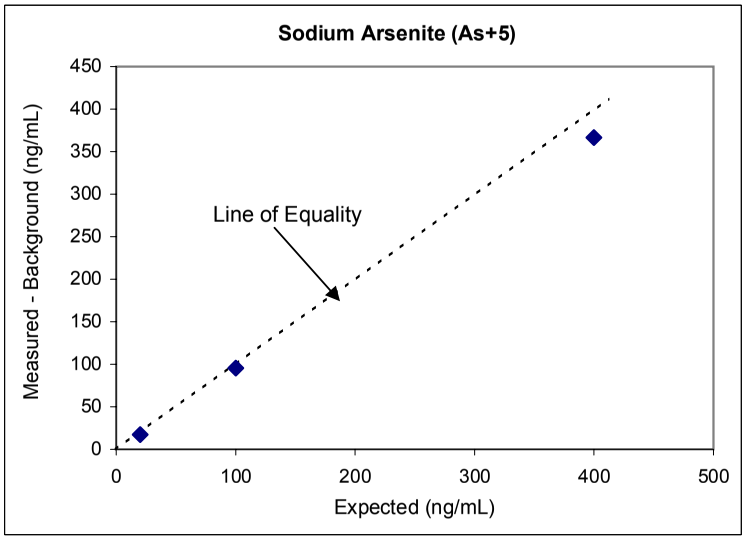
FIGURE D-6 URINARY ARSENIC BLIND DUPLICATES

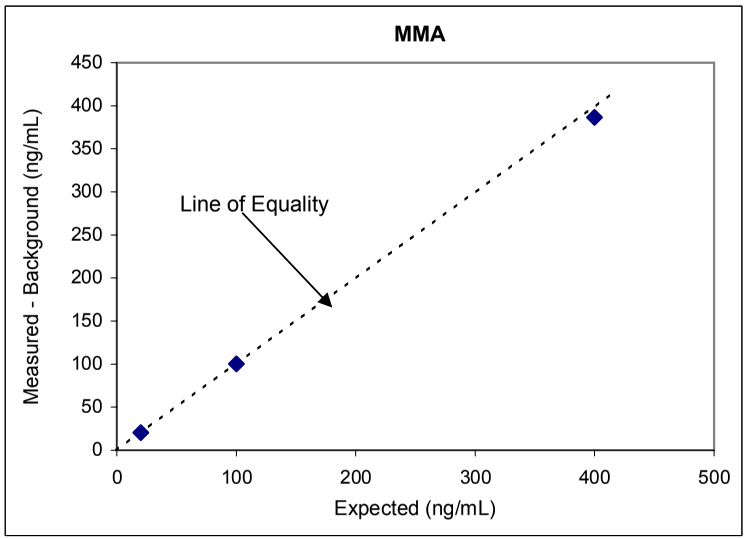


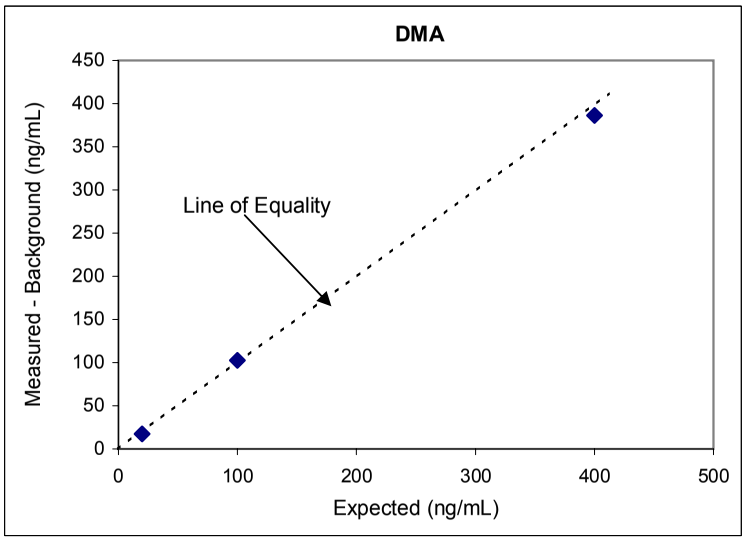
4_Navy1 Lab QC analysis.xls (Fig2-2_BlindDupes)T

FIGURE D-7 PERFORMANCE EVALUATION SAMPLES





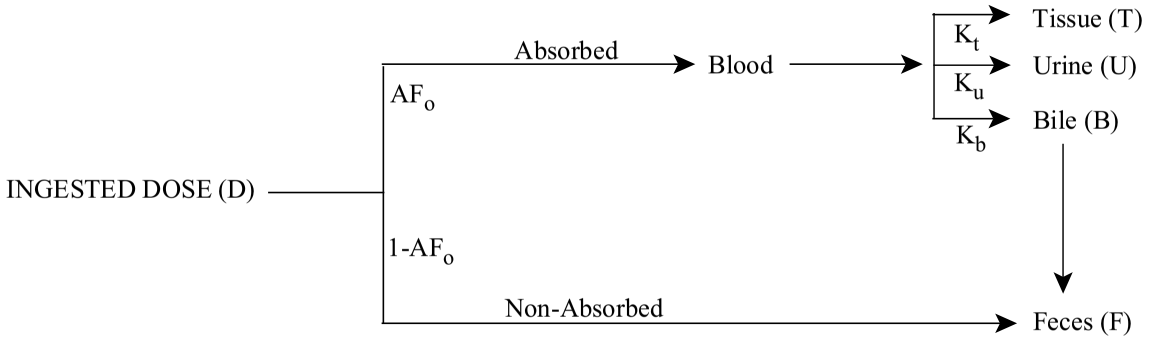




Note: The MMA result of 103 ng/mL was intended to be a sample of the 400 ng/mL stock solution. Based on the result of 103 ng/mL, it is presumed that it was actually from the 100 ng/mL stock solution, not 400. However, this change was not documented so is not known for certain which stock solution was used for this sample.

4_Navy1 Lab QC analysis.xls (Fig2-3_PE)

Figure D-8. Conceptual Model for Arsenic Toxicokinetics



where:

D = Ingested dose (ug)
AF_o = Oral Absorption Fraction
K_t = Fraction of absorbed arsenic which is retained in tissues
K_u = Fraction of absorbed arsenic which is excreted in urine
K_b = Fraction of absorbed arsenic which is excreted in the bile

BASIC EQUATIONS:

Amount Absorbed (ug) $= D \times AF_o$

Amount Excreted (ug) $=$ Amount absorbed $\times K_u$

$= D \times AF_o \times K_u$

Urinary Excretion Fraction (UEF) $=$ Amount excreted / Amount Ingested

$= (D \times AF_o \times K_u) / D$

$= AF_o \times K_u$

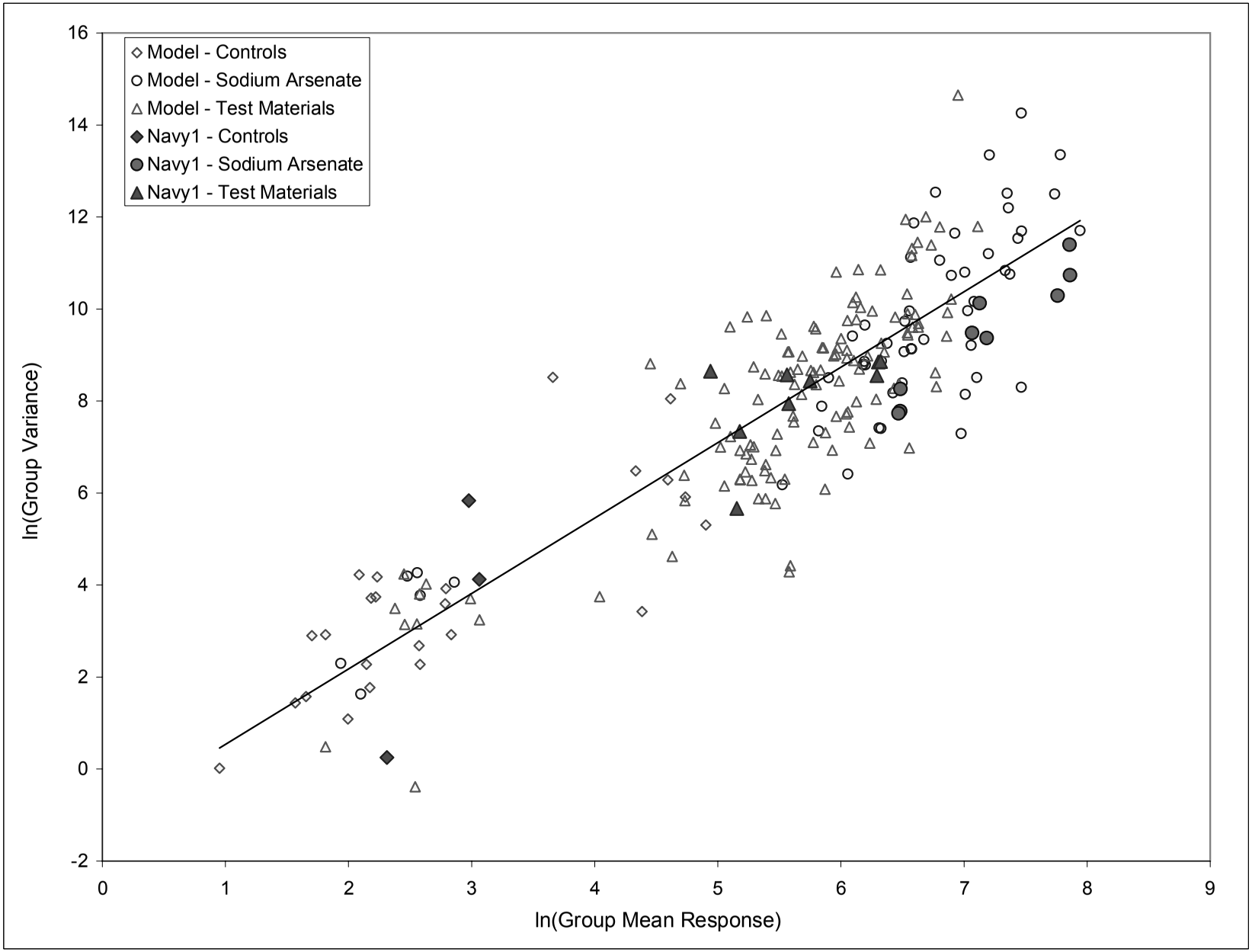
Relative Bioavailability (x vs. y) $= UEF(x) / UEF(y)$

$= (AF_o(x) \times K_u) / (AF_o(y) \times K_u)$

$= AF_o(x) / AF_o(y)$

Fig 3-1_Toxicokinetics.wpd

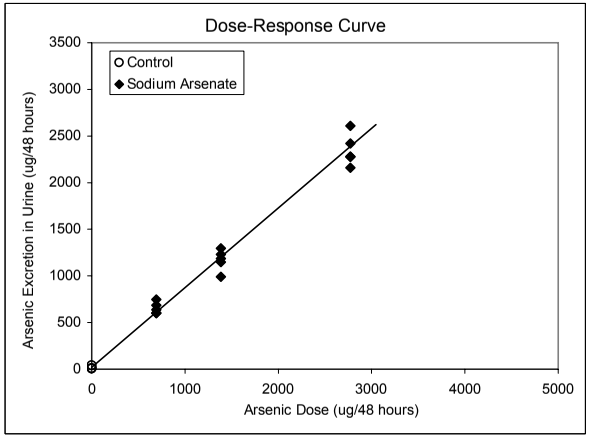
FIGURE D-9 URINARY ARSENIC VARIANCE MODEL

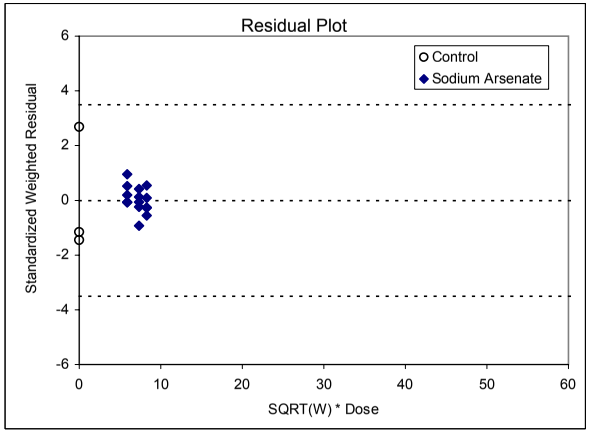


3_Navy1 Variance.xls (Fig 4-1)

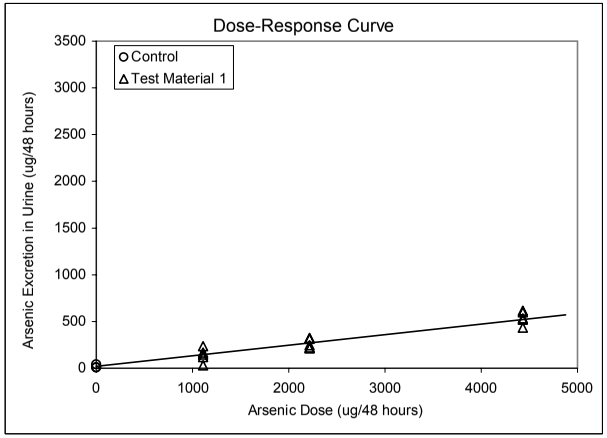
FIGURE D-10 URINARY EXCRETION OF ARSENIC: Days 6/7 (All Data)

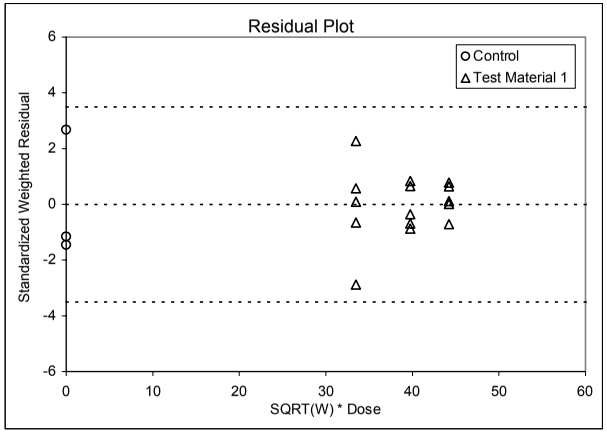
Reference Material (Sodium Arsenate)



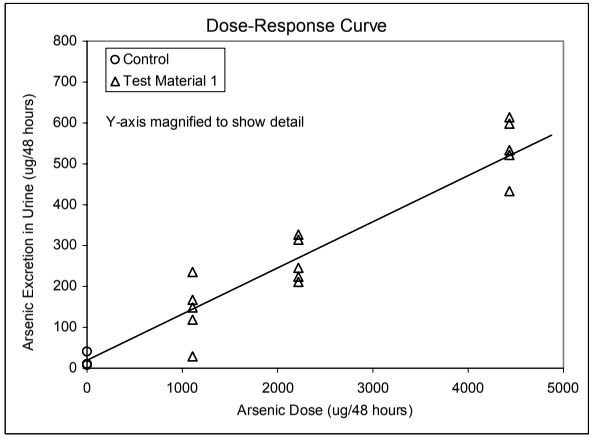


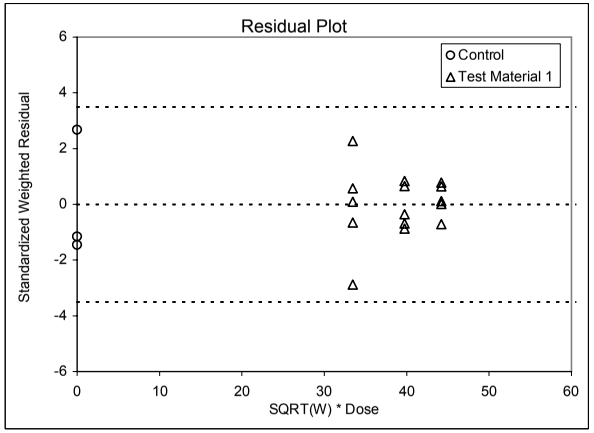
Test Material 1 (Deseret Chemical Depot)





Test Material: Magnified Scale





Summary of Fitting[a]

Parameter	Estimate	Standard Error
a	19.5	4.7
b_r	0.85	0.04
b_{t1}	0.11	0.01
b_{t2}	--	--
Covariance (b_r,b_{t1})	0.0197	--
Covariance (b_r,b_{t2})	--	--
Degrees of Freedom	31	--

[a] $y = a + b_r{*}x_r + b_{t1}{*}x_{t1} + b_{t2}{*}x_{t2}$

where r = Reference Material, t1 = Test Material 1, and t2 = Test Material 2

ANOVA

Source	SSE	DF	MSE
Fit	831.63	2	415.82
Error	46.91	30	1.56
Total	878.54	32	27.45

Statistic	Estimate
F	265.913
p	< 0.001
Adjusted R^2	0.9430

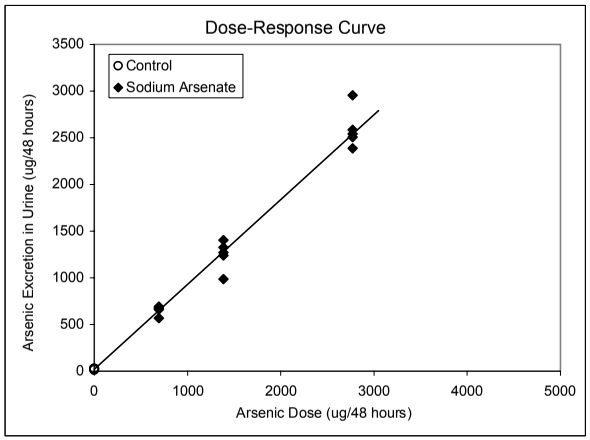
RBA and Uncertainty

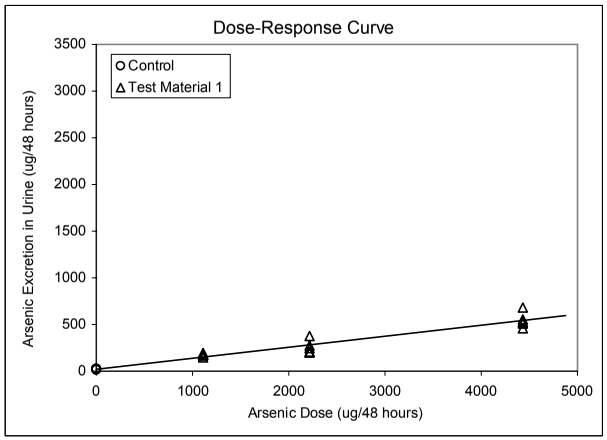
	Test Material 1	Test Material 2
RBA	0.13	--
Lower bound[b]	0.11	--
Upper bound[b]	0.15	--
Standard Error[b]	0.012	--

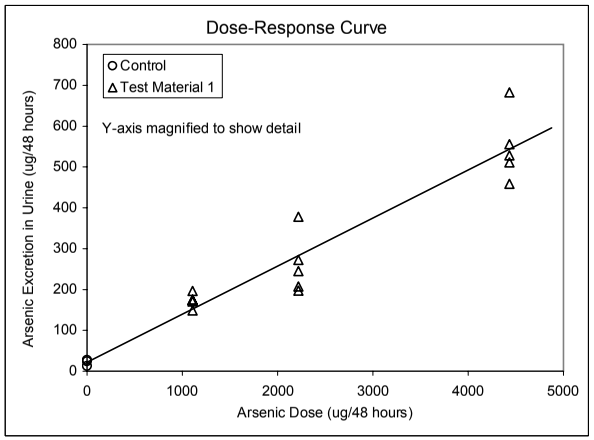
[b] Calculated using Fieller's theorem

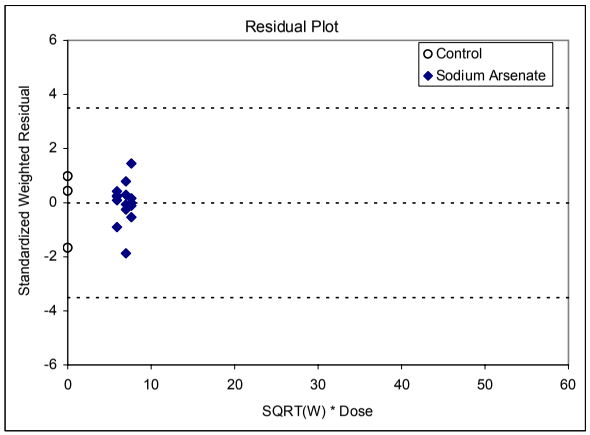
FIGURE D-11 URINARY EXCRETION OF ARSENIC: Days 9/10 (All Data)

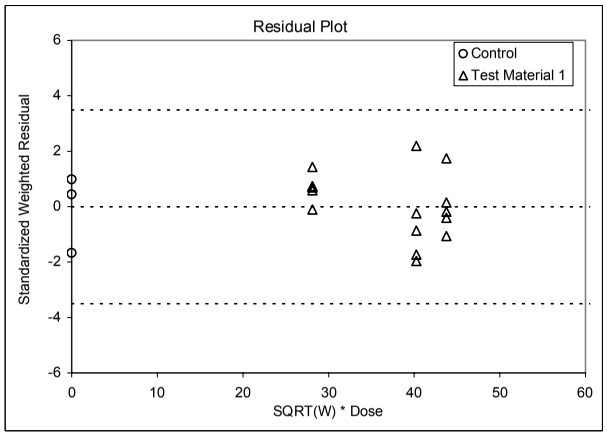
Reference Material (Sodium Arsenate) | **Test Material 1 (Deseret Chemical Depot)** | ***Test Material: Magnified Scale***

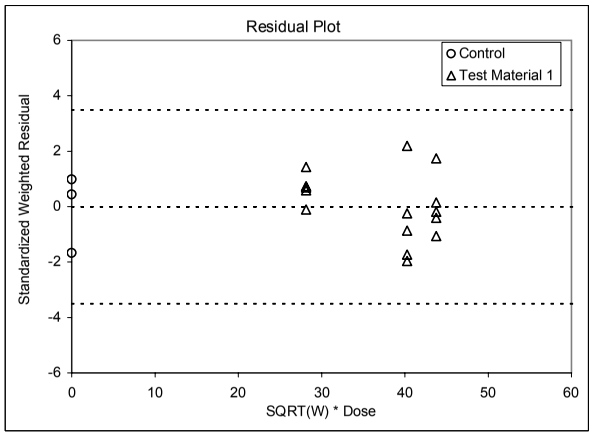












Summary of Fitting[a]

Parameter	Estimate	SE
a	21.9	3.3
b_r	0.91	0.03
b_{t1}	0.12	0.01
b_{t2}	--	--
Covariance (b_r,b_{t1})	0.0202	--
Covariance (b_r,b_{t2})	--	--
Degrees of Freedom	31	--

[a] $y = a + b_r{*}x_r + b_{t1}{*}x_{t1} + b_{t2}{*}x_{t2}$

where r = Reference Material, t1 = Test Material 1, and t2 = Test Material 2

ANOVA

Source	SSE	DF	MSE
Fit	846.04	2	423.02
Error	19.99	30	0.67
Total	866.03	32	27.06

Statistic	Estimate
F	634.759
p	< 0.001
Adjusted R^2	0.9754

RBA and Uncertainty

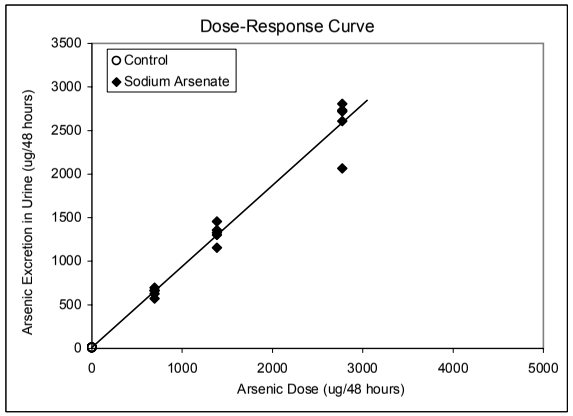
	Test Material 1	Test Material 2
RBA	0.13	--
Lower bound[b]	0.12	--
Upper bound[b]	0.14	--
Standard Error[b]	0.008	--

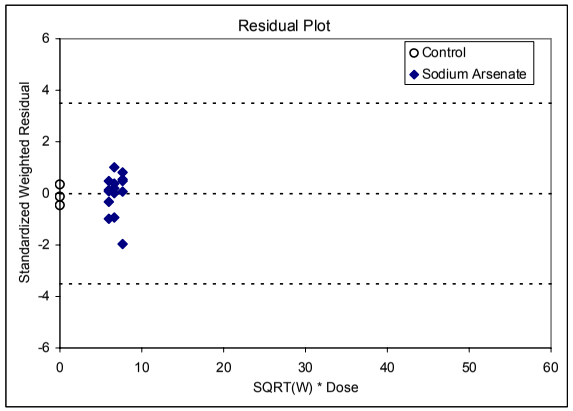
[b] Calculated using Fieller's theorem

2_Navy1 RBA Calcs_all data.xls (Graph_2)

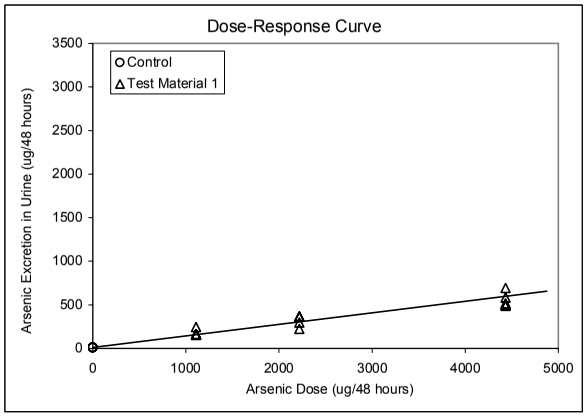
FIGURE D-12 URINARY EXCRETION OF ARSENIC: Days 12/13 (All Data)

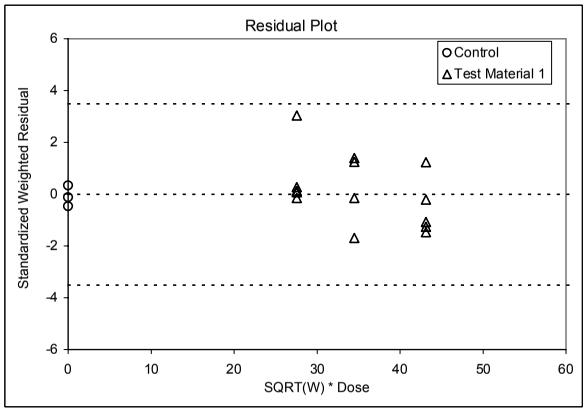
Reference Material (Sodium Arsenate)



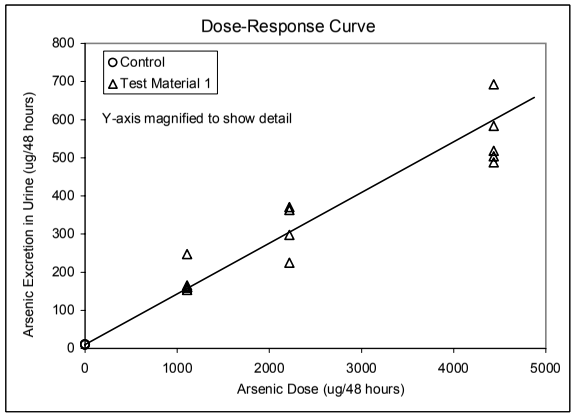


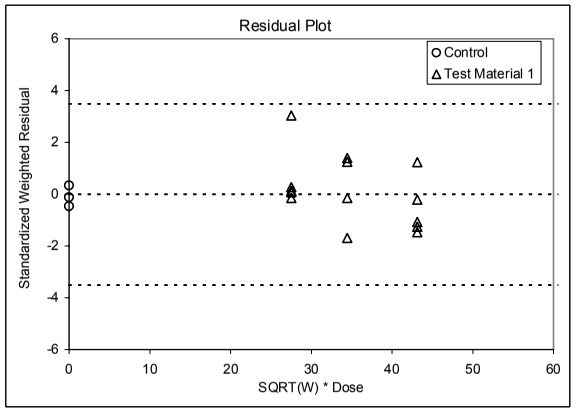
Test Material 1 (Deseret Chemical Depot)





Test Material: Magnified Scale





Summary of Fitting[a]

Parameter	Estimate	SE
a	10.3	1.7
b_r	0.93	0.03
b_{t1}	0.13	0.01
b_{t2}	--	--
Covariance (b_r,b_{t1})	0.0055	--
Covariance (b_r,b_{t2})	--	--
Degrees of Freedom	30	--

[a] $y = a + b_r*x_r + b_{t1}*x_{t1} + b_{t2}*x_{t2}$

where r = Reference Material, t1 = Test Material 1, and t2 = Test Material 2

ANOVA

Source	SSE	DF	MSE
Fit	903.83	2	451.92
Error	16.71	29	0.58
Total	920.54	31	29.69

Statistic	Estimate
F	784.248
p	< 0.001
Adjusted R^2	0.9806

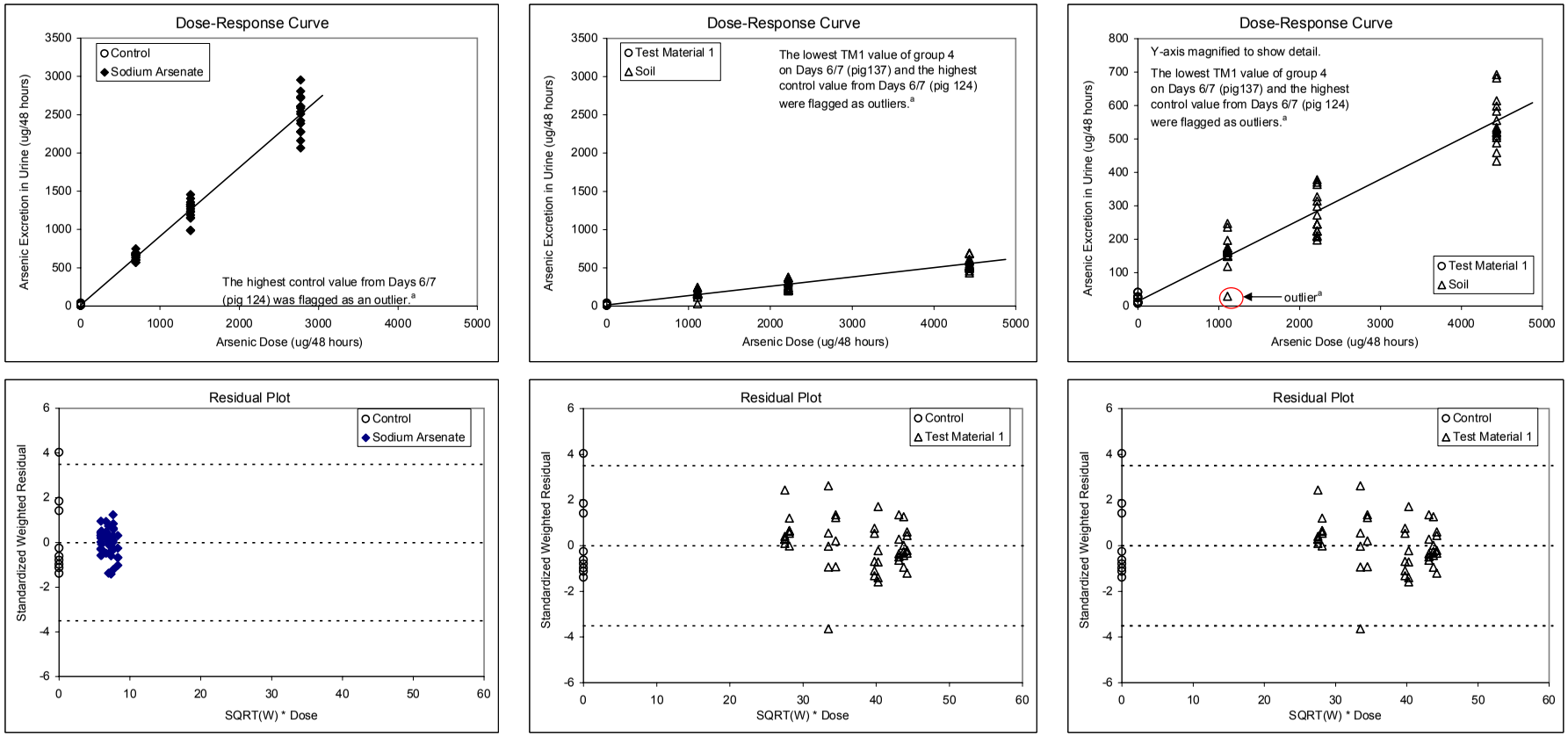
RBA and Uncertainty

	Test Material 1	Test Material 2
RBA	0.14	--
Lower bound[b]	0.13	--
Upper bound[b]	0.16	--
Standard Error[b]	0.008	--

[b] Calculated using Fieller's theorem

FIGURE D-13 URINARY EXCRETION OF ARSENIC: All Days (All Data)

Reference Material (Sodium Arsenate) | **Test Material 1 (Deseret Chemical Depot)** | ***Test Material: Magnified Scale***



D-23

[a] Note that the data from this figure were refitted with the outliers excluded (see Figure 4-5b); these outliers were excluded from the final evaluation for arsenic RBA.

Summary of Fitting[b]

Parameter	Estimate	SE
a	14.3	1.7
b_r	0.90	0.02
b_{t1}	0.12	0.00
b_{t2}	--	--
Covariance (b_r,b_{t1})	0.0113	--
Covariance (b_r,b_{t2})	--	--
Degrees of Freedom	96	--

[b] $y = a + b_r{*}x_r + b_{t1}{*}x_{t1} + b_{t2}{*}x_{t2}$

where r = Reference Material, t1 = Test Material 1, and t2 = Test Material 2

ANOVA

Source	SSE	DF	MSE
Fit	2627.64	2	1313.82
Error	96.86	95	1.02
Total	2724.49	97	28.09

Statistic	Estimate
F	1288.642
p	< 0.001
Adjusted R^2	0.9637

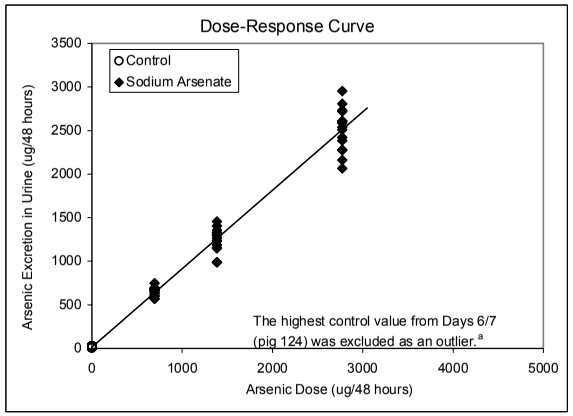
RBA and Uncertainty

	Test Material 1	Test Material 2
RBA	0.14	--
Lower bound[c]	0.13	--
Upper bound[c]	0.14	--
Standard Error[c]	0.006	--

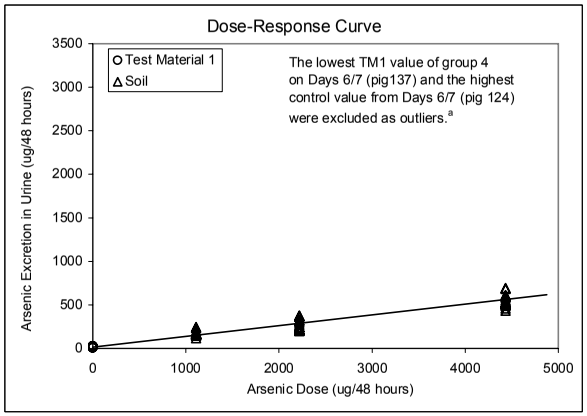
[c] Calculated using Fieller's theorem

FIGURE D-14 URINARY EXCRETION OF ARSENIC: All Days (Outliers Excluded)

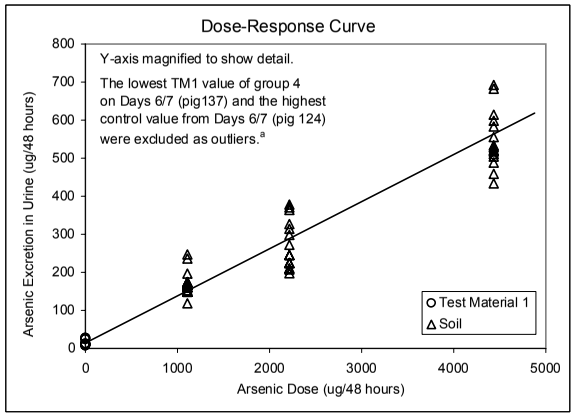
Reference Material (Sodium Arsenate)

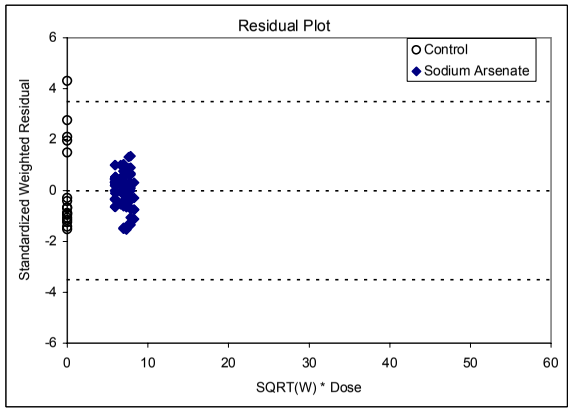


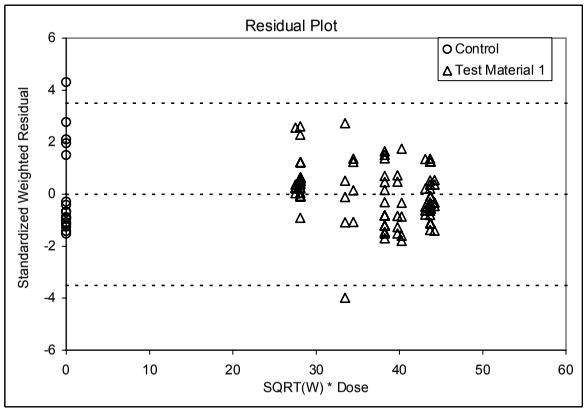
Test Material 1 (Deseret Chemical Depot)

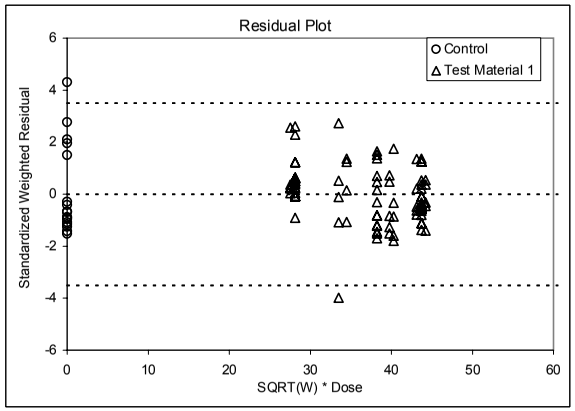


Test Material: Magnified Scale









[a] The outliers were identified in the initial fitting (see Figure 4-5a); the data are plotted here (Figure 4-5b) with the outliers excluded. These results, with the outliers excluded, were used in the final evaluation for arsenic RBA.

Summary of Fitting[b]

Parameter	Estimate	SE
a	14.4	1.5
b_r	0.90	0.02
b_{t1}	0.12	0.00
b_{t2}	--	--
Covariance (b_r,b_{t1})	0.0111	--
Covariance (b_r,b_{t2})	--	--
Degrees of Freedom	192	--

[b] $y = a + b_r{*}x_r + b_{t1}{*}x_{t1} + b_{t2}{*}x_{t2}$

where r = Reference Material, t1 = Test Material 1, and t2 = Test Material 2

ANOVA

Source	SSE	DF	MSE
Fit	5296.37	2	2648.18
Error	83.47	93	0.90
Total	5379.84	95	56.63

Statistic	Estimate
F	2950.448
p	< 0.001
Adjusted R^2	0.9842

RBA and Uncertainty

	Test Material 1	Test Material 2
RBA	0.14	--
Lower bound[c]	0.13	--
Upper bound[c]	0.15	--
Standard Error[c]	0.005	--

[c] Calculated using Fieller's theorem

TABLE D-7 SCHEDULE

Study Day	Day	Date	Feed Special Diet	Cull Pigs/ Assign Dose Group	Weigh	Dose Preparation	Dose Administration	Urine Collection[a]	Sacrifice/ Necropsy
-6	Tuesday	09/26/06							
-5	Wednesday	09/27/06			X				
-4	Thursday	09/28/06	transition	X					
-3	Friday	09/29/06	transition						
-2	Saturday	09/30/06	transition						
-1	Sunday	10/01/06	transition		X	X			
0	Monday	10/02/06	X				X		
1	Tuesday	10/03/06	X				X		
2	Wednesday	10/04/06	X		X		X		
3	Thursday	10/05/06	X			X	X		
4	Friday	10/06/06	X				X		
5	Saturday	10/07/06	X		X		X		
6	Sunday	10/08/06	X				X	U-1	
7	Monday	10/09/06	X				X		
8	Tuesday	10/10/06	X		X	X[b]	X		
9	Wednesday	10/11/06	X				X	U-2	
10	Thursday	10/12/06	X				X		
11	Friday	10/13/06	X		X		X		
12	Saturday	10/14/06	X				X	U-3	
13	Sunday	10/15/06	X				X		
14	Monday	10/16/06	X		X				X

[a] Urine was collected over a period of 48 hours.

[a] The original schedule called for dose preparation to occur on days -1, 3, 7, and 11. However, due to scheduling conflicts, the day 7 and 11 preparations were replaced with a single preparation on day 8.

TABLE D-8 GROUP ASSIGNMENTS

Pig Number	Dose Group	Material Administered	Target Dose of Arsenic (µg/kg-day)
101 103 110 114 132	1	Sodium Arsenate	25
105 107 121 130 133	2	Sodium Arsenate	50
104 113 125 126 138	3	Sodium Arsenate	100
102 116 129 134 137	4	Test Material 1	40
106 117 118 122 127	5	Test Material 1	80
108 120 123 128 131	6	Test Material 1	160
109 124 135	7	Control	0

TABLE D-9 BODY WEIGHTS AND ACTUAL ADMINISTERED DOSES, BY DAY

Body weights were measured on days -1, 2, 5, 8, 11, and 14. Weights for other days are estimated, based on linear interpolation between measured values.

Group	Pig #	Day -1		Day 0		Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7		Day 8		Day 9		Day 10		Day 11		Day 12		Day 13		Day 14		Days 0-14
		BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	Mean As Dose (µg/kg-d)
1	101	9.8	0.00	10.1	34.18	10.5	33.09	10.8	32.07	11.2	30.93	11.6	29.86	12.0	28.86	12.4	28.01	12.7	27.20	13.1	26.44	13.6	25.47	14.1	24.56	14.6	23.72	15.0	23.07	15.4	22.44	15.9	21.85	28.17
1	103	10.2	0.00	10.6	32.83	10.9	31.78	11.3	30.79	11.7	29.73	12.1	28.74	12.5	27.82	12.9	26.82	13.4	25.88	13.9	25.01	14.3	24.16	14.8	23.38	15.3	22.64	15.7	22.04	16.1	21.47	16.6	20.93	26.99
1	110	10.2	0.00	10.5	32.93	10.9	31.83	11.3	30.79	11.6	29.86	12.0	28.98	12.3	28.16	12.7	27.20	13.2	26.31	13.6	25.47	14.1	24.59	14.6	23.78	15.1	23.01	15.5	22.35	16.0	21.72	16.4	21.12	27.23
1	114	10.0	0.00	10.2	34.07	10.4	33.36	10.6	32.68	11.0	31.49	11.4	30.38	11.8	29.35	12.1	28.55	12.5	27.78	12.8	27.06	13.3	26.14	13.7	25.28	14.2	24.48	14.6	23.75	15.0	23.07	15.5	22.42	28.66
1	132	10.8	0.00	11.0	31.49	11.3	30.79	11.5	30.12	11.8	29.31	12.1	28.55	12.5	27.82	12.9	26.92	13.3	26.08	13.7	25.28	14.1	24.54	14.5	23.83	15.0	23.17	15.5	22.42	16.0	21.72	16.5	21.06	26.79
2	105	9.8	0.00	10.2	68.25	10.6	65.66	11.0	63.26	11.2	61.85	11.5	60.50	11.7	59.21	12.0	57.81	12.3	56.47	12.6	55.20	13.1	53.01	13.6	51.00	14.1	49.13	14.5	47.77	14.9	46.49	15.3	45.28	57.31
2	107	9.5	0.00	9.9	69.97	10.3	67.26	10.7	64.74	11.1	62.41	11.5	60.24	11.9	58.21	12.3	56.24	12.7	54.40	13.2	52.68	13.7	50.69	14.2	48.84	14.7	47.12	15.0	46.08	15.4	45.08	15.7	44.12	56.79
2	121	9.8	0.00	10.2	67.69	10.7	64.94	11.1	59.29	11.4	60.59	11.8	58.87	12.1	57.25	12.4	56.02	12.6	54.83	12.9	53.70	13.4	51.89	13.8	50.20	14.3	48.61	14.7	47.23	15.1	45.93	15.5	44.69	55.92
2	130	10.2	0.00	10.5	65.87	10.8	63.94	11.2	62.13	11.5	60.24	11.9	58.46	12.2	56.78	12.6	54.98	13.0	53.29	13.4	51.70	14.0	49.66	14.5	47.77	15.1	46.03	15.5	44.60	16.0	43.25	16.5	41.98	54.77
2	133	10.6	0.00	10.8	63.94	11.1	62.60	11.3	61.30	11.7	59.38	12.0	57.57	12.4	55.87	12.8	53.98	13.3	52.22	13.7	50.56	14.1	49.30	14.4	48.11	14.8	46.96	15.3	45.42	15.8	43.98	16.3	42.63	54.12
3	104	10.5	0.00	10.8	128.09	11.2	123.89	11.6	119.95	12.1	114.82	12.6	110.10	13.1	105.76	13.5	103.01	13.8	100.40	14.2	97.91	14.6	95.22	15.0	92.67	15.4	90.26	15.8	87.87	16.2	85.61	16.6	83.46	105.07
3	113	11.2	0.00	11.4	121.35	11.7	118.58	12.0	115.94	12.3	112.79	12.6	109.81	13.0	106.98	13.3	103.91	13.7	101.01	14.1	98.26	14.5	95.66	14.9	93.19	15.3	90.85	15.8	87.69	16.4	84.74	16.9	81.98	103.59
3	125	9.8	0.00	10.2	136.50	10.6	131.32	11.0	126.53	11.2	123.33	11.5	120.30	11.8	117.41	12.2	113.56	12.6	109.96	13.0	106.57	13.4	103.26	13.8	100.15	14.3	97.22	14.7	94.14	15.2	91.25	15.7	88.53	113.28
3	126	9.7	0.00	10.1	137.86	10.5	132.58	10.9	127.69	11.3	122.97	11.7	118.58	12.1	114.50	12.6	109.96	13.1	105.76	13.6	101.87	14.0	99.20	14.3	96.66	14.7	94.25	15.1	91.55	15.6	89.00	16.0	86.59	111.58
3	138	10.1	0.00	10.2	136.05	10.3	134.95	10.4	133.86	10.7	129.28	11.1	125.00	11.5	121.00	11.9	116.26	12.4	111.88	12.9	107.82	13.3	104.04	13.8	100.52	14.3	97.22	14.8	93.61	15.4	90.26	15.9	87.14	115.59
4	102	10.7	0.00	11.1	50.08	11.4	48.47	11.8	46.96	12.2	45.42	12.6	43.98	13.0	42.63	13.4	41.36	13.8	40.16	14.2	39.03	14.7	37.79	15.1	36.62	15.6	35.52	16.0	34.56	16.5	33.65	16.9	32.79	41.59
4	116	9.9	0.00	10.1	55.05	10.3	53.89	10.5	52.78	10.9	51.08	11.2	49.48	11.6	47.98	12.0	46.38	12.4	44.87	12.8	43.47	13.2	42.14	13.6	40.90	14.0	39.73	14.4	38.48	14.9	37.32	15.3	36.22	46.42
4	129	9.7	0.00	10.1	55.14	10.5	53.03	10.9	51.08	11.1	49.85	11.4	48.68	11.7	47.57	12.1	45.67	12.6	43.92	13.1	42.30	13.5	41.10	13.9	39.97	14.3	38.89	14.8	37.49	15.3	36.18	15.9	34.96	45.49
4	134	9.9	0.00	10.2	54.42	10.5	52.95	10.8	51.55	11.1	49.93	11.5	48.40	11.8	46.96	12.2	45.49	12.6	44.10	13.0	42.79	13.5	41.00	14.1	39.35	14.7	37.83	15.2	36.58	15.7	35.41	16.2	34.31	45.24
4	137	10.0	0.00	10.2	54.24	10.5	52.86	10.8	51.55	11.1	50.15	11.4	48.83	11.7	47.57	12.2	45.55	12.7	43.69	13.2	41.98	13.6	40.70	14.0	39.49	14.5	38.35	15.0	37.03	15.5	35.79	16.0	34.64	45.32
5	106	10.1	0.00	10.4	106.23	10.8	102.47	11.2	98.96	11.5	96.24	11.8	93.66	12.2	91.22	12.5	88.43	12.9	85.81	13.3	83.34	13.6	81.50	13.9	79.74	14.2	78.05	14.6	75.83	15.0	73.73	15.5	71.74	88.97
5	117	10.3	0.00	10.7	103.91	11.0	100.46	11.4	97.22	11.8	94.06	12.2	91.10	12.6	88.32	13.0	85.48	13.4	82.82	13.8	80.32	14.5	76.61	15.1	73.24	15.8	70.15	16.2	68.28	16.7	66.50	17.1	64.82	85.27
5	118	10.0	0.00	10.2	108.84	10.4	106.40	10.7	104.07	11.1	100.30	11.5	96.80	11.9	93.53	12.3	89.99	12.8	86.70	13.3	83.65	13.7	80.90	14.2	78.33	14.6	75.92	15.0	74.14	15.3	72.44	15.7	70.82	90.64
5	122	10.6	0.00	10.9	101.84	11.2	98.81	11.6	95.96	11.9	93.27	12.2	90.73	12.6	88.32	13.0	85.15	13.5	82.20	14.0	79.45	14.2	78.24	14.4	77.06	14.6	75.92	15.2	73.00	15.8	70.30	16.4	67.79	85.57
5	127	9.9	0.00	10.2	108.31	10.6	104.40	11.0	100.76	11.3	97.80	11.7	95.00	12.0	92.36	12.4	89.14	12.9	86.14	13.3	83.34	13.7	80.90	14.1	78.61	14.5	76.44							
6	108	10.4	0.00	10.8	205.25	11.2	197.92	11.6	191.10	12.0	184.73	12.4	178.77	12.8	173.18	13.3	167.30	13.7	161.80	14.2	156.66	14.5	152.88	14.9	149.27	15.2	145.84	15.8	140.00	16.5	134.62	17.1	129.63	168.41
6	120	9.6	0.00	10.0	222.79	10.4	214.18	10.8	206.21	11.2	197.63	11.7	189.73	12.2	182.45	12.5	176.87	12.9	171.62	13.3	166.67	13.9	160.05	14.4	153.94	15.0	148.28	15.4	144.26	15.8	140.45	16.2	136.84	179.14
6	123	10.4	0.00	10.8	205.89	11.1	199.11	11.5	192.76	11.9	185.76	12.4	179.25	12.8	173.18	13.3	166.46	13.8	160.25	14.4	154.48	14.8	149.44	15.3	144.73	15.8	140.30	16.2	136.55	16.7	133.00	17.1	129.63	168.03
6	128	9.8	0.00	10.1	219.12	10.5	211.45	10.9	204.31	11.3	196.17	11.8	188.66	12.2	181.70	12.7	174.55	13.2	167.93	13.7	161.80	14.2	155.74	14.8	150.12	15.3	144.88	15.7	141.19	16.1	137.68	16.5	134.35	176.58
6	131	10.3	0.00	10.7	207.17	11.1	199.71	11.5	192.76	11.9	187.07	12.2	181.70	12.6	176.63	12.9	171.84	13.3	167.30	13.6	162.99	14.0	158.34	14.4	153.94	14.8	149.78	15.4	144.41	15.9	139.42	16.5	134.76	172.15
7	109	10.0	0.00	10.3	0.00	10.7	0.00	11.0	0.00	11.4	0.00	11.7	0.00	12.1	0.00	12.6	0.00	13.0	0.00	13.5	0.00	14.0	0.00	14.5	0.00	15.0	0.00	15.5	0.00	15.9	0.00	16.4	0.00	0.00
7	124	10.2	0.00	10.6	0.00	10.9	0.00	11.3	0.00	11.6	0.00	11.9	0.00	12.3	0.00	12.8	0.00	13.3	0.00	13.8	0.00	14.1	0.00	14.4	0.00	14.7	0.00	15.2	0.00	15.7	0.00	16.2	0.00	0.00
7	135	10.3	0.00	10.6	0.00	11.0	0.00	11.3	0.00	11.7	0.00	12.0	0.00	12.4	0.00	12.8	0.00	13.2	0.00	13.7	0.00	14.0	0.00	14.4	0.00	14.8	0.00	15.3	0.00	15.9	0.00	16.4	0.00	0.00

Missed Doses:

Day 2 - Pig 121 did not eat entire PM dose (ate approximately 90%). Daily dose adjusted to 95%.

On day 11 (10/13/2006), pig 127 was noted during weighing to have a rectal prolapse and was euthanized.

TABLE D-10 ANIMAL HEALTH

Naxcel Treatment[a]

First Day of Treatment	Pig	Group	Treatment Duration
Day 0 (10/2/06)	101	1	3 Days
	109	7	6 Days
Day 1 (10/3/06)	118	5	3 Days
	126	3	
	133	2	
	138	3	
Day 3 (10/5/06)	114	1	3 Days
	125	3	
Day 5 (10/7/06)	124	7	3 Days
Day 6 (10/8/06)	120	6	3 Days
	105	2	3 Days[b]
Day 7 (10/9/06)	110	1	3 Days
	118	5	

[a] All treatments are 1mL/day of Naxcel

[b] Received two doses in the day (on in the morning, one in the evening

Necropsy

Pig 131 (group 6) was noted to have a small umbilical abcess.

Other

On day 11 (10/13/06), pig 127 (group 5) was noted during weighing to have a rectal prolapse and was euthanized.

TABLE D-11 URINE VOLUMES

Group	Pig Number	Urine Collection[a]		
		U-1 Days 6-7	U-2 Days 9-10	U-3 Days 12-13
1	101	10720	9460	16980
	103	6280	15650	24520
	110	6152	8000	12800
	114	18049	16090	11200
	132	8400	5920	6730
2	105	2610	4575	5660
	107	4590	5190	6850
	121	9200	11050	10520
	130	17460	14950	22740
	133	11500	19780	17960
3	104	5270	8920	11880
	113	18107	12845	11860
	125	11000	9940	17530
	126	9480	7600	14300
	138	7125	5080	6080
4	102	12750	21860	23000
	116	26183	26330	19020
	129	13500	9930	17040
	134	16840	8116	8067
	137	7880	9220	11420
5	106	10137	12200	24230
	117	5080	3500	13240
	118	7270	9880	9000
	122	5990	5980	5050
	127	4600	3890	no sample[b]
6	108	7490	5660	8840
	120	5160	8660	5880
	123	5500	8520	13620
	128	10860	12920	19920
	131	11290	14520	36450
7	109	3900	4340	4510
	124	6940	13680	19960
	135	1340	2710	5640

Units = milliliters

[a] Urine was collected over 48-hour periods.

[a] Pig 127 was euthanized on day 11 due to a rectal prolapse.

TABLE D-12 URINARY ARSENIC ANALYTICAL RESULTS FOR STUDY SAMPLES

Sample Number	Tag Number	Pig Number	Group	Material Administered	Urine Collection Days	48-hr Dose (ug/48hr)	48-hr BWAdj Dose (ug/kg-48hr)	Q	Reported Conc (ng/mL)	AdjConc*(ng/mL)	Urine Volume (mL)	Total Excreted (ug/48hrs)
Navy1As-114-U1	Navy1As-109	114	1	Sodium Arsenate	6/7	692.73	56.33		38	38	18049	686
Navy1As-132-U1	Navy1As-133	132	1	Sodium Arsenate	6/7	692.73	52.99		76	76	8400	638
Navy1As-101-U1	Navy1As-106	101	1	Sodium Arsenate	6/7	692.73	55.21		56	56	10720	600
Navy1As-110-U1	Navy1As-125	110	1	Sodium Arsenate	6/7	692.73	53.51		98	98	6152	603
Navy1As-103-U1	Navy1As-141	103	1	Sodium Arsenate	6/7	692.73	52.7		119	119	6280	747
Navy1As-105-U1	Navy1As-138	105	2	Sodium Arsenate	6/7	1385.45	114.28		380	380	2610	992
Navy1As-107-U1	Navy1As-114	107	2	Sodium Arsenate	6/7	1385.45	110.65		250	250	4590	1148
Navy1As-121-U1	Navy1As-108	121	2	Sodium Arsenate	6/7	1385.45	110.85		141	141	9200	1297
Navy1As-130-U1	Navy1As-102	130	2	Sodium Arsenate	6/7	1385.45	108.27		68	68	17460	1187
Navy1As-133-U1	Navy1As-137	133	2	Sodium Arsenate	6/7	1385.45	106.19		107	107	11500	1231
Navy1As-104-U1	Navy1As-118	104	3	Sodium Arsenate	6/7	2770.91	203.4		410	410	5270	2161
Navy1As-113-U1	Navy1As-105	113	3	Sodium Arsenate	6/7	2770.91	204.91		144	144	18107	2607
Navy1As-125-U1	Navy1As-131	125	3	Sodium Arsenate	6/7	2770.91	223.52		220	220	11000	2420
Navy1As-126-U1	Navy1As-113	126	3	Sodium Arsenate	6/7	2770.91	215.72		240	240	9480	2275
Navy1As-138-U1	Navy1As-104	138	3	Sodium Arsenate	6/7	2770.91	228.14		320	320	7125	2280
Navy1As-134-U1	Navy1As-121	134	4	Test Material 1	6/7	1108.36	89.59		14	14	16840	236
Navy1As-137-U1	Navy1As-128	137	4	Test Material 1	6/7	1108.36	89.24		3.7	3.7	7880	29
Navy1As-116-U1	Navy1As-120	116	4	Test Material 1	6/7	1108.36	91.25		6.4	6.4	26183	168
Navy1As-102-U1	Navy1As-130	102	4	Test Material 1	6/7	1108.36	81.51		9.3	9.3	12750	119
Navy1As-129-U1	Navy1As-139	129	4	Test Material 1	6/7	1108.36	89.6		11	11	13500	149
Navy1As-127-U1	Navy1As-112	127	5	Test Material 1	6/7	2216.73	175.29		71	71	4600	327
Navy1As-122-U1	Navy1As-126	122	5	Test Material 1	6/7	2216.73	167.35		41	41	5990	246
Navy1As-118-U1	Navy1As-103	118	5	Test Material 1	6/7	2216.73	176.69		29	29	7270	211
Navy1As-117-U1	Navy1As-124	117	5	Test Material 1	6/7	2216.73	168.29		44	44	5080	224
Navy1As-106-U1	Navy1As-135	106	5	Test Material 1	6/7	2216.73	174.24		31	31	10137	314
Navy1As-131-U1	Navy1As-134	131	6	Test Material 1	6/7	4433.45	339.14		53	53	11290	598
Navy1As-128-U1	Navy1As-115	128	6	Test Material 1	6/7	4433.45	342.48		48	48	10860	521
Navy1As-123-U1	Navy1As-107	123	6	Test Material 1	6/7	4433.45	326.71		97	97	5500	534
Navy1As-120-U1	Navy1As-101	120	6	Test Material 1	6/7	4433.45	348.48		84	84	5160	433
Navy1As-108-U1	Navy1As-122	108	6	Test Material 1	6/7	4433.45	329.11		82	82	7490	614
Navy1As-135-U1	Navy1As-111	135	7	Control	6/7	0	0		7.6	7.6	1340	10
Navy1As-124-U1	Navy1As-132	124	7	Control	6/7	0	0		5.9	5.9	6940	41
Navy1As-109-U1	Navy1As-136	109	7	Control	6/7	0	0		2	2	3900	8
Navy1As-101-U2	Navy1As-147	101	1	Sodium Arsenate	9/10	692.73	50.03		73	73	9460	691
Navy1As-103-U2	Navy1As-170	103	1	Sodium Arsenate	9/10	692.73	47.54		43	43	15650	673
Navy1As-110-U2	Navy1As-160	110	1	Sodium Arsenate	9/10	692.73	48.37		71	71	8000	568
Navy1As-114-U2	Navy1As-146	114	1	Sodium Arsenate	9/10	692.73	51.42		41	41	16090	660
Navy1As-132-U2	Navy1As-150	132	1	Sodium Arsenate	9/10	692.73	48.37		114	114	5920	675
Navy1As-121-U2	Navy1As-151	121	2	Sodium Arsenate	9/10	1385.45	102.09		115	115	11050	1271
Navy1As-130-U2	Navy1As-167	130	2	Sodium Arsenate	9/10	1385.45	97.43		83	83	14950	1241
Navy1As-107-U2	Navy1As-161	107	2	Sodium Arsenate	9/10	1385.45	99.53		190	190	5190	986
Navy1As-105-U2	Navy1As-163	105	2	Sodium Arsenate	9/10	1385.45	104.01		290	290	4575	1327
Navy1As-133-U2	Navy1As-155	133	2	Sodium Arsenate	9/10	1385.45	97.41		71	71	19780	1404
Navy1As-104-U2	Navy1As-177	104	3	Sodium Arsenate	9/10	2770.91	187.89		290	290	8920	2587
Navy1As-113-U2	Navy1As-164	113	3	Sodium Arsenate	9/10	2770.91	188.85		230	230	12845	2954
Navy1As-125-U2	Navy1As-142	125	3	Sodium Arsenate	9/10	2770.91	203.42		240	240	9940	2386
Navy1As-126-U2	Navy1As-152	126	3	Sodium Arsenate	9/10	2770.91	195.86		330	330	7600	2508
Navy1As-138-U2	Navy1As-156	138	3	Sodium Arsenate	9/10	2770.91	204.56		500	500	5080	2540
Navy1As-102-U2	Navy1As-180	102	4	Test Material 1	9/10	1108.36	74.41		9	9	21860	197
Navy1As-137-U2	Navy1As-145	137	4	Test Material 1	9/10	1108.36	80.19		19	19	9220	175
Navy1As-116-U2	Navy1As-171	116	4	Test Material 1	9/10	1108.36	83.04		6.6	6.6	26330	174
Navy1As-134-U2	Navy1As-172	134	4	Test Material 1	9/10	1108.36	80.35		21	21	8116	170
Navy1As-129-U2	Navy1As-166	129	4	Test Material 1	9/10	1108.36	81.07		15	15	9930	149
Navy1As-106-U2	Navy1As-178	106	5	Test Material 1	9/10	2216.73	161.24		31	31	12200	378
Navy1As-117-U2	Navy1As-157	117	5	Test Material 1	9/10	2216.73	149.85		70	70	3500	245
Navy1As-118-U2	Navy1As-173	118	5	Test Material 1	9/10	2216.73	159.23		21	21	9880	207
Navy1As-122-U2	Navy1As-175	122	5	Test Material 1	9/10	2216.73	155.3		33	33	5980	197
Navy1As-127-U2	Navy1As-168	127	5	Test Material 1	9/10	2216.73	159.51		70	70	3890	272
Navy1As-108-U2	Navy1As-153	108	6	Test Material 1	9/10	4433.45	302.15		81	81	5660	458
Navy1As-131-U2	Navy1As-162	131	6	Test Material 1	9/10	4433.45	312.28		47	47	14520	682
Navy1As-128-U2	Navy1As-148	128	6	Test Material 1	9/10	4433.45	305.86		43	43	12920	556
Navy1As-120-U2	Navy1As-169	120	6	Test Material 1	9/10	4433.45	313.99		59	59	8660	511
Navy1As-123-U2	Navy1As-165	123	6	Test Material 1	9/10	4433.45	294.17		62	62	8520	528
Navy1As-109-U2	Navy1As-174	109	7	Control	9/10	0	0		5.6	5.6	4340	24
Navy1As-124-U2	Navy1As-159	124	7	Control	9/10	0	0		2	2	13680	27
Navy1As-135-U2	Navy1As-143	135	7	Control	9/10	0	0		4.6	4.6	2710	12
ENSR1-445-U2	ENSR1-183	445	6	Test Material 2	9/10	3495.42	224.11		70	70	20740	1452

Sample Number	Tag Number	Pig Number	Group	Material Administered	Urine Collection Days	48-hr Dose (ug/48hr)	48-hr BWAdj Dose (ug/kg-48hr)	Q	Reported Conc (ng/mL)	AdjConc*(ng/mL)	Urine Volume (mL)	Total Excreted (ug/48hrs)
ENSR1-452-U2	ENSR1-165	452	7	Control	9/10	0	0	<	1	0.5	23980	12
ENSR1-461-U2	ENSR1-144	461	7	Control	9/10	0	0		1	1	7600	8
ENSR1-464-U2	ENSR1-174	464	7	Control	9/10	0	0		2	2	5140	10
ENSR1-467-U2	ENSR1-147	467	7	Control	9/10	0	0	<	1	0.5	7160	4
ENSR1-432-U2	ENSR1-180	432	7	Control	9/10	0	0	<	1	0.5	14560	7
Navy1As-101-U3	Navy1As-188	101	1	Sodium Arsenate	12/13	692.73	45.51		41	41	16980	696
Navy1As-132-U3	Navy1As-215	132	1	Sodium Arsenate	12/13	692.73	44.13		99	99	6730	666
Navy1As-114-U3	Navy1As-184	114	1	Sodium Arsenate	12/13	692.73	46.82		51	51	11200	571
Navy1As-110-U3	Navy1As-192	110	1	Sodium Arsenate	12/13	692.73	44.06		49	49	12800	627
Navy1As-103-U3	Navy1As-195	103	1	Sodium Arsenate	12/13	692.73	43.51		27	27	24520	662
Navy1As-133-U3	Navy1As-222	133	2	Sodium Arsenate	12/13	1385.45	89.41		74	74	17960	1329
Navy1As-130-U3	Navy1As-216	130	2	Sodium Arsenate	12/13	1385.45	87.85		64	64	22740	1455
Navy1As-121-U3	Navy1As-221	121	2	Sodium Arsenate	12/13	1385.45	93.16		110	110	10520	1157
Navy1As-107-U3	Navy1As-205	107	2	Sodium Arsenate	12/13	1385.45	91.16		190	190	6850	1302
Navy1As-105-U3	Navy1As-218	105	2	Sodium Arsenate	12/13	1385.45	94.27		240	240	5660	1358
Navy1As-113-U3	Navy1As-193	113	3	Sodium Arsenate	12/13	2770.91	172.42		220	220	11860	2609
Navy1As-138-U3	Navy1As-190	138	3	Sodium Arsenate	12/13	2770.91	183.87		340	340	6080	2067
Navy1As-125-U3	Navy1As-200	125	3	Sodium Arsenate	12/13	2770.91	185.39		160	160	17530	2805
Navy1As-104-U3	Navy1As-220	104	3	Sodium Arsenate	12/13	2770.91	173.48		230	230	11880	2732
Navy1As-126-U3	Navy1As-194	126	3	Sodium Arsenate	12/13	2770.91	180.55		190	190	14300	2717
Navy1As-134-U3	Navy1As-187	134	4	Test Material 1	12/13	1108.36	71.99		20	20	8067	161
Navy1As-137-U3	Navy1As-210	137	4	Test Material 1	12/13	1108.36	72.82		14	14	11420	160
Navy1As-129-U3	Navy1As-202	129	4	Test Material 1	12/13	1108.36	73.67		9	9	17040	153
Navy1As-116-U3	Navy1As-219	116	4	Test Material 1	12/13	1108.36	75.8		13	13	19020	247
Navy1As-102-U3	Navy1As-209	102	4	Test Material 1	12/13	1108.36	68.22		7.2	7.2	23000	166
Navy1As-117-U3	Navy1As-206	117	5	Test Material 1	12/13	2216.73	134.78		28	28	13240	371
Navy1As-118-U3	Navy1As-214	118	5	Test Material 1	12/13	2216.73	146.58		25	25	9000	225
Navy1As-122-U3	Navy1As-204	122	5	Test Material 1	12/13	2216.73	143.3		59	59	5050	298
Navy1As-127-U3	Navy1As-199	127	5	Test Material 1	12/13							
Navy1As-106-U3	Navy1As-201	106	5	Test Material 1	12/13	2216.73	149.56		15	15	24230	363
Navy1As-131-U3	Navy1As-186	131	6	Test Material 1	12/13	4433.45	283.83		19	19	36450	693
Navy1As-123-U3	Navy1As-198	123	6	Test Material 1	12/13	4433.45	269.56		37	37	13620	504
Navy1As-120-U3	Navy1As-196	120	6	Test Material 1	12/13	4433.45	284.7		83	83	5880	488
Navy1As-128-U3	Navy1As-217	128	6	Test Material 1	12/13	4433.45	278.88		26	26	19920	518
Navy1As-108-U3	Navy1As-185	108	6	Test Material 1	12/13	4433.45	274.62		66	66	8840	583
Navy1As-109-U3	Navy1As-189	109	7	Control	12/13	0	0		2	2	4510	9
Navy1As-124-U3	Navy1As-203	124	7	Control	12/13	0	0	<	1	0.5	19960	10
Navy1As-135-U3	Navy1As-207	135	7	Control	12/13	0	0		2	2	5640	11

Q = Data qualifier
*Non-detects taken at one-half the detection limit.

TABLE D-13 ARSENIC ANALYTICAL RESULTS FOR QUALITY CONTROL SAMPLES

Blind Duplicates

Tag Number	Group	Event	DL	Duplicate Conc	Original Pig #
Navy1As-110	7	U1	1	7.6	135
Navy1As-127	2	U1	1	105	133
Navy1As-117	6	U1	1	89	120
Navy1As-179	1	U2	1	74	101
Navy1As-154	3	U2	10	470	138
Navy1As-149	5	U2	1	37	122
Navy1As-183	2	U3	5	200	107
Navy1As-211	4	U3	1	6.4	116
Navy1As-197	6	U3	1	16	131

Laboratory Control Standards

Reference Material	Certified Mean	DL	Measured Conc
NIST 2670a-L	(3)	3	5
NIST 2670a-H	220 ± 10	5	220
NIST 2670a-H	220 ± 10	5	210
NIST 2670a-L	(3)	3	<3
NIST 2670a-H	220 ± 10	5	220
NIST 2670a-H	220 ± 10	5	210
NIST 1640	0.0267 +/-0.0004	0.001	0.029
NRCC TORT-2	21.6 +/-1.8	0.2	22.3

Blanks

Tag Number	DL	Measured Conc
Blank-1	1	<1
Blank-2	1	<1
Blank-3	1	<1
Blank-4	1	<1
Blank-5	1	<1
Blank-6	1	<1

PE Sample Analysis

Tag Number	QC Sample	Nominal Conc	Measured Conc	DL
Navy1As-212	Control Urine	0	2	1
Navy1As-116	Control Urine	0	3	1
Navy1As-158	Sodium arsenate	20	21	1
Navy1As-123	Sodium arsenate	100	104	1
Navy1As-191	Sodium arsenate	400	400	10
Navy1As-208	Sodium arsenite	20	21	1
Navy1As-176	Sodium arsenite	100	99	1
Navy1As-129	Sodium arsenite	400	370	10
Navy1As-119	Dimethyl arsenic acid	20	24	1
Navy1As-213	Dimethyl arsenic acid	100	104	1
Navy1As-181	Dimethyl arsenic acid	400	390	10
Navy1As-140	Disodium methylarsenate	20	21	1
Navy1As-144	Disodium methylarsenate	100	106	1
Navy1As-182	Disodium methylarsenate	400	390	10

Duplicates

Tag Number	DL	Duplicate Result	Original Sample Conc
Navy1-103	1	30	29
Navy1-113	5	240	240
Navy1-123	1	106	104
Navy1-135	1	31	31
Navy1-142	5	270	240
Navy1-152	10	340	330
Navy1-163	10	300	290
Navy1-173	1	22	21
Navy1-182	10	400	390
Navy1-192	1	49	49
Navy1-204	1	63	59
Navy1-212	1	2	2
Navy1-221	1	109	110

Spikes (nominal spike: 200 ng/mL)

Tag Number	DL	Original Sample Conc	Spiked Result
Navy1-108	1	141	360
Navy1-116	1	3	210
Navy1-128	1	19	210
Navy1-139	1	11	210
Navy1-147	1	73	280
Navy1-157	1	70	280
Navy1-168	1	70	280
Navy1-177	10	290	520*
Navy1-187	1	20	230
Navy1-198	1	37	240
Navy1-208	1	21	230
Navy1-216	1	64	250

DL = Detection limit
Units: ng/mL (urine), ug/mL (water), ug/g (tissue)
*Nominal spike amount too low (less than original sample concentration)

Relative bioavailable Cr of soil from the McClellan Air Force Base

A study using juvenile swine as test animals was performed to measure the gastrointestinal absorption of chromium from a soil sample taken in the vicinity of McClellan Air Force Base The soil sample contained a chromium concentration of 593 ug/g. The relative bioavailability of chromium was assessed by comparing the absorption of chromium from the test material to that of a reference material (chromium chloride). Groups of five swine were given oral doses of chromium chloride or the test materials twice a day for 14 days; a group of three non-treated swine served as a control. The amount of chromium absorbed by each animal was evaluated by measuring the amount of chromium excreted in the urine (collected over 48-hour periods beginning on days 6, 9, and 12). The urinary excretion fraction (UEF) (the ratio of the amount excreted per 48 hours divided by the dose given per 48 hours) was calculated for both the test soil and chromium chloride using linear regression analysis. The relative bioavailability (RBA) of chromium in the test soil compared to that in chromium chloride was calculated as follows:

$$\text{RBA} = \frac{\text{UEF (test material)}}{\text{UEF (chromium chloride)}}$$

Results are summarized in Table D-3. Support detailed information of experimental data used to derive the RBA Cr for the McClellan soil follows Table D-3. The chromium RBA estimates are approximately 107% for the test material. This indicates that the chromium in this material is as well absorbed as soluble chromium. This relative bioavailability estimate may be used to improve accuracy and decrease uncertainty in estimating human health risks from exposure to this site-specific test material. It is not clear why the estimate based on the day 6/7 urine collection has such wide variation. A plausible explanation might be that fecal contamination of the urine sample resulting in falsely elevated levels.

Table D-13. Relative Bioavailable Cr of the McClellan Soil

Navy2 Chromium - McClellan Air Force Base

Measurement Endpoint	Estimated RBA (90% Confidence Interval)
Days 6/7	1.27 (0.60 - -16.91)
Days 9/10	0.75 (0.44 - 1.18)
Days 12/13	0.77 (0.16 - 2.06)
All Days	1.07 (0.76 - 1.69)

TABLE D-14 DOSING PROTOCOL

Group	Number of Animals	Dose Material Administered	Chromium Dose (µg/kg-day)	
			Target	Actual [a]
1	5	Chromium Chloride	250	306.9
2	5	Chromium Chloride	500	593.7
3	5	Chromium Chloride	750	866.7
4	5	Test Material 2	400	426.7
5	5	Test Material 2	650	729.4
6	5	Test Material 2	900	999.7
7	3	Control	0	0.0

[a] Calculated as the administered daily dose divided by the measured or extrapolated daily body weight, averaged over days 0-14 for each animal and each group.

Doses were administered in two equal portions given at 9:00 AM and 3:00 PM each day. Doses were held constant based on a body weight of 13.0 kg, the expected mean weight during the exposure interval (14 days). Actual mean body weight across all animals during the exposure interval was 11.7 kg.

TABLE D-15 TYPICAL FEED COMPOSITION

TestDiet 5TXP: Porcine Grower Purified Diet with Low Lead[1]

INGREDIENTS

Corn Starch, %	25.2	Potassium Phosphate, %	0.87
Sucrose, %	20.9648	Calcium Carbonate, %	0.7487
Glucose, %	16	Salt, %	0.501
Soy Protein Isolate, %	14.9899	Magnesium Sulfate, %	0.1245
Casein - Vitamin Free, %	8.5	DL-Methionine, %	0.0762
Powdered Cellulose, %	6.7208	Choline Chloride, %	0.0586
Corn Oil, %	3.4046	Vitamin/Mineral Premix, %	0.0577
Dicalcium Phosphate, %	1.7399	Sodium Selenite, %	0.0433

NUTRITIONAL PROFILE[2]

Protein, %	**21**	**Fat, %**		**3.5**
Arginine, %	1.42	Cholesterol, ppm		0
Histidine, %	0.61	Linoleic Acid, %		1.95
Isoleucine, %	1.14	Linolenic Acid, %		0.03
Leucine, %	1.95	Arachidonic Acid, %		0
Lysine, %	1.56	Omega-3 Fatty Acids, %		0.03
Methionine, %	0.49	Total Saturated Fatty Acids, %		0.43
Cystine, %	0.23	Total Monounsaturated Fatty Acids, %		0.82
Phenylalanine, %	1.22	Polyunsaturated Fatty Acids, %		1.98
Tyrosine, %	1.03			
Threonine, %	0.88			
Tryptophan, %	0.32	**Fiber (max), %**		**6.8**
Valine, %	1.16			
Alanine, %	0.95	**Carbohydrates, %**		**62.2**
Aspartic Acid, %	2.33			
Glutamic Acid, %	4.96	**Energy (kcal/g)[3]**		**3.62**
Glycine, %	0.79	*From:*	*kcal*	*%*
Proline, %	1.83	Protein	0.84	23.1
Serine, %	1.25	Fat (ether extract)	0.315	8.7
Taurine, %	0	Carbohydrates	2.487	68.3

Minerals		**Vitamins**	
Calcium, %	0.8	Vitamin A, IU/g	1.7
Phosphorus, %	0.72	Vitamin 0-3 (added), IU/g	0.2
Phosphorus (available), %	0.4	Vitamin E, IU/kg	11
Potassium, %	0.27	Vitamin K (as menadione), ppm	0.52
Magnesium, %	0.04	Thiamin Hydrochloride, ppm	1
Sodium, %	0.3	Ribonavin, ppm	3.1
Chlorine, %	0.31	Niacin, ppm	13
Fluorine, ppm	0	Pantothenic Acid, ppm	9
Iron, ppm	82	Folic Acid, ppm	0.3
Zinc, ppm	84	Pyridoxine, ppm	1.7
Manganese, ppm	3	Biotin, ppm	0.1
Copper, ppm	4.9	Vitamin B-12, mcg/kg	15
Cobalt, ppm	0.1	Choline Chloride, ppm	410
Iodine, ppm	0.15	Ascorbic Acid, ppm	0
Chromium, ppm	0		
Molybdenum, ppm	0.01		
Selenium, ppm	0.26		

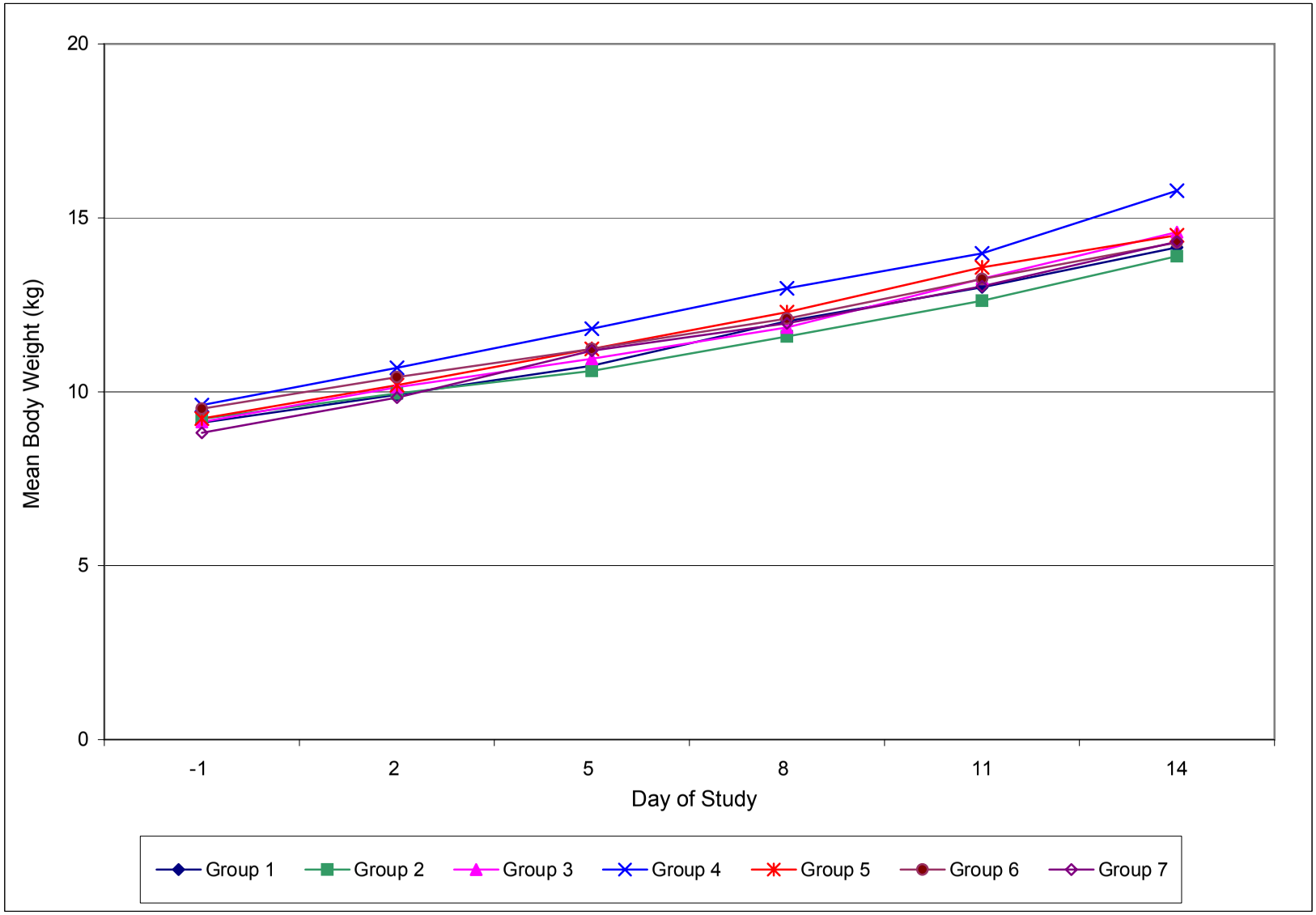
FOOTNOTES

[1] This special purified diet was originally developed for lead RBA studies.

[2] Based on the latest ingredient analysis information. Since nutrient composition of natural ingredients varies, analysis will differ accordingly. Nutrients expressed as percent of ration on an As Fed basis except where otherwise indicated.

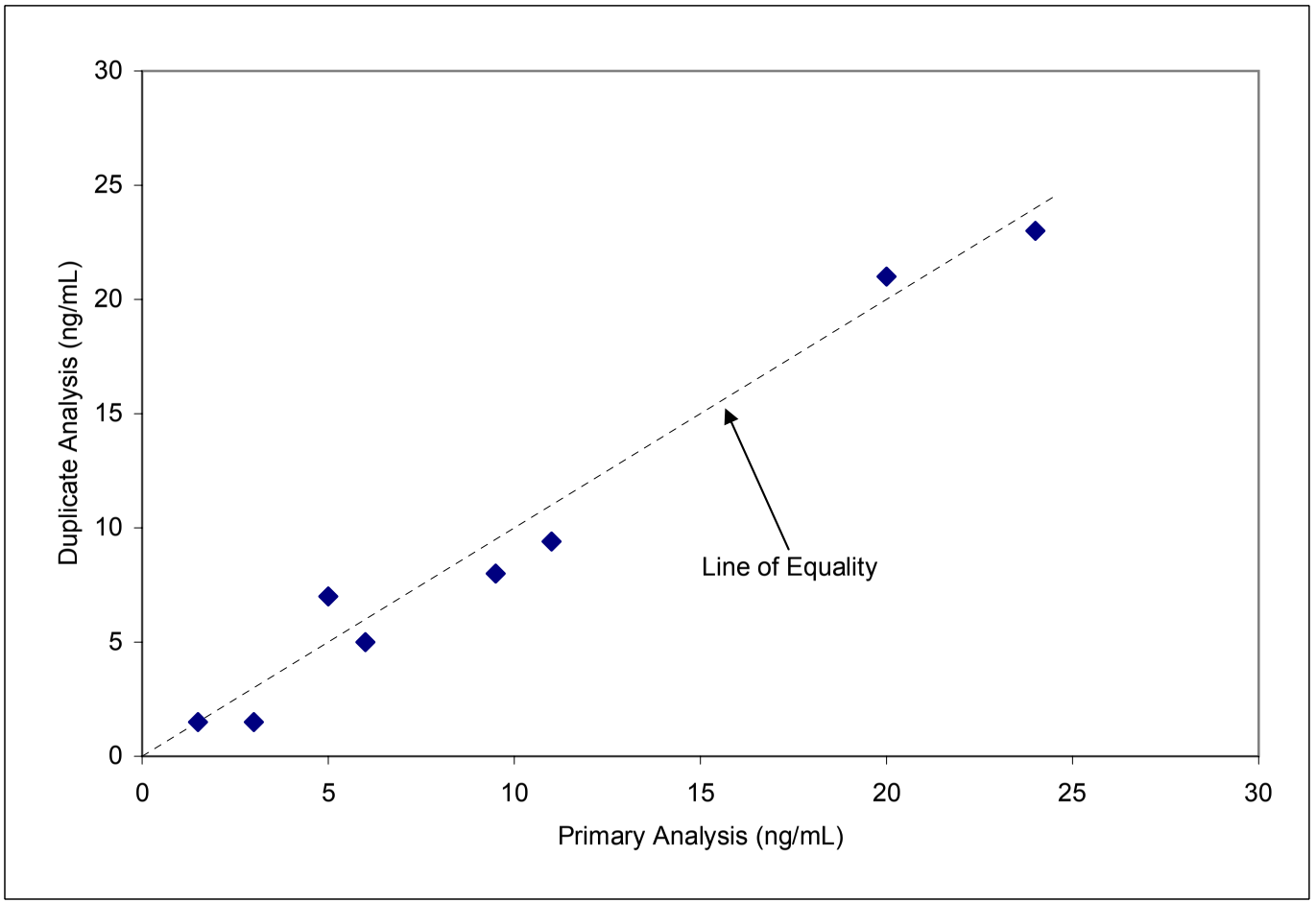
[3] Energy (kcal/gm) - Sum of decimal fractions of protein, fat and carbohydrate x 4,9,4 kcal/gm respectively.

FIGURE D-15 BODY WEIGHT GAIN



1_Navy2 Weights.xls (Fig2-1_group weights)

FIGURE D-16 URINARY CHROMIUM BLIND DUPLICATES



4_Navy2 Lab QC analysis.xls (Fig2-2_BlindDupes)

FIGURE D-17 PERFORMANCE EVALUATION SAMPLES

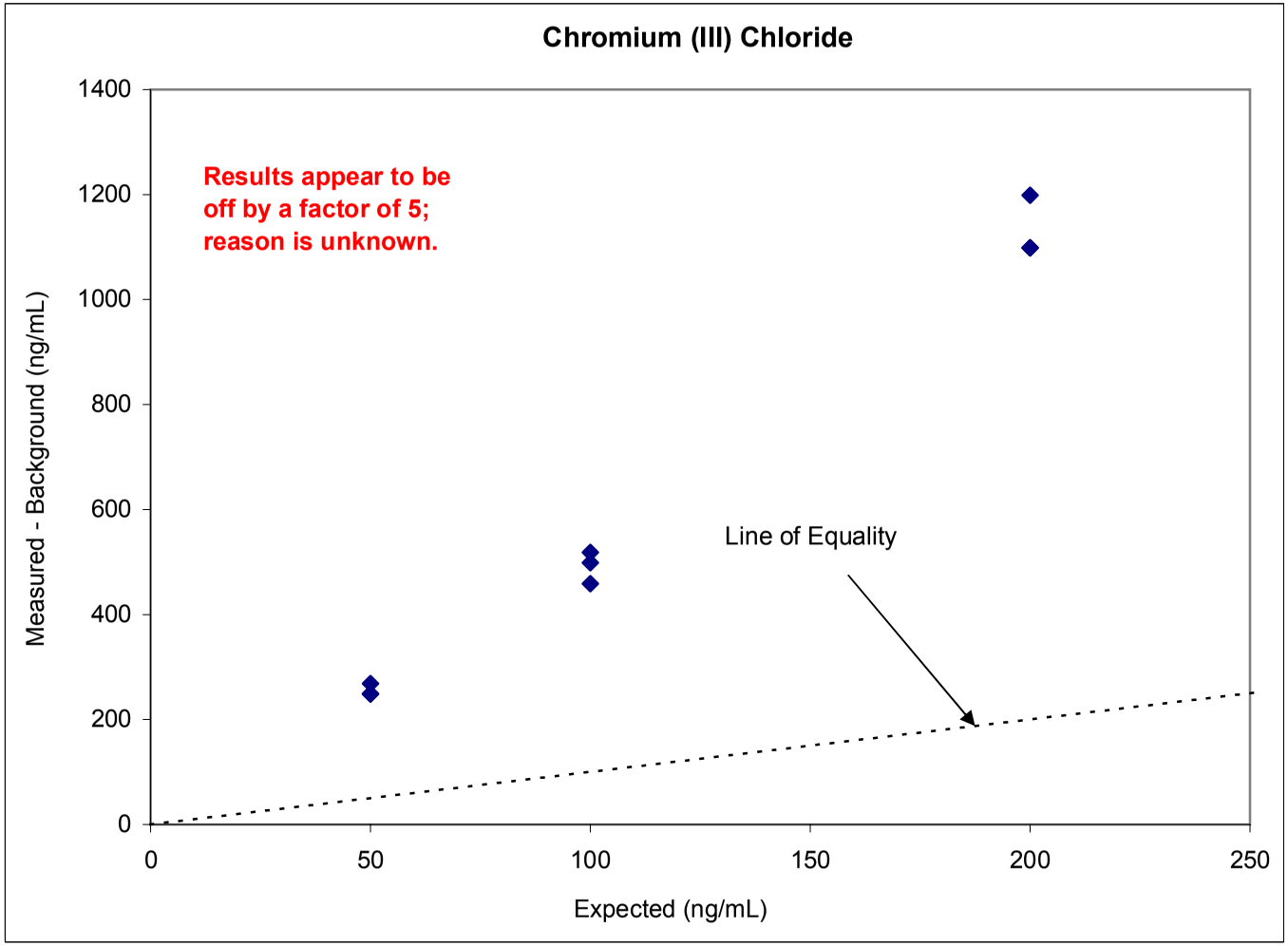
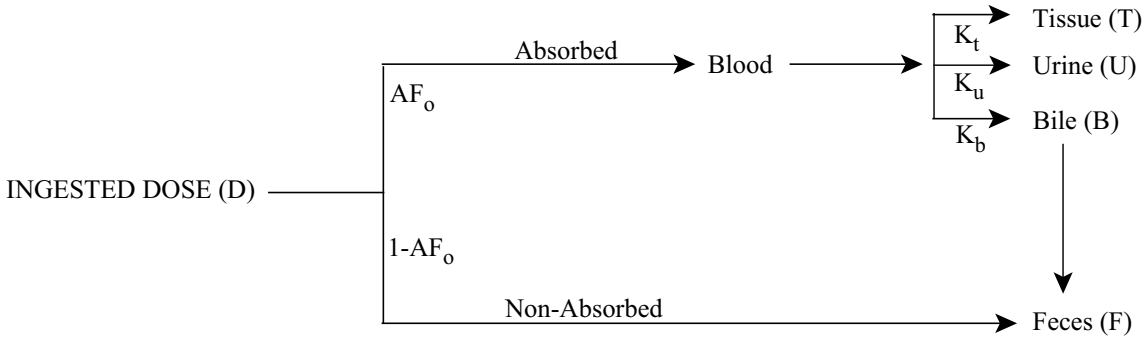


Figure D-18. Conceptual Model for Chromium Toxicokinetics



where:

D = Ingested dose (ug)
AF_o = Oral Absorption Fraction
K_t = Fraction of absorbed chromium which is retained in tissues
K_u = Fraction of absorbed chromium which is excreted in urine
K_b = Fraction of absorbed chromium which is excreted in the bile

BASIC EQUATIONS:

Amount Absorbed (ug) $= D \times AF_o$

Amount Excreted (ug) $=$ Amount absorbed $\times K_u$

$= D \times AF_o \times K_u$

Urinary Excretion Fraction (UEF) $=$ Amount excreted / Amount Ingested

$= (D \times AF_o \times K_u) / D$

$= AF_o \times K_u$

Relative Bioavailability (x vs. y) $= UEF(x) / UEF(y)$

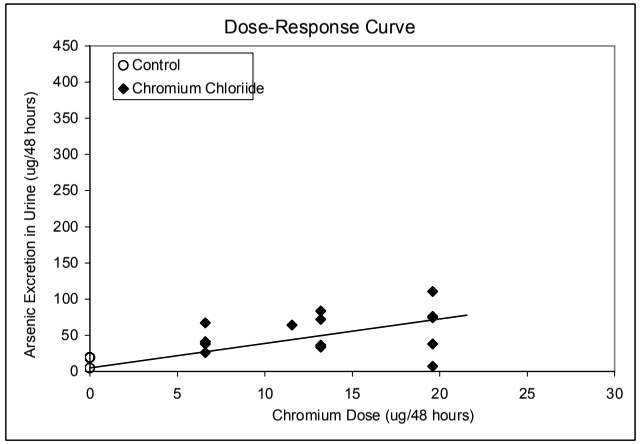
$= (AF_o(x) \times K_u) / (AF_o(y) \times K_u)$

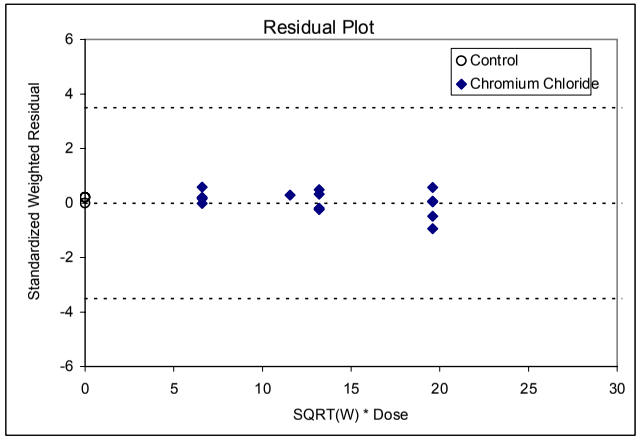
$= AF_o(x) / AF_o(y)$

Fig 3-1_Toxicokinetics.wpd

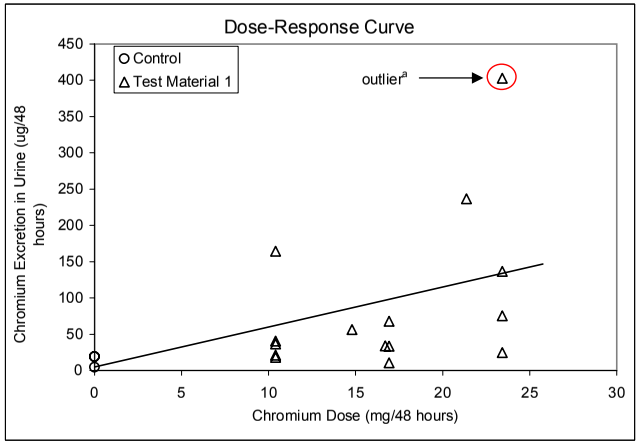
FIGURE D-19 URINARY EXCRETION OF CHROMIUM: Days 6/7 (All Data)

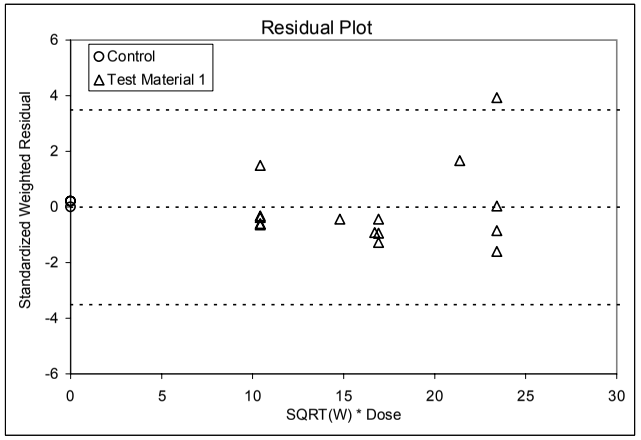
Reference Material (Chromium Chloride)





Test Material 2 (McClellan Air Force Base)





[a] Note that the data from this figure were refitted with the outlier excluded (see Figure 4-1b); this outlier was excluded from the final RBA evaluation.

Summary of Fitting[a]

Parameter	Estimate	Standard Error
a	4.94E+00	2.85E+01
b_r	3.39E+00	2.26E+00
b_{t2}	5.50E+00	1.88E+00
Covariance (b_r,b_{t2})	0.6768	--
Degrees of Freedom	30	--

[a] $y = a + b_r \cdot x_r + b_{t2} \cdot x_{t2}$

ANOVA

Source	MSE
Fit	84360.64
Error	5009.26
Total	10128.70

Statistic	Estimate
F	16.841
p	< 0.001
Adjusted R^2	0.5054

RBA and Uncertainty

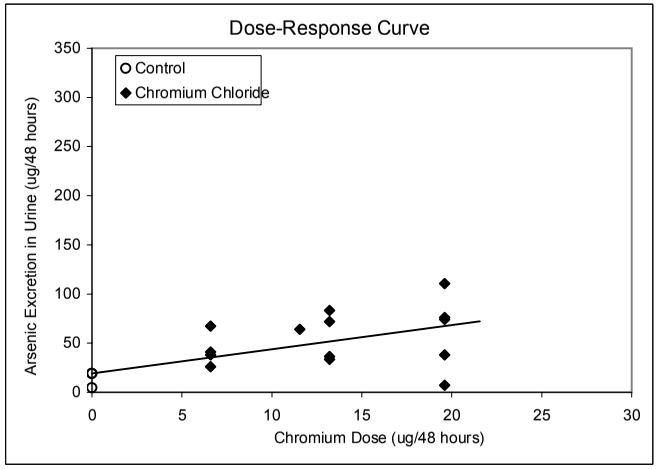
	Test Material 1
RBA	1.62
Lower bound[b]	0.86
Upper bound[b]	-7.27
Standard Error[b]	0.817**

[b] Calculated using Fieller's theorem

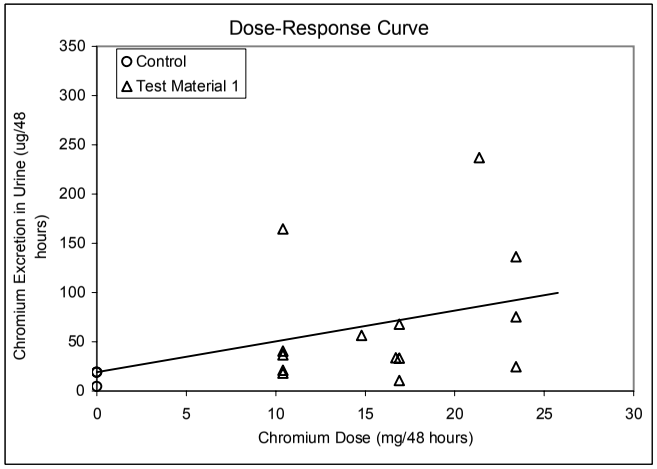
** *g ≥ 0.05, estimate is uncertain*

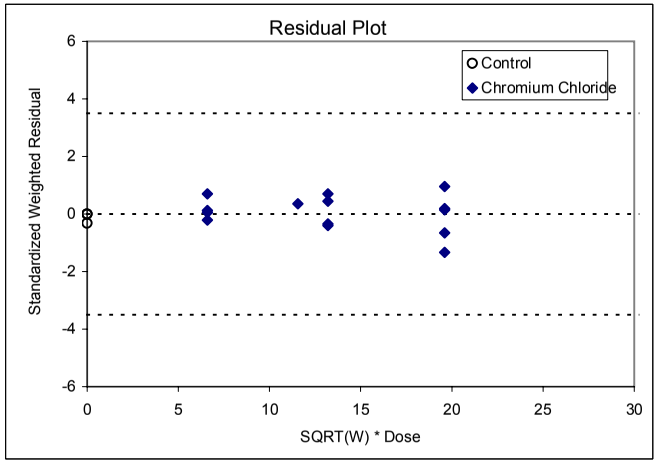
FIGURE D-20 URINARY EXCRETION OF CHROMIUM: Days 6/7 (Outliers Excluded)

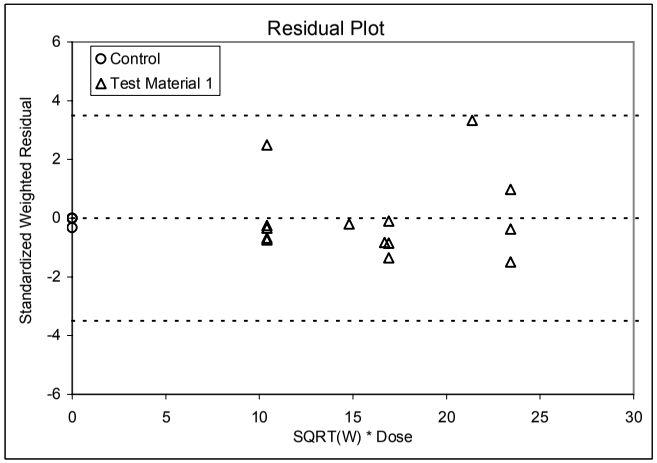
Reference Material (Chromium Chloride)



Test Material 2 (McClellan Air Force Base)







Summary of Fitting[a]

Parameter	Estimate	Standard Error
a	1.93E+01	1.91E+01
b_r	2.46E+00	1.50E+00
b_{t2}	3.12E+00	1.31E+00
Covariance (b_r,b_{t2})	0.6728	--
Degrees of Freedom	29	--

[a] $y = a + b_r \cdot x_r + b_{t2} \cdot x_{t2}$

ANOVA

Source	MSE
Fit	48881.13
Error	2201.01
Total	5313.02

Statistic	Estimate
F	22.209
p	< 0.001
Adjusted R^2	0.5857

RBA and Uncertainty

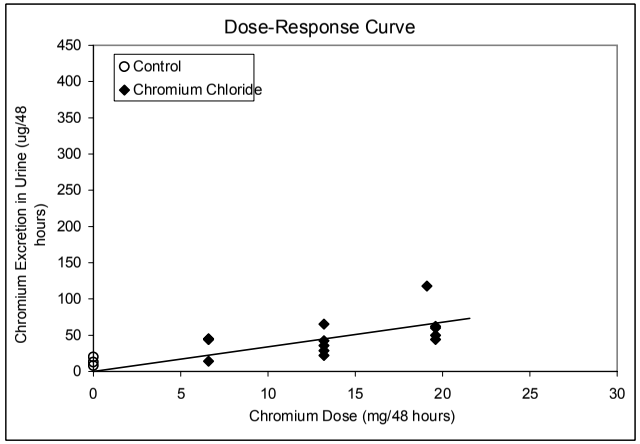
	Test Material 1
RBA	1.27
Lower bound[b]	0.60
Upper bound[b]	-16.91
Standard Error[b]	0.572**

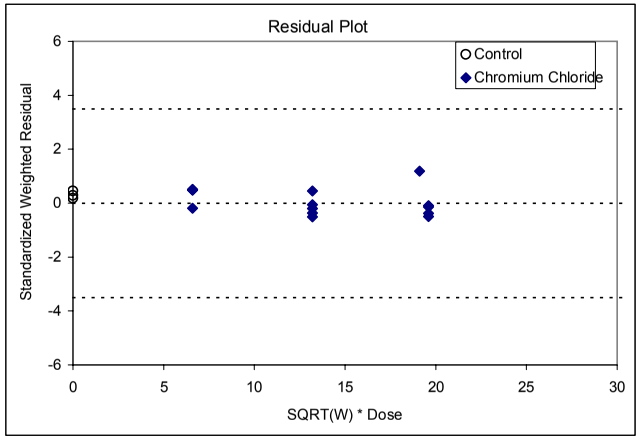
[b] Calculated using Fieller's theorem

*** g ≥ 0.05, estimate is uncertain*

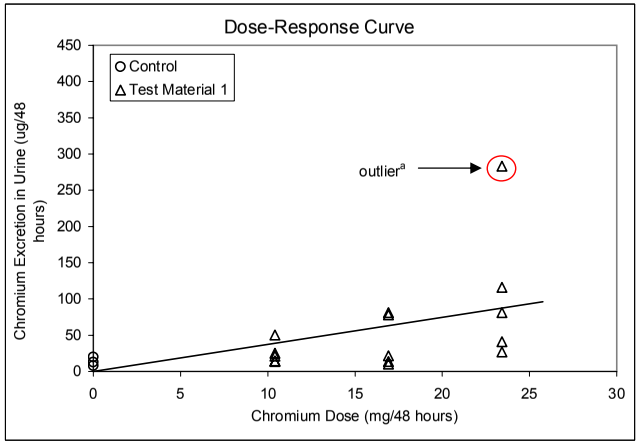
FIGURE D-21 URINARY EXCRETION OF CHROMIUM: Days 9/10 (All Data)

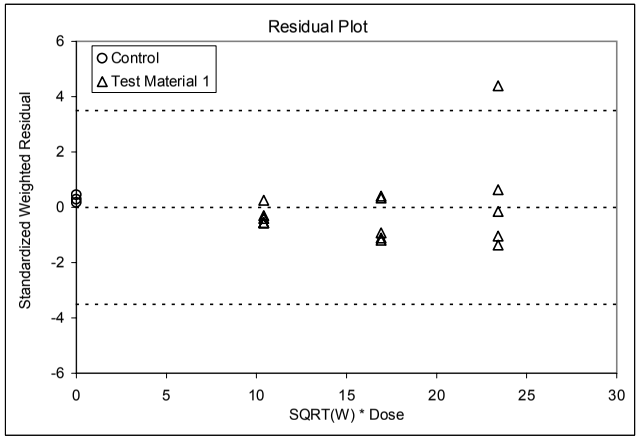
Reference Material (Chromium Chloride)





Test Material 2 (McClellan Air Force Base)





[a] Note that the data from this figure were refitted with the outlier excluded (see Figure 4-2b); this outlier was excluded from the final RBA evaluation.

Summary of Fitting[a]

Parameter	Estimate	SE
a	1.01E-01	1.86E+01
b_r	3.40E+00	1.47E+00
b_{t2}	3.73E+00	1.21E+00
Covariance (b_r,b_{t2})	0.6788	--
Degrees of Freedom	30	--

[a] $y = a + b_r \cdot x_r + b_{t2} \cdot x_{t2}$

ANOVA

Source	MSE
Fit	43676.28
Error	2120.72
Total	4801.72

Statistic	Estimate
F	20.595
p	< 0.001
Adjusted R^2	0.5583

RBA and Uncertainty

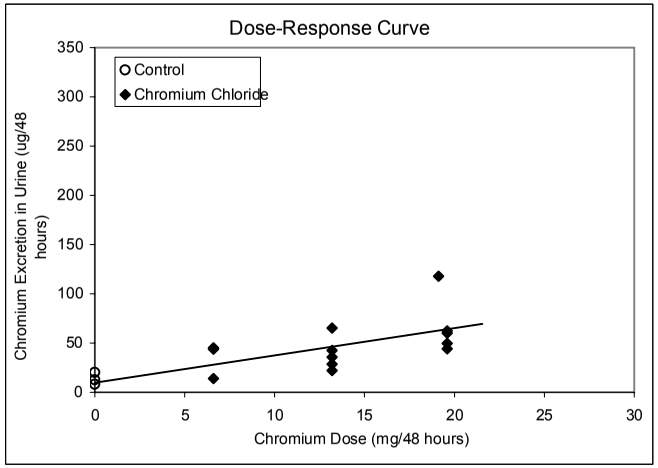
	Test Material 1
RBA	1.10
Lower bound[b]	0.65
Upper bound[b]	2.81
Standard Error[b]	0.350**

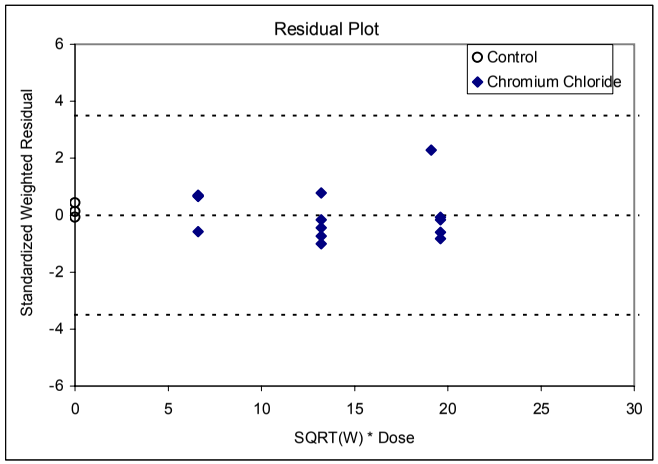
[b] Calculated using Fieller's theorem

** *g ≥ 0.05, estimate is uncertain*

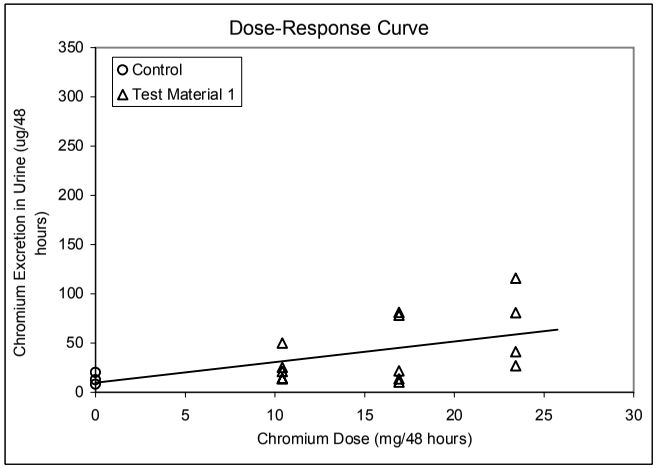
FIGURE D-22 URINARY EXCRETION OF CHROMIUM: Days 9/10 (Outliers Excluded)

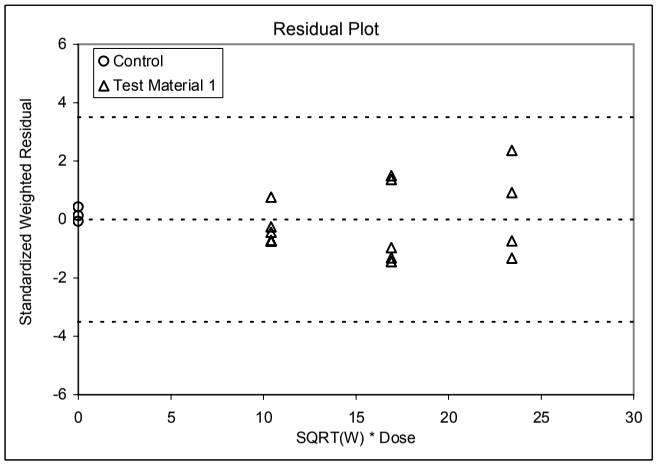
Reference Material (Chromium Chloride)





Test Material 2 (McClellan Air Force Base)





Summary of Fitting[a]

Parameter	Estimate	SE
a	9.67E+00	1.02E+01
b_r	2.78E+00	8.02E-01
b_{t2}	2.10E+00	6.83E-01
Covariance (b_r,b_{t2})	0.6741	--
Degrees of Freedom	29	--

[a] $y = a + b_r \cdot x_r + b_{t2} \cdot x_{t2}$

ANOVA

Source	MSE
Fit	27554.04
Error	625.41
Total	2420.65

Statistic	Estimate
F	44.057
p	< 0.001
Adjusted R^2	0.7416

RBA and Uncertainty

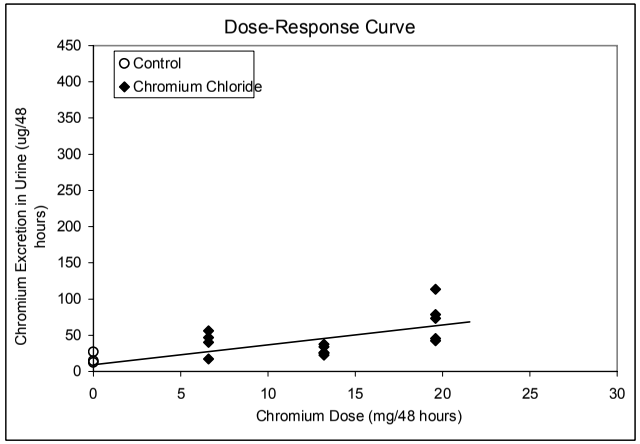
	Test Material 1
RBA	0.75
Lower bound[b]	0.44
Upper bound[b]	1.18
Standard Error[b]	0.189**

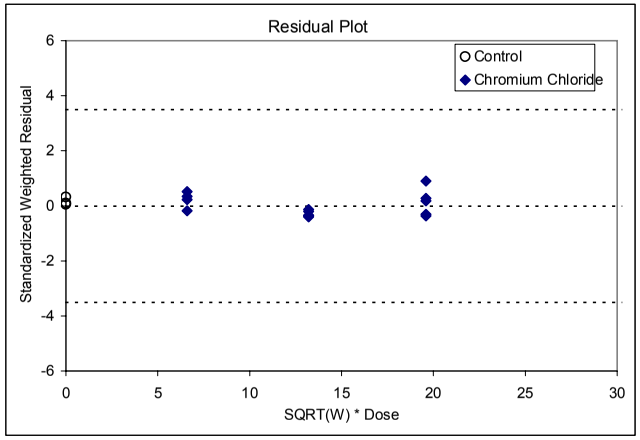
[b] Calculated using Fieller's theorem

*** g ≥ 0.05, estimate is uncertain*

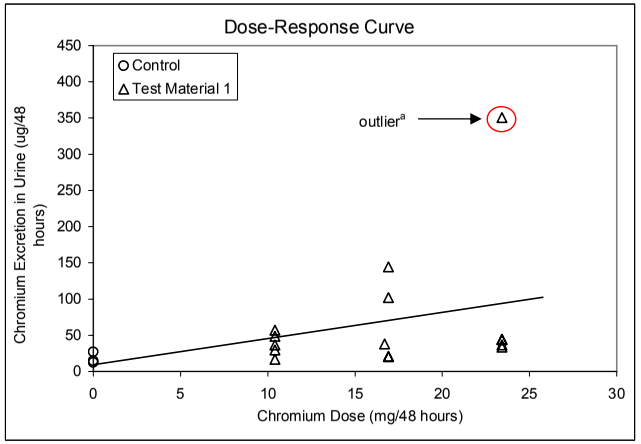
FIGURE D-23 URINARY EXCRETION OF CHROMIUM: Days 12/13 (All Data)

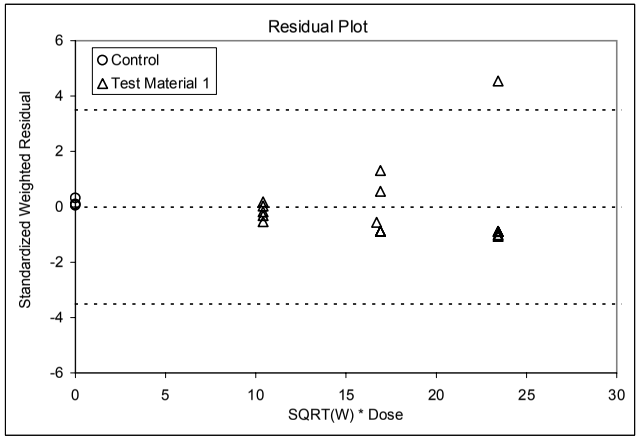
Reference Material (Chromium Chloride)





Test Material 2 (McClellan Air Force Base)





[a] Note that the data from this figure were refitted with the outlier excluded (see Figure 4-3b); this outlier was excluded from the final RBA evaluation.

Summary of Fitting[a]

Parameter	Estimate	SE
a	9.26E+00	2.35E+01
b_r	2.75E+00	1.85E+00
b_{t2}	3.62E+00	1.53E+00
Covariance (b_r,b_{t2})	0.6780	--
Degrees of Freedom	30	--

[a] $y = a + b_r \cdot x_r + b_{t2} \cdot x_{t2}$

ANOVA

Source	MSE
Fit	49358.54
Error	3394.64
Total	6360.05

Statistic	Estimate
F	14.540
p	< 0.001
Adjusted R^2	0.4663

RBA and Uncertainty

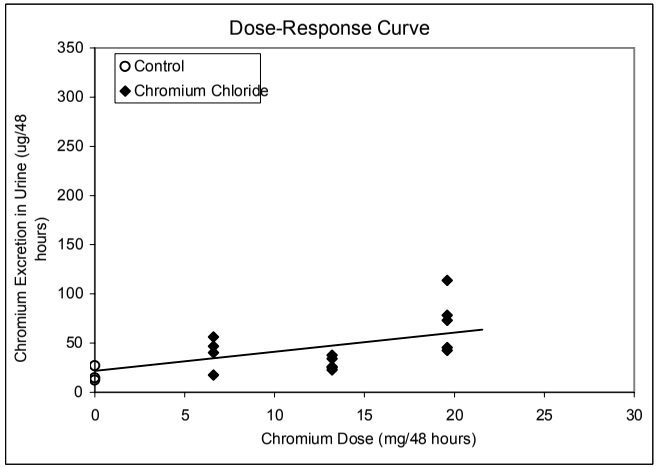
	Test Material 1
RBA	1.32
Lower bound[b]	0.62
Upper bound[b]	-4.42
Standard Error[b]	0.656**

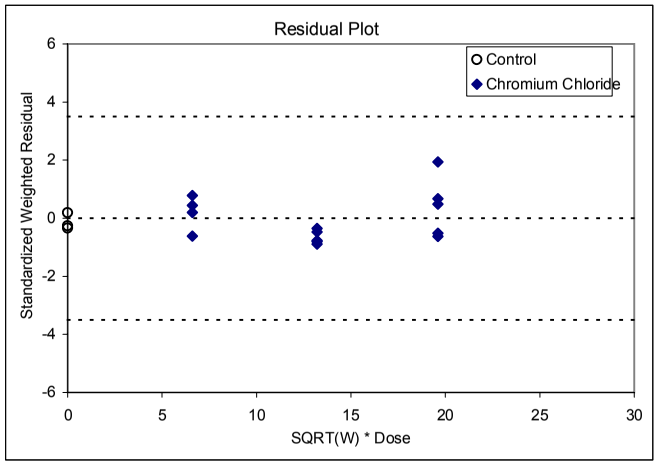
[b] Calculated using Fieller's theorem

*** g ≥ 0.05, estimate is uncertain*

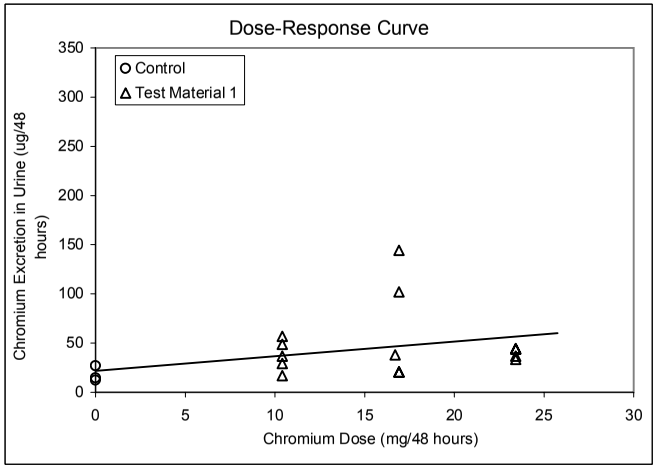
FIGURE D-24 URINARY EXCRETION OF CHROMIUM: Days 12/13 (Outliers Excluded)

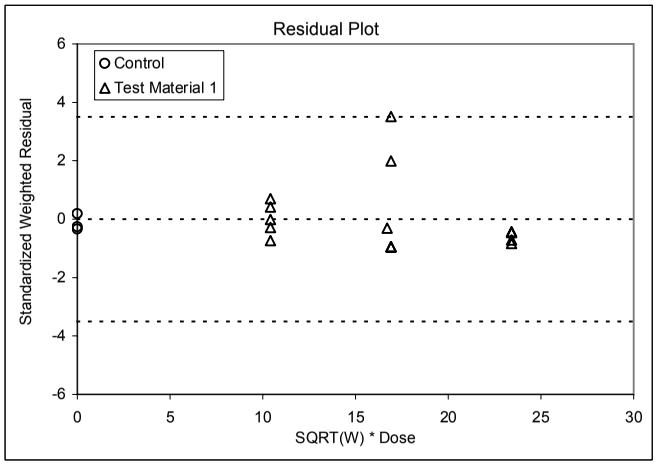
Reference Material (Chromium Chloride)





Test Material 2 (McClellan Air Force Base)





Summary of Fitting[a]

Parameter	Estimate	SE
a	2.18E+01	1.17E+01
b_r	1.94E+00	9.16E-01
b_{t2}	1.49E+00	7.84E-01
Covariance (b_r,b_{t2})	0.6733	--
Degrees of Freedom	29	--

[a] $y = a + b_r \cdot x_r + b_{t2} \cdot x_{t2}$

ANOVA

Source	MSE
Fit	28300.32
Error	824.39
Total	2656.12

Statistic	Estimate
F	34.329
p	< 0.001
Adjusted R^2	0.6896

RBA and Uncertainty

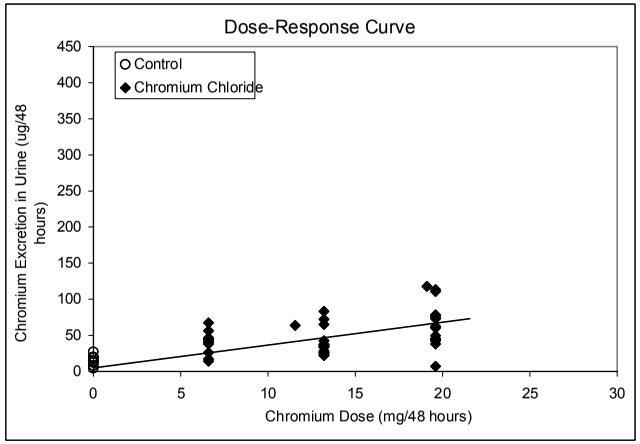
	Test Material 1
RBA	0.77
Lower bound[b]	0.16
Upper bound[b]	2.06
Standard Error[b]	0.312**

[b] Calculated using Fieller's theorem

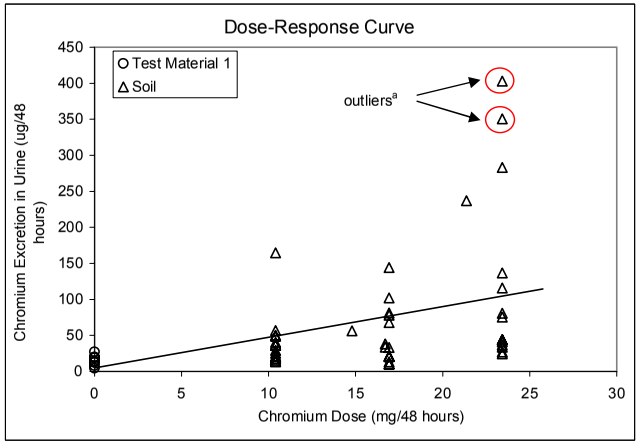
*** g ≥ 0.05, estimate is uncertain*

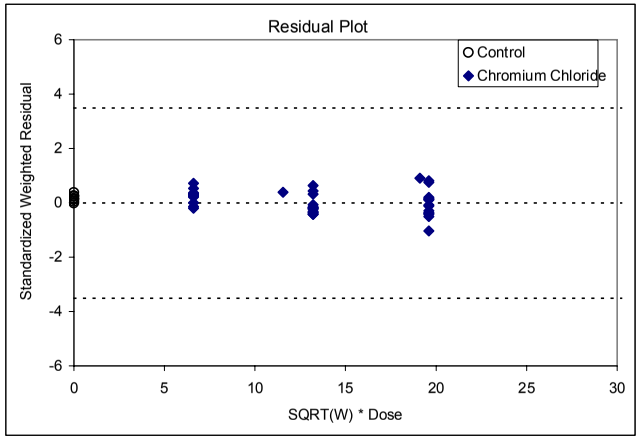
FIGURE D-25 URINARY EXCRETION OF CHROMIUM: All Days (All Data)

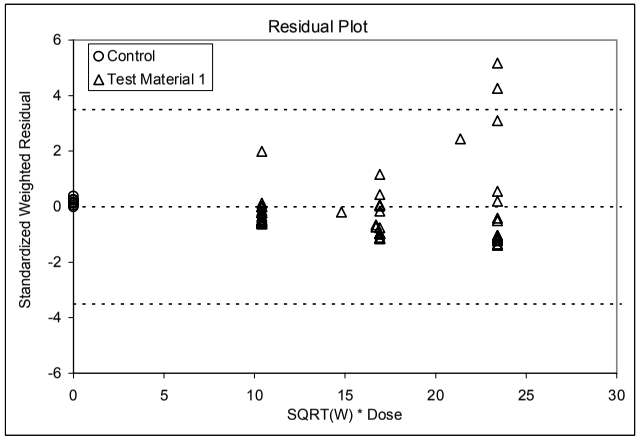
Reference Material (Chromium Chloride)



Test Material 2 (McClellan Air Force Base)







[a] Note that the data from this figure were refitted with the outliers excluded (see Figure 4-4b); these outliers were excluded from the final RBA evaluation.

Summary of Fitting[b]

Parameter	Estimate	SE
a	5.08E+00	1.36E+01
b_r	3.15E+00	1.07E+00
b_{t2}	4.25E+00	8.87E-01
Covariance (b_r,b_{t2})	0.6778	--
Degrees of Freedom	94	--

[a] $y = a + b_r \cdot x_r + b_{t2} \cdot x_{t2}$

ANOVA

Source	MSE
Fit	128993.74
Error	3402.52
Total	6046.55

Statistic	Estimate
F	37.911
p	< 0.001
Adjusted R^2	0.4373

RBA and Uncertainty

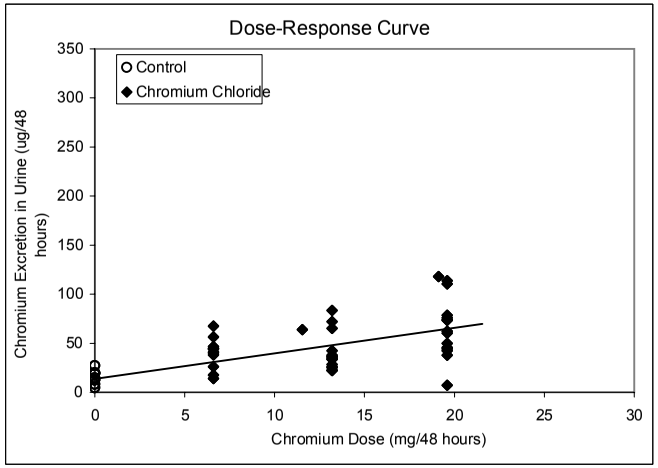
	Test Material 1
RBA	1.35
Lower bound[c]	0.94
Upper bound[c]	2.49
Standard Error[c]	0.338**

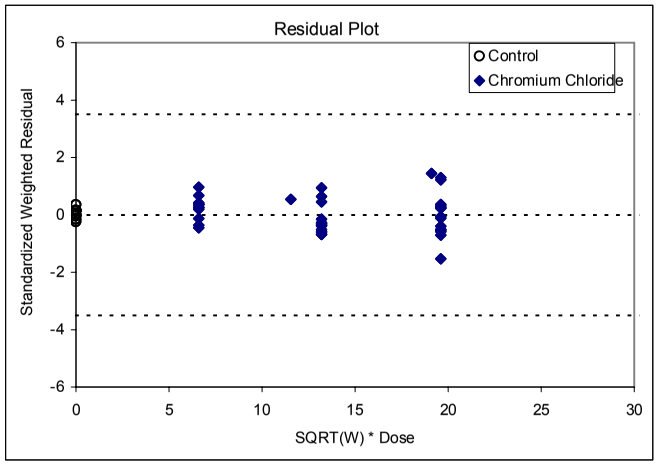
[c] Calculated using Fieller's theorem

** *g ≥ 0.05, estimate is uncertain*

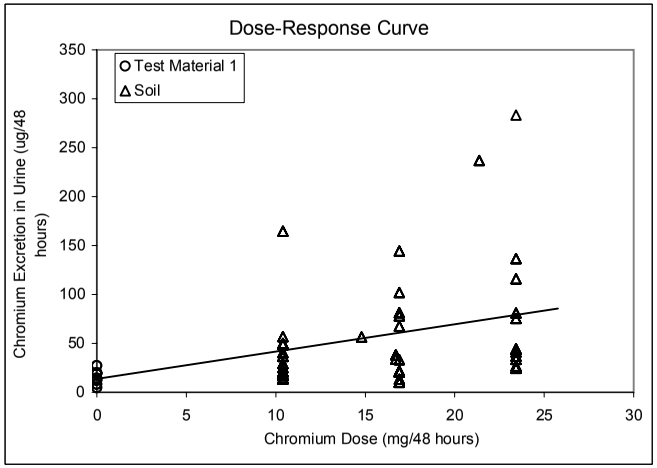
FIGURE D-26 URINARY EXCRETION OF CHROMIUM: All Days (Outliers Excluded)

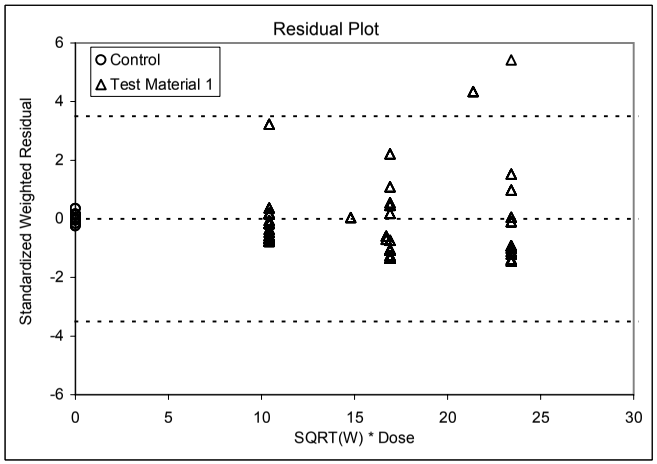
Reference Material (Chromium Chloride)





Test Material 2 (McClellan Air Force Base)





Summary of Fitting[b]

Parameter	Estimate	SE
a	1.38E+01	9.60E+00
b_r	2.60E+00	7.57E-01
b_{t2}	2.78E+00	6.41E-01
Covariance (b_r,b_{t2})	0.6748	--
Degrees of Freedom	185	--

[a] $y = a + b_r \cdot x_r + b_{t2} \cdot x_{t2}$

ANOVA

Source	MSE
Fit	98889.66
Error	1436.52
Total	2484.40

Statistic	Estimate
F	68.840
p	< 0.001
Adjusted R^2	0.4218

RBA and Uncertainty

	Test Material 1
RBA	1.07
Lower bound[c]	0.76
Upper bound[c]	1.69
Standard Error[c]	0.234**

[c] Calculated using Fieller's theorem

*** g ≥ 0.05, estimate is uncertain*

TABLE D-16 SCHEDULE

Study Day	Day	Date	Feed Special Diet	Cull Pigs/ Assign Dose Group	Weigh	Dose Preparation	Dose Administration	Bleed	Urine Collection[a]	Sacrifice/ Necropsy
-5	Wednesday	10/25/06		Cull Pigs	X					
-4	Thursday	10/26/06	transition							
-3	Friday	10/27/06	transition	Assign Dose Groups						
-2	Saturday	10/28/06	transition							
-1	Sunday	10/29/06	transition		X	X				
0	Monday	10/30/06	X				X	X		
1	Tuesday	10/31/06	X				X			
2	Wednesday	11/01/06	X		X		X			
3	Thursday	11/02/06	X			X	X			
4	Friday	11/03/06	X				X			
5	Saturday	11/04/06	X		X		X			
6	Sunday	11/05/06	X				X		U-1	
7	Monday	11/06/06	X				X			
8	Tuesday	11/07/06	X		X	X	X	X		
9	Wednesday	11/08/06	X				X		U-2	
10	Thursday	11/09/06	X				X			
11	Friday	11/10/06	X		X	X	X			
12	Saturday	11/11/06	X				X		U-3	
13	Sunday	11/12/06	X				X			
14	Monday	11/13/06			X			X		X

[a] Urine was collected over a period of 48 hours.

TABLE D-17 GROUP ASSIGNMENTS

Pig Number	Dose Group	Material Administered	Target Dose of Chromium (µg/kg-day)
208 210 212 219 234	1	Chromium Chloride	250
201 205 221 229 232	2	Chromium Chloride	500
203 204 209 211 240	3	Chromium Chloride	750
214 224 231 235 242	4	Test Material 2	400
220 222 226 228 241	5	Test Material 2	650
202 207 217 223 225	6	Test Material 2	900
227 233 237	7	Control	0

TABLE D-18 BODY WEIGHTS AND ACTUAL ADMINISTERED DOSES, BY DAY

Body weights were measured on days -1, 2, 5, 8, 11, and 14. Weights for other days are estimated, based on linear interpolation between measured values.

Group	Pig #	Day -1		Day 0		Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7		Day 8		Day 9		Day 10		Day 11		Day 12		Day 13		Day 14		Days 0-14
		BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	Mean As Dose (µg/kg-d)
1	208	9.9	0.00																													
1	210	9.4	0.00	9.8	368.60	10.1	355.26	10.5	342.86	10.7	336.97	10.9	303.68	11.1	298.64	11.4	289.05	11.8	280.06	12.2	271.60	12.5	264.00	12.9	256.81	13.2	250.00	13.6	242.94	14.0	236.28	14.4	229.97	296.33
1	212	8.3	0.00	8.7	416.18	9.1	397.79	9.5	380.95	9.8	367.97	10.1	326.19	10.5	315.79	11.1	296.41	11.8	279.27	12.5	264.00	12.6	261.56	12.7	259.16	12.9	256.81	13.2	250.63	13.5	244.75	13.8	239.13	314.92
1	219	9.7	0.00	9.8	366.72	10.0	360.60	10.2	354.68	10.5	344.50	10.8	306.98	11.1	298.64	11.4	289.90	11.7	281.65	12.1	273.86	12.5	264.71	12.9	256.14	13.3	248.12	13.8	240.00	14.2	232.39	14.7	225.26	297.93
1	234	8.4	0.00	8.8	411.43	9.2	393.44	9.6	376.96	9.9	365.48	10.2	325.12	10.5	315.79	10.8	306.50	11.1	297.74	11.4	289.47	11.8	279.27	12.2	269.75	12.7	260.87	13.0	253.20	13.4	245.96	13.8	239.13	318.35
2	201	8.5	0.00	8.9	744.36	9.2	714.80	9.6	687.50	9.9	665.55	10.2	644.95	10.6	625.59	10.9	604.58	11.3	584.93	11.7	566.52	12.0	549.24	12.4	532.97	12.8	517.65	13.2	500.63	13.6	484.70	14.1	469.75	607.82
2	205	9.1	0.00	9.3	707.14	9.6	689.90	9.8	673.47	10.0	657.81	10.3	642.86	10.5	628.57	10.9	607.36	11.2	587.54	11.6	568.97	12.0	550.76	12.4	533.69	12.8	517.65	13.1	503.18	13.5	489.49	13.9	476.53	602.73
2	221	9.1	0.00	9.3	713.51	9.5	698.41	9.7	512.95	9.8	674.62	9.9	632.27	10.1	656.72	10.4	475.96	10.8	613.95	11.1	594.59	11.5	574.75	11.9	556.18	12.3	538.78	12.7	520.37	13.1	503.18	13.6	487.08	590.30
2	229	9.9	0.00	10.2	646.00	10.6	623.62	11.0	602.74	11.2	587.54	11.5	573.08	11.8	559.32	12.2	542.47	12.5	526.60	12.9	511.63	13.1	503.18	13.3	495.00	13.6	487.08	14.0	471.99	14.4	457.80	14.9	444.44	545.60
2	232	9.6	0.00	9.7	683.94	9.7	680.41	9.8	676.92	9.9	668.92	10.0	661.10	10.1	653.47	10.3	640.78	10.5	628.57	10.7	616.82	11.1	596.39	11.4	577.26	11.8	559.32	12.3	538.04	12.7	518.32	13.2	500.00	622.27
3	203	9.5	0.00	9.7	1006.85	10.0	978.37	10.3	951.46	10.7	920.19	11.0	890.91	11.4	863.44	11.7	836.42	12.1	811.03	12.5	787.15	12.9	757.73	13.4	730.43	13.9	705.04	14.4	680.56	14.9	657.72	15.4	636.36	835.04
3	204	9.5	0.00	9.7	1010.31	10.0	984.92	10.2	960.78	10.5	936.31	10.7	913.04	11.0	890.91	11.3	864.71	11.7	840.00	12.0	816.67	12.4	790.32	12.8	765.63	13.2	742.42	13.6	720.59	14.0	700.00	14.4	680.56	859.90
3	209	9.2	0.00	9.6	1020.83	10.1	975.12	10.5	933.33	10.8	906.01	11.1	880.24	11.5	855.90	11.8	834.04	12.1	813.28	12.4	793.52	13.0	755.78	13.6	721.47	14.2	690.14	14.6	669.70	15.1	650.44	15.5	632.26	830.39
3	211	8.7	0.00	9.0	1084.87	9.4	1046.26	9.7	1010.31	10.0	981.64	10.3	954.55	10.6	928.91	10.8	910.22	11.0	892.26	11.2	875.00	11.6	842.41	12.1	771.55	12.5	784.00	13.0	756.76	13.4	731.34	13.9	707.58	906.64
3	240	8.9	0.00	9.3	1059.46	9.6	1020.83	10.0	984.92	10.1	970.30	10.3	956.10	10.4	942.31	10.7	917.32	11.0	893.62	11.3	871.11	11.7	841.20	12.1	813.28	12.5	787.15	12.9	759.69	13.4	734.08	13.8	710.14	901.66
4	214	10.0	0.00	10.4	500.34	10.9	467.60	11.3	460.49	11.7	446.66	12.0	433.63	12.4	421.34	12.7	410.81	13.0	400.79	13.3	391.24	13.1	398.74	12.8	406.53	12.6	414.63	13.7	381.21	14.8	352.78	15.9	328.30	416.27
4	224	10.0	0.00	10.3	504.38	10.7	487.07	11.1	470.91	11.4	457.79	11.7	445.38	12.0	433.63	12.4	420.77	12.7	408.66	13.1	397.22	13.7	381.21	14.2	366.45	14.8	352.78	15.1	345.37	15.4	338.26	15.7	331.44	420.07
4	231	8.6	0.00	9.0	579.24	9.4	552.59	9.9	528.28	10.2	509.32	10.6	491.67	11.0	475.21	11.4	456.45	11.9	439.12	12.3	423.05	12.7	409.19	13.1	396.21	13.6	384.03	14.1	369.05	14.7	355.19	15.2	342.34	460.13
4	235	9.5	0.00	9.9	527.39	10.2	508.49	10.6	490.90	11.0	473.77	11.4	457.79	11.8	442.86	12.2	428.28	12.6	414.63	13.0	401.82	13.5	386.88	14.0	373.01	14.5	360.11	14.9	349.23	15.4	338.99	15.8	329.34	430.29
4	242	10.2	0.00	10.3	504.38	10.5	446.73	10.7	366.45	11.1	468.79	11.6	450.52	12.0	433.63	12.4	419.64	12.8	406.53	13.2	394.21	13.7	380.75	14.1	368.18	14.6	356.41	15.2	342.71	15.8	330.03	16.4	318.26	406.82
5	220	9.9	0.00	10.1	835.80	10.3	818.27	10.6	801.47	10.8	743.77	11.1	650.42	11.3	748.27	11.7	541.25	12.1	696.88	12.6	673.74	13.0	648.76	13.5	625.56	14.0	603.96	14.2	596.86	14.3	589.92	14.5	583.14	689.98
5	222	8.8	0.00	9.2	922.42	9.5	886.94	9.9	854.09	10.2	826.27	10.6	800.20	10.9	775.73	11.3	748.27	11.7	722.69	12.1	698.80	12.4	680.98	12.7	664.04	13.1	647.93	13.4	631.01	13.8	614.94	14.1	599.68	756.75
5	226	9.2	0.00	9.5	890.05	9.8	862.80	10.1	837.18	10.5	806.56	10.9	778.11	11.3	751.60	11.6	732.08	11.9	713.54	12.2	695.92	12.7	667.54	13.2	641.38	13.7	617.19	14.0	602.53	14.4	588.55	14.7	575.20	736.18
5	228	9.5	0.00	9.9	858.42	10.2	828.97	10.6	801.47	10.9	773.37	11.3	747.17	11.7	722.69	12.1	699.76	12.5	678.25	12.9	658.01	13.3	638.15	13.7	619.45	14.1	601.81	14.4	587.19	14.8	558.92	15.1	559.97	706.18
5	241	8.8	0.00	9.1	927.47	9.5	891.61	9.9	858.42	10.2	826.27	10.6	796.43	11.0	768.68	11.3	750.49	11.5	714.81	11.8	716.57	12.2	691.18	12.7	667.54	13.1	645.46	13.4	629.44	13.8	614.20	14.1	599.68	757.84
6	202	9.2	0.00	9.4	1121.03	9.6	853.75	9.8	925.93	10.1	1128.42	10.4	1122.23	10.8	1089.17	11.0	935.62	11.2	997.59	11.4	1031.59	11.7	999.31	12.1	968.99	12.5	940.45	12.9	908.82	13.3	879.24	13.8	851.53	987.08
6	207	9.3	0.00	9.6	1215.42	10.0	1168.91	10.4	1125.83	10.6	1101.12	10.9	1077.48	11.1	1054.83	11.4	1030.08	11.6	1006.47	11.9	983.91	12.3	955.80	12.6	929.25	13.0	904.14	13.3	882.56	13.6	861.98	13.9	842.34	1029.24
6	217	9.8	0.00	10.2	1151.66	10.6	1106.32	11.0	1064.42	11.3	1034.63	11.6	1006.47	12.0	979.80	12.3	954.50	12.6	930.48	12.9	907.64	13.3	881.45	13.7	856.73	14.1	833.35	14.4	814.04	14.7	795.60	15.1	777.98	960.30
6	223	10.0	0.00	10.3	1136.76	10.6	1049.35	10.9	1074.18	11.2	1048.53	11.4	1024.07	11.7	1000.73	12.1	970.32	12.4	941.71	12.8	914.73	13.2	885.90	13.6	858.82	14.1	833.35	14.4	812.16	14.8	792.01	15.2	772.84	961.45
6	225	9.4	0.00	9.6	1223.89	9.8	1196.79	10.0	1170.86	10.2	1146.03	10.4	1122.23	10.7	1099.40	11.0	1069.28	11.3	1040.76	11.6	1013.73	11.9	981.17	12.3	950.63	12.7	921.94	13.0	900.66	13.3	880.34	13.6	860.93	1060.41
7	227	8.4	0.00	8.7	0.00	9.1	0.00	9.4	0.00	10.0	0.00	10.6	0.00	11.2	0.00	11.3	0.00	11.4	0.00	11.6	0.00	12.0	0.00	12.4	0.00	12.8	0.00	13.2	0.00	13.5	0.00	13.9	0.00	0.00
7	233	9.0	0.00	9.3	0.00	9.7	0.00	10.0	0.00	10.4	0.00	10.7	0.00	11.1	0.00	11.4	0.00	11.7	0.00	12.0	0.00	12.2	0.00	12.5	0.00	12.8	0.00	13.2	0.00	13.7	0.00	14.2	0.00	0.00
7	237	9.2	0.00	9.5	0.00	9.8	0.00	10.1	0.00	10.5	0.00	10.9	0.00	11.3	0.00	11.7	0.00	12.0	0.00	12.4	0.00	12.8	0.00	13.2	0.00	13.6	0.00	14.0	0.00	14.5	0.00	14.9	0.00	0.00

Missed Doses:

Day 0 - Pig 202 did not eat entire PM dose (ate approximately 80%). Daily dose adjusted to 90%.
Day 1 - Pig 202 did not eat entire AM or PM dose (ate approximately 90% and 50%, respectively). Daily dose adjusted to 70%.
Day 1 - Pig 223 did not eat entire AM dose (ate approximately 90%). Daily dose adjusted to 95%.
Day 1 - Pig 242 did not eat entire AM dose (ate approximately 80%). Daily dose adjusted to 90%.
Day 1 - Pig 214 did not eat entire PM dose (ate approximately 95%). Daily dose adjusted to 97.5%.
Day 2 - Pig 202 did not eat entire AM or PM dose (ate approximately 80% and 75%, respectively). Daily dose adjusted to 78%.
Day 2 - Pig 242 did not eat entire AM dose (ate approximately 50%). Daily dose adjusted to 75%.
Day 2 - Pig 221 did not eat entire PM dose (ate approximately 50%). Daily dose adjusted to 75%.
Day 3 - Pig 202 did not eat entire PM dose (ate approximately 95%). Daily dose adjusted to 97.5%.
Day 3 - Pig 220 did not eat entire PM dose (ate approximately 90%). Daily dose adjusted to 95%.
Day 4 - Pig 221 did not eat entire PM dose (ate approximately 90%). Daily dose adjusted to 95%.
Day 4 - Pig 220 did not eat entire PM dose (ate approximately 70%). Daily dose adjusted to 85%.
Day 5 - Pig 220 did not eat entire PM dose; uneaten dose was not quantified, so daily dose could not be adjusted.
Day 6 - Pig 202 did not eat entire PM dose (ate approximately 75%). Daily dose adjusted to 87.5%.
Day 6 - Pig 221 did not eat entire PM dose (ate approximately 50%). Daily dose adjusted to 75%.
Day 6 - Pig 220 did not eat entire PM dose (ate approximately 50%). Daily dose adjusted to 75%.
Day 7 - Pig 202 did not eat entire PM dose (ate approximately 90%). Daily dose adjusted to 95%.
Day 7 - Pig 241 did not eat entire PM dose (ate approximately 95%). Daily dose adjusted to 97.5%.
Day 10 - Pig 211 did not eat entire AM dose (ate approximately 90%). Daily dose adjusted to 95%.
Day 13 - Pig 228 did not eat entire AM dose (ate approximately 95%). Daily dose adjusted to 97.5%.

Instances of late consumption of doses are shown in Table A-5 (no adjustments necessary).
On day 1 (10/31/2006), pig 208 was found dead in the morning.

TABLE D-19 ANIMAL HEALTH

Naxcel Treatment[a]

First Day of Treatment	Pig	Group	Treatment Duration
Day 1 (10/31/06)	233	7	3 Days
Day 3 (11/2/06)	210	1	4 Days
	229	2	11 Days
	240	3	3 Days
Day 4 (11/3/06)	221	2	3 Days
Day 6 (11/5/06)	212	1	3 Days
Day 7 (11/6/06)	232	2	7 Days
	209	3	3 Days
	235	4	3 Days
Day 10 (11/9/06)	221	2	4 Days

[a] Pigs were dosed with 1 mL/day Naxcel for diarhea, inappetance, fever, vomiting, and general illness.

Other

On day 1 (10/31/06), pig 208 (group 1) was found dead in the morning; the animal was necropsied and tissue samples were taken for pathology. No significant pathological organisms were recovered and the necropsy was inconclusive, although the animal had a history of diarrhea.

TABLE D-20 LATE DOSE CONSUMPTION

Study Day	Pig	Dose*
Day 2	220	PM
	223	PM
	225	PM
	240	PM
Day 3	202	AM
	220	AM
	242	AM
Day 4	209	AM
	220	AM
Day 5	202	PM
	223	PM
Day 6	221	AM
Day 7	202	AM
	220	AM
	223	PM
Day 8	202	AM and PM
	209	PM
	211	PM
	223	AM and PM
Day 9	202	PM
Day 10	202	PM
	223	PM

*Dose was consumed by feeding time, but not immediately; typically, dose was eaten over the hour following dosing.

See Table A-3 for missed doses.

TABLE D-21 URINE VOLUMES

Group	Pig Number	Urine Collection[a]		
		U-1 Days 6-7	**U-2** Days 9-10	**U-3** Days 12-13
1	208	no sample[b]	no sample[b]	no sample[b]
	210	16830	29280	37500
	212	1280	4095	4370
	219	4320	4700	4360
	234	3170	4036	5190
2	201	12000	14810	16720
	205	11180	9500	15070
	221	6660	9300	12560
	229	2800	3540	5210
	232	4630	7160	5690
3	203	1910	1310	2830
	204	3620	3010	4580
	209	4096	4920	9800
	211	12640	12410	14200
	240	2470	3460	3800
4	214	4500	8430	11350
	224	5260	9000	11330
	231	41140	33400	32500
	235	5220	9560	9200
	242	6064	5250	9790
5	220	11290	14550	13890
	222	6690	8220	13130
	226	4840	4063	3190
	228	7080	9010	9520
	241	5640	6850	6840
6	202	5040	10540	8920
	207	16790	15740	17525
	217	3500	3740	5600
	223	7620	7360	6143
	225	4095	6720	3990
7	227	3040	5340	9690
	233	12580	13350	18000
	237	12740	8480	8163

Units = milliliters

[a] Urine was collected over 48-hour periods.

[b] Pig 208 died on day 1, before urine collections occurred.

TABLE D-22 URINARY CHROMIUM ANALYTICAL RESULTS FOR STUDY SAMPLES

Sample Number	Tag Number	Pig Number	Group	Material Administered	Urine Collection Days	48-hr Dose (ug/48hr)	48-hr BWAdj Dose (ug/kg-48hr)	Q	Reported Conc (ng/mL)	AdjConc*(ng/mL)	Urine Volume (mL)	Total Excreted (ug/48hrs)
Navy2Cr-208-U3	Navy2Cr-204	208	1	Chromium Chloride	12/13	No data -- pig was found dead on day 1.						
Navy2Cr-210-U3	Navy2Cr-211	210	1	Chromium Chloride	12/13	6600	479.22	<	3	1.5	37500	56
Navy2Cr-212-U3	Navy2Cr-213	212	1	Chromium Chloride	12/13	6600	495.38		9.2	9.2	4370	40
Navy2Cr-219-U3	Navy2Cr-191	219	1	Chromium Chloride	12/13	6600	472.39		4	4	4360	17
Navy2Cr-234-U3	Navy2Cr-197	234	1	Chromium Chloride	12/13	6600	499.16		9	9	5190	47
Navy2Cr-232-U3	Navy2Cr-183	232	2	Chromium Chloride	12/13	13200	1056.37		6	6	5690	34
Navy2Cr-201-U3	Navy2Cr-193	201	2	Chromium Chloride	12/13	13200	985.33	<	3	1.5	16720	25
Navy2Cr-205-U3	Navy2Cr-209	205	2	Chromium Chloride	12/13	13200	992.67	<	3	1.5	15070	23
Navy2Cr-221-U3	Navy2Cr-185	221	2	Chromium Chloride	12/13	13200	1023.54		3	3	12560	38
Navy2Cr-229-U3	Navy2Cr-217	229	2	Chromium Chloride	12/13	13200	929.79		5	5	5210	26
Navy2Cr-240-U3	Navy2Cr-194	240	3	Chromium Chloride	12/13	19600	1493.77		12	12	3800	46
Navy2Cr-211-U3	Navy2Cr-202	211	3	Chromium Chloride	12/13	19600	1488.1		8	8	14200	114
Navy2Cr-209-U3	Navy2Cr-205	209	3	Chromium Chloride	12/13	19600	1320.15		8	8	9800	78
Navy2Cr-204-U3	Navy2Cr-179	204	3	Chromium Chloride	12/13	19600	1420.59		16	16	4580	73
Navy2Cr-203-U3	Navy2Cr-212	203	3	Chromium Chloride	12/13	19600	1338.27		15	15	2830	42
Navy2Cr-231-U3	Navy2Cr-215	231	4	Test Material 2	12/13	10407.1	724.24	<	3	1.5	32500	49
Navy2Cr-224-U3	Navy2Cr-208	224	4	Test Material 2	12/13	10407.1	683.63	<	3	1.5	11330	17
Navy2Cr-214-U3	Navy2Cr-192	214	4	Test Material 2	12/13	10407.1	734		5	5	11350	57
Navy2Cr-242-U3	Navy2Cr-186	242	4	Test Material 2	12/13	10407.1	672.75		3	3	9790	29
Navy2Cr-235-U3	Navy2Cr-203	235	4	Test Material 2	12/13	10407.1	688.23		4	4	9200	37
Navy2Cr-226-U3	Navy2Cr-216	226	5	Test Material 2	12/13	16910.95	1191.08		32	32	3190	102
Navy2Cr-228-U3	Navy2Cr-200	228	5	Test Material 2	12/13	16699.56	1146.11		4	4	9520	38
Navy2Cr-220-U3	Navy2Cr-189	220	5	Test Material 2	12/13	16910.95	1186.77	<	3	1.5	13890	21
Navy2Cr-222-U3	Navy2Cr-214	222	5	Test Material 2	12/13	16910.95	1245.95		11	11	13130	144
Navy2Cr-241-U3	Navy2Cr-180	241	5	Test Material 2	12/13	16910.95	1243.64		3	3	6840	21
Navy2Cr-225-U3	Navy2Cr-199	225	6	Test Material 2	12/13	23417.16	1781		11	11	3990	44
Navy2Cr-202-U3	Navy2Cr-181	202	6	Test Material 2	12/13	23417.16	1788.06		5	5	8920	45
Navy2Cr-207-U3	Navy2Cr-195	207	6	Test Material 2	12/13	23417.16	1744.54		20	20	17525	351
Navy2Cr-217-U3	Navy2Cr-187	217	6	Test Material 2	12/13	23417.16	1609.64		6	6	5600	34
Navy2Cr-223-U3	Navy2Cr-210	223	6	Test Material 2	12/13	23417.16	1604.17		6	6	6143	37
Navy2Cr-227-U3	Navy2Cr-188	227	7	Control	12/13	0	0	<	3	1.5	9690	15
Navy2Cr-233-U3	Navy2Cr-184	233	7	Control	12/13	0	0	<	3	1.5	18000	27
Navy2Cr-237-U3	Navy2Cr-182	237	7	Control	12/13	0	0	<	3	1.5	8163	12
Navy2Cr-210-U1	Navy2Cr-131	210	1	Chromium Chloride	6/7	6600	569.11		4	4	16830	67
Navy2Cr-208-U1	Navy2Cr-124	208	1	Chromium Chloride	6/7	No data -- pig was found dead on day 1.						
Navy2Cr-234-U1	Navy2Cr-101	234	1	Chromium Chloride	6/7	6600	604.25		12	12	3170	38
Navy2Cr-212-U1	Navy2Cr-130	212	1	Chromium Chloride	6/7	6600	575.67		32	32	1280	41
Navy2Cr-219-U1	Navy2Cr-115	219	1	Chromium Chloride	6/7	6600	571.55		6	6	4320	26
Navy2Cr-232-U1	Navy2Cr-120	232	2	Chromium Chloride	6/7	13200	1269.35		18	18	4630	83
Navy2Cr-229-U1	Navy2Cr-133	229	2	Chromium Chloride	6/7	13200	1069.06		13	13	2800	36
Navy2Cr-221-U1	Navy2Cr-116	221	2	Chromium Chloride	6/7	11550	1089.92		9.6	9.6	6660	64
Navy2Cr-201-U1	Navy2Cr-126	201	2	Chromium Chloride	6/7	13200	1189.51		6	6	12000	72
Navy2Cr-205-U1	Navy2Cr-137	205	2	Chromium Chloride	6/7	13200	1194.9		3	3	11180	34
Navy2Cr-211-U1	Navy2Cr-112	211	3	Chromium Chloride	6/7	19600	1802.48		3	3	12640	38
Navy2Cr-203-U1	Navy2Cr-129	203	3	Chromium Chloride	6/7	19600	1647.45		3.7	3.7	1910	7
Navy2Cr-240-U1	Navy2Cr-122	240	3	Chromium Chloride	6/7	19600	1810.93		30	30	2470	74
Navy2Cr-209-U1	Navy2Cr-109	209	3	Chromium Chloride	6/7	19600	1647.32		27	27	4096	111
Navy2Cr-204-U1	Navy2Cr-118	204	3	Chromium Chloride	6/7	19600	1704.71		21	21	3620	76
Navy2Cr-231-U1	Navy2Cr-128	231	4	Test Material 2	6/7	10407.1	895.57		4	4	41140	165
Navy2Cr-224-U1	Navy2Cr-104	224	4	Test Material 2	6/7	10407.1	829.43		4	4	5260	21
Navy2Cr-242-U1	Navy2Cr-113	242	4	Test Material 2	6/7	10407.1	826.17		3	3	6064	18
Navy2Cr-235-U1	Navy2Cr-127	235	4	Test Material 2	6/7	10407.1	842.9		7	7	5220	37
Navy2Cr-214-U1	Navy2Cr-135	214	4	Test Material 2	6/7	10407.1	811.59		9	9	4500	41
Navy2Cr-228-U1	Navy2Cr-102	228	5	Test Material 2	6/7	16910.95	1378.01	<	3	1.5	7080	11
Navy2Cr-241-U1	Navy2Cr-111	241	5	Test Material 2	6/7	16699.56	1465.29		6	6	5640	34
Navy2Cr-226-U1	Navy2Cr-139	226	5	Test Material 2	6/7	16910.95	1445.62		14	14	4840	68
Navy2Cr-220-U1	Navy2Cr-132	220	5	Test Material 2	6/7	14797.08	1238.13		5	5	11290	56
Navy2Cr-222-U1	Navy2Cr-107	222	5	Test Material 2	6/7	16910.95	1470.96		5	5	6690	33
Navy2Cr-223-U1	Navy2Cr-136	223	6	Test Material 2	6/7	23417.16	1912.03		9.9	9.9	7620	75
Navy2Cr-202-U1	Navy2Cr-123	202	6	Test Material 2	6/7	21368.16	1933.21		47	47	5040	237
Navy2Cr-207-U1	Navy2Cr-105	207	6	Test Material 2	6/7	23417.16	2036.55		24	24	16790	403
Navy2Cr-217-U1	Navy2Cr-119	217	6	Test Material 2	6/7	23417.16	1884.99		39	39	3500	137
Navy2Cr-225-U1	Navy2Cr-103	225	6	Test Material 2	6/7	23417.16	2110.04		6	6	4095	25
Navy2Cr-237-U1	Navy2Cr-134	237	7	Control	6/7	0	0	<	3	1.5	12740	19
Navy2Cr-233-U1	Navy2Cr-106	233	7	Control	6/7	0	0	<	3	1.5	12580	19
Navy2Cr-227-U1	Navy2Cr-108	227	7	Control	6/7	0	0	<	3	1.5	3040	5
Navy2Cr-234-U2	Navy2Cr-166	234	1	Chromium Chloride	9/10	6600	549.02		11	11	4036	44
Navy2Cr-208-U2	Navy2Cr-163	208	1	Chromium Chloride	9/10	No data -- pig was found dead on day 1.						
Navy2Cr-210-U2	Navy2Cr-164	210	1	Chromium Chloride	9/10	6600	520.81	<	3	1.5	29280	44

Sample Number	Tag Number	Pig Number	Group	Material Administered	Urine Collection Days	48-hr Dose (ug/48hr)	48-hr BWAdj Dose (ug/kg-48hr)	Q	Reported Conc (ng/mL)	AdjConc*(ng/mL)	Urine Volume (mL)	Total Excreted (ug/48hrs)
Navy2Cr-212-U2	Navy2Cr-157	212	1	Chromium Chloride	9/10	6600	520.72		11	11	4095	45
Navy2Cr-219-U2	Navy2Cr-171	219	1	Chromium Chloride	9/10	6600	520.85		3	3	4700	14
Navy2Cr-205-U2	Navy2Cr-150	205	2	Chromium Chloride	9/10	13200	1084.46		3	3	9500	29
Navy2Cr-221-U2	Navy2Cr-161	221	2	Chromium Chloride	9/10	13200	1130.93		7	7	9300	65
Navy2Cr-229-U2	Navy2Cr-168	229	2	Chromium Chloride	9/10	13200	998.18		12	12	3540	42
Navy2Cr-232-U2	Navy2Cr-175	232	2	Chromium Chloride	9/10	13200	1173.65		5	5	7160	36
Navy2Cr-201-U2	Navy2Cr-141	201	2	Chromium Chloride	9/10	13200	1082.21	<	3	1.5	14810	22
Navy2Cr-211-U2	Navy2Cr-147	211	3	Chromium Chloride	9/10	18620	1571.83		9.5	9.5	12410	118
Navy2Cr-209-U2	Navy2Cr-145	209	3	Chromium Chloride	9/10	19600	1477.26		9	9	4920	44
Navy2Cr-240-U2	Navy2Cr-154	240	3	Chromium Chloride	9/10	19600	1654.48		18	18	3460	62
Navy2Cr-204-U2	Navy2Cr-152	204	3	Chromium Chloride	9/10	19600	1555.95		20	20	3010	60
Navy2Cr-203-U2	Navy2Cr-165	203	3	Chromium Chloride	9/10	19600	1488.17		38	38	1310	50
Navy2Cr-214-U2	Navy2Cr-151	214	4	Test Material 2	9/10	10407.1	805.27		3	3	8430	25
Navy2Cr-224-U2	Navy2Cr-174	224	4	Test Material 2	9/10	10407.1	747.66	<	3	1.5	9000	14
Navy2Cr-231-U2	Navy2Cr-149	231	4	Test Material 2	9/10	10407.1	805.4	<	3	1.5	33400	50
Navy2Cr-235-U2	Navy2Cr-146	235	4	Test Material 2	9/10	10407.1	759.9	<	3	1.5	9560	14
Navy2Cr-242-U2	Navy2Cr-178	242	4	Test Material 2	9/10	10407.1	748.92		4	4	5250	21
Navy2Cr-226-U2	Navy2Cr-173	226	5	Test Material 2	9/10	16910.95	1308.91		20	20	4063	81
Navy2Cr-228-U2	Navy2Cr-170	228	5	Test Material 2	9/10	16910.95	1257.6	<	3	1.5	9010	14
Navy2Cr-222-U2	Navy2Cr-172	222	5	Test Material 2	9/10	16910.95	1345.02		9.5	9.5	8220	78
Navy2Cr-220-U2	Navy2Cr-169	220	5	Test Material 2	9/10	16910.95	1274.32	<	3	1.5	14550	22
Navy2Cr-241-U2	Navy2Cr-176	241	5	Test Material 2	9/10	16910.95	1358.72	<	3	1.5	6850	10
Navy2Cr-202-U2	Navy2Cr-167	202	6	Test Material 2	9/10	23417.16	1968.3		11	11	10540	116
Navy2Cr-207-U2	Navy2Cr-177	207	6	Test Material 2	9/10	23417.16	1885.06		18	18	15740	283
Navy2Cr-217-U2	Navy2Cr-160	217	6	Test Material 2	9/10	23417.16	1738.17		11	11	3740	41
Navy2Cr-223-U2	Navy2Cr-142	223	6	Test Material 2	9/10	23417.16	1744.72		11	11	7360	81
Navy2Cr-225-U2	Navy2Cr-155	225	6	Test Material 2	9/10	23417.16	1931.8		4	4	6720	27
Navy2Cr-227-U2	Navy2Cr-143	227	7	Control	9/10	0	0	<	3	1.5	5340	8
Navy2Cr-233-U2	Navy2Cr-158	233	7	Control	9/10	0	0	<	3	1.5	13350	20
Navy2Cr-237-U2	Navy2Cr-162	237	7	Control	9/10	0	0	<	3	1.5	8480	13

Q = Data qualifier
*Non-detects taken at one-half the detection limit.

TABLE D-23 CHROMIUM ANALYTICAL RESULTS FOR QUALITY CONTROL SAMPLES

Blind Duplicates

Tag Number	Group	Event	DL	Duplicate Conc	Original Pig #
Navy2Cr-117	2	U1	3	5	201
Navy2Cr-121	6	U1	3	23	207
Navy2Cr-138	7	U1	3	<3	237
Navy2Cr-148	3	U2	3	21	204
Navy2Cr-140	5	U2	3	8	222
Navy2Cr-190	2	U3	3	<3	221
Navy2Cr-201	4	U3	3	7	214
Navy2Cr-207	6	U3	3	9.4	225

Laboratory Control Standards

Reference Material	Certified Mean	DL	Measured Conc
NIST 1640	38.6 ± 1.6	1	36
NIST 1640	38.6 ± 1.6	1	34
NIST 1640	38.6 ± 1.6	1	37
Fluka-40-Cr	40 ± 4	1	40
Fluka-40-Cr	40 ± 4	1	35
Fluka-40-Cr	40 ± 4	1	43

Blanks

Tag Number	DL	Measured Conc
Blank-1	3	<3
Blank-2	3	<3
Blank-3	3	<3
Blank-4	3	<3
Blank-5	3	<3
Blank-6	3	<3

PE Sample Analysis

Tag Number	QC Sample	Nominal Conc	Measured Conc	DL
Navy2Cr-110	Chromium (III) chloride	50	250	3
Navy2Cr-159	Chromium (III) chloride	50	250	3
Navy2Cr-206	Chromium (III) chloride	50	270	3
Navy2Cr-125	Chromium (III) chloride	100	520	6
Navy2Cr-153	Chromium (III) chloride	100	460	6
Navy2Cr-196	Chromium (III) chloride	100	500	6
Navy2Cr-114	Chromium (III) chloride	200	1100	20
Navy2Cr-156	Chromium (III) chloride	200	1100	20
Navy2Cr-198	Chromium (III) chloride	200	1200	20

Duplicates

Tag Number	DL	Duplicate Result	Original Sample Conc
Navy2Cr-103	3	6	6
Navy2Cr-112	3	3	3
Navy2Cr-132	3	6	5
Navy2Cr-141	3	<3	<3
Navy2Cr-150	3	<3	3
Navy2Cr-161	3	7	7
Navy2Cr-170	3	<3	<3
Navy2Cr-179	3	15	16
Navy2Cr-188	3	<3	<3
Navy2Cr-199	3	9	11
Navy2Cr-209	3	3	<3

Spikes (nominal spike: 300 ng/mL)

Tag Number	DL	Original Sample Conc	Spiked Result
Navy2Cr-108	3	<3	320
Navy2Cr-116	3	9.6	260
Navy2Cr-128	3	4	330
Navy2Cr-136	3	9.9	270
Navy2Cr-146	3	<3	340
Navy2Cr-155	3	4	260
Navy2Cr-166	3	11	310
Navy2Cr-174	3	<3	260
Navy2Cr-184	3	<3	260
Navy2Cr-193	3	<3	270
Navy2Cr-214	3	11	260

DL = Detection limit
Units: ng/mL

APPENDIX E

ABILITY OF SOIL PROPERTIES AND IN VITRO GASTROINTESTINAL EXTRACTIONS TO PREDICT BIOAVAILABILITY AND BIOACCESSIBILITY OF AS, PB, AND /OR CR IN SOIL

ABILITY OF SOIL PROPERTIES TO PREDICT CONTAMINANT BIOAVAILABILITY IN ESTCP SOILS

Key soil physical and chemical properties (e.g. particle size, CEC, Fe-oxides, TOC/TIC, pH) were identified as controlling the extent of toxic metals bioaccessibility as measured using an in-vitro Physiologically-Based Extraction Test (PBET) that simulated the digestive system of humans. Statistical models were developed and incorporated into a predictive tool known as Soil BioAccessibility Tool (SBAT). The bioaccessibility results (in-vitro) were found to be in excellent agreement with molecular-level metal speciation studies and in-vivo swine metal bioavailability studies, which confirmed that key soil properties control metal bioavailability.

Prediction of in vivo RBA from in vitro bioaccessibility

The main objective of the project was to determine the ability of in vitro gastrointestinal methods (i.e., bioaccessibility methods) to predict measured contaminant bioavailability in contaminated soils from study sites. Equations used to predict bioavailability from bioaccessibility methods are available for Pb and As. Results are summarized and discussed in the following sections.

Lead

Drexler and Brattin (2007) reported the following prediction equations to calculate relative bioavailability of Pb from their relative bioaccessibility leaching procedure (RBALP). The RBALP method is the same as the PBET method used in this study.

RBA Pb (%) = 1.1368 IVBA (pH 1.5) – 7.79 $r^2 = 0.8241$

RBA Pb (%) = 1.3409 IVBA (pH 2.5) – 1.607 $r^2 = 0.7531$

Relative bioavailable Pb was determined for the Portsmouth soil in our study . Comparison of measured and predicted RBA Pb for the Portsmouth soil are shown in Table E-1.

Table E-1. Comparison of measured and predicted RBA Pb for the Portsmouth soil

		Predicted Pb RBA				
Measured Pb RBA, %		PBET pH 1.5		PBET pH 2.5		OSU IVG pH 1.8
Mean	90 % CI†	IVBA, %	RBA, %	IVBA, %	RBA, %	IVBA, %
99	70 - 127	83.3	86.9	80.4	106.2	102.5

† CI = confidence interval

The PBET methods (pH 1.5 and 2.5) were able to accurately predict in vivo RBA for the Portsmouth soil. The predicted RBA for the PBET method at pH 2.5 was closer to actual in vivo RBA than pH 1.5. However both methods predict RBA Pb within the 90% C.I. The OSU IVG method IVBA Pb was very close to the in vivo RBA Pb. However, information on the ability of the OSU IVG method to predict RBA Pb is very limited whereas in depth validation studies have been conducted for the RBALP (i.e., PBET) method.

These results support the PBET methods of pH 1.5 and 2.5 can accurately predict in vivo RBA Pb. Although the study was limited to determination of in vivo Pb RBA in soil, results support use of the PBET method. Future validation studies where this approach is expanded from the Portsmouth soil will increase the confidence of using in vitro methods to predict in vivo RBA Pb. Results suggest that similar "PBET" in vitro methods (i.e., OSU IVG) may also be able to estimate RBA Pb in contaminated soils.

Arsenic

Several studies have reported correlation between bioaccessible As and bioavailable As measured from juvenile swine dosing trials. These include the OSU IVG method (Basta et al., 2007; Rodriguez et al., 1999) and the SBET method (Juhasz et al. 2009). The OSU IVG method reported by Rodriguez et al. (1999) incorporated the dosing vehicle used in the swine dosing trial study into its in vitro solution for 14 As contaminated soils. Basta et al. (2007) reported results from the OSU IVG method with and without dosing vehicle for a subset of 9 soils used in Rodriguez et al. (1999). In this study, the following regression equations used to predict RBA As were determined from the OSU IVG procedure without dosing vehicle (Basta et al., 2007) for the 14 soils used in Rodriguez et al. (1999).

Bioaccessible As measured under gastric conditions:
RBA As (%) = 0.906 IVBA As + 7.37 r^2 =0.85

Bioaccessible As measured under intestinal conditions:
RBA As (%) = 1.02 IVBA As + 7.55 r^2 =0.82

The SBRC method of Juhasz et. al (2009) is identical to the PBET pH of 1.5 method used in our study. The following regression from the SBRC method (Juhasz et al., 2009) was used to predict the RBA As for the Deseret soil using PBET pH 1.5 IVBA As data.

RBA As (%) = 1.656 IVBA As + 0.992 r^2 =0.75

Comparison of measured and predicted RBA As for the Deseret soil are shown in Table E-2.

Table E-2. Comparison of measured and predicted RBA As for the Deseret soil

		Predicted As RBA					
Measured As RBA, %		OSU IVG gastric		OSU IVG intestinal		SBET gastric	
Mean	90 % CI†	IVBA, %	RBA, %	IVBA, %	RBA, %	IVBA, %	RBA, %
14	13-15	8.45	15.0	8.47	16.2	10.6	12.2

† CI = confidence interval

In general, all of the in vitro methods predicted in vivo RBA As with 90% confidence.

These results support that the OSU IVG methods (gastric and intestinal) and the SBRC method (PBET, pH 1.5) can accurately predict in vivo RBA As. However, the study was limited to determination of in vivo As RBA in one soil. The source of As contamination in the Desert soil was associated with previous mining activities. Soils contaminated with As from mining activities were used to derive the RBA As prediction equations for the OSU IVG method.

The effect of arsenic contaminant speciation on the ability of in vitro methods to predict RBA As in not known. The contaminant chemical speciation of arsenic of the mining soils used in the OSU IVG is likely to be more similar to speciation in the mining contaminated Desert soil than other contaminant sources (i.e., arsenical pesticides). Therefore application of an in vivo RBA As prediction equation from in vitro bioaccessible As developed using mining soils may be more accurate for the Desert soil than using prediction equations developed using different contaminant sources.

The 12 As contamination sources used to derive the RBA As prediction equations for the SBRC method were more diverse. The number of soils, in parentheses, from contaminant sources reported in Juhasz et al. (2009) were: railway corridors (6), dip sites (2), mine sites (2), and gossans (2). However, the SBRC method provided an accurate prediction of RBA As in the Deseret soil. Juhasz et al. (2009) reported RBA As prediction equations derived from the OSU IVG method for the 12 soils in their study.

Future validation studies where this approach is expanded from the Deseret soil to other soils contaminated with arsenic will increase the confidence of using in vitro methods to predict in vivo RBA As. Predicted RBA As by the OSU IVG method determined using Juhasz et al. (2009) is 21.5% for gastric phase and 23.3% for the intestinal phase. These results suggest OSU IVG would overpredict bioavailable As by 6.5% (gastric) or 8.3% (intestinal). Overprediction may be viewed as a desirable conservative measure by regulators. However, the correlation between the OSU IVG method and RBA As reported by Juhasz et al. (2009) of r2=0.57 (gastric and intestinal) was much weaker than reported by Basta et al. (2003) of r2=0.85 (gastric) and r2=(0.81). Therefore, the regression with the higher prediction power was used to predict RBA As for our study soil. It is possible that the weaker correlation for the OSU IVG method reported by Juhasz et al. (2009) was due to different contaminant speciation in a few of the non-mining soils. Further research on the effect of contaminant speciation of different contaminant sources on the ability of various in vitro methods to predict RBA As is needed and is underway.

Results from our study show both the OSU IVG and SBRC method was able to predict RBA As in the Desert soil. The predicted RBA As by all methods ranged from 12.2 % to 16.2% which is comparable to the in vivo RBA As 14%. Further validation studies of these methods for other contaminated soils from different contaminant sources are warranted.

Chromium

A study investigating the relationship between in vitro IVBA Cr and in vivo RBA Cr has not been reported. Thus, it was not possible to evaluate the ability of bioaccessible Cr to predict in vivo RBA Cr. In our study, a novel immature swine dosing model was used to determine the in vivo RBA Cr for the McClellan soil. RBA Cr was 107% with a 90% confidence interval ranging

from 76% to 169%. In vitro IVBA Cr PBET method, used to measure bioaccessible Cr at pH 1.5 and at pH 2.5, was 10.1% and 19.0%, respectively. The in vitro IVBA values were much lower than the in vivo RBA Cr. Further research is needed before IVBA can be used to predict in vivo RBA Cr.

Prediction of in vitro bioaccessibility using soil properties

Study of the determination of soil properties on in vivo bioavailability or in vitro bioaccessibility is very limited. To our knowledge, these relationships has not been reported for Pb and limited studies exist for As and Cr. The ability of soil properties to predict bioaccessible As and Cr is discussed below.

Arsenic

Yang et al. (2002) reported the following relationship between in vitro bioaccessible As (IVBA As) and soil properties.

IVBA As (%) = 11.3 pH – 30.5 log Fe

Where pH is the soil pH and Fe is soil Fe extracted with citrate-bicarbonate-dithionite solution (CBD) in g/kg (Mehra and Jackson, 1960). Using the pH reported by Rodriguez et al. (1999) and CBD extractable Fe measured on these samples, the equation derived to predict IVBA As was able to predict RBA As within a root square mean error of 9.5%.

Similarly, Whitacre (2009) reported Fe oxide content and pH in 19 soils were able to predict IVBA As measured by the OSU IVG gastric and Intestinal phases. The following prediction equations were reported:

gastric conditions:
IVBA As (%) = 30.7 – 22.6 log Feox + 12.3 pH $R^2 = 0.84$
IVBA As (%) = 88.2 - 33.2 log FeCBD + 12.2 pH $R^2 = 0.92$

Intestinal conditions:
IVBA As (%) = 36.4 – 23.4 log Feox + 12.0 pH $R^2 = 0.83$
IVBA As (%) = 95.7 -34.3 log FeCBD + 11.9 pH $R^2 = 0.92$

where Feox is soil Fe determined by extraction with acid ammonium oxalate solution (in mg/kg) and FeCBD is soil Fe extracted with citrate bicarbonate dithionite (in mg/kg).

Use of above regression equations allow prediction of IVBA from soil properties. Comparison of measured and calculated IVBA As using soil property predictive models of Yang et al. (2002) and Whitacre (2008) for As contaminated study soils are summarized in Table E-3.

Table E-3. Comparison of measured and predicted IVBA As (%) for study soils with As contamination.

	Gastric					Gastrointestinal		
Soil	M1	P1	P2	M2	P1	M3	P4	P5
Concord	18.6	30.8	33.3	22.1	41.7	18.4	31.6	34.3
Deseret	8.45	76.7	75.9	10.6	81.0	8.47	77.2	76.4
Hilo	11.8	-0.1	9.8	15.1	20.0	11.1	0.3	10.6
PC	14.6	13.5	27.5	43.3	36.4	13.8	13.3	27.9

M1 = measured IVBA As using OSU IVG
M2 = measured IVBA using PBET, pH 1.5
M3 = measured IVBA using OSU IVG
P1 = predicted using IVBA As (%) = 30.7 – 22.6 log Feox + 12.3 pH (Whitacre, 2008)
P2 = predicted using IVBA As (%) = 88.2 - 33.2 log FeCBD + 12.2 pH (Whitacre, 2008)
P3 = predicted using IVBA As (%) = 11.3 pH – 30.5 log Fe (Yang et al. (2002)
P4 = predicted using IVBA As (%) = 36.4 – 23.4 log Feox + 12.0 pH (Whitacre, 2008)
P5 = predicted using IVBA As (%) = 95.7 -34.3 log FeCBD + 11.9 pH (Whitacre, 2008)

The predicted IVBA As by Whitacre using the OSU IVG method using CBD Fe were very close to the IVBA predicted from the equation reported by Yang et al. (2002) using the PBET pH 1.5 method. In general, predicted IVBA were similar between predictive equations using Feox or CBD Fe.

Soil properties of the Deseret soil were not predictive of the measured IVBA As. However, predicted and measured IVBA for the Concord, Hilo and PC were in good agreement. Soils with a wide range of properties were spiked with As in Yang et al. (2002) and in Whitacre (2009). Soluble As in the spike solution reacted with soil clays and other components that sorb the arsenic. Arsenical pesticide was the contaminant source in the Concord, Hilo and PC soils. Solid phase arsenic speciation (Table E-4) showed As was associated with Fe and possibly Al oxides for these 3 soils. Soluble arsenical pesticide likely reacted (i.e., sorbed, precipitated) to soil reactive components similar to soluble As spike solution.

However, the arsenic source was mining waste in the Deseret soil. Unlike the previous 3 soils, solid phase arsenic speciation (Table E-4) showed As associated with Fe was highly variable. This finding suggests arsenic may occur as discrete minerals from the mining operation. It is likely the insoluble As minerals in the mining waste did not appreciably dissolve and react with soil components. Therefore, its chemical speciation and IVBA solubility will depend on the mining waste mineral not soil property.

It is very possible that the poor prediction of soil properties to predict IVBA As in the Deseret soil may be due to different arsenic speciation in this mining contaminant soil than the spiked soils. Further research on the effect of contaminant speciation of different contaminant sources on the ability of various in vitro methods to predict RBA As is needed and is underway. Further validation studies of these methods for other contaminated soils from different contaminant sources are warranted.

The root square mean error (RSME) was used to evaluate the ability of each soil property driven model to predict its respective IVBA (Table E-5) .

Table E-4. Metal(loid) contaminant speciation of Study Soils

Site Name	Site Location	Suspected contaminant source(s)	Metal-(loid)	Contaminant Speciation
MCAS Cherry Point	Cherry Point, NC	incinerator	Cr Pb	Not chromite; associated with Mn, Bi Non-crystalline β-PbO
Concord Naval Weapons	Concord, CA	pesticide use	As	Assoc. with Fe, Mn oxides Not consistent with discrete mineral phases (e.g., scorodite, schultenite, arseniosiderite)
Deseret Chemical Depot	Tooele, UT	alluvial mine tailings	As	Highly variable assoc. with Fe
Hill AFB	Ogden, UT	Water treatment sludge drying bed	As Cd Cr	Nothing reported?
Sugar Cane Farm	Hilo, HI	pesticide use	As	Assoc. with Fe and Al oxides
McClellan AFB	Sacramento, CA	Wastewater treatment lagoon	Cr Pb	Not chromite; heterogeneous distribution associated with Mn, Fe and organic matter
NSA	Mechanicsburg, PA	lead ingot storage area	Pb	Non-crystalline β-PbO; similar to Travis Elemental Pb Not associated with Fe, Mn oxides
ORNL Firing Range	Oak Ridge, TN	small arms fire	Pb	Adsorbed to Fe, Mn oxides Similar to McClellan
Pearl Harbor Fuel Depot	Pearl City, HI	pesticides	As Pb	Both As(III) and As(V), predominantely As(III); Arsenite sorbed to ferrihyrite
Portsmouth Naval Shipyard	Kittery, ME	Lead battery cells	Pb	Inconsistent assoc. with Fe
Travis AFB	Fairfield, CA	small arms fire	Pb	Associated with Fe, Cr, Mn Poorly crystalline Pb oxides

Table E-5. Comparison of root square mean errors (RSME) for predictive soil property models.

	RSME, %				
	Gastric			Gastrointestinal	
	PBET	OSU IVG		OSU IVG	
	CBD Fe	Feox	CBD Fe	Feox	CBD Fe
All soils	36.8	35.2	35.1	35.4	44.5
All soils excluding Deseret	3.50	3.42	3.42	3.44	3.85

RSME > 25% showed poor agreement between measured and predicted values for all prediction models. However, very low RSME values of less than 4% showed excellent agreement between measured and predicted values was found for all models when the Deseret soil was excluded from the statistical analysis. This clearly shows the ability of soil properties to predictive IVBA As is contaminant source dependent. Good prediction was achieved for the non-mining arsenic source Concord, Hilo, and PC soils. However, soil property models were not able to predict IVBA As in the mining arsenic source Deseret soil.

Chromium

Stewart et al. (2003) reported the following relationships between in vitro bioaccessible Cr (IVBA Cr) and soil properties.

IVBA Cr (%) = 16.02 + (0.426 x %clay) - (9.56 x % TIC)

IVBA Cr (%) = 15.54 + (0.4908 x % clay) - (3.78 x %TOC)

where TIC is the soil inorganic carbon content and TOC is the soil organic C content.

Use of above regression equations allow prediction of IVBA from soil properties. Comparison of measured and calculated IVBA Cr using soil property predictive models of Stewart et al. (2003) are summarized in Table E-6.

Table E-6. Comparison of measured and predicted IVBA Cr (%) for study soils with Cr contamination.

	IVBA Cr, %		
Soil	M1	P1	P2
Cherry Pt	24.7	2.0	-3.6
Hill	9.8	10.7	16.0
McClellan	10.1	26.3	10.1

M1 = measured IVBA using PBET, pH 1.5
P1 = predicted using IVBA Cr (%) = 16.02 + (0.426 x %clay) - (9.56 x % TIC)
P2 = predicted using IVBA Cr (%) = 15.54 + (0.4908 x % clay) - (3.78 x %TOC)

Good agreement between the measured IVBA Cr and predicted IVBA Cr by the P2 model were found for Hill and McClellan soils. Poor agreement between the measured IVBA Cr and IVBA Cr predicted by P1 and P2 models was found for the Cherry Point soil. Differences in Cr chemical speciation in soil may offer an explanation. Water or wastewater treatment was the contaminant source for the Hill and McClellan soils. Incinerator ash was the contaminant source for the Cherry Point soil.

The root square mean error (RSME) was used to evaluate the ability of each soil property driven model to predict its respective IVBA (Table E-7).

Table E-7. Comparison of root square mean errors (RSME) for IVBA Cr predictive soil property models.

	RSME (%) for prediction models	
Soils	P1	P2
all	16.5	12.2
Hill and McClellan	17.6	7.9

RSME values of less than 20% showed agreement between measured and predicted values was found for all models. The RSME reduced to <10% for the P2 model when the Cherry Point soil was excluded from the statistical analysis. This suggests the ability of soil properties to predictive IVBA Cr is contaminant source dependent.

APPENDIX F

Soil Properties and Metal(loid) Contaminant Concentrations

Soils from 12 study sites were collected. Contaminated and allegedly uncontaminated reference soil was collected at each study site (Table F-1). Soil samples from both contaminated and reference materials, ranging from 60 kg to 240 kg, were sent to The Ohio State University for processing and homogenization (see SOP in Materials and Methods). Two size fractions, < 250 um and < 2 mm, of each soil were prepared at OSU and shipped to research team members. The < 2 mm size fraction, defined as whole soil, was used for ecological plant and earthworm bioassay studies and < 250 um size fraction was used for human risk in vivo swine dosing and in vitro gastrointestinal extraction studies. Soil properties, important to plant and earthworm bioassays and contaminant bioavailability, were determined for both < 2 mm and < 250 um fractions. Elemental content of major soil constituents and/or soil metal(loid) contaminants were determined for both soil size fractions. Total contaminant concentration in < 2 mm soil (Table F-2) and < 250 um (Table F-3) are summarized as follows.

Total contaminant concentration in < 2 mm soil (Table F-2) and < 250 um (Table F-3) found showed 4 soils contaminated with As, 3 soils contaminated with Cd, 3 soils contaminated with Cr, 8 soils contaminated with Pb, and 6 soils contaminated with Zn (Table F-4). In some soils, large differences were found between contaminant concentration in < 2 mm and < 250 um fractions. These differences underscore the importance of using contaminant concentration data from different size fractions when performing human or ecological risk assessments. In this case, contaminant concentration data for the < 2 mm fraction was used for ecological assessment evaluated with plants and earthworms. Contaminant concentration data for the < 250 um fraction was used for human risk assessment evaluated through the soil ingestion pathway.

Higher concentrations in the < 250 um fraction is consistent with reaction between the soil surfaces and dissolved metal contaminant. The finer < 250 um fraction has greater specific surface area (surface area/weight) than the < 2 mm fraction. The greater surface area in the < 250 um vs. < 2 mm soil will adsorb greater amounts of metal contaminant and result in higher contaminant concentration in the finer < 250 um fraction. Soil fractions with different surface areas but similar metal contaminant concentrations suggests the metal contaminant may not have reacted with soil surfaces and occurs as discrete soil mineral precipitates or as chemically unweathered contaminant.

Table F-1. Site names, location and contaminant sources.

Site Name	Site Location	Suspected Contaminant(s)	Suspected contaminant source(s)	Soil Type
MCAS Cherry Point	Cherry Point, NC	Cr, Pb	incinerator	Entisol
Concord Naval Weapons	Concord, CA	As	pesticide use	Vertisol
Deseret Chemical Depot	Tooele, UT	As	alluvial mine tailings	Aridisol
Hill AFB	Ogden, UT	As, Cd, Cr, Pb	Water treatment sludge drying bed	Entisol
Sugar Cane Farm	Hilo, HI	As	pesticide use	Andisol
McClellan AFB	Sacramento, CA	Cd, Cr, Pb	Wastewater treatment lagoon	Alfisol
NSA	Mechanicsburg, PA	Pb	lead ingot storage area	Ultisol
ORNL Firing Range	Oak Ridge, TN	Pb	small arms fire	Ultisol
Fuel Depot	Pearl City, HI	As, Pb	pesticides	Mollisol
Portsmouth Naval Shipyard	Kittery, ME	Pb	Lead battery cells	Inceptisol
Travis AFB	Fairfield, CA	Pb	small arms fire	Alfisol
Naval Base Point Loma	San Diego, CA	As, Pb	??	??

Table F-2. Total Elemental Content† of contaminated soil (C) and reference (i.e. uncontaminated) soil (R) for the study sites. All soils are < 2 mm fraction.

		Study Site										
		Cherry Pt		Concord		Deseret		Hill‡	Hilo		McCllelan	
	units	C	R	C	R	C	R	C	C	R	C	R
Al	g/kg	14.7	9.20	39.0	37.8	16.8	25.3	21.7	25.6	16.7	40.1	6.66
As	mg/kg	6.89	1.73	220	7.84	438	11.2	19.5	660	21.7	9.88	6.08
Ba	mg/kg	200	38.6	250	241	529	191	164	292	38.4	333	0.119
Cd	mg/kg	18.9	<1.0	<1.0	<1.0	<1.0	<1.0	43.8	5.90	1.29	21.9	0.652
Co	mg/kg	<1.0	<1.0	16.5	17.0	5.19	6.51	9.12	< 0.2	7.77	7.55	1.44
Cr	mg/kg	876	13.3	76.6	79.0	23.6	26.7	239	140	120.2	699	126
Cu	mg/kg	167	<1.0	54.3	50.1	12.6	15.2	81.8	224	69.2	241	0.360
Fe	g/kg	10.4	4.11	39.8	38.2	17.5	21.2	28.9	74.3	45.6	28.2	0.42
Mg	g/kg	633	823	10.4	10.4	8.56	10.4	12.9	22.1	26.3	3.66	<0.1
Mn	mg/kg	48.4	27.0	843	835	538	515	553	914	503	128	14.7
Mo	mg/kg	4.83	<1.0	<1.0	<1.0	1.13	<1.0	7.69	< 2	<2	2.26	12.0
Ni	mg/kg	77.6	3.49	91.5	97.9	16.3	16.9	40.0	417	561	87.0	59.9
P	mg/kg	6,482	197	606	390	740	550	784	4318	796	1068	25.2
Pb	mg/kg	114	16.7	22.4	15.8	18.6	19.5	51.1	2134	153	193	14.9
Se	mg/kg	2.59	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<2	<2	<1.0	1.71
Tl	mg/kg	<1.0	<1.0	<1.0	<1.0	15.7	<1.0	<1.0	<2	<2	<1.0	93.1
V	mg/kg	69.6	14.8	117	113	33.6	39.8	48.2	70.2	45.7	109	558
Zn	mg/kg	486	31.7	112	101	85.2	83.2	203	1889	282	448	32.0

† Acid Digestion (USEPA 3051a) followed by analysis using high resolution ICP OES.

‡ Hill AFB did not have a reference soil

Table F-2 (continued). Total Elemental Content† of contaminated soil (C) and reference (i.e. uncontaminated) soil (R) of the study sites. All soils are < 2 mm fraction.

	Units	Mechanicsburg		ORNL		Pearl City		Portsmouth		Travis		Point Loma§
		C	R	C	R	C	R	C	R	C	R	C
Al	g/kg	43.8	53.8	12.6	49.5	44.6	60.4	11.1	10.6	26.0	30.0	15.0
As	mg/kg	14.6	16.8	5.01	13.9	619	4.08	11.3	10.0	10.9	8.11	3.67
Ba	mg/kg	127	149	309	66.4	221	103	133	74.4	300	211	18.9
Cd	mg/kg	<1.0	<1.0	<1.0	<1.0	3.63	1.41	1.14	<1.0	<1.0	<1.0	<1.0
Co	mg/kg	11.1	15.7	<1.0	<1.0	<0.2	<0.2	6.30	37.9	9.13	9.14	<1.0
Cr	mg/kg	39.3	55.8	16.0	48.0	185	233	10.9	13.7	42.4	42.8	22.8
Cu	mg/kg	25.4	18.7	65.0	13.9	423	110	185	12.3	1477	18.9	10.6
Fe	g/kg	29.5	36.5	11.9	28.1	118	92.9	19.8	10.7	27.1	24.0	20.8
Mg	g/kg	13.0	6.32	711	3.45	7.21	7.78	1.90	1.48	33.3	3.22	4.26
Mn	mg/kg	651	1126	88.3	60.8	1384	701	231	163	513	534	244
Mo	mg/kg	1.63	<1.0	<1.0	<1.0	18.6	<2	2.85	<1.0	<1.0	<1.0	<1.0
Ni	mg/kg	28.8	35.5	4.22	14.7	196	182	61.5	8.42	28.5	22.9	6.83
P	mg/kg	362	443	75.1	94.6	1121	1001	436	392	289	207	233
Pb	mg/kg	120	32.9	966	12.2	1466	13.1	3069	47.9	2034	16.9	8.65
Se	mg/kg	<1.0	<1.0	<1.0	<1.0	<2.0	<2.0	<1.0	<1.0	<1.0	<1.0	<1.0
Tl	mg/kg	<1.0	<1.0	<1.0	<1.0	<2.0	<2.0	<1.0	<1.0	<1.0	<1.0	<1.0
V	mg/kg	58.2	76.7	28.6	75.7	197	205	37.0	16.3	69.3	73.8	58.0
Zn	mg/kg	97.8	96.8	30.1	85.2	1804	133	500	59.5	225	69.9	60.8

§ Point Loma soil was not contaminated.

Table F-3. Total Elemental Content† of contaminated soil of the study sites. All soils are < 250 um fraction.

		Study Site					
		Cherry Pt	Concord	Deseret	Hill	Hilo	McCllelan
Al	g/kg	23.3	38.8	14.1	28.6	32.4	39.9
As	mg/kg	11.4	222	521	21.8	904	10.4
Ba	mg/kg	308	254	602	214	468	285
Cd	mg/kg	82.3	<1	<1	66.0	7.06	22.1
Co	mg/kg	<1	<1	5.23	10.7	<0.2	7.75
Cr	mg/kg	1456	85.2	21.1	369	155	593
Cu	mg/kg	276	62.6	12.6	114	339	232
Fe	g/kg	17.3	39.9	17.3	30.9	70.0	28.9
Mg	g/kg	1.01	10.4	7.97	13.6	21.3	3.64
Mn	mg/kg	80.9	848	511	725	1025	126
Mo	mg/kg	8.16	<2	<2	11.9	<2	<2
Ni	mg/kg	125	93.1	16.3	51.2	300	76.1
P	mg/kg	10823	622	693	863	5528	856
Pb	mg/kg	189	23.9	18.7	77.7	3182	164
Se	mg/kg	4.10	<2	<2	2	<2	<2
Tl	mg/kg	<2	<2	18.1	<2	<2	<2
V	mg/kg	112	114	30.6	59.0	91.3	112
Zn	mg/kg	778	107	85.1	278	2525	445

† Acid Digestion (USEPA 3051a) followed by analysis using ICP OES.

Table F-3 (continued). Total Elemental Content† of contaminated soil of the study sites. All soils are < 250 um fraction.

		Mechanicsburg	ORNL	Pearl City	Portsmouth	Travis
Al	g/kg	41.8	14.0	59.8	14.6	32.4
As	mg/kg	13.0	4.69	464	16.6	11.8
Ba	mg/kg	133	395	240	132	358
Cd	mg/kg	<1	<1	3.46	1.43	<1
Co	mg/kg	9.46	<1	<0.2	8.26	9.47
Cr	mg/kg	35.8	16.5	215	55.2	48.3
Cu	mg/kg	26.6	79.0	399	256	250
Fe	g/kg	25.1	11.0	118	23.5	28.7
Mg	g/kg	12.1	0.77	6.06	2.43	3.61
Mn	mg/kg	447	66.2	1466	336	537
Mo	mg/kg	<2	<2	5.94	4.45	<2
Ni	mg/kg	27.6	5.00	240	116	29.9
P	mg/kg	334	75.3	1252	649	320
Pb	mg/kg	223	1127	1616	4113	2416
Se	mg/kg	<2	<2	<2	<2	<2
Tl	mg/kg	<2	5.34	<2	<2	<2
V	mg/kg	52.4	25.0	213	58.5	77.6
Zn	mg/kg	98.5	34.0	1559	757	117

.

Table F-4. Summary of metal(loid) contaminated study sites. Contaminant concentrations are in mg/kg in < 2 mm soil from study sites

		Ch Pt	Con-cord	Desert	Hill	Hilo	McCle	Mechanic	ORNL	Pearl City	Port	Travis
As	< 2 mm		220	438		660				619		
	<250 um		222	521		904				464		
Cd	< 2 mm	18.9			43.8		21.9					
	<250 um	82.3			66.0		22.1					
Cr	< 2 mm	876			239		699					
	<250 um	1456			369		593					
Pb	< 2 mm	114				2134	193	120	966	1466	3069	2034
	<250 um	189				3182	164	223	1127	1616	4113	2416
Zn	< 2 mm	486				1889	448			1804	500	225
	<250 um	778				2525	445			1559	757	117

Select soil properties, known to affect soil contaminant bioavailability and plant growth, were determined for both soil size fractions. Soil properties < 2 mm soil (Table F-5) and < 250 um (Table F-6) are summarized as follows. There was a wide range in properties for contaminated soils (Table F-7). The best attempt to obtain control soils with properties similar to contaminated soils was made. However, the source of the contamination did alter key soil properties (i.e., soil pH, reactive oxide content, carbon content) for some of the contaminated soils. In these cases, differences in key soil properties may alter contaminant bioavailability and affect plant dry matter production. The low soil pH (<5.0) of the ORNL and the McClellan soils will likely results in aluminum phytotoxicity and prevent plant bioassays from being conducted on these soils.

There was a wide range in properties of the < 250 um soil fraction used to assess human risk from soil ingestion (Table F-8). Summary statistics of properties of < 250 um soil are listed in Table F-9.

Table F-5. Select Soil Properties of contaminated soil (C) and reference (i.e. uncontaminated) soil (R). All soils are < 2 mm fraction.

		Cherry Pt		Concord		Deseret		Hill	Hilo		McCllelan	
	units	C	R	C	R	C	R	C	C	R	C	R
Soil pH, water		5.50	7.43	6.67	6.34	9.28	7.84	7.22	5.88	4.71	4.31	6.66
Soil pH, $CaCl_2$		5.01	6.96	6.15	5.89	7.49	6.91	7.08	5.74	4.73	4.32	6.08
EC	dS/m	0.892	0.353	0.111	0.189	0.544	0.480	0.989	0.820	1.53	0.276	0.119
Alox	mg/kg	6061	909	1522	1672	786	1207	1175	21344	5917	2175	487
Feox	mg/kg	7506	797	3664	4519	863	681	956	25678	7535	4805	804
Mnox	mg/kg	32.2	<25	641	659	313	381	333	484	85.7	<25	125
Org C	%	3.71	0.758	3.13	2.17	0.645	0.792	1.50	7.77	5.69	4.36	0.360
Total C	%	4.54	1.94	3.04	2.13	2.32	1.52	2.66	8.44	5.50	4.66	0.42
CEC	$cmol_c/kg$	9.14	3.94	27.9	27.7	8.37	13.4	11.0	17.1	10.1	13.4	12.0
Sand	%	79.7	80.0	18.4	19.9	36.6	27.5	52.3	61.1	72.3	25.7	59.9
Silt	%	13.5	12.2	40.9	44.3	54.7	53.2	31.3	25.3	17.8	50.2	25.2
Clay	%	6.8	7.8	40.7	35.8	8.7	19.3	16.4	7.8	2.6	24.1	14.9

Soil pH (water): pH measured in 1:1 soil:deionized water suspension
Soil pH ($CaCl_2$): pH measured in 1:2 soil: 0.01 M $CaCl_2$ suspension
EC: electrical conductivity measured in 1:1 soil:deionized water suspension
Alox, Feox, Mnox: reactive oxide fraction measured using acid ammonium oxalate extraction

Table F-5 (continued).

		Mechanicsburg		ORNL		Pearl City		Portsmouth		Travis	
		C	R	C	R	C	R	C	R	C	**R**
Soil pH, water		8.04	7.46	4.1	3.81	7.34	7.65	6.2	6.2	7.04	6.02
Soil pH, $CaCl_2$		7.04	7.12	3.53	3.14	7.28	7.47	6.04	5.72	6.46	5.63
EC	dS/m	0.209	0.291	0.184	0.152	0.995	0.929	0.089	0.183	0.247	0.261
Alox	mg/kg	1615	2050	388	851	3502	2046	3764	4149	799	885
Feox	mg/kg	1407	2492	507	798	44900	1977	5758	2682	3088	4569
Mnox	mg/kg	290	944	27.4	<25	1014	492	124	70.1	405	547
Org C	%	0.640	1.22	0.326	0.222	2.34	0.29	1.64	1.44	1.09	1.32
Total C	%	4.49	1.43	0.38	0.17	3.33	2.01	2.57	1.72	1.22	1.39
CEC	$cmol_c/kg$	9.74	9.58	2.79	7.90	25.9	39.4	2.73	2.68	17.3	10.8
Sand	%	29.9	9.90	45.7	9.0	48.7	54.7	89.0	86.5	47.6	29.9
Silt	%	36.6	50.0	36.5	33.4	29.2	26.9	8.5	9.6	26.3	44.3
Clay	%	33.5	40.1	17.8	57.6	22.1	18.4	2.5	3.9	26.1	25.8

Soil pH (water): pH measured in 1:1 soil:deionized water suspension
Soil pH ($CaCl_2$): pH measured in 1:2 soil: 0.01 M $CaCl_2$ suspension
EC: electrical conductivity measured in 1:1 soil:deionized water suspension
Alox, Feox, Mnox: reactive oxide fraction measured using acid ammonium oxalate extraction

Table F-6. Select Properties of ESTCP Contaminated soils (C) and Reference (uncontaminated) soils (R). All soils are < 250 µm fraction.

		Cherry Pt		Concord		Deseret		Hill	Hilo		McCllelan	
	units	**C**	**R**	**C**	**R**	**C**	**R**	**C**	**C**	**R**	**C**	**R**
Alox	mg/kg	10897	988	1746	1765	747	1251	1548	28692	none	3415	650
Feox	mg/kg	13216	821	4207	4752	1037	763	1358	30671	none	6248	1482
Mnox	mg/kg	54.3	<25	634	621	293	224	413	635	none	<25	125
Org C	%	5.94	0.97	2.59	1.79	0.48	0.73	2.02	9.42	none	4.56	0.52
Total C	%	7.71	1.62	3.18	2.11	2.00	1.33	3.31	10.6	none	4.42	0.548
CBD Fe	mg/kg	10824	---	12749	---	6044	---	4530	29606	---	6030	---

Alox, Feox, Mnox: reactive oxide fraction measured using acid ammonium oxalate extraction

CBD Fe: citrate-bicarbonate-dithionite extractable Fe

Table F-6 (continued). Select Properties of ESTCP Contaminated soils (C) and Reference (uncontaminated) soils (R). All soils are < 250 µm fraction.

		Mechanicsburg		ORNL		Pearl City		Portsmouth		Travis	
		C	R	C	R	C	R	C	R	C	R
Alox	**mg/kg**	2182	2160	473	828	4155	none	5739	6481	1016	902
Feox	**mg/kg**	1993	2692	556	786	52796	none	9177	4291	3630	4697
Mnox	**mg/kg**	257	914	32.7	<25	1192	none	178	104	402	424
Org C	**%**	0.83	1.37	0.33	0.22	3.22	none	2.78	2.41	1.12	1.16
Total C	**%**	4.41	1.69	0.365	0.205	3.21	none	3.07	2.78	1.28	1.36
CBD Fe	**mg/kg**	16348	---	8715	---	31795	---	11992	---	11247	---

Alox, Feox, Mnox: reactive oxide fraction measured using acid ammonium oxalate extraction

CBD Fe: citrate-bicarbonate-dithionite extractable Fe

Table F-7. Summary Statistics of Soil Properties of contaminated soils. All soils are < 2 mm fraction.

Property	Units	Min	Max	Mean	Median	90th percentile
Soil pH, water		4.10	9.28	6.51	6.67	8.04
Soil pH, $CaCl_2$		3.53	7.49	6.10	6.15	7.28
EC	dS/m	0.089	0.995	0.487	0.276	0.989
Alox	mg/kg	388	21344	3921	1615	6061
Feox	mg/kg	507	44900	9012	3664	25678
Mnox	mg/kg	27.4	1014	366	323	678
Org C	%	0.326	7.77	2.47	1.64	4.36
Total C	%	0.38	8.44	3.42	3.04	4.66
CEC	$cmol_c/kg$	2.73	27.9	13.2	11.0	25.9
Sand	%	18.4	89.0	48.6	47.6	79.7
Silt	%	8.5	54.7	32.1	31.3	50.2
Clay	%	2.5	40.7	18.8	17.8	33.5

Table F-8. Select Soil Properties of contaminated soils. All soils are < 250 μm fraction

	units	Ch Pt	Concord	Deseret	Hill	Hilo	McCllelan
Alox	mg/kg	10897	1746	747	1548	28692	3415
Feox	mg/kg	13216	4207	1037	1358	30671	6248
Mnox	mg/kg	54.3	634	293	413	635	<25
Org C	%	5.94	2.59	0.48	2.02	9.42	4.56
Total C	%	7.71	3.18	2.00	3.31	10.6	4.42
CBD Fe	mg/kg	10824	12749	6044	4530	29606	6030

Alox, Feox, Mnox: reactive oxide fraction measured using acid ammonium oxalate extraction
CBD Fe: citrate-bicarbonate-dithionite extractable Fe.

Table F-8 (continued). Select Soil Properties of contaminated soils. All soils are < 250 μm fraction

		Mechanicsburg	ORNL	Pearl City	Portsmouth	Travis
Alox	mg/kg	2182	473	4155	5739	1016
Feox	mg/kg	1993	556	52796	9177	3630
Mnox	mg/kg	257	32.7	1192	178	402
Org C	%	0.83	0.33	3.22	2.78	1.12
Total C	%	4.41	0.365	3.21	3.07	1.28
CBD Fe	mg/kg	16348	8715	31795	11992	11247

Alox, Feox, Mnox: reactive oxide fraction measured using acid ammonium oxalate extraction
CBD Fe: citrate-bicarbonate-dithionite extractable Fe.

Table F-9. Summary Statistics of Soil Properties of contaminated soils. All soils are < 250 μm fraction

		Minimum	Maximum	Mean	Median	90th percentile
Alox	mg/kg	473	28692	5510	2182	10897
Feox	mg/kg	556	52796	11354	4207	30671
Mnox	mg/kg	32.7	1192	409	347	691
Org C	%	0.33	9.42	3.03	2.59	5.94
Total C	%	0.365	10.6	3.95	3.21	7.71
CBD Fe	mg/kg	4530	31795	13625	11247	29606