Exponent®

Final Technical Report: Development of Extraction Tests for Determining the Bioavailability of Metals in Soil

SERDP Project Number CU-1165

Final Technical Report: Development of Extraction Tests for Determining the Bioavailability of Metals in Soil

SERDP Project Number CU-1165

Prepared for

Strategic Environmental Research and
Development Program
Arlington, Virginia 22203

Prepared by

Yvette W. Lowney and Michael V. Ruby
Exponent
4940 Pearl East Circle, Suite 300
Boulder, Colorado 80301

June 2005

© Exponent

Doc. no. 8601191.002 0101 0405 R289

Contents

	Page
List of Figures	**v**
List of Tables	**vii**
Acronyms and Abbreviations	**viii**
Acknowledgments	**ix**
Executive Summary	**x**
Metals that Drive Remedial Decisions at DoD Sites	x
Relative Bioavailability of Metals – Human Receptors	xi
Relative Oral Bioavailability of Arsenic	xi
Relative Oral Bioavailability of Cadmium	xi
Percutaneous Absorption of Arsenic from Soils	xii
Percutaneous Absorption of Cadmium from Soils	xii
Relative Bioavailability of Metals – Wildlife Receptors	xiii
Avian Receptors	xiii
Mammalian Receptor	xiii
In Vitro Research	xiii
Human Exposure – Oral	xiv
Human Exposure – Dermal	xiv
Wildlife Exposure	xv
Wildlife Receptors	xv
1 Project Overview	**1**
1.1 Objective	1
1.2 Project Background	1
1.2.1 Purpose	1
1.2.2 New Guidance from U.S. Environmental Protection Agency (EPA)	2
1.3 Technical Approach	4
1.4 Organization of the Final Report	5
1.5 References	5

2 Soil Characterization and Geochemistry 6

3 Determining Metals that Drive Health-Based Remedial Decisions for Soils at U.S. Department of Defense Sites 7

3.1 Overview 7

3.2 Objectives 7

3.3 Methods 8

3.3.1 Database Construction 8

3.3.2 Use of Screening Criteria to Assess and Compare Risks 8

3.4 Results 9

3.4.1 What metals potentially drive risk-based remedial decisions at DoD facilities? 9

3.4.2 For facilities where more than one metal exceeds risk-based screening criteria, what are the metals of concern, and how do they compare in perceived importance? 9

3.4.3 For the metals that most often exceed the screening criteria, what is the receptor of greatest concern (human or ecological)? 9

3.4.4 Agency Staff Interviews 10

3.4.5 Overall Conclusions 10

3.5 References 11

4 Oral Bioavailability of Metals to Humans 12

4.1 Arsenic 12

4.1.1 Introduction 13

4.1.2 Materials and Methods 14

4.1.3 Results 15

4.1.4 Discussion 16

4.1.5 References 18

4.2 Cadmium 18

4.2.1 Background 18

4.2.2 Results 19

4.2.3 Discussion 21

4.2.4 References 22

5 Dermal Absorption of Metals by Humans 24

5.1 Arsenic 24

5.1.1 *In Vivo* Model for Percutaneous Absorption 25

5.1.2 Study Design 26

5.1.3 Results 27
5.1.4 Discussion 27
5.1.5 References 28

5.2 Cadmium 29

6 Bioavailability of Metals to Wildlife 30

6.1 Relative Oral Bioavailability of Metals in Soil to the American Robin 30
6.1.1 Objectives 30
6.1.2 Methods 30
6.1.3 Results 33
6.1.4 Overall Conclusions 34

6.2 Least Shrew Bioavailability Research 35
6.2.1 Introduction 35
6.2.2 Study Design 35
6.2.3 Results 37
6.2.4 Conclusions 39
6.2.5 References 40

7 Summary of *In Vitro* Research 42

7.1 Human Exposure – Oral 42
7.1.1 Arsenic 42
7.1.2 Cadmium 46

7.2 Human Exposure – Dermal 48
7.2.1 Arsenic 48

7.3 Wildlife Exposure 49
7.3.1 Arsenic 49
7.3.2 Cadmium 50
7.3.3 Chromium 50
7.3.4 Lead 50
7.3.5 Summary of *In Vitro* Testing for Wildlife Receptors 51

7.4 References 51

Supplemental Materials for Section 3
Supplemental Materials for Section 4
Supplemental Materials for Section 5
Supplemental Materials for Section 6
Supplemental Materials for Section 7

List of Figures

Figures and tables are inserted at the end of each corresponding section.

Figure 3-1. Indicates that lead, arsenic, chromium, cadmium, and antimony most commonly exceed residential and industrial human health screening criteria

Figure 3-2. Suggests that lead, zinc, mercury, chromium, selenium, and cadmium most commonly exceed avian and mammalian ecological screening criteria

Figure 5-1. Urinary arsenic mass excretion (corrected) in 24-hour increments

Figure 6-1. Cadmium concentration in avian tissue versus dose (individual bird data plotted)

Figure 6-2. Lead concentration in avian tissue versus dose (individual bird data plotted)

Figure 6-3. Chromium concentration in avian tissue versus dose (individual bird data plotted)

Figure 6-4. Dose-response for lead in all four test soils dosed to shrew

Figure 6-5. Dose-response graph for arsenic in the DoD-DP soil

Figure 6-6. Dose-response graph for arsenic in the Orchard soil

Figure 6-7. Dose-response graph for cadmium in the DoD-DP soil

Figure 6-8. Dose-response graph for chormium in the DoD-DP soil

Figure 6-9. Dose-response for chromium in the Smelter soil

Figure 7-1. IV:IVT correlation for relative bioavailability of arsenic in cynomolgus monkeys: SBRC method at pH 1.5 and 2.5

Figure 7-2. IV:IVT correlation for relative bioavailability of arsenic in cynomolgus monkeys: SBRC method at pH 1.5 and 2.5 without soil samples CDV and CAMT

Figure 7-3. IV:IVT correlation of relative bioavailability of arsenic in cynomolgus monkeys: SBRC + 500 mg/L PO_4

Figure 7-4. IV:IVT correlation of relative oral bioavailability of arsenic in cynomolgus monkeys: 0.4 M KH_2PO_4 in place of glycine buffer

Figure 7-5. IV:IVT correlation for relative oral bioavailability of arsenic in cynomolgus monkeys: Highest bioaccessibility of pH 2.5 SBRC method or 0.4 M KH_2PO_4

Figure 7-6. Regression of predicted vs. measured oral arsenic RBAs

Figure 7-7. IV:IVT correlation for relative bioavailability of cadmium in swine: SBRC method at pH 1.5 and 2.5

Figure 7-8. IV:IVT correlation for relative bioavailability of arsenic in shrews: SBRC method at pH 1.5, 2.5, 3.5, and 4.5

Figure 7-9. IV:IVT correlation for relative bioavailability of cadmium in shrews: SBRC method at ph 1.5, 2.5, 3.5, and 4.5

Figure 7-10. IV:IVT correlation for relative bioavailability of lead in shrews: SBRC method at ph 1.5, 2.5, 3.5, and 4.5 without soil sample DoD-PM

List of Tables

Figures and tables are inserted at the end of each corresponding section.

Table 2-1. Summary of soils used in development of extraction tests for determining the bioavailability of metals in soil

Table 2-2. SERDP — arsenic in vivo study substrates

Table 2-3. SERDP — cadmium in vivo study substrates

Table 2-4. SERDP — arsenic dermal study substrates

Table 2-5. SERDP — wildlife study substrates

Table 4-1. Soil arsenic mineralogy data (frequency % / arsenic mass distribution %)

Table 4-2. Relative oral bioavailability of arsenic from 10 soil samples

Table 4-3. Arsenic recovery and RBA from a spiked soil sample

Table 4-4. Urinary and fecal recovery of arsenic after a gavage dose of sodium arsenate

Table 4-5. Cadmium relative bioavailability estimates

Table 5-1. Summary of applied arsenic doses and percent absorption for dermal absorption studies

Table 6-1. Characterization data for soils dosed to shrews

Table 6-2. Shrew mortality rates

Table 6-3. Average doses (μg/kg-day) received by each shrew dose group

Table 6-4. Shrew body-weight changes

Table 6-5. Summary of relative bioavailability estimates for shrews

Table 7-1. *In vitro* arsenic extraction of SERDP soil research substrates

Table 7-2. Impact of phosphate buffering on *in vitro* bioaccessibility of arsenic from soils

Table 7-3. Extraction of arsenic from soils using human sweat

Acronyms and Abbreviations

AUC	area under the curve
CBR	critical body residue
CCA	chromated copper arsenate
CEC	cation exchange capacity
CERCLIS	Comprehensive Environmental Response, Compensation and Liability Information System
COV	coefficient of variance
Cr(III)	trivalent chromium
Cr(VI)	hexavalent chromium
DCB	dithionite citrate bicarbonate
DoD	Department of Defense
EAE	environmentally acceptable endpoint
eco-SSLs	Ecological Soil Screening Level
EPA	U.S. Environmental Protection Agency
FFRRO	Federal Facilities Restoration and Reuse Office
GFAAS	graphite furnace atomic absorption spectrometry
ICP/MS	inductively coupled plasma/mass spectroscopy
ICP-AES	inductively coupled plasma–atomic emission spectrometry
IV:IVT	*in vivo* to *in vitro*
NOAEL	no-observed-adverse-effect level
PBET	physiologically based extraction test
RBA	relative bioavailability adjustment
SBRC	Solubility/Bioavailability Research Consortium
SD	standard deviation
SERDP	Strategic Environmental Research and Development Program
TIC	total inorganic carbon
TOC	total organic carbon
UCSF	University of California–San Francisco
WER	water effect ratio

Acknowledgments

Exponent acknowledges the many researchers who contributed to this effort over the course of the three-year program. At the risk of unintentionally omitting valued contributors, we thank the following persons, and others in their labs, who collaborated with Exponent staff on research design and conduct, and review of the results:

Mark Barnett, Auburn University
Jeff Borchardt, Genesis Labs
Susan Brauning, Battelle Columbus Laboratories
Technical staff at Columbia Analytical Services
Eric Crecelius, Battelle Northwest Laboratories
Ron Wester, University of California, San Francisco
Stan Casteel, University of Missouri
Nissar Darmani, Western University of Health Sciences
Rob Pastorok, Integral Consulting
Stephen Roberts, University of Florida
Rosalind Schoof, Integral Consulting

The authors thank the technical and support staff within Exponent, without whom the project would not have been possible. These staff members include (but are not limited to):

Brad Bessinger
Catherine Boykin-Sena
Bonnie Clipson
Colleen Cushing
Melanie Edwards
Wendy Hawkins
Rick Nelson
Larry Peterson
Linda Peterson
Jo Salatas
Linda Ziccardi

Private-sector co-funding for this research was provided by FMC Corporation and Georgia-Pacific.

Executive Summary

The research conducted under this project is designed to yield a database that establishes whether site- or soil-specific factors affect the bioavailability of target metals from soils. Where the database (from *in vivo* research) identifies that these types of factors are operating, an additional goal of the research has been to develop simple extraction tests that are inexpensive to perform and that are predictive of metals bioavailability from soil. These tools can then be available to U.S. Department of Defense (DoD) personnel for site-specific evaluation of metals bioavailability from soil at field sites and will result in more accurate exposure and risk estimates that are still protective of human health and the environment.

Exposure pathways and receptors of concern were established to target the *in vivo* research, and focused on three distinct areas: oral bioavailability to humans, dermal bioavailability to humans, and oral bioavailability to wildlife. The project was designed in this manner, because each of these receptor/pathway combinations requires a different approach, due to the differences in the mechanism of absorption and/or differences in risk assessment methods.

The research was undertaken in three phases. First, research was conducted to understand which metals are risk drivers at DoD sites. Second, *in vivo* testing was conducted on soils to understand whether there are site- or soil-specific parameters that control absorption, and if so, to generate a database of information upon which to base development and validation of *in vitro* approaches to assessing bioavailability. The third phase, conducted once the *in vivo* data were compiled, involved determining whether *in vitro* methods for approximating bioavailability can be supported.

Metals that Drive Remedial Decisions at DoD Sites

Prior to initiating animal research, it was necessary to determine which particular metals should be evaluated. This research was structured to answer the following specific questions:

What metals potentially drive risk-based remedial decisions at DoD facilities?

For facilities where more than one metal exceeds risk-based screening criteria, what are the metals of concern, and how do they compare in perceived importance?

For the metals that most often exceed the screening criteria, what is the receptor of greatest concern (human or ecological)?

Screening criteria for ecological receptors (mammalian and avian) were exceeded at more sites than those for human receptors (residential and industrial). The results could be interpreted to indicate that ecological receptors are at greater risk from metals present in soil at DoD sites than are humans, but these results more likely reflect the conservative nature and uncertainty associated with the ecological screening criteria.

Based on this research, lead was the most frequent soil contaminant associated with DoD sites that exceeded screening criteria, for both human health and ecological scenarios. Other metals that have been determined to be of concern for human health include arsenic, chromium, cadmium, and antimony. The most frequent metals of concern based on the ecological screening criteria were lead, zinc, mercury, chromium, and selenium for birds, and arsenic for mammals.

Relative Bioavailability of Metals – Human Receptors

For human receptors, three major areas of investigation were pursued: relative oral bioavailability of arsenic, relative oral bioavailability of cadmium, and percutaneous absorption of arsenic from soil. The primary findings from each aspect of research are summarized below.

Relative Oral Bioavailability of Arsenic

Samples of arsenic-contaminated soil were obtained from 10 different sites. The mean RBA values for the 10 soil samples varied from 5% to 31%. Because the RBA values for the various soil samples tested were all relatively low, an additional experiment was conducted to verify that this monkey model is capable of measuring high oral bioavailability. For this experiment, a low-arsenic-content soil was spiked with sodium arsenate. Urinary recovery of arsenic from the spiked soil high, indicating that this research model is capable of demonstrating high RBA values for soluble forms of arsenic in soil.

The presence of arsenic in insoluble mineralogic forms is likely a factor in controlling the RBA. It should be noted that this *in vivo* research continues under funding from an industrial source, and four more arsenic-bearing soils are slated to be tested for arsenic bioavailability in the cynomolgus monkey model. These new data will be added to the existing database, and a manuscript discussing all findings will be prepared during the first half of 2005.

Relative Oral Bioavailability of Cadmium

A juvenile swine model was used to assess the relative oral bioavailability of cadmium in soil from four sites with varying soil characteristics. The reference material was soluble cadmium chloride, administered at doses of 10, 60, or 320 μg Cd/kg day. Concentrations of cadmium in blood, liver, and kidney were evaluated to determine the bioavailability of cadmium from soils relative to the reference material. Results indicate that soil-specific factors control the relative bioavailability of cadmium. In order to understand the factors controlling the bioavailability of cadmium, each test sample was evaluated for soil chemistry, including cadmium mineralogy.

In this research, the three soils with the greatest cadmium concentrations demonstrated modest reductions in bioavailability relative to cadmium chloride. In contrast, the fourth soil yielded a considerably lower cadmium RBA of 0.18. An examination of soil characteristics and cadmium mineralogy suggests that this outcome may be due to the more basic soil pH and high clay content of this soil, and the occurrence of a cadmium form not found in the other soils. This study provides further evidence of the value of the juvenile swine model in assessing the relative

bioavailability of soil cadmium, and reinforces the importance of including soil characterization and mineralogical analyses in these studies. The three soils with similar chemical and physical characteristics yielded similar kidney RBA values, ranging from 0.60 to 0.89. In contrast, the alkaline soil with a cadmium sulfate phase had a much lower RBA, despite having the smallest mean particle size. This finding suggests that the solubility of the predominant cadmium phases may be a more significant factor in controlling relative bioavailability than is particle size.

Percutaneous Absorption of Arsenic from Soils

Female Rhesus monkeys were selected for the research on percutaneous absorption of arsenic because of their ability to duplicate the biodynamics of percutaneous absorption in humans, and because previous studies of percutaneous arsenic absorption have used this same model. As with humans, significant exposure to arsenic occurs from the normal diet of monkeys. Therefore, a significant component of the method development work included identifying a mechanism for ensuring a low-arsenic diet so that the "signal" from dermally-absorbed arsenic could be detected above pre-dosing levels. Topical doses of arsenic in soil from two sites, and a reference dose of soluble arsenic in solution, were applied to each monkey. During interim review of preliminary results, reviewers expressed an interest in understanding whether hydration levels controlled absorption of arsenic. Therefore, study design was altered to re-investigate the same two soils, while adding water to the material on the skin surface.

For the soluble dose, calculated absorption rates averaged 2.9% for the group. These results are consistent with earlier research that utilized a radioactive marker, and indicated that the research model was effective at detecting dermally-absorbed arsenic without radiolabel. Converse to the results for soluble arsenic, data from dermal application of arsenic in soils indicate virtually no absorption, irrespective of hydration level.

Percutaneous Absorption of Cadmium from Soils

Dermal absorption of cadmium in soil was studied in human cadaver skin at the Dermatology Department at the University of California–San Francisco (UCSF).Following a considerable amount of pilot work conducted with UCSF, it became apparent that limitations of the research design would not permit generation of meaningful data on the dermal absorption rate for cadmium in soil. This occurred because of the amount and variability of background concentrations of cadmium in the cadaver skin, and candidate receptor fluids that are used in the test method. As a result, the values that this test system provided for dermally absorbed cadmium from soil are well above values that would be meaningful, given what is already known about absorption of soluble forms of cadmium (i.e., the method is not adequately sensitive). Given these preliminary results, Exponent conferred with Battelle and SERDP, and all parties agreed that discontinuation of the dermal absorption research on cadmium would be appropriate.

Relative Bioavailability of Metals – Wildlife Receptors

Only limited research has been conducted on the bioavailability of metals from soil to wildlife. Given this lack of information, ecological risk assessments generally assume that metals in soil are equally bioavailable as in the critical toxicity study, potentially resulting in overestimates of risk. Research was undertaken for SERDP to begin to address this data gap.

Avian Receptors

Exponent designed a research program to evaluate the bioavailability of metals in birds exposed to soil via the oral pathway. The first portion of this research involved selecting an appropriate receptor species to study. The American robin was determined to be an appropriate species for evaluating metals bioavailability. Surrogate avian receptors were determined to be inappropriate test species because of dietary or physiological differences from the target species.

The results from the avian research did not provide meaningful information due to highly variable food ingestion by the birds, and the corresponding variability in metal doses administered. Although the data that emerged from this component of the SERDP research indicate that there may be dose-related increases in body burdens of the dosed metals, the variability in food consumption among the birds precludes any meaningful interpretation regarding the relative bioavailability of metals from soil.

Mammalian Receptor

Small mammals such as shrews are among the wildlife receptors for which ecological risk assessment models consistently indicate the greatest level of potential exposure to metals in soil. The research conducted under the SERDP project involved the development of a novel animal model for assessing the relative bioavailability of metals from soil using the least shrew.

Results indicate that the relative bioavailability of arsenic, cadmium, and lead ranged from 7% to 49%, 13% to 81%, and 21% to 60%, respectively. Cr(III) was not absorbed from soil, even at very high doses, and Cr(VI) was absorbed to a slight extent from a soil that was spiked with a high concentration of Cr(VI). Based on the study results, it is clear that arsenic, cadmium, and lead are absorbed to varying extents from different soils in this shrew model, and that site-specific (or soil-specific) factors affect the relative absorption of the metals.

In Vitro Research

In order to evaluate the potential for development of an *in vitro* method for each receptor/pathway combination investigated in the *in vivo* research component of this project, soils were tested *in vitro* under a variety of conditions, and the results were evaluated for correlation to the *in vivo* results. These results are discussed below, as the "*in vivo* to *in vitro*" (IV:IVT) correlations.

Human Exposure – Oral

Arsenic

Research conducted prior to this study suggests that the extent of arsenic dissolution during an acidic gastric-like extraction is predictive of RBAs in the juvenile swine model. Our findings, however, were that the gastric-type extraction method provided predictive information for the relative oral bioavailability of most, but not all samples tested in the monkey model. Modifications of the extraction protocol were undertaken, primarily focused on the addition of phosphate to the extraction system.

Based on these findings, a preliminary protocol has been developed that specifies parallel extraction of soil samples in a glycine-buffered system and a phosphate-buffered system. Using this approach, a good correlation (r2 = 0.745) between the in vivo and in vitro data can be achieved.

Additional soils are currently being tested for relative oral bioavailability in the cynomolgus monkey model. Although the funding for testing these samples is being provided by an alternative source, the results will be made available to augment the database developed for SERDP, and will be included in publication of the *in vivo* research results. Once *in vivo* testing of those soils has been completed, the IV:IVT correlation will be re-examined.

Cadmium

In addition to the four test soils evaluated in the swine research conducted for SERDP, a fifth soil that had been evaluated previously for cadmium bioavailability in the swine model at the University of Missouri was obtained and was also subjected to the *in vitro* testing.

Based on this previous research, the PBET developed by the SBRC was used as a starting point for evaluating cadmium bioaccessibility, and was run at pH values of 1.5 and 2.5. The major limitation to a strong IV:IVT correlation for cadmium in this study is the limited *in vivo* data set, and the fact that four of the five RBA values are clustered at high relative bioavailability values (78% to 94%; see Figure 7-7). Despite this, the *in vitro* test results at both pH 1.5 and 2.5 yielded strong, and equivalent, IV:IVT correlations (r^2 values of 0.94 and 0.95, respectively).

Human Exposure – Dermal

Arsenic

For assessing an in vitro system to approximate dermal absorption, the soils tested in monkeys were subjected to an extraction procedure using human sweat. The results from the sweat extraction indicate that the amount of arsenic extractable in human sweat is more than an order of magnitude higher than the average fraction of arsenic that is actually dermally absorbed. In addition to the two site soils, arsenic extraction in sweat was also evaluated for a Yolo County soil that was spiked with soluble arsenic. For this soil, the *in vivo* results indicate dermal absorption of 3.2% to 4.5% of the applied dose. In comparison, the sweat extraction of a

similarly prepared sample yielded a 72% extraction efficiency. This finding is consistent with the results for the site soils, in that the sweat extraction was more than an order of magnitude higher than the dermal absorption efficiency.

Possibly more important than providing the basis for evaluation of a predictive *in vitro* method, the results of the *in vivo* testing of dermal absorption of arsenic suggest that absorption from "field-derived" arsenic-containing soils does not result in urinary arsenic levels that are distinguishable from background, and establishes that dermal absorption of arsenic from environmental soils is significantly lower than the default assumption recommended by EPA (3%, based on testing of soluble arsenic freshly mixed with soil). These results suggest that percutaneous absorption of arsenic from environmental soils does not contribute significantly to total arsenic exposures, and can be appropriately excluded from exposure evaluations.

Wildlife Exposure

Oral bioavailability to the shrew of arsenic, cadmium, chromium, and lead from four test soils was studied. For the *in vitro* research, the PBET developed by the SBRC was used as a starting point for evaluating the bioaccessibility of target metals in soil, and was run at pH values of 1.5, 2.5, 3.5, and 4.5.

Wildlife Receptors

Arsenic

The shrew research yielded RBA values for arsenic for three soils, so the *in vivo* data set on which to base an IV:IVT correlation is a limited one. Comparison of the *in vivo* RBA results against the *in vitro* values at the four different pH values indicates that the SBRC *in vitro* test run at a pH value of 2.5 yields the best IV:IVT correlation ($r^2 = 0.83$). The slope of the pH 2.5 IV:IVT correlation of close to 0.7 indicates that this *in vitro* test slightly overestimates the shrew-based RBA values for arsenic at this pH value. The fact that the slope of the line for the IV:IVT correlation does not equal one does not mean that the model is not predictive, but rather only that the *in vitro* results cannot be used directly without adjustment to account for the slope of the line. These results indicate that, based on a limited *in vivo* data set, the SBRC *in vitro* test run at a pH of 2.5 is capable of predicting the shrew-based RBA values for arsenic in soil.

Cadmium

The shrew research yielded three RBA values for cadmium, so the *in vivo* data set on which to base an IV:IVT correlation is a limited one. Comparison of the *in vivo* RBA results against the *in vitro* values at the four different pH values indicates that the SBRC *in vitro* test run at a pH of 4.5 yields the best IV:IVT correlation ($r^2 = 0.998$), while the extraction at pH 3.5 also yielded a reasonable correlation ($r^2 = 0.88$). The slope of the pH 4.5 IV:IVT correlation of 3.2 indicates that the PBET underestimates the shrew-based RBA values for cadmium by about a factor of about three, and this must be accounted for in the use of *in vitro* data.

Based on this data set, the SBRC *in vitro* test run at a pH of 4.5 is recommended for estimating the oral bioavailability of cadmium in soil to shrew.

Chromium

The limited data set that is available suggests that there is little, if any, dose-response relation between the administered dose of chromium and the absorbed dose (or body burden) of chromium in the exposed animal. This suggests that some factor is regulating the uptake of chromium into the tissues, and that relative oral bioavailability may not be a concern for this metal in soil. No *in vitro* method was developed for this metal.

Lead

The shrew research yielded four RBA values for lead. Comparison of the *in vivo* RBA results against the *in vitro* values at the four different pH values indicates a lack of IV:IVT correlation when all four data points are included. This is odd because the SBRC *in vitro* test correlates strongly with oral lead bioavailability in the juvenile swine model at both pH 1.5 and 2.5 (r^2 values of 0.87 and 0.85, respectively, with 15 soils tested both *in vivo* and *in vitro*). Given the uncertainty associated with the *in vivo* data for one of the soils, this soil was eliminated from the IV:IVT analysis. When this change was made, the IV:IVT correlation improved for both pH 1.5 and 2.5 (r^2 values of 0.69 and 0.66, respectively). Thus, it appears that the SBRC *in vitro* test, which is highly effective for predicting the oral bioavailability of lead to juvenile swine, is also useful for predicting this endpoint in shrew. Based on these results, the SBRC *in vitro* test, run at a pH value of 2.5, is recommended for estimating the oral bioavailability of soil lead to shrew.

1 Project Overview

1.1 Objective

The primary objective of this research project is to develop a suite of simple and easy-to-use extraction tests to predict human and ecological exposures to metals in soil. These tests are designed to provide inexpensive and rapid tools for establishing the relative bioavailability of metals in soils at hazardous waste sites, on a site-specific basis. For the purpose of this research, "relative bioavailability" has been defined as the fraction of a metal absorbed into systemic circulation, relative to the absorption of soluble forms of the metal. Soils used in the project were characterized for metal species and soil parameters to provide a mechanistic basis for any differences in metals bioavailability among the samples. Therefore, results from the project will also provide an understanding of how various species of a metal may differ in bioavailability, and how various soil properties may affect metals bioavailability and the stability of the measured bioavailability estimates.

1.2 Project Background

1.2.1 Purpose

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

The research conducted under this project is designed to yield a database that establishes whether or not site- or soil-specific factors affect the bioavailability of target metals from soils. Where the database (from *in vivo* research) identifies that these types of factors are operating, then a secondary goal of the research has been to develop simple extraction tests that are inexpensive to perform, that produce reliable results, and that are predictive of metals bioavailability from soil. In the context of this research, the test is "predictive" if it can be used to estimate the bioavailability of a soil, as measured in the *in vivo* studies. These tools can then be available to DoD personnel for site-specific evaluation of metals bioavailability from soil at field sites and will result in more accurate exposure and risk estimates that are still protective of human health and the environment.

1.2.2 New Guidance from U.S. Environmental Protection Agency (EPA)

EPA's Framework for Inorganic Metals Risk Assessment (U.S. EPA 2004) discusses key issues and processes that differentiate risk assessments for inorganic metal compounds from those conducted for other chemicals. This document specifically acknowledges that one of the challenges in metals risk assessment is to measure the differences in bioavailability between the multiple forms taken by metals in the environment. The processes that affect metal speciation and the effects of speciation on metal bioavailability must be understood and quantified to better characterize risks to human and ecological receptors. EPA (2004) addresses the issue of bioavailability within the framework document and also in the "Issue Paper on the Bioavailability and Bioaccumulation of Metals" (McGreer et al. 2004). These documents identify the issues regarding bioavailability in the risk assessment context, and also summarize the current state of the science and current practices that are used to address bioavailability in human and ecological risk assessments. Recommendations for incorporating bioavailability into risk assessment practices and research needs are also identified.

The current practice under EPA guidance is to assume that the relative bioavailability of a chemical is equal in food, water, or soil, and that the bioavailability of the metal exposure on the site is the same as the bioavailability used to derive the toxicity value on which the risk estimate is based (McGreer et al. 2004). In some situations, site-specific adjustments are being made using data from *in vitro* juvenile swine studies—for lead and arsenic, for example. Also, in the aquatic environment, the water effect ratio (WER) procedure has been employed in making site-specific bioavailability adjustments to water quality criteria. The WER is derived by comparing toxicity measurements made in site water to those made in laboratory water, and the WER is then used to adjust the national criterion to reflect site-specific bioavailability. Mechanistic-based approaches for assessing metals bioavailability, such as the biotic ligand model, are also being developed and incorporated. The framework addresses rather extensively the methods and recommendations for addressing bioavailability in aquatic risk assessment. Terrestrial considerations for human and ecological risk assessment are summarized below.

The framework identifies the key parameters that affect metals bioavailability in soil as pH, soil sorptive properties, organic matter content, the content of iron and alumimun oxyhydroxides, and the soil clay mineral content. In terrestrial systems, understanding metals speciation is important in characterizing bioavailability and exposure to human and ecological receptors. The framework recommends a variety of analytical and chemical methods for characterizing metal speciation (e.g., x-ray absorption spectroscopy, x-ray diffraction, particle-induced x-ray emission). Models are also recommended for predicting speciation and metals transport. In addressing wildlife exposure specifically, incorporating information on critical body residues (CBRs) is recommended, because they account for site-specific bioavailability. The limitation to this approach, however, is the paucity of information for metal CBRs in terrestrial wildlife, with the exception of methyl mercury, lead, selenium, and cadmium. The notion of incorporating dietary bioavailability into food-chain models is also discussed. However, information is limited, particularly due to differences in digestive physiology and anatomy across the range of ecological receptors. Values derived for human health risk assessments, such as for lead, may be used for animal species that have digestive systems similar to humans. Uncertainties associated with species-to-species extrapolations would have to be described.

The following methods for addressing bioavailability in terrestrial risk assessments (human and/or ecological) are recommended in the framework (U.S. EPA 2004):

- Soil toxicity testing
- Plant bioassays
- Biomarkers
- Estimating relative bioavailability based on pH and organic matter content
- Plant and animal critical tissue residues (toxic thresholds)
- Terrestrial biotic ligand model (biotic ligand model, currently being developed)
- *In vitro* methods
- Adjusting toxicity data by the organic matter content of soils, or as a function of organic matter plus clay content.
- The following, specifically for human health risk assessment (McGreer et al. 2004):
  - Epidemiological or human subject studies to estimate the ingestion of soil and bioavailability of metals, such as arsenic via urine samples and lead via blood samples, with stable isotope dilution
  - Fecal measures
  - Tissue measures
  - Animal models.

The framework and the bioavailability issues paper both address future research needs in the area of addressing bioavailability in terrestrial risk assessments. Recommendations for future research include (U.S. EPA 2004 and McGreer et al. 2004):

- Develop extraction techniques that are useful for assessing bioavailability and/or metals mobility
- Develop laboratory tests for soil systems that better reflect the actual forms of metals encountered in the field
- Develop and validate empirical and mechanistic models that link soil physicochemical characteristics, metal speciation, and toxic effects and bioaccumulation in soil invertebrates (e.g., biotic ligand model for soil organisms)
- Develop and validate kinetics models that describe metal bioaccumulation in soil invertebrates

- Conduct basic research on the physiology of metal metabolism in various groups of soil invertebrates; evaluate the relevance of soil pore water or diet to exposure and partitioning of metals in soil invertebrates
- Identify risks to predators associated with the consumption of soil invertebrates that contain metals
- Develop metal-specific biomarkers that are capable of quantitatively detecting magnitude and species of metal exposure.

1.3 Technical Approach

The research was undertaken in three phases. First, research was conducted to understand which metals are risk drivers at DoD sites. Second, *in vivo* testing was conducted on soils to understand whether there are site- or soil-specific parameters that control absorption, and if so, to generate a database of information upon which to base development and validation of *in vitro* approaches to assessing bioavailability. The third phase, conducted once the *in vivo* data were compiled, involved determining whether *in vitro* methods for approximating bioavailability can be supported. The research conducted under all three phases of this effort is summarized in this Final Report. Because much original research was conducted, manuscripts presenting the research were prepared, where appropriate, and submitted to peer-reviewed publications. Copies of publications and manuscripts produced during the course of this research are included as supplements to the relevant report sections, because these Strategic Environmental Research and Development Program (SERDP)-funded work products provide additional details or perspectives on the research.

Exposure pathways and receptors of concern were established to target the *in vivo* research, and focused on three distinct areas: oral bioavailability to humans, dermal bioavailability to humans, and oral bioavailability to wildlife. The project was designed in this manner, because each of these receptor/pathway combinations requires a different approach, due to the differences in the mechanism of absorption and/or differences in risk assessment methods.

Research protocols were developed to study the relative bioavailability of metals from soils for each target receptor. For human health endpoints, the protocols focus on establishing the relative bioavailability of metals from soil using animal models that are the most predictive of humans. For the human receptor, the data provide an evaluation of the relative bioavailability of soil in appropriate, defined surrogates. These data allow for the development of relative bioavailability adjustments (RBAs) for these metals that could be applied in risk assessments. If these RBAs are highly variable, and appear to depend on site-specific considerations (e.g., soil or source characteristics), it would indicate a need for development of a simple *in vitro* extraction test that is predictive of metals bioavailability for each receptor.

For wildlife receptors, study protocols were developed that are specifically targeted at evaluating the bioavailability of metals from soils to sentinel receptors. Preliminary exposure modeling conducted to support the EPA Ecological Soil Screening Level (eco-SSL) (U.S. EPA 2000) effort indicated that the receptors that have the greatest potential for exposure to soil are

small mammals (short-tailed shrew and cottontail rabbit) and two avian species (American robin and woodcock). These receptors may receive soil exposure from either direct soil ingestion or consumption of earthworms. Therefore, this research was designed to use the least shrew (as a surrogate for the target species of short-tailed shrew) and the American robin to study the bioavailability of metals through these two pathways (i.e., direct soil ingestion, and consumption of earthworms). Research defined in the protocols has been ongoing during the past year, using metal-contaminated soils from selected DoD sites. This phase of the effort consists of full characterization of the soil, and testing for the relative bioavailability of metals from the selected site soils to the target receptors.

1.4 Organization of the Final Report

A description and detailed synopsis of each aspect of the research undertaken under this project are provided below. As mentioned above, formal reports, manuscripts, or published articles that were developed on the basis of this research are also attached, because they provide additional details and represent work products sponsored by SERDP.

The first section below presents results of soil characterization and geochemical analysis that were conducted on the soils included in this research. These results are presented here to provide a comprehensive compilation of the data generated by this project. However, discussion of these parameters as they influence the absorption of metals is included in the sections specific to the three receptor/pathway combinations, because the issues relevant to absorption can be specific to a particular metal and/or receptor.

The information following the soil characterization data is organized by the specific receptor/pathway combinations, similar to the scheme set forth in the original proposal and which directed the research needs. Specifically, preliminary research to understand what metals drive soil remediation decisions at DoD facilities is presented first. This is followed by research related to oral bioavailability in humans, dermal bioavailability in humans, and oral bioavailability in wildlife. Finally, information pertaining to the development of *in vitro* methods that provide predictors of *in vivo* findings is presented at the end.

1.5 References

McGreer, J., G. Henningson, R. Lanno, N. Fisher, K. Sappington, and J. Drexler. 2004. Issue paper on the bioavailability and bioaccumulation of metals. Available at http://cfpub.epa.gov/ncea/raf/recordisplay.cfm?deid=86119

U.S. EPA. 2000. Ecological soil screening level guidance, draft. U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response, Washington, DC.

U.S. EPA. 2004. Framework for inorganic metals risk assessment. Risk assessment forum. EPA/630/P-04/068B. Available at http://cfpub2.epa.gov/ncea/raf/recordisplay.cfm?deid=88903.

2 Soil Characterization and Geochemistry

The soils that formed the basis of all the research undertaken for this project are listed below in Table 2-1. This table lists all the soils used, along with an indication of which specific soils (and particle size fraction) were incorporated into the specific receptor/pathway research efforts. All of the soils used in the SERDP research were characterized for a consistent set of soil parameters, which include pH, total organic carbon (TOC), total inorganic carbon (TIC), cation exchange capacity (CEC), particle size distribution (i.e., sand, silt, clay), DCB (dithionite-citrate-bicarbonate)-extractable iron, and metals concentrations. Data collected are presented in Tables 2-2 through 2-5, arranged by target metal or study type. (Table 2-2 presents data on soils used for the oral/human arsenic research, Table 2-3 presents data on soils used for the oral/human cadmium research, Table 2-4 presents data on soils used for the dermal/human studies, and Table 2-5 presents the data on soils used for the oral/wildlife studies.) As results from the various metals bioavailability studies were finalized, the soil geochemistry data were used to establish whether specific soil parameters control or influence the bioavailability of particular metals to specific receptors.

Table 2-1. Summary of soils used in development of extraction tests for determining the bioavailability of metals in soil

			Study (pathway, metal, animal)				
Description	Abbreviation	Grain Size	Oral Arsenic Monkey	Oral Cadmium Swine	Dermal Arsenic Monkey	Oral Metals Robin	Oral Metals Shrew
Pt. Mugu soil	PTMG	<250 µm		X			
Naval Weapons Air Station, Point Mugu, CA	DoD-PM	<500 µm				X	X
CO smelter soil	COSCS	<250 µm	X	X			
Colorado smelter composite soil	Smelter	<500 µm					X
OK smelter soil	OK-SS	<250 µm		X			
Dugway soil	DPGC	<250 µm		X			
Mixture from Dugway Proving Grounds & Picatinny arsenal	DoD-DP	<500 µm					X
Washington orchard soil	WAOS	<250 µm	X				
Washington orchard soil	Orchard	<500 µm					X
Colorado residential soil	CORS	<150 µm			X		
Colorado residential soil	CORS	<250 µm	X				
Montana Smelter Soil	MTSS	<250 µm	X				
Florida cattle dip vat soil	FLCDV	<250 µm	X				
Western iron slag soil	WISS	<250 µm	X				
California mine tailings	CAMT	<250 µm	X				
New York orchard soil	NYOS	<250 µm	X				
Colorado smelter soil	COSS	<250 µm	X				
Florida chemical plant soil	FLCPS	<250 µm	X				
New York pesticide facility	A1B21	<150 µm			X		
New York pesticide facility[a]	T5E3	<250 µm	X[a]				
New York pesticide facility[a]	T15E4	<250 µm	X[a]				
New York pesticide facility[a]	A1B20	<250 µm	X[a]				

[a] Experiments are scheduled for these samples during the second half of 2005.

Table 2-2. SERDP — arsenic in vivo study substrates

Chemical	Units	Florida CCA Soil <250 µm 12/3/2002	Florida Cattle Dip Vat Soil <250 µm 12/3/2002	Florida Power Co. #1 Soil <250 µm 12/3/2002	Florida Pesticide #1 Soil <250 µm 12/3/2002	Florida Pesticide #1 Soil <250 µm 12/20/2004	Florida Pesticide #2 Soil <250 µm 12/3/2002	Florida Pesticide #2 Soil <250 µm 12/20/2004	California Mine Tailings <250 µm 2/12/2001	Montana Smelter Soil <250 µm 8/23/2002	Rodriguez #8[a] <250 µm 3/2/2004
Conventionals											
pH	s.u.	8.60	6.17	8.22	8.28	--	8.26	--	7.4	7.22	7.33
Total organic carbon	%	0.95	1.18	0.795	8.02	--	2.78	--	12.5	4.31	1.54
Total inorganic carbon	%	--	--	--	--	--	--	--	--	--	--
Total carbon	%	--	--	--	--	--	--	--	--	--	--
Cation exchange capacity	meq/100g	--	--	--	--	--	--	--	--	--	--
Total solids	%	--	--	--	--	--	--	--	--	--	--
DCB extractable iron	mg/kg	2,071 [f]	5,881 [f]	12,290 [f]	12,039 [f]	--	5,408 [f]	--	22,461 [f]	15,382 [f]	--
Particle Size Distribution											
Medium gravel (> 4,750 µm)	%	0	0	0	0	--	0	--	0	0	--
Fine gravel (2,000 – 4,750 µm)	%	0	0	0	0	--	0	--	0	0	--
Very coarse sand (850 – 2,000 µm)	%	0	0.01	0	0	--	0.07	--	0	0.06	--
Coarse sand (425 – 850 µm)	%	0.06	0.05	0.02	0.05	--	0.05	--	0.04	0.07	--
Medium sand (250 – 425 µm)	%	0.18	0.31	0.13	0.72	--	0.3	--	0.45	0.85	--
Fine sand (106 – 250 µm)	%	34.5	57.1	54.9	49.9	--	41.5	--	36.7	38.3	--
Very fine sand (75 – 106 µm)	%	28.0	16.5	13.4	10.8	--	9.33	--	14.3	11.2	--
Percent silt (4 – 75 µm)	%	34.4	24.2	27.4	32.9	--	39.5	--	42.7	43.5	--
Percent clay (<4 µm)	%	1.57	1.47	3.49	4.07	--	7.84	--	4.3	6.24	--
Other											
Chromium, hexavalent	mg/kg	--	--	--	--	--	--	--	--	--	--
Cyanide	mg/kg	--	--	--	--	--	--	--	--	--	--
Organic sulfur	%	--	--	--	--	--	--	--	--	--	--
Sulfate	%	--	--	--	--	--	--	--	--	--	--
Sulfides	%	--	--	--	--	--	--	--	--	--	--
Sulfur	%	--	--	--	--	--	--	--	--	--	--
Replicate Arsenic Analyses											
Replicate - 1	mg/kg	96.4	166	223	270	260	546	654	303	656	1,390
Replicate - 2	mg/kg	93.0	145	219	273	274 [k]	650	650 [k]	295	655	1,390
Replicate - 3	mg/kg	76.9	145	225	277	232	671	--	302	671	1,420
Replicate - 4	mg/kg	85.3	147	240	275	303	694	--	296	643	1,470
Replicate - 5	mg/kg	81.5	148	245	289	--	694	--	303	626	1,370
Replicate - 6	mg/kg	84.5	151	226	277	--	666	--	--	630	1,430
Average Arsenic Concentration:	**mg/kg**	**86.3 [d]**	**150 [d]**	**230 [d]**	**277 [d]**	**267 [d]**	**654 [d]**	**652 [d]**	**300 [d]**	**647 [d]**	**1,412 [d]**

Table 2-2. (cont.)

Chemical	Units	Florida CCA Soil <250 µm 12/3/2002	Florida Cattle Dip Vat Soil <250 µm 12/3/2002	Florida Power Co. #1 Soil <250 µm 12/3/2002	Florida Pesticide #1 Soil <250 µm 12/3/2002	Florida Pesticide #1 Soil <250 µm 12/20/2004	Florida Pesticide #2 Soil <250 µm 12/3/2002	Florida Pesticide #2 Soil <250 µm 12/20/2004	California Mine Tailings <250 µm 2/12/2001	Montana Smelter Soil <250 µm 8/23/2002	Rodriguez #8[a] <250 µm 3/2/2004
Other metals											
Aluminum	mg/kg	--	--	--	--	--	--	--	--	--	--
Antimony	mg/kg	10 *U*	10 *U*	10 *U*	11.8	--	10 *U*	--	10 *U*	18.7	95.4
Barium	mg/kg	--	--	--	--	--	--	--	--	--	--
Beryllium	mg/kg	1.0 *U*	1.0 *U*	1.0 *U*	1.0 *U*	--	1.0 *U*	--	1.0 *U*	1.0 *U*	1.0 *U*
Cadmium	mg/kg	1.0 *U*	1.0 *U*	7.5	11.1	--	4.1	--	1.8	10	126
Calcium	mg/kg	--	--	--	--	--	--	--	--	--	--
Chromium	mg/kg	75.8	8.0	16.0	32.1	--	86.8	--	41.6	17.9	30.1
Cobalt	mg/kg	--	--	--	--	--	--	--	--	--	--
Copper	mg/kg	18.9	6.5	51.9	133	--	267	--	61.6	2,190	2,720
Iron	mg/kg	--	--	--	--	--	--	--	--	--	--
Lead	mg/kg	24.6	20 *U*	77.5	388	--	81.2	--	35.8	435	6,810
Magnesium	mg/kg	--	--	--	--	--	--	--	--	--	--
Manganese	mg/kg	--	--	--	--	--	--	--	--	--	--
Mercury	mg/kg	0.03	0.02 *U*	0.29	0.22	--	1.33	--	0.51	0.53	6.32
Nickel	mg/kg	4.0 *U*	4.0 *U*	4.0 *U*	14.2	--	14.6	--	29.9	9.4	23.4
Phosphorus	mg/kg	--	--	--	--	--	--	--	--	--	--
Potassium	mg/kg	--	--	--	--	--	--	--	--	--	--
Selenium	mg/kg	1.0 *U*	1.0 *U*	1.0 *U*	1.0 *U*	--	1.0 *U*	--	1.0 *U*	2.0 *U*	9.2
Silver	mg/kg	2.0 *U*	2.0 *U*	2.0 *U*	2.0 *U*	--	2.0 *U*	--	2.0 *U*	16.5	36.1
Sodium	mg/kg	--	--	--	--	--	--	--	--	--	--
Thallium	mg/kg	1.0 *U*	1.0 *U*	1.0 *U*	1.0 *U*	--	1.0 *U*	--	1.0 *U*	2.0 *U*	10.0 *U*
Vanadium	mg/kg	--	--	--	--	--	--	--	--	--	--
Zinc	mg/kg	146	3.6	355	545	--	269	--	107	1,160	2,710

Table 2-2. (cont.)

Chemical	Units	Washington Orchard Soil <500 µm 12/3/2002	Washington Orchard Soil <500 µm 2/10/2004	Washington Orchard Soil <250 µm 8/23/2002	Washington Orchard Soil <180 µm 10/23/2002	New York Orchard Soil <250 µm 8/23/2002	Colorado Smelter Soil (Smeltertown) <500 µm 2/20/2003	Colorado Smelter Soil (Smeltertown) <250 µm 2/20/2003	VB/I-70 Soil[b] <2 mm 2/19/2004	VB/I-70 Soil[b] <250 µm 2/19/2004	VB/I-70 Soil[b] <150 µm 2/19/2004	VB/I-70 Soil[b] <150 µm 12/20/2004
Conventionals												
pH	s.u.	--	5.92	5.28	--	5.77	6.64	7.01 [d]	--	5.33	--	--
Total organic carbon	%	--	2.98	3.40	--	6.22	2.15	1.78 [d]	--	2.76	--	--
Total inorganic carbon	%	--	1.27	--	--	--	--	--	--	--	--	--
Total carbon	%	--	4.25	--	--	--	--	--	--	--	--	--
Cation exchange capacity	meq/100g	--	74.0	--	--	--	--	--	--	--	--	--
Total solids	%	98.2 [e]	--	--	--	--	--	--	--	--	--	--
DCB extractable iron	mg/kg	--	5,630 [g]	8,413 [f]	--	9,472 [f]	24,748 [f]	--	--	--	--	--
Particle Size Distribution												
Medium gravel (> 4,750 µm)	%	--	--	0	--	0	0	--	0	--	--	--
Fine gravel (2,000 – 4,750 µm)	%	--	--	0	--	0.08	0	--	2.55	--	--	--
Very coarse sand (850 – 2,000 µm)	%	--	--	0.06	--	0.17	0.01	--	4.88	--	--	--
Coarse sand (425 – 850 µm)	%	--	--	0.835	--	0.33	5.91	--	11.7	--	--	--
Medium sand (250 – 425 µm)	%	--	--	4.55	--	1.72	20.7	--	17.4	--	--	--
Fine sand (106 – 250 µm)	%	--	--	32.9	--	15.9	25.1	--	32.5	--	--	--
Very fine sand (75 – 106 µm)	%	--	--	11.2	--	8.15	7.72	--	9.24	--	--	--
Percent silt (4 – 75 µm)	%	--	--	47.1	--	68.3	35.1	--	20.6	--	--	--
Percent clay (<4 µm)	%	--	--	3.29	--	5.58	5.15	--	1.14	--	--	--
Other												
Chromium, hexavalent	mg/kg	--	0.5 *U*	--	--	--	--	--	--	--	--	--
Cyanide	mg/kg	0.50 *U* [e]	--	--	--	--	--	--	--	--	--	--
Organic sulfur	%	--	--	--	--	--	--	--	--	--	--	--
Sulfate	%	--	--	--	--	--	--	--	--	--	--	--
Sulfides	%	--	--	--	--	--	--	--	--	--	--	--
Sulfur	%	--	--	--	--	--	--	--	--	--	--	--
Replicate Arsenic Analyses												
Replicate - 1	mg/kg	280 [e]	284	310	320	117	1,250	1,440	--	1,100	1,230	--
Replicate - 2	mg/kg	--	--	294	327	127	1,240	1,430	--	672	--	--
Replicate - 3	mg/kg	--	--	306	333	119	--	1,510	--	835	--	--
Replicate - 4	mg/kg	--	--	295	--	126	--	1,520	--	--	--	--
Replicate - 5	mg/kg	--	--	289	--	126	--	1,560	--	--	--	--
Replicate - 6	mg/kg	--	--	311	--	124	--	--	--	--	--	--
Average Arsenic Concentration:	**mg/kg**	**280** [e]	**284**	**301** [d]	**327** [d]	**123** [d]	**1,245** [d]	**1,492** [d]	--	**869**	**1,230**	--

Table 2-2. (cont.)

Chemical	Units	Washington Orchard Soil <500 µm 12/3/2002	Washington Orchard Soil <500 µm 2/10/2004	Washington Orchard Soil <250 µm 8/23/2002	Washington Orchard Soil <180 µm 10/23/2002	New York Orchard Soil <250 µm 8/23/2002	Colorado Smelter Soil (Smeltertown) <500 µm 2/20/2003	Colorado Smelter Soil (Smeltertown) <250 µm 2/20/2003	VB/I-70 Soil[b] <2 mm 2/19/2004	VB/I-70 Soil[b] <250 µm 2/19/2004	VB/I-70 Soil[b] <150 µm 2/19/2004	VB/I-70 Soil[b] <150 µm 12/20/2004
Other metals												
Aluminum	mg/kg	17,200 [e]	--	--	--	--	--	--	--	--	--	--
Antimony	mg/kg	0.60 [e]	10 *U*	10 *U*	--	10 *U*	28.8 [d]	30.1 [d]	--	10.0	--	--
Barium	mg/kg	128 [e]	--	--	--	--	--	--	--	--	--	--
Beryllium	mg/kg	0.20 *U* [e]	1.0 *U*	1.0 *U*	--	1.0 *U*	1.0 *U* [d]	1.0 *U* [d]	--	1.0 *U*	--	--
Cadmium	mg/kg	0.33 [e]	2.40	1.0 *U*	--	1.0 *U*	46.4 [d]	52.8 [d]	--	5.0	--	--
Calcium	mg/kg	5,410 [e]	--	--	--	--	--	--	--	--	--	--
Chromium	mg/kg	31 [e]	36.0	33.6	--	25.8	11.5 [d]	11.9 [d]	--	54.5	--	51.8
Cobalt	mg/kg	9.0 [e]	--	--	--	--	--	--	--	--	--	--
Copper	mg/kg	37 [e]	35.5	36.5	--	41.6	1,675 [d]	1,950 [d]	--	30.4	--	--
Iron	mg/kg	20,900 [e]	22,300	--	--	--	--	--	--	--	--	13,650
Lead	mg/kg	2,380 [e]	2,640	2,890	--	695	10,250 [d]	11,550 [d]	--	469	--	--
Magnesium	mg/kg	6,530 [e]	--	--	--	--	--	--	--	--	--	--
Manganese	mg/kg	365 [e]	394	--	--	--	--	--	--	--	--	--
Mercury	mg/kg	0.090 [e]	0.0400	0.040	--	0.14	52.1 [d]	60.2 [d]	--	0.80	--	--
Nickel	mg/kg	15 [e]	16.2	17	--	11.8	68.9 [d]	80.8 [d]	--	11.2	--	--
Phosphorus	mg/kg	--	887	--	--	--	--	--	--	--	--	--
Potassium	mg/kg	6,240 [e]	--	--	--	--	--	--	--	--	--	--
Selenium	mg/kg	200 *U* [e]	1.0 *U*	2.0 *U*	--	2.0 *U*	4.78 [d]	4.85 [d]	--	2.5 *U*	--	--
Silver	mg/kg	0.060 [e]	2.0 *U*	2.0 *U*	--	2.0 *U*	73.6 [d]	88.1 [d]	--	2.0 *U*	--	--
Sodium	mg/kg	140 [e]	--	--	--	--	--	--	--	--	--	--
Thallium	mg/kg	0.25 [e]	1.0 *U*	2.0 *U*	--	2.0 *U*	1.8 [d]	2.0 [d]	--	10.0 *U*	--	--
Vanadium	mg/kg	46 [e]	--	--	--	--	--	--	--	--	--	--
Zinc	mg/kg	275 [e]	286	312	--	71.8	13,200 [d]	15,500 [d]	--	314	--	--

Table 2-2. (cont.)

		Colorado Smelter Composite Soil (Globeville)						
		<2 mm	<500 µm		<250 µm		<150 µm	
Chemical	Units	4/10/2003	4/25/2003	2/10/2004	4/10/2003	2/10/2004	4/10/2003	2/10/2004
Conventionals								
pH	s.u.	7.48	--	7.53	--	7.52	--	7.48
Total organic carbon	%	2.51	--	2.09	--	2.21	--	2.27
Total inorganic carbon	%	--	--	0.05 *U*	--	0.05 *U*	--	0.27
Total carbon	%	--	--	2.11	--	2.20	--	2.54
Cation exchange capacity	meq/100g	--	--	52.3	--	54.1	--	61.9
Total solids	%	100	--	--	100	--	100	--
DCB extractable iron	mg/kg	--	--	5,110 [g]	--	--	--	--
Particle Size Distribution								
Medium gravel (> 4,750 µm)	%	0	--	--	--	--	--	--
Fine gravel (2,000 – 4,750 µm)	%	0	--	--	--	--	--	--
Very coarse sand (850 – 2,000 µm)	%	9.08	--	--	--	--	--	--
Coarse sand (425 – 850 µm)	%	10.7	--	--	--	--	--	--
Medium sand (250 – 425 µm)	%	12.8	--	--	--	--	--	--
Fine sand (106 – 250 µm)	%	25.7	--	--	--	--	--	--
Very fine sand (75 – 106 µm)	%	9.97	--	--	--	--	--	--
Percent silt (4 – 75 µm)	%	27.5	--	--	--	--	--	--
Percent clay (<4 µm)	%	3.18	--	--	--	--	--	--
Other								
Chromium, hexavalent	mg/kg	--	1,355 [d]	0.5 *U*	--	--	--	--
Cyanide	mg/kg	--	--	--	--	--	--	--
Organic sulfur	%	--	--	--	--	--	--	--
Sulfate	%	--	--	--	--	--	--	--
Sulfides	%	--	--	--	--	--	--	--
Sulfur	%	--	--	--	--	--	--	--
Replicate Arsenic Analyses								
Replicate - 1	mg/kg	--	303	393	412	416	452	474
Replicate - 2	mg/kg	--	297	--	379	--	470	--
Replicate - 3	mg/kg	--	--	--	388	--	471	--
Replicate - 4	mg/kg	--	--	--	401	--	--	--
Replicate - 5	mg/kg	--	--	--	391	--	--	--
Replicate - 6	mg/kg	--	--	--	--	--	--	--
Average Arsenic Concentration:	**mg/kg**	**--**	**300 [d]**	**393**	**394 [d]**	**416**	**464 [d]**	**474**

Table 2-2. (cont.)

		Colorado Smelter Composite Soil (Globeville)						
		<2 mm	<500 µm		<250 µm		<150 µm	
Chemical	Units	4/10/2003	4/25/2003	2/10/2004	4/10/2003	2/10/2004	4/10/2003	2/10/2004
Other metals								
Aluminum	mg/kg	--	--	--	--	--	--	--
Antimony	mg/kg	--	--	16.1	12.9	19.3	--	18.7
Barium	mg/kg	--	--	--	--	--	--	--
Beryllium	mg/kg	--	--	1.0 *U*	1.0 *U*	1.0 *U*	--	1.0 *U*
Cadmium	mg/kg	--	423 [d]	424	437 [d]	437	497 [d]	497 [d]
Calcium	mg/kg	--	--	--	--	--	--	--
Chromium	mg/kg	--	1,835 [d]	18.8	23.1	26.0	--	31.4
Cobalt	mg/kg	--	--	--	--	--	--	--
Copper	mg/kg	--	--	80.0	85.0	89.3	--	93.9
Iron	mg/kg	--	--	15,800	--	20,500	--	22,700
Lead	mg/kg	--	584 [d]	587	655	642	--	713
Magnesium	mg/kg	--	--	--	--	--	--	--
Manganese	mg/kg	--	--	479	--	510	--	525
Mercury	mg/kg	--	--	7.38	7.67	8.04	--	9.69
Nickel	mg/kg	--	--	13.2	16.3	16.7	--	18.7
Phosphorus	mg/kg	--	--	673	--	804	--	871
Potassium	mg/kg	--	--	--	--	--	--	--
Selenium	mg/kg	--	--	14.8	14.0 [d]	14.1	--	17.6
Silver	mg/kg	--	--	2.0 *U*	2.55	2.50	--	2.10
Sodium	mg/kg	--	--	--	--	--	--	--
Thallium	mg/kg	--	--	13.6	15.2 [d]	14.3	--	16.3
Vanadium	mg/kg	--	--	--	--	--	--	--
Zinc	mg/kg	--	--	1,200	1,200	1,310	--	1,420

Table 2-2. (cont.)

Chemical	Units	Point Mugu #1B <2 mm 2/10/2004	<500 µm 8/23/2002	<500 µm 2/10/2004	<250 µm 2/10/2004	<150 µm 2/10/2004	(unknown)[c] 10/2/2002	FL Inglis <250 µm 10/14/2004
Conventionals								
pH	s.u.	--	--	7.61	7.43	7.20	--	7.30
Total organic carbon	%	--	--	0.75	1.90	4.01	--	1.93
Total inorganic carbon	%	--	--	0.53	0.99	1.21	--	--
Total carbon	%	--	--	1.28	2.89	5.22	--	--
Cation exchange capacity	meq/100g	--	--	46.2	65.9	103	--	--
Total solids	%	--	--	--	--	--	98.5 [e]	--
DCB extractable iron	mg/kg	--	--	3,830 [g]	--	--	--	--
Particle Size Distribution								
Medium gravel (> 4,750 µm)	%	0.01	--	--	--	--	--	--
Fine gravel (2,000 – 4,750 µm)	%	0.05	--	--	--	--	--	--
Very coarse sand (850 – 2,000 µm)	%	11.8	--	--	--	--	--	--
Coarse sand (425 – 850 µm)	%	30.5	--	--	--	--	--	--
Medium sand (250 – 425 µm)	%	30.3	--	--	--	--	--	--
Fine sand (106 – 250 µm)	%	20.4	--	--	--	--	--	--
Very fine sand (75 – 106 µm)	%	1.91	--	--	--	--	--	--
Percent silt (4 – 75 µm)	%	2.22	--	--	--	--	--	--
Percent clay (<4 µm)	%	3.34	--	--	--	--	--	--
Other								
Chromium, hexavalent	mg/kg	--	1.1 [d]	0.5 *U*	--	--	--	--
Cyanide	mg/kg	--	--	--	--	--	100 [e]	--
Organic sulfur	%	--	--	--	--	--	0.040 [e]	--
Sulfate	%	--	--	--	--	--	0.16 [e]	--
Sulfides	%	--	--	--	--	--	0.16 [e]	--
Sulfur	%	--	--	--	--	--	0.36 [e]	--
Replicate Arsenic Analyses								
Replicate - 1	mg/kg	--	79.4	77.7	165	285 [h]	70 [e]	249
Replicate - 2	mg/kg	--	82.6	--	--	--	--	282
Replicate - 3	mg/kg	--	88	--	--	--	--	274
Replicate - 4	mg/kg	--	--	--	--	--	--	--
Replicate - 5	mg/kg	--	--	--	--	--	--	--
Replicate - 6	mg/kg	--	--	--	--	--	--	--
Average Arsenic Concentration:	**mg/kg**	**--**	**83.3 [d]**	**77.7**	**165**	**285 [h]**	**70 [e]**	**268**

Table 2-2. (cont.)

Chemical	Units	Point Mugu #1B <2 mm 2/10/2004	<500 µm 8/23/2002	<500 µm 2/10/2004	<250 µm 2/10/2004	<150 µm 2/10/2004	(unknown)[c] 10/2/2002	FL Inglis <250 µm 10/14/2004
Other metals								
Aluminum	mg/kg	--	--	--	--	--	2,530 [e]	--
Antimony	mg/kg	--	8.56 [d]	10 *U*	10 *U*	10 *U*	9 [e]	110
Barium	mg/kg	--	--	--	--	--	1,110 [e]	--
Beryllium	mg/kg	--	--	1.0 *U*	1.0 *U*	1.0 *U*	0.2 *U*[e]	1.0 *U*
Cadmium	mg/kg	--	1,753 [d]	1,760	3,897 [d, i]	7,625 [d]	1,420 [e]	1.0 *U*
Calcium	mg/kg	--	--	--	--	--	13,400 [e]	--
Chromium	mg/kg	--	8,380 [d]	8,310	16,300	30,200	6,600 [e]	9.4
Cobalt	mg/kg	--	--	--	--	--	9 [e]	--
Copper	mg/kg	--	--	943	1,950	3,310	790 [e]	154
Iron	mg/kg	--	--	9,330	15,600	26,900	9,550 [e]	--
Lead	mg/kg	--	573 [d]	555	1,140	1,980	643 [e]	3,770
Magnesium	mg/kg	--	--	--	--	--	2,380 [e]	--
Manganese	mg/kg	--	--	78.0	138	220	72.5 [e]	--
Mercury	mg/kg	--	0.88 [d]	0.850	1.85	3.15	0.86 [e]	1.39
Nickel	mg/kg	--	--	1,870	3,850	6,740	1,520 [e]	4.7
Phosphorus	mg/kg	--	--	1,710	3,310	5,100	--	--
Potassium	mg/kg	--	--	--	--	--	850 [e]	--
Selenium	mg/kg	--	2.0 *U*[d]	1.0 *U*	1.80	2.30	30 *U*[e]	2.5
Silver	mg/kg	--	--	171	362	615	173 [e]	13.9
Sodium	mg/kg	--	--	--	--	--	3,620 [e]	--
Thallium	mg/kg	--	--	1.0 *U*	1.0 *U*	1.0 *U*	1 *U*[e]	11.6
Vanadium	mg/kg	--	--	--	--	--	11.6 [e]	--
Zinc	mg/kg	--	738 [d]	673	1,370	2,380	589 [e]	127

(notes appear on following page)

Table 2-2. (cont.)

Note: Analysis performed by Columbia Analytical Services unless noted otherwise
-- – Not applicable or not available
U – undetected; value represents reporting limit for CAS analyses; value represent detection limit for ACZ analyses

[a] Soil #8 from Rodriguez et al. (1999), obtained from Nick Basta (Ohio State University).
[b] Soil obtained from Bill Brattin.
[c] Unknown grainsize.
[d] Average of field replicates
[e] Analysis by ACZ Laboratories
[f] Analysis by Auburn University for 6/19/2003 samples.
[g] Analysis by Auburn University (samples sent 3/31/2004).
[h] Average of two results by different methods: 300 mg/kg using method 6010B and 269 mg/kg using method 7060A.
[i] One of the averaged samples was sent to CAS by Stan Casteel (Exponent tag # 57171, orginally sent to Stan on 5/2/2003).
[k] HCl was added

Table 2-3. SERDP — cadmium in vivo study substrates

		Point Mugu #1B					
		< 2 mm	< 500 µm		< 250 µm	< 150 µm	(unknown)[d]
Chemical	Units	2/10/2004	8/23/2002	2/10/2004	2/10/2004	2/10/2004	10/2/2002
Conventionals							
pH	s.u.	--	--	7.61	7.43	7.20	--
Total organic carbon	%	--	--	0.75	1.90	4.01	--
Total inorganic carbon	%	--	--	0.53	0.99	1.21	--
Total carbon	%	--	--	1.28	2.89	5.22	--
Cation exchange capacity	meq/100g	--	--	46.2	65.9	103	--
Total solids	%	--	--	--	--	--	98.5 [d]
DCB extractable iron	mg/kg	--	--	3,830 [g]	--	--	--
Particle Size Distribution							
Medium gravel (> 4,750 µm)	%	0.01	--	--	--	--	--
Fine gravel (2,000 – 4,750 µm)	%	0.05	--	--	--	--	--
Very coarse sand (850 – 2,000 µm)	%	11.8	--	--	--	--	--
Coarse sand (425 – 850 µm)	%	30.5	--	--	--	--	--
Medium sand (250 – 425 µm)	%	30.3	--	--	--	--	--
Fine sand (106 – 250 µm)	%	20.4	--	--	--	--	--
Very fine sand (75 – 106 µm)	%	1.91	--	--	--	--	--
Percent silt (4 – 75 µm)	%	2.22	--	--	--	--	--
Percent clay (< 4 µm)	%	3.34	--	--	--	--	--
Other							
Chromium, hexavalent	mg/kg	--	1.1 [e]	0.5 *U*	--	--	--
Cyanide	mg/kg	--	--	--	--	--	100 [d]
Organic sulfur	%	--	--	--	--	--	0.040 [d]
Sulfate	%	--	--	--	--	--	0.16 [d]
Sulfides	%	--	--	--	--	--	0.16 [d]
Sulfur	%	--	--	--	--	--	0.36 [d]
Replicate Cadmium Analyses							
Replicate - 1	mg/kg	--	1,770	1,760	3,860	7,480	1,420 [d]
Replicate - 2	mg/kg	--	1,630	--	4,000	7,740	--
Replicate - 3	mg/kg	--	1,860	--	3,830 [h]	7,580	--
Replicate - 4	mg/kg	--	--	--	--	7,700	--
Average Cadmium Concentration:	**mg/kg**	**--**	**1,753 [e]**	**1,760**	**3,897 [e]**	**7,625 [e]**	**1,420 [d]**

Table 2-3. (cont.)

		Point Mugu #1B					
		< 2 mm	< 500 µm		< 250 µm	< 150 µm	(unknown)[d]
Chemical	Units	2/10/2004	8/23/2002	2/10/2004	2/10/2004	2/10/2004	10/2/2002
Other Metals							
Aluminum	mg/kg	--	--	--	--	--	2,530 [d]
Antimony	mg/kg	--	8.56 [e]	10 *U*	10 *U*	10 *U*	9 [d]
Arsenic	mg/kg	--	83.3 [e]	77.7	165	285 [f]	70 [d]
Barium	mg/kg	--	--	--	--	--	1,110 [d]
Beryllium	mg/kg	--	--	1.0 *U*	1.0 *U*	1.0 *U*	0.2 *U* [d]
Calcium	mg/kg	--	--	--	--	--	13,400 [d]
Chromium	mg/kg	--	8,380 [e]	8,310	16,300	30,200	6,600 [d]
Cobalt	mg/kg	--	--	--	--	--	9 [d]
Copper	mg/kg	--	--	943	1,950	3,310	790 [d]
Iron	mg/kg	--	--	9,330	15,600	26,900	9,550 [d]
Lead	mg/kg	--	573 [e]	555	1,140	1,980	643 [d]
Magnesium	mg/kg	--	--	--	--	--	2,380 [d]
Manganese	mg/kg	--	--	78.0	138	220	72.5 [d]
Mercury	mg/kg	--	0.88 [e]	0.850	1.85	3.15	0.86 [d]
Nickel	mg/kg	--	--	1,870	3,850	6,740	1,520 [d]
Phosphorus	mg/kg	--	--	1,710	3,310	5,100	--
Potassium	mg/kg	--	--	--	--	--	850 [d]
Selenium	mg/kg	--	2.0 *U* [e]	1.0 *U*	1.80	2.30	30 *U* [d]
Silver	mg/kg	--	--	171	362	615	173 [d]
Sodium	mg/kg	--	--	--	--	--	3,620 [d]
Thallium	mg/kg	--	--	1.0 *U*	1.0 *U*	1.0 *U*	1 *U* [d]
Vanadium	mg/kg	--	--	--	--	--	11.6 [d]
Zinc	mg/kg	--	738 [e]	673	1,370	2,380	589 [d]

Table 2-3. (cont.)

		Colorado Smelter Composite Soil (Globeville)						
		< 2 mm	< 500 µm		< 250 µm		< 150 µm	
Chemical	Units	4/10/2003	4/25/2003	2/10/2004	4/10/2003	2/10/2004	4/10/2003	2/10/2004
Conventionals								
pH	s.u.	7.48	--	7.53	--	7.52	--	7.48
Total organic carbon	%	2.51	--	2.09	--	2.21	--	2.27
Total inorganic carbon	%	--	--	0.05 *U*	--	0.05 *U*	--	0.27
Total carbon	%	--	--	2.11	--	2.20	--	2.54
Cation exchange capacity	meq/100g	--	--	52.3	--	54.1	--	61.9
Total solids	%	100	--	--	100	--	100	--
DCB extractable iron	mg/kg	--	--	5,110 [g]	--	--	--	--
Particle Size Distribution								
Medium gravel (> 4,750 µm)	%	0	--	--	--	--	--	--
Fine gravel (2,000 – 4,750 µm)	%	0	--	--	--	--	--	--
Very coarse sand (850 – 2,000 µm)	%	9.08	--	--	--	--	--	--
Coarse sand (425 – 850 µm)	%	10.7	--	--	--	--	--	--
Medium sand (250 – 425 µm)	%	12.8	--	--	--	--	--	--
Fine sand (106 – 250 µm)	%	25.7	--	--	--	--	--	--
Very fine sand (75 – 106 µm)	%	9.97	--	--	--	--	--	--
Percent silt (4 – 75 µm)	%	27.5	--	--	--	--	--	--
Percent clay (< 4 µm)	%	3.18	--	--	--	--	--	--
Other								
Chromium, hexavalent	mg/kg	--	1,355 [e]	0.5 *U*	--	--	--	--
Cyanide	mg/kg	--	--	--	--	--	--	--
Organic sulfur	%	--	--	--	--	--	--	--
Sulfate	%	--	--	--	--	--	--	--
Sulfides	%	--	--	--	--	--	--	--
Sulfur	%	--	--	--	--	--	--	--
Replicate Cadmium Analyses								
Replicate - 1	mg/kg	--	455	424	444	437	501	510
Replicate - 2	mg/kg	--	390	--	410	--	497	517
Replicate - 3	mg/kg	--	--	--	449	--	493	493
Replicate - 4	mg/kg	--	--	--	443	--	--	468
Average Cadmium Concentration:	**mg/kg**	**--**	**423** [e]	**424**	**437** [e]	**437**	**497** [e]	**497** [e]

Table 2-3. (cont.)

		Colorado Smelter Composite Soil (Globeville)						
		< 2 mm	< 500 µm		< 250 µm		< 150 µm	
Chemical	Units	4/10/2003	4/25/2003	2/10/2004	4/10/2003	2/10/2004	4/10/2003	2/10/2004
Other Metals								
Aluminum	mg/kg	--	--	--	--	--	--	--
Antimony	mg/kg	--	--	16.1	12.9	19.3	--	18.7
Arsenic	mg/kg	--	300 [e]	393	394 [e]	416	464 [e]	474
Barium	mg/kg	--	--	--	--	--	--	--
Beryllium	mg/kg	--	--	1.0 *U*	1.0 *U*	1.0 *U*	--	1.0 *U*
Calcium	mg/kg	--	--	--	--	--	--	--
Chromium	mg/kg	--	1,835 [e]	18.8	23.1	26.0	--	31.4
Cobalt	mg/kg	--	--	--	--	--	--	--
Copper	mg/kg	--	--	80.0	85.0	89.3	--	93.9
Iron	mg/kg	--	--	15,800	--	20,500	--	22,700
Lead	mg/kg	--	584 [e]	587	655	642	--	713
Magnesium	mg/kg	--	--	--	--	--	--	--
Manganese	mg/kg	--	--	479	--	510	--	525
Mercury	mg/kg	--	--	7.38	7.67	8.04	--	9.69
Nickel	mg/kg	--	--	13.2	16.3	16.7	--	18.7
Phosphorus	mg/kg	--	--	673	--	804	--	871
Potassium	mg/kg	--	--	--	--	--	--	--
Selenium	mg/kg	--	--	14.8	14.0 [e]	14.1	--	17.6
Silver	mg/kg	--	--	2.0 *U*	2.55	2.50	--	2.10
Sodium	mg/kg	--	--	--	--	--	--	--
Thallium	mg/kg	--	--	13.6	15.2 [e]	14.3	--	16.3
Vanadium	mg/kg	--	--	--	--	--	--	--
Zinc	mg/kg	--	--	1,200	1,200	1,310	--	1,420

Table 2-3. (cont.)

		Oklahoma Smelter Soil #1[a]					OK Smelter Soil #2[b]	Dugway Composite[c]		
		< 2 mm	< 500 µm	< 250 µm		< 150 µm	< 250 µm	< 2 mm	< 250 µm	
Chemical	Units	4/10/2003	4/10/2003	4/10/2003	2/10/2004	4/10/2003	3/2/2004	2/10/2004	5/5/2003	2/10/2004
Conventionals										
pH	s.u.	7.61	--	--	7.55	--	--	--	--	9.06
Total organic carbon	%	4.64	--	--	4.98	--	--	--	--	2.87
Total inorganic carbon	%	--	--	--	0.74	--	--	--	--	1.51
Total carbon	%	--	--	--	5.72	--	--	--	--	4.38
Cation exchange capacity	meq/100g	--	--	--	70.1	--	--	--	--	52.2
Total solids	%	100	100	100	--	100	--	--	--	--
DCB extractable iron	mg/kg	--	--	--	--	--	--	--	--	--
Particle Size Distribution										
Medium gravel (> 4,750 µm)	%	0	--	--	--	--	--	0	--	--
Fine gravel (2,000 – 4,750 µm)	%	0.175	--	--	--	--	--	0.38	--	--
Very coarse sand (850 – 2,000 µm)	%	12.9	--	--	--	--	--	10.6	--	--
Coarse sand (425 – 850 µm)	%	15.7	--	--	--	--	--	8.58	--	--
Medium sand (250 – 425 µm)	%	15.1	--	--	--	--	--	8.18	--	--
Fine sand (106 – 250 µm)	%	16.5	--	--	--	--	--	29.8	--	--
Very fine sand (75 – 106 µm)	%	4.45	--	--	--	--	--	9.87	--	--
Percent silt (4 – 75 µm)	%	31.8	--	--	--	--	--	3.31	--	--
Percent clay (< 4 µm)	%	1.73	--	--	--	--	--	29.7	--	--
Other										
Chromium, hexavalent	mg/kg	--	--	--	--	--	--	--	--	--
Cyanide	mg/kg	--	--	--	--	--	--	--	--	--
Organic sulfur	%	--	--	--	--	--	--	--	--	--
Sulfate	%	--	--	--	--	--	--	--	--	--
Sulfides	%	--	--	--	--	--	--	--	--	--
Sulfur	%	--	--	--	--	--	--	--	--	--
Replicate Cadmium Analyses										
Replicate - 1	mg/kg	--	86.2	96.3	101	102	410	--	43.0	45.0
Replicate - 2	mg/kg	--	--	99.4	--	103	--	--	44.1	--
Replicate - 3	mg/kg	--	--	99.6	--	106	--	--	41.9	--
Replicate - 4	mg/kg	--	--	98.3	--	107	--	--	--	--
Average Cadmium Concentration:	**mg/kg**	**--**	**86.2**	**98.4 [e]**	**101**	**105 [e]**	**410**	**--**	**43.0 [e]**	**45.0**

Table 2-3. (cont.)

		Oklahoma Smelter Soil #1[a]					OK Smelter Soil #2[b]	Dugway Composite[c]		
		< 2 mm	< 500 µm	< 250 µm		< 150 µm	< 250 µm	< 2 mm	< 250 µm	
Chemical	Units	4/10/2003	4/10/2003	4/10/2003	2/10/2004	4/10/2003	3/2/2004	2/10/2004	5/5/2003	2/10/2004
Other Metals										
Aluminum	mg/kg	--	--	--	--	--	--	--	--	--
Antimony	mg/kg	--	10 *U*	10 *U*	10 *U*	10 *U*	--	--	--	10 *U*
Arsenic	mg/kg	--	66.2	81.2 [e]	77.2	92 [e]	--	--	--	8.50
Barium	mg/kg	--	--	--	--	--	--	--	--	--
Beryllium	mg/kg	--	1.0 *U*	1.0 *U*	1.0 *U*	1.0 *U*	--	--	--	1.0 *U*
Calcium	mg/kg	--	--	--	--	--	--	--	--	--
Chromium	mg/kg	--	21.2	21.4	19.4	22.2	--	--	--	41.8
Cobalt	mg/kg	--	--	--	--	--	--	--	--	--
Copper	mg/kg	--	992	1,190	1,300	1,300	--	--	--	45.2
Iron	mg/kg	--	--	--	22,500	--	--	--	--	14,100
Lead	mg/kg	--	756	919	1,000	957	540	--	--	71.3
Magnesium	mg/kg	--	--	--	--	--	--	--	--	--
Manganese	mg/kg	--	--	--	804	--	--	--	--	266
Mercury	mg/kg	--	0.74	0.87	0.900	1.15	--	--	--	5.95
Nickel	mg/kg	--	32.7	42.4	45.1	46.4	--	--	--	24.1
Phosphorus	mg/kg	--	--	--	790	--	--	--	--	1,150
Potassium	mg/kg	--	--	--	--	--	--	--	--	--
Selenium	mg/kg	--	1.2	1.2	1.30	1.3	--	--	--	1.0 *U*
Silver	mg/kg	--	23.5	31.6	35.8	35.6	--	--	--	2.50
Sodium	mg/kg	--	--	--	--	--	--	--	--	--
Thallium	mg/kg	--	1.0 *U*	1.0 *U*	1.0 *U*	1.0 *U*	--	--	--	1.0 *U*
Vanadium	mg/kg	--	--	--	--	--	--	--	--	--
Zinc	mg/kg	--	19,600	24,500	28,500	27,700	17,600	--	368 [e]	394

(notes appear on following page)

Table 2-3. (cont.)

Note: Analysis performed by Columbia Analytical Services unless noted otherwise
-- – Not applicable or not available
U – undetected; value represents reporting limit for CAS analyses; value represent detection limit for ACZ analyses

[a] Collected from field north of Blackwell smelter by Exponent.
[b] Blackwell soil obtained from Nick Basta (Ohio State University).
[c] Dugway Proving Grounds #3B and #1 Composite
[d] Analysis by ACZ Laboratories (10/2/2002 Pt Mugu #1B sample sent to ACZ)
[e] Average of field replicates
[f] Average of two results by different methods: 300 mg/kg using method 6010B and 269 mg/kg using method 7060A
[g] Analysis by Auburn University (samples sent 3/31/2004)
[h] Sample sent to CAS by Stan Casteel (Exponent tag # 57171, orginally sent to Stan on 5/2/2003)

Table 2-4. SERDP — arsenic dermal study substrates

Chemical	Units	Colorado Residential Soil[a] <2 mm 2/19/04	<250 µm 2/19/04	<150 µm 2/19/04	<150 µm 12/20/04	New York Pesticide Facility Soil (A1B21) <2 mm 10/2/03	<2 mm 10/6/04	<250 µm 10/2/03	<250 µm 10/6/04	<150 µm[b] 10/30/03	<150 µm 10/30/03
Conventionals											
pH	s.u.	--	5.33	--	--	--	--	--	5.24	5.34	5.61
Total organic carbon	%	--	2.76	--	--	--	--	--	5.08	4.77	4.25
Cation exchange capacity	meq/100g	--	--	--	--	--	--	--	--	--	81.0
DCB extractable iron	mg/kg	--	--	--	--	--	--	--	--	--	9,720
Particle Size Distribution											
Medium gravel (>4,750 µm)	%	0	--	--	--	0.03	0.0	--	--	--	--
Fine gravel (2,000 – 4,750 µm)	%	2.55	--	--	--	0.59	0.03	--	--	--	--
Very coarse sand (850 – 2,000 µm)	%	4.88	--	--	--	23.6	22.8	--	--	--	--
Coarse sand (425 – 850 µm)	%	11.7	--	--	--	23.9	18.1	--	--	--	--
Medium sand (250 – 425 µm)	%	17.4	--	--	--	15.1	11.8	--	--	--	--
Fine sand (106 – 250 µm)	%	32.5	--	--	--	18.1	13.7	--	--	--	--
Very fine sand (75 – 106 µm)	%	9.24	--	--	--	3.95	4.02	--	--	--	--
Percent silt (4 – 75 µm)	%	20.6	--	--	--	1.86	2.02	--	--	--	--
Percent clay (<4 µm)	%	1.14	--	--	--	14.9	15.8	--	--	--	--
Replicate Arsenic Analyses											
Replicate - 1	mg/kg	--	1,100	1,230	--	--	1,500	1,580	1,750	1,610	1,350
Replicate - 2	mg/kg	--	672	--	--	--	--	--	--	--	1,360
Replicate - 3	mg/kg	--	835	--	--	--	--	--	--	--	1,360
Average Arsenic Concentration:	**mg/kg**	**--**	**869**	**1,230**	**--**	**--**	**1,500**	**1,580**	**1,750**	**1,610**	**1,400**
Other metals											
Antimony	mg/kg	--	10.0	--	--	--	--	--	15.7	--	10 *U*
Beryllium	mg/kg	--	1.0 *U*	--	--	--	--	--	1.0 *U*	--	1 *U*
Cadmium	mg/kg	--	5.0	--	--	--	--	--	8.4	--	1.7
Chromium	mg/kg	--	54.5	--	51.8	--	--	--	19.0	17.3	16.2
Copper	mg/kg	--	30.4	--	--	--	--	--	61.6	60.1	62.3
Iron	mg/kg	--	--	--	13,650	--	--	--	--	16,950	15,050
Lead	mg/kg	--	469	--	--	--	--	--	416	374	399
Manganese	mg/kg	--	--	--	--	--	--	--	--	661	645
Mercury	mg/kg	--	0.80	--	--	--	--	--	2.11	--	0.44
Nickel	mg/kg	--	11.2	--	--	--	--	--	12.9	--	13.7
Selenium	mg/kg	--	2.5 *U*	--	--	--	--	--	2.2	--	1.9
Silver	mg/kg	--	2.0 *U*	--	--	--	--	--	2.0 *U*	--	2 *U*
Thallium	mg/kg	--	10.0 *U*	--	--	--	--	--	1.0 *U*	--	1 *U*
Zinc	mg/kg	--	314	--	--	--	--	--	254	--	244

Note: -- – Not applicable or not available
U – undetected; value represents detection or reporting limit

[a] Soil obtained from Bill Brattin.
[b] Average of duplicates.

Table 2-5. SERDP — wildlife study substrates

		Point Mugu #1B						Dugatinny[a]	
		< 2 mm	< 500 µm		< 250 µm	< 150 µm	(unknown)[b]	< 500 µm	
Chemical	Units	2/10/2004	8/23/2002	2/10/2004	2/10/2004	2/10/2004	10/2/2002	10/28/2002	12/3/2002
Conventionals									
pH	s.u.	--	--	7.61	7.43	7.20	--	8.00	--
Total organic carbon	%	--	--	0.75	1.90	4.01	--	3.26	--
Total inorganic carbon	%	--	--	0.53	0.99	1.21	--	1.02	--
Total carbon	%	--	--	1.28	2.89	5.22	--	4.28	--
Cation exchange capacity	meq/100g	--	--	46.2	65.9	103	--	71.3	--
Total solids	%	--	--	--	--	--	98.5 [b]	--	98.3 [b]
DCB extractable iron	mg/kg	--	--	3,830 [f]	--	--	--	12,240 [f]	--
Particle Size Distribution									
Medium gravel (> 4,750 µm)	%	0.01	--	--	--	--	--	0	--
Fine gravel (2,000 – 4,750 µm)	%	0.05	--	--	--	--	--	0.08	--
Very coarse sand (850 – 2,000 µm)	%	11.8	--	--	--	--	--	0.25	--
Coarse sand (425 – 850 µm)	%	30.5	--	--	--	--	--	7.73	--
Medium sand (250 – 425 µm)	%	30.3	--	--	--	--	--	19.4	--
Fine sand (106 – 250 µm)	%	20.4	--	--	--	--	--	36.5	--
Very fine sand (75 – 106 µm)	%	1.91	--	--	--	--	--	8.96	--
Percent silt (4 – 75 µm)	%	2.22	--	--	--	--	--	1.67	--
Percent clay (< 4 µm)	%	3.34	--	--	--	--	--	24.7	--
Other									
Chromium, hexavalent	mg/kg	--	1.1 [c]	0.5 *U*	--	--	--	0.5 *U*	--
Cyanide	mg/kg	--	--	--	--	--	100 [b]	--	0.50 *U* [b]
Organic sulfur	%	--	--	--	--	--	0.040 [b]	--	--
Sulfate	%	--	--	--	--	--	0.16 [b]	--	--
Sulfides	%	--	--	--	--	--	0.16 [b]	--	--
Sulfur	%	--	--	--	--	--	0.36 [b]	--	--

Table 2-5. (cont.)

Chemical	Units	Point Mugu #1B < 2 mm 2/10/2004	Point Mugu #1B < 500 µm 8/23/2002	Point Mugu #1B < 500 µm 2/10/2004	Point Mugu #1B < 250 µm 2/10/2004	Point Mugu #1B < 150 µm 2/10/2004	Point Mugu #1B (unknown)[b] 10/2/2002	Dugatinny[a] < 500 µm 10/28/2002	Dugatinny[a] < 500 µm 12/3/2002
Replicate Arsenic Analyses									
Replicate - 1	mg/kg	--	79.4	77.7	165	285 [d]	70 [b]	60.4	56 [b]
Replicate - 2	mg/kg	--	82.6	--	--	--	--	--	--
Replicate - 3	mg/kg	--	88	--	--	--	--	--	--
Replicate - 4	mg/kg	--	--	--	--	--	--	--	--
Replicate - 5	mg/kg	--	--	--	--	--	--	--	--
Replicate - 6	mg/kg	--	--	--	--	--	--	--	--
Average Arsenic Concentration:	**mg/kg**	**--**	**83.3 [c]**	**77.7**	**165**	**285 [d]**	**70 [b]**	**60.4**	**56 [b]**
Replicate Cadmium Analyses									
Replicate - 1	mg/kg	--	1,770	1,760	3,860	7,480	1,420 [b]	14.0	8.98 [b]
Replicate - 2	mg/kg	--	1,630	--	4,000	7,740	--	--	--
Replicate - 3	mg/kg	--	1,860	--	3,830 [e]	7,580	--	--	--
Replicate - 4	mg/kg	--	--	--	--	7,700	--	--	--
Average Cadmium Concentration:	**mg/kg**	**--**	**1,753 [c]**	**1,760**	**3,897 [c]**	**7,625 [c]**	**1,420 [b]**	**14.0**	**8.98 [b]**
Replicate Chromium Analyses									
Replicate - 1	mg/kg	--	8,410	8,310	16,300	30,200	6,600 [b]	79.4	71 [b]
Replicate - 2	mg/kg	--	7,960	--	--	--	--	--	--
Replicate - 3	mg/kg	--	8,770	--	--	--	--	--	--
Average Chromium Concentration:	**mg/kg**	**--**	**8,380 [c]**	**8,310**	**16,300**	**30,200**	**6,600 [b]**	**79.4**	**71 [b]**
Replicate Lead Analyses									
Replicate - 1	mg/kg	--	561	555	1,140	1,980	643 [b]	257	282 [b]
Replicate - 2	mg/kg	--	557	--	--	--	--	--	--
Replicate - 3	mg/kg	--	602	--	--	--	--	--	--
Average Lead Concentration:	**mg/kg**	**--**	**573 [c]**	**555**	**1,140**	**1,980**	**643 [b]**	**257**	**282 [b]**

Table 2-5. (cont.)

		Point Mugu #1B						Dugatinny[a]	
		< 2 mm	< 500 µm		< 250 µm	< 150 µm	(unknown)[b]	< 500 µm	
Chemical	Units	2/10/2004	8/23/2002	2/10/2004	2/10/2004	2/10/2004	10/2/2002	10/28/2002	12/3/2002
Other Metals									
Aluminum	mg/kg	--	--	--	--	--	2,530 [b]	--	12,700 [b]
Antimony	mg/kg	--	8.56 [c]	10 *U*	10 *U*	10 *U*	9 [b]	10 *U*	0.90 [b]
Barium	mg/kg	--	--	--	--	--	1,110 [b]	--	159 [b]
Beryllium	mg/kg	--	--	1.0 *U*	1.0 *U*	1.0 *U*	0.2 *U*[b]	1.0 *U*	0.40 [b]
Calcium	mg/kg	--	--	--	--	--	13,400 [b]	--	47,400 [b]
Cobalt	mg/kg	--	--	--	--	--	9 [b]	--	84 [b]
Copper	mg/kg	--	--	943	1,950	3,310	790 [b]	57.3	65 [b]
Iron	mg/kg	--	--	9,330	15,600	26,900	9,550 [b]	20,900	21,600 [b]
Magnesium	mg/kg	--	--	--	--	--	2,380 [b]	--	11,200 [b]
Manganese	mg/kg	--	--	78.0	138	220	72.5 [b]	498	413 [b]
Mercury	mg/kg	--	0.88 [c]	0.850	1.85	3.15	0.86 [b]	11.3	14.9 [b]
Nickel	mg/kg	--	--	1,870	3,850	6,740	1,520 [b]	41.1	39 [b]
Phosphorus	mg/kg	--	--	1,710	3,310	5,100	--	1,560	--
Potassium	mg/kg	--	--	--	--	--	850 [b]	--	1,960 [b]
Selenium	mg/kg	--	2.0 *U*[c]	1.0 *U*	1.80	2.30	30 *U*[b]	1.0 *U*	20 *U*[b]
Silver	mg/kg	--	--	171	362	615	173 [b]	3.70	3.16 [b]
Sodium	mg/kg	--	--	--	--	--	3,620 [b]	--	410 [b]
Thallium	mg/kg	--	--	1.0 *U*	1.0 *U*	1.0 *U*	1 *U*[b]	1.0 *U*	0.17 [b]
Vanadium	mg/kg	--	--	--	--	--	11.6 [b]	--	26.3 [b]
Zinc	mg/kg	--	738 [c]	673	1,370	2,380	589 [b]	356	369 [b]

Table 2-5. (cont.)

Chemical	Units	Washington Orchard Soil < 500 µm 12/3/2002	< 500 µm 2/10/2004	< 250 µm 8/23/2002	< 180 µm 10/23/2002
Conventionals					
pH	s.u.	--	5.92	5.28	--
Total organic carbon	%	--	2.98	3.40	--
Total inorganic carbon	%	--	1.27	--	--
Total carbon	%	--	4.25	--	--
Cation exchange capacity	meq/100g	--	74.0	--	--
Total solids	%	98.2 [b]	--	--	--
DCB extractable iron	mg/kg	--	5,630 [f]	--	--
Particle Size Distribution					
Medium gravel (> 4,750 µm)	%	--	--	0	--
Fine gravel (2,000 – 4,750 µm)	%	--	--	0	--
Very coarse sand (850 – 2,000 µm)	%	--	--	0.06	--
Coarse sand (425 – 850 µm)	%	--	--	0.835	--
Medium sand (250 – 425 µm)	%	--	--	4.55	--
Fine sand (106 – 250 µm)	%	--	--	32.9	--
Very fine sand (75 – 106 µm)	%	--	--	11.2	--
Percent silt (4 – 75 µm)	%	--	--	47.1	--
Percent clay (< 4 µm)	%	--	--	3.29	--
Other					
Chromium, hexavalent	mg/kg	--	0.5 *U*	--	--
Cyanide	mg/kg	0.50 *U* [b]	--	--	--
Organic sulfur	%	--	--	--	--
Sulfate	%	--	--	--	--
Sulfides	%	--	--	--	--
Sulfur	%	--	--	--	--

Table 2-5. (cont.)

Chemical	Units	Washington Orchard Soil < 500 µm 12/3/2002	< 500 µm 2/10/2004	< 250 µm 8/23/2002	< 180 µm 10/23/2002
Replicate Arsenic Analyses					
Replicate - 1	mg/kg	280 [b]	284	310	321
Replicate - 2	mg/kg	--	--	294	327
Replicate - 3	mg/kg	--	--	306	326
Replicate - 4	mg/kg	--	--	295	--
Replicate - 5	mg/kg	--	--	289	--
Replicate - 6	mg/kg	--	--	311	--
Average Arsenic Concentration:	**mg/kg**	**280** [b]	**284**	**301** [c]	**325** [c]
Replicate Cadmium Analyses					
Replicate - 1	mg/kg	0.33 [b]	2.40	1.0 *U*	--
Replicate - 2	mg/kg	--	--	--	--
Replicate - 3	mg/kg	--	--	--	--
Replicate - 4	mg/kg	--	--	--	--
Average Cadmium Concentration:	**mg/kg**	**0.33** [b]	**2.40**	**1.0** ***U***	**--**
Replicate Chromium Analyses					
Replicate - 1	mg/kg	31 [b]	36.0	33.6	--
Replicate - 2	mg/kg	--	--	--	--
Replicate - 3	mg/kg	--	--	--	--
Average Chromium Concentration:	**mg/kg**	**31** [b]	**36.0**	**33.6**	**--**
Replicate Lead Analyses					
Replicate - 1	mg/kg	2,380 [b]	2,640	2,890	--
Replicate - 2	mg/kg	--	--	--	--
Replicate - 3	mg/kg	--	--	--	--
Average Lead Concentration:	**mg/kg**	**2,380** [b]	**2,640**	**2,890**	**--**

Table 2-5. (cont.)

Chemical	Units	Washington Orchard Soil < 500 µm 12/3/2002	< 500 µm 2/10/2004	< 250 µm 8/23/2002	< 180 µm 10/23/2002
Other Metals					
Aluminum	mg/kg	17,200 [b]	--	--	--
Antimony	mg/kg	0.60 [b]	10 *U*	10 *U*	--
Barium	mg/kg	128 [b]	--	--	--
Beryllium	mg/kg	0.20 *U* [b]	1.0 *U*	1.0 *U*	--
Calcium	mg/kg	5,410 [b]	--	--	--
Cobalt	mg/kg	9.0 [b]	--	--	--
Copper	mg/kg	37 [b]	35.5	36.5	--
Iron	mg/kg	20,900 [b]	22,300	--	--
Magnesium	mg/kg	6,530 [b]	--	--	--
Manganese	mg/kg	365 [b]	394	--	--
Mercury	mg/kg	0.090 [b]	0.0400	0.040	--
Nickel	mg/kg	15 [b]	16.2	17	--
Phosphorus	mg/kg	--	887	--	--
Potassium	mg/kg	6,240 [b]	--	--	--
Selenium	mg/kg	200 *U* [b]	1.0 *U*	2.0 *U*	--
Silver	mg/kg	0.060 [b]	2.0 *U*	2.0 *U*	--
Sodium	mg/kg	140 [b]	--	--	--
Thallium	mg/kg	0.25 [b]	1.0 *U*	2.0 *U*	--
Vanadium	mg/kg	46 [b]	--	--	--
Zinc	mg/kg	275 [b]	286	312	--

Table 2-5. (cont.)

		Colorado Smelter Composite Soil (Globeville)						
		< 2 mm	< 500 µm		< 250 µm		< 150 µm	
Chemical	Units	4/10/2003	4/25/2003	2/10/2004	4/10/2003	2/10/2004	4/10/2003	2/10/2004
Conventionals								
pH	s.u.	7.48	--	7.53	--	7.52	--	7.48
Total organic carbon	%	2.51	--	2.09	--	2.21	--	2.27
Total inorganic carbon	%	--	--	0.05 *U*	--	0.05 *U*	--	0.27
Total carbon	%	--	--	2.11	--	2.20	--	2.54
Cation exchange capacity	meq/100g	--	--	52.3	--	54.1	--	61.9
Total solids	%	100	--	--	100	--	100	--
DCB extractable iron	mg/kg	--	--	5,110 [f]	--	--	--	--
Particle Size Distribution								
Medium gravel (> 4,750 µm)	%	0	--	--	--	--	--	--
Fine gravel (2,000 – 4,750 µm)	%	0	--	--	--	--	--	--
Very coarse sand (850 – 2,000 µm)	%	9.08	--	--	--	--	--	--
Coarse sand (425 – 850 µm)	%	10.7	--	--	--	--	--	--
Medium sand (250 – 425 µm)	%	12.8	--	--	--	--	--	--
Fine sand (106 – 250 µm)	%	25.7	--	--	--	--	--	--
Very fine sand (75 – 106 µm)	%	9.97	--	--	--	--	--	--
Percent silt (4 – 75 µm)	%	27.5	--	--	--	--	--	--
Percent clay (< 4 µm)	%	3.18	--	--	--	--	--	--
Other								
Chromium, hexavalent	mg/kg	--	1,355 [c]	0.5 *U*	--	--	--	--
Cyanide	mg/kg	--	--	--	--	--	--	--
Organic sulfur	%	--	--	--	--	--	--	--
Sulfate	%	--	--	--	--	--	--	--
Sulfides	%	--	--	--	--	--	--	--
Sulfur	%	--	--	--	--	--	--	--

Table 2-5. (cont.)

		Colorado Smelter Composite Soil (Globeville)						
		< 2 mm	< 500 µm		< 250 µm		< 150 µm	
Chemical	Units	4/10/2003	4/25/2003	2/10/2004	4/10/2003	2/10/2004	4/10/2003	2/10/2004
Replicate Arsenic Analyses								
Replicate - 1	mg/kg	--	303	393	412	416	452	474
Replicate - 2	mg/kg	--	297	--	379	--	470	--
Replicate - 3	mg/kg	--	--	--	388	--	471	--
Replicate - 4	mg/kg	--	--	--	401	--	--	--
Replicate - 5	mg/kg	--	--	--	391	--	--	--
Replicate - 6	mg/kg	--	--	--	--	--	--	--
Average Arsenic Concentration:	**mg/kg**	**--**	**300 [c]**	**393**	**394 [c]**	**416**	**464 [c]**	**474**
Replicate Cadmium Analyses								
Replicate - 1	mg/kg	--	455	424	444	437	501	510
Replicate - 2	mg/kg	--	390	--	410	--	497	517
Replicate - 3	mg/kg	--	--	--	449	--	493	493
Replicate - 4	mg/kg	--	--	--	443	--	--	468
Average Cadmium Concentration:	**mg/kg**	**--**	**423 [c]**	**424**	**437 [c]**	**437**	**497 [c]**	**497 [c]**
Replicate Chromium Analyses								
Replicate - 1	mg/kg	--	1,850	18.8	23.1	26.0	--	31.4
Replicate - 2	mg/kg	--	1,820	--	--	--	--	--
Replicate - 3	mg/kg	--	--	--	--	--	--	--
Average Chromium Concentration:	**mg/kg**	**--**	**1,835 [c]**	**18.8**	**23.1**	**26.0**	**--**	**31.4**
Replicate Lead Analyses								
Replicate - 1	mg/kg	--	571	587	655	642	--	713
Replicate - 2	mg/kg	--	597	--	--	--	--	--
Replicate - 3	mg/kg	--	--	--	--	--	--	--
Average Lead Concentration:	**mg/kg**	**--**	**584 [c]**	**587**	**655**	**642**	**--**	**713**

Table 2-5. (cont.)

		Colorado Smelter Composite Soil (Globeville)						
		< 2 mm	< 500 µm		< 250 µm		< 150 µm	
Chemical	Units	4/10/2003	4/25/2003	2/10/2004	4/10/2003	2/10/2004	4/10/2003	2/10/2004
Other Metals								
Aluminum	mg/kg	--	--	--	--	--	--	--
Antimony	mg/kg	--	--	16.1	12.9	19.3	--	18.7
Barium	mg/kg	--	--	--	--	--	--	--
Beryllium	mg/kg	--	--	1.0 *U*	1.0 *U*	1.0 *U*	--	1.0 *U*
Calcium	mg/kg	--	--	--	--	--	--	--
Cobalt	mg/kg	--	--	--	--	--	--	--
Copper	mg/kg	--	--	80.0	85.0	89.3	--	93.9
Iron	mg/kg	--	--	15,800	--	20,500	--	22,700
Magnesium	mg/kg	--	--	--	--	--	--	--
Manganese	mg/kg	--	--	479	--	510	--	525
Mercury	mg/kg	--	--	7.38	7.67	8.04	--	9.69
Nickel	mg/kg	--	--	13.2	16.3	16.7	--	18.7
Phosphorus	mg/kg	--	--	673	--	804	--	871
Potassium	mg/kg	--	--	--	--	--	--	--
Selenium	mg/kg	--	--	14.8	14.0 [c]	14.1	--	17.6
Silver	mg/kg	--	--	2.0 *U*	2.55	2.50	--	2.10
Sodium	mg/kg	--	--	--	--	--	--	--
Thallium	mg/kg	--	--	13.6	15.2 [c]	14.3	--	16.3
Vanadium	mg/kg	--	--	--	--	--	--	--
Zinc	mg/kg	--	--	1,200	1,200	1,310	--	1,420

Note: Analysis performed by Columbia Analytical Services unless noted otherwise

-- – Not applicable or not available

U – undetected; value represents reporting limit for CAS analyses; value represent detection limit for ACZ analyses

[a] 50/50 mix of Dugway Proving Grounds #3B and Picatinny Arsenal.

[b] Analysis by ACZ Laboratories (10/2/2002 Pt Mugu #1B sample sent to ACZ).

[c] Average of field replicates.

[d] Average of two results by different methods: 300 mg/kg using method 6010B and 269 mg/kg using method 7060A.

[e] Sample sent to CAS by Stan Casteel (Exponent tag # 57171, orginally sent to Stan on 5/2/2003).

[f] Analysis by Auburn University (samples sent 3/31/2004).

3 Determining Metals that Drive Health-Based Remedial Decisions for Soils at U.S. Department of Defense Sites

3.1 Overview

The primary objective of research that Exponent conducted for SERDP was to develop bench-scale extraction tests to predict human and ecological exposures to metals in soil. Using this research, the tests developed would yield inexpensive tools for quickly determining the bioavailability of metals in soils at hazardous waste sites. However, before the planned research could begin, it was necessary to decide which particular metals should be evaluated. By identifying the target metals, Exponent could focus the research on the metals that typically drive remedial decisions at DoD sites, while also providing SERDP with information to aid in focusing future research efforts. This phase of the project culminated in development of a white paper that was submitted to SERDP, as well as a published manuscript. Both of these work products are provided in Supplemental Materials for Section 3.

3.2 Objectives

This section details how Exponent decided which metals are most prevalent at DoD sites throughout the country, and which metals drive the need for remediation at these sites. Remediation decisions are typically driven by the amount of metal present in soils that could actually be absorbed by human or ecological receptors (i.e., the bioavailability of the metal). SERDP's immediate needs would be best served by tailoring the research to a few specific metals.

This research was structured to answer the following specific questions:

- What metals potentially drive risk-based remedial decisions at DoD facilities?
- For facilities where more than one metal exceeds risk-based screening criteria, what are the metals of concern, and how do they compare in perceived importance?
- For the metals that most often exceed the screening criteria, what is the receptor of greatest concern (human or ecological)?

3.3 Methods

3.3.1 Database Construction

To determine which metals are driving remedial decisions at DoD sites, it was necessary first to solicit information, such as databases, from various organizations that are involved with remediation. These organizations included the Army, Navy, Air Force, EPA, Federal Facilities Restoration and Reuse Office (FFRRO), Comprehensive Environmental Response, Compensation, and Liability Information System (CERCLIS), EPA Records of Decision, and the DoD Environmental Cleanup Office.

Individuals were contacted at the various organizations to identify and gain access to databases that could help determine which metals are of most concern. No single database existed that contains the entirety of the information we sought. Five databases were deemed to contain relevant information, and although other databases were identified, they were not suitable due to their generalized format or the cumbersome extraction approach that would have been required. Even among the five databases that were used, it was necessary to extract the relevant information, combine the extracted data into a single database, and format it for appropriate comparisons so that it could be queried.

3.3.2 Use of Screening Criteria to Assess and Compare Risks

The compiled database contained soil concentration data for sites that are known to have metals in soil. The concentrations were compared to human and ecological risk-based screening criteria to determine which metals most frequently exceeded the criteria. The criteria are risk based (i.e., derived from toxicity information and assumptions regarding potential exposure levels), and are used to determine whether additional study is warranted. Exceeding screening levels suggests the potential need for further evaluation, or the screening levels may serve as preliminary remediation goals. Therefore, by comparing the standardized data to the screening criteria, preliminary conclusions could be made for SERDP regarding which metals may warrant potential remediation at DoD sites.

As mentioned above, metal concentration data were compared against two sets of screening criteria—human health and ecological. The human health screening criteria were obtained from EPA's Soil Screening Guidance, which is a tool that the Agency developed to help standardize and accelerate the evaluation and cleanup of contaminated soils at sites on the National Priorities List for which future land use is slated as residential. The ecological screening criteria were based on Ecological Soil Screening Levels (U.S. EPA 2000) for mammals and birds, if available; if not, the Preliminary Remediation Goals for Ecological Endpoints (Efroymson et al. 1997) for American woodcock were used. Please refer to the manuscript (Supplemental Materials for Section 3) for a more detailed discussion of the methods and criteria that were used.

3.4 Results

3.4.1 What metals potentially drive risk-based remedial decisions at DoD facilities?

The first set of analyses was conducted to answer the above question. After analyzing the database that was created by assembling data from five different sources, the database was first queried to determine which metals most frequently exceeded the health-based screening criteria. For the human health screening, the metal concentrations were compared to residential and industrial criteria, and for the ecological screening, the metal concentrations were compared to mammalian and avian criteria. The results denoting the percentage of sites that exceeded criteria are presented in Figures 3-1 and 3-2.

Figure 3-1 indicates that lead, arsenic, chromium, cadmium, and antimony most commonly exceed residential and industrial human health screening criteria. Figure 3-2 suggests that lead, zinc, mercury, chromium, selenium, and cadmium most commonly exceed avian and mammalian ecological screening criteria.

3.4.2 For facilities where more than one metal exceeds risk-based screening criteria, what are the metals of concern, and how do they compare in perceived importance?

In the second set of analyses, the five sites presenting the highest potential concern were selected from the compiled database. For those five sites with the highest overall ratio of screening level to site soil concentrations, we determined what metals were present at concentrations above screening values. This portion of the research was undertaken to determine the relative contribution from metals in soil at facilities where multiple metals exceed screening criteria, thereby helping to distinguish how metals at DoD facilities compare in perceived importance.

The results indicated that lead consistently appears as a metal that exceeds screening criteria for human receptors, and selenium consistently appeared as the metal that exceeded screening criteria when avian receptors were the focus of the screening assessments. Other than that, none of the metals shows an ordered pattern in terms of driving exceedances.

3.4.3 For the metals that most often exceed the screening criteria, what is the receptor of greatest concern (human or ecological)?

The final analysis was designed to determine what receptor (human in a residential setting, or human in an industrial setting, or ecological mammalian or ecological avian) is of primary importance for the metals associated with the highest exceedance of screening criteria, across all DoD sites evaluated. Screening criteria for ecological receptors (mammalian and avian) were exceeded at more sites than those for human receptors (residential and industrial). The results could be interpreted to indicate that ecological receptors are at greater risk from metals present

in soil at DoD sites than are humans, but these results more likely reflect the conservative nature and uncertainty associated with the ecological screening criteria.

3.4.4 Agency Staff Interviews

In addition to the database that was created to provide SERDP with information regarding what metals drive remedial decisions at DoD sites, we also contacted professional staff (either regional toxicologists or the Regional Contact for the FFRRO) within each EPA region. Five specific questions were posed to each contact:

- Which DoD facilities present risks from potential exposures to metals in soils?
- Which specific metals are of concern?
- Which receptors (human or ecological) are of concern for metals in soils?
- Which human and ecological exposure pathways are potentially of concern for metals in soils?
- Which human exposure scenarios (e.g., workers, residents, trespassers) are potentially of concern for exposure to metals in soils?

The information from these individuals was generally anecdotal, and none of the individuals had compiled information from the DoD sites within their region. For some regions, anecdotal evidence suggests that metals are not driving risks at DoD facilities, but rather, organic compounds are the primary concern, and in nearly all instances, human receptors were driving remedial decisions, and ingestion of soils was the exposure pathway of concern. Ecological receptors or other exposure pathways were rarely mentioned.

3.4.5 Overall Conclusions

Based on this research that was conducted for SERDP, lead was the most frequent soil contaminant associated with DoD sites that exceeded screening criteria, for both human health and ecological scenarios. Agency staff interviews also indicated that lead was the top metal of concern for human health. Other metals that have been determined to be of concern for human health include arsenic, chromium, cadmium, and antimony (Figure 3-1). The most frequent metals of concern based on the ecological screening criteria were lead, zinc, mercury, chromium, and selenium for birds, and arsenic for mammals (Figure 3-2). For a more detailed report on these findings, please refer to the paper that was published in the *International Journal of Human and Ecological Risk Assessment* (Supplemental Materials for Section 3).

3.5 References

U.S. EPA. 2000. Ecological soil screening level guidance, draft. U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response, Washington, DC.

Efroymson, R.A., G.W. Suter II, B.E. Sample, and D.S. Jones. 1997. Preliminary remediation goals for ecological endpoints. ES/ER/TM-162/R2. U.S. Department of Energy, Office of Environmental Management, Oak Ridge National Laboratory.

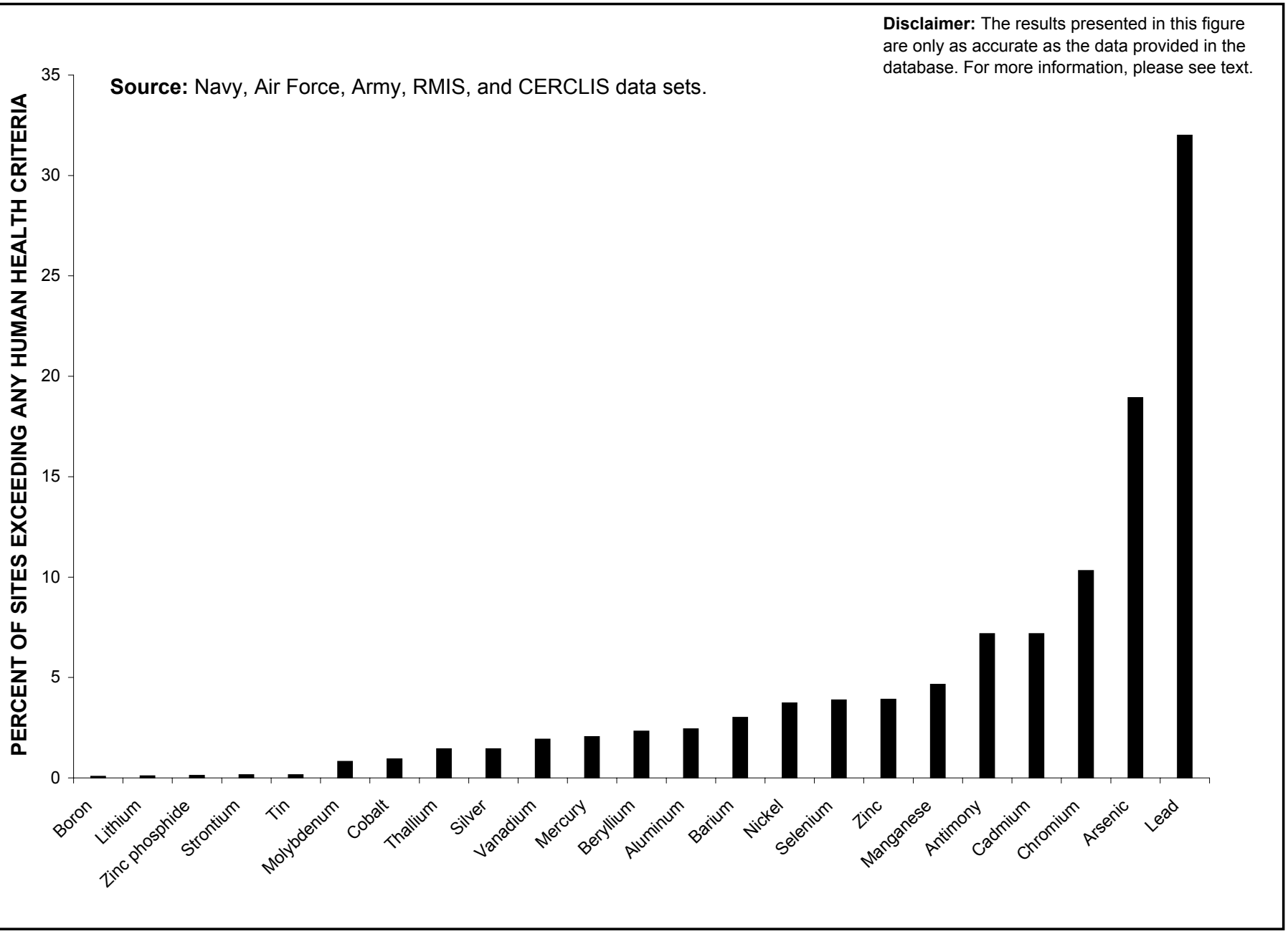


Figure 3-1. Indicates that lead, arsenic, chromium, cadmium, and antimony most commonly exceed residential and industrial human health screening criteria

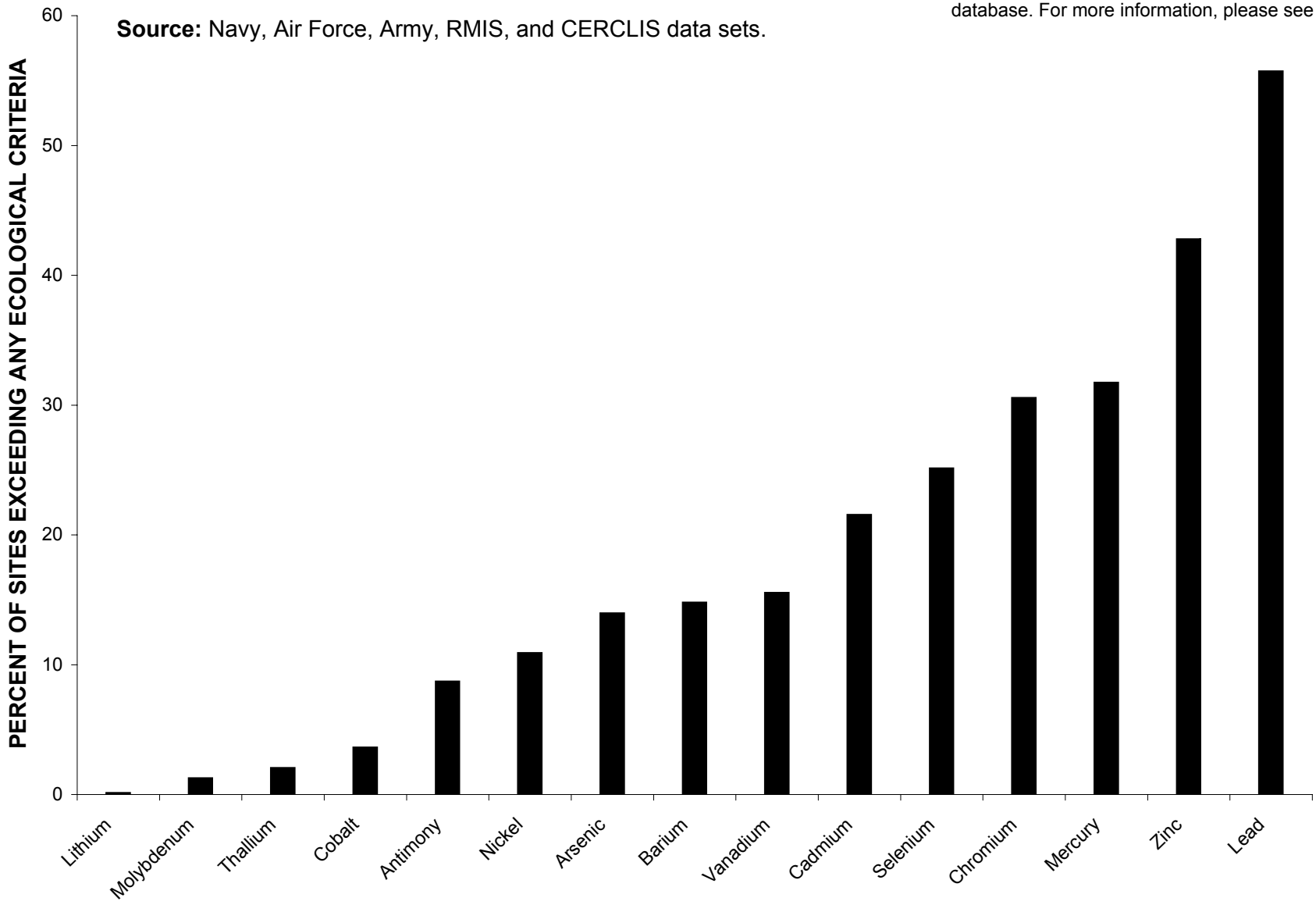


Figure 3-2. Suggests that lead, zinc, mercury, chromium, selenium, and cadmium most commonly exceed avian and mammalian ecological screening criteria

4 Oral Bioavailability of Metals to Humans

Based on the research that was conducted for SERDP and reported in Section 3, lead was the most frequent soil contaminant associated with DoD sites that exceeded screening criteria for human health exposure scenarios. Other metals that were determined to be of concern for human health include arsenic, cadmium, chromium, and antimony. Because EPA and others have already devoted substantial effort to developing research methods and *in vitro* approaches to estimate the relative oral bioavailability of lead, this metal was not deemed a priority for the SERDP research for human receptors. Instead, efforts were focused on evaluating some of the other metals that were identified as priorities—specifically, arsenic and cadmium. Initially, some effort was also directed toward understanding absorption associated with exposures to chromium. However, early evaluations indicated that bioavailability was a less important issue for chromium than understanding the form of chromium (i.e., trivalent or hexavalent) that is absorbed by biological systems. Because elucidating the form of absorbed chromium was beyond the scope of this SERDP-funded research, attention was instead focused on arsenic and cadmium.

4.1 Arsenic

The protocol for the oral arsenic study (included in Supplemental Materials for Section 4) was finalized and received DoD approval. Originally, the intent of the research design was to measure arsenic bioavailability from soils using the *Cebus* monkey, as described previously (Roberts et al. 2002). The *Cebus* monkeys used in the published study were being retired from research, and replacements were needed. We experienced difficulty finding a source for *Cebus* monkeys at the time, and consequently explored the possibility of changing monkey species. Ultimately, cynomolgus monkeys were selected for the replacements, for a number of reasons:

1. They were readily available from commercial suppliers.
2. A previous study (Freeman et al. 1995) had used cynomolgus monkeys successfully to measure the relative oral bioavailability of arsenic from soils.
3. The *Cebus* monkeys were prone to vomit their doses, and several of the dosings had to be repeated. Experience of others with cynomolgus monkeys suggested that vomiting of doses would not be a problem, allowing more expeditious completion of the experiments.
4. Cynomolgus monkeys could be obtained that were chair-trained, offering the opportunity to modify the experimental protocol to eliminate some of the sedative drugs that were used in the *Cebus* protocol. Reducing the use of sedative drugs was considered desirable, in that it would more closely mimic circumstances of human exposure.

Seven cynomolgus monkeys were received at the University of Florida and passed an initial quarantine period. All monkeys were successfully dosed via gavage, and urine samples were

collected and sent to Battelle for analysis. Battelle conducted trial analyses on the urine and changed the analytical procedure from graphite furnace atomic absorption spectrometry (GFAAS) to inductively coupled plasma/mass spectroscopy (ICP/MS), to decrease by two orders of magnitude the practical quantitation limit for arsenic in monkey urine.

Initial problems related to poor urinary arsenic recovery from Plexiglass trays positioned beneath the metabolism cages were resolved, and the improved procedure for recovery from Plexiglass was developed and implemented. The 0.5-mg/kg body weight and 0.1-mg/kg body weight dose of sodium arsenate were administered to the monkeys. Data from these trials indicated acceptable recovery rates, and are included in the Supplemental Materials for Section 4, as are the research protocol and the DoD approval form. The testing of soil samples commenced in January 2004.

Below is a summary of the research, including methods and results. Included in the Supplemental Materials for Section 4 is a preliminary draft manuscript of the research, which provides additional detail. Additional soil samples were obtained recently from industrial entities that co-funded this research. Once these additional soils have been dosed to the monkeys and the results obtained from the laboratory, a total of 13 soil substrates will have been tested in this research model. At that time, the latest data will be incorporated into the draft manuscript. Analyses are expected to be complete by July 2005, with submission of a manuscript for journal publication prior to September 2005.

4.1.1 Introduction

When assessing potential risks from arsenic contamination in soil, contemporary models and assumptions generally regard incidental soil ingestion as the dominant route of exposure. The default assumption typically used in risk assessments is that the extent of gastrointestinal absorption of arsenic from soil is equivalent to its absorption from water. Absorption from water is the relevant comparison in the case of arsenic, because the cancer slope factor used to estimate excess cancer risks was developed from studies of individuals exposed to arsenic in drinking water. Assuming equivalent absorption is the same as stating that the relative oral bioavailability (RBA) of arsenic from soil [compared to water] is 1.0, or 100%.

Although the principal of reduced bioavailability of arsenic from soils is well established, understanding of the factors that dictate bioavailability is limited. One of the obstacles in conducting research on factors that influence arsenic bioavailability is the limited number of soil samples for which bioavailability has been measured. Many of the soil samples for which bioavailability data have been published are no longer available or are inaccessible for research for other reasons. Consequently, there is a need for characterization of additional soils in terms of arsenic bioavailability, not only to support additional research on this topic, but also to better define the range of arsenic bioavailabilities that may exist in contaminated soils. For this project, RBA values for arsenic in soil from 10 different contaminated sites were measured in cynomolgus monkeys. Four of the 10 samples were from sites where the arsenic RBA in soils had been reported previously, allowing a comparison of RBA results in two different experimental models. Although there are obvious limitations in such a comparison, it

nonetheless offers the first look at the comparability of the *in vivo* bioavailability measurements in swine and monkey.

4.1.2 Materials and Methods

4.1.2.1 Animals and Animal Care

Seven young adult male cynomolgus (*Macaca fascicularis*) monkeys, 4–5 kg body weight, were purchased from Primate Products, Inc. (Miami, Florida). Prior to and during the studies, the monkeys were fed a low-arsenic pelletized diet (Bio-Serv, Frenchtown, New Jersey). This diet was necessary because background exposures to arsenic from the diet can obscure detection of arsenic from soils. All procedures involving the animals were approved by the Institutional Animal Care and Use Committee of the University of Florida.

4.1.2.2 Soil Samples

Some of the soil samples tested had been included in previous studies of arsenic bioavailability or bioaccessibility. These soil samples had been previously sieved to 250 μm, and no further processing was conducted. Other samples were collected from selected arsenic-contaminated sites. Soils were dried for at least 3 days at 30–38 °C, and sieved to 250 μm or less. The total arsenic concentration in an aliquot of the 250-μm sieved soil was measured using EPA Method 6010.

4.1.2.3 Animal Dosing and Sampling

At the beginning of each experiment, monkeys were fed a low-arsenic diet starting five days prior to the arsenic dose. Three days after initiating the diet, the animals were sedated and transferred to metal-free metabolic cages, where urine was collected for baseline arsenic levels prior to dosing. Each monkey was fasted overnight before dosing, but the low-arsenic diet was restored four hours after the animal was dosed and continued while the animal remained in the metabolism cage. Soil doses were administered as a slurry in metal-free, deionized water from a 60-mL irrigating syringe attached to the gastric tube. The mass of soil administered did not exceed 1 g per kg body weight. Sodium arsenate was administered from a 1.0-mg As/mL stock solution in deionized water, and the volume was adjusted to provide a dose no greater than 1.0 mg As/kg body weight. The syringe and gastric tube were flushed twice with metal-free, deionized water to ensure complete transfer of the dose to the stomach. Urine and feces were then collected for 4 days. After collection of urine and feces was complete, each animal was returned to its home cage for a period of 3 weeks before the next dosing period. This “wash-out” period ensured that urinary and fecal arsenic concentrations returned to baseline levels. Evaluation of pre-dosing urine samples collected over the course of the study confirmed that no arsenic was carried over from one dose to the next under these conditions.

In one experiment, each animal was administered intravenously a single dose of sodium arsenate (1 mg As, as sodium arsenate, per kg body weight in sterile saline). Animals were placed in the metabolism cage and fed a low-arsenic diet as detailed above. At the time of dosing, an intravenous line was placed in the leg via the saphenous vein. The arsenic dose was

introduced through the intravenous line over a period of about five minutes. The animal was then returned to the metabolism cage, where urine and feces were collected.

4.1.2.4 Quantification of Arsenic in Plasma, Urine, and Feces

Baseline urine samples were analyzed by ICP/MS. The limit of quantification for arsenic in urine was 0.3 μg/L. Urine samples collected after the dose, and all fecal samples, were analyzed by inductively coupled plasma–atomic emission spectrometry (ICP-AES). The limits of quantification for urine and feces using this method were 2.3 μg/L and 0.5 μg/g, respectively.

4.1.2.5 Calculation of Bioavailability

The relative oral bioavailability of arsenic from each test soil was measured in five individual animals using urinary excretion data. As described in Section 4.1.3 - Results, each animal received, on separate occasions, three doses of sodium arsenate by gavage—0.25, 0.5, and 1.0 mg/kg (as arsenic). Measurements of arsenic in urine over two days prior to dosing were used to establish the baseline arsenic excretion due to diet for each subject in each experiment. This "background" was subtracted from the total arsenic excreted in urine after the dose to calculate the excretion resulting from arsenic administration. The percent of arsenic dose recovered in urine following each of the sodium arsenate doses was averaged for each animal. This average recovery was used as the reference value to adjust for urinary recovery following administration of arsenic in soil. For each soil sample, five animals were selected randomly, and a dose of the test soil was administered by gavage. The percent of administered arsenic in soil (based on arsenic concentration and administered soil volume) was divided by the sodium arsenate reference value for that animal to calculate the relative bioavailability of arsenic from soil. When total arsenic recovery was less than 70% after a soil dose in an animal, the RBA value was flagged, and the soil sample was re-administered. In all such instances, total recovery from the subsequent dose was greater than 70%, and the resultant RBA replaced the original, low-recovery value.

4.1.3 Results

Samples of arsenic-contaminated soil were obtained from 10 different sites. Information regarding the mineralogy of the soil was available for some, but not all, of the samples (Table 4-1). Total arsenic content for the soils tested ranged from 125 to 1,500 mg/kg (Table 4-1). The relative oral bioavailability (RBA) of arsenic in the soil sample was calculated for each subject. Mean (±SD) values obtained for each soil are presented in Table 4-2, along with the coefficient of variation (COV). The mean RBA values for the 10 soil samples varied from 5% to 31%. The COVs were less than 50%, except for the soil with the lowest RBA, which had a COV of 81% (RBA ranged from 0% to 11%). The RBA for arsenic for some of the soil samples had been determined previously in other animal models. For those soil samples, the RBA, as determined elsewhere, is also shown for comparison.

Because the RBA values for the various soil samples tested were all relatively low, and in some instances were less than that observed for the same or similar soil samples in the swine, an additional experiment was conducted to verify that this monkey model is, in fact, capable of

measuring high oral bioavailability. For this experiment, a low-arsenic-content soil (3.6 mg/kg) was spiked with sodium arsenate three hours before dosing. The spiked soil was administered to three animals by gavage in the same manner as the test soils. Urinary recovery of arsenic from the spiked soil (Table 4-3) was much higher than that observed for the test soils from contaminated sites, resulting in RBA values that averaged about 0.8, indicating that this research model is capable of demonstrating high RBA values for soluble forms of arsenic in soil.

4.1.4 Discussion

Two previous studies have used primates to evaluate the relative bioavailability of arsenic from soil. A Battelle study measured the relative oral bioavailability of arsenic from one soil and one house-dust sample collected near a Montana smelter site in female cynomolgus monkeys (Freeman et al. 1995). Roberts et al. (2002) used five male *Cebus* monkeys to measure the relative bioavailability of arsenic from five soil samples collected from contaminated sites in Florida. The urinary and fecal recovery of intravenously administered arsenic in female cynomolgus monkeys in the Battelle study matched closely the recoveries observed in male cynomolgus monkeys reported here. In the Battelle study, 76.5% ± 2.5% of the arsenic dose was recovered in urine, 3.2% ± 1.9% was recovered in feces, and total arsenic recovery was 79.7% ± 4.0% (mean ±SD). *Cebus* monkeys in the Florida study also excreted almost the entire intravenous dose in the urine, with an average total recovery for four animals of 66.8 ± 6.5%.

The percent of arsenic dose recovered in urine following a gavage dose of sodium arsenate was about 40% in cynomolgus monkeys in this study, compared with about 50% in *Cebus* monkeys in the Florida study, and almost 70% on average for cynomolgus monkeys in the Battelle study. The reason for the substantial difference in urinary excretion following oral sodium arsenate doses, particularly between studies using the same monkey species, is unclear. Total arsenic recoveries were also different, although the margin was smaller (about 80% in this study versus 95% in the Battelle study), suggesting that at least part of the difference lies in lower gastrointestinal absorption of arsenic in water by the monkeys used in this study. The difference cannot be explained by dose— the Battelle study used a gavage dose (0.62 mg/kg) in the middle of the range of doses (0.25–1.0 mg/kg) used in the study reported here. It is also difficult to explain based on experimental protocol. Both studies administered the sodium arsenate dose by gavage tube without anesthesia, followed by recovery of urine and feces in metabolism cages for similar lengths of time (five days in the Battelle study and four days in this study). Cage washes recovered nearly 90% of arsenic in urine (see Section 4.1.2 - Materials and Methods), so under-recovery of arsenic from the metabolism cages can be ruled out. The differences might be due to the sex of the animals (females in the Battelle study versus males in this study). Unfortunately, there are no studies of arsenic bioavailability that have included animals of both sexes to examine this possibility. It is also possible that different cynomolgus monkey strains were used in the two studies. Even though urinary arsenic recoveries following dosing with sodium arsenate in water vary among studies, each serves as a valid point of comparison within the study.

Among the 10 soil samples tested in this study, the mean RBA values ranged from 0.05 to 0.31. For some soil samples, the RBA values obtained from different subjects were variable, and the coefficient of variation was near 50% for half of the samples. Based on the variability in

recoveries following gavage treatment with sodium arsenate (Table 4-4), much of this variability may be intra-subject; that is, reflecting differences in absorption of arsenic on different experiment days. However, it is interesting to note that variability was small for the cattle dip vat soil, and that when tested previously in *Cebus* monkeys, this soil sample also produced very tight results. This suggests that some attribute of the soil may also influence variability in RBA results among different experimental subjects.

Four soil samples included in the study had RBA estimates available from other studies or models (Table 4-2). The Florida cattle dip vat soil was also tested in a previous study using the *Cebus* monkey (Roberts et al. 2002), with similar results (0.25 ± 0.03 in the *Cebus* versus 0.31 ± 0.04 in the cynomolgus monkey). The RBA for a Montana smelter soil, measured in the Battelle cynomolgus monkey study, was not substantially different from a soil sample from the same site measured here. The differences were larger for soils from two sites previously assessed using the swine model, with lower RBAs measured in the monkey. For the Colorado residential soil, the differences might simply reflect the fact that soil samples from different locations at the site were compared. In fact, in the swine studies conducted on soils from this site, the overall average estimate of RBA was determined to be 0.31; however, for the five soils from different areas of the site that were evaluated in the swine study, the range of RBA estimates extended from 0.18 to 0.45. The lowest RBA ("best estimate") of 0.18 from the samples from this site that were tested in the swine model, is quite close to the 0.17 (average) measured here in the monkey. It is not clear whether the difference between the findings from monkeys represents a different result, or consistent results but site variability. The difference for the Western iron slag sample is more difficult to explain, because splits from the same soil sample were used in both models.[1] The swine model uses a somewhat different protocol, involving multiple doses of arsenic in soil, but there is no reason to suspect *a priori* that the frequency of administration would affect absorption across the gastrointestinal tract. One important difference between the monkey and swine protocols is the volume of soil administered relative to body weight, with larger volumes administered to the monkey. To test whether this soil volume might interfere with arsenic absorption, leading to underestimates of relative bioavailability, spiked soil samples were tested in the monkey model. These spiked samples showed high bioavailability (Table 4-3), as would be expected, suggesting that the higher soil volume in the monkey model is not affecting the RBA estimates from this model. Strict comparisons between the two models are difficult to make based on the samples dosed in both models. Therefore, conclusions regarding tendencies or accuracy of the monkey and swine model are premature.

This study demonstrates reduced bioavailability of arsenic from soil samples from 10 sites, with RBA values ranging from 5% to about 30%. This is consistent with previous reports of the relative bioavailability of arsenic from soils in the literature. Soil samples differed in the RBA for arsenic due to properties that have yet to be completely elucidated. However, the presence of arsenic in insoluble mineralogy forms is likely a factor in controlling the RBA. The measurements provided in this study, and reference soils created, will be a valuable asset in understanding the variables that control the oral bioavailability of arsenic, as predictive tools to enhance the risk assessment of arsenic-contaminated sites.

---

[1] Note, the sample tested in this study was a split from the same bulk soil that was tested in swine, but it was not a split of the sieved fraction that was dosed to swine.

4.1.5 References

Casteel, S.W., T. Evans, M.E. Dunsmore, et al. 2001. Relative bioavailability of arsenic in soils from the VB I70 site. Final report. U.S. Environmental Protection Agency, Region VIII, Denver, CO.

Freeman, G.B., Schoof, R.A., Ruby, M.V, Davis, A.O., Dill, J.A., Liao, S.C., Lapin, C.A., and Bergstrom, P.D. (1995) Bioavailability of arsenic in soil and house dust impacted by smelter activities following oral administration in cynomolgus monkeys. Fund. Appl. Toxicol. 28:215-222.

Roberts, S.M., Weimer, W.R., Vinson, J.R.T., Munson, J.W., and Bergeron, R.J. (2002) Measurement of arsenic bioavailability in soil using a primate model. Toxicol. Sci. 67:303-310.

Rodriguez, R.R., and Basta, N.T. (1999) An in vitro gastrointestinal method to estimate bioavailable arsenic in contaminated soils and solid media. Environ. Sci. Technol. 33:642-649.

4.2 Cadmium

4.2.1 Background

Cadmium is a ubiquitous soil constituent, and may be present at higher concentrations in lead, zinc, and copper ores. Although ingestion of cadmium in food is the primary exposure route for humans, residents and workers in the vicinity of smelter sites and other industrial operations that use cadmium-containing materials may incur additional exposure to cadmium in soil. The study reported herein examined the influence of cadmium mineralogy and soil characteristics on the bioavailability of ingested soil cadmium relative to the bioavailability of a water-soluble form of cadmium (i.e., the relative bioavailability), using a swine model.

It has been estimated that environmental exposure to cadmium results in renal disease in 1% to 7% of the world's population (Klaassen et al. 1999; Satarug et al. 2000), principally due to consumption of rice and other foods that accumulate cadmium, with smokers being at higher risk due to having higher body burdens. Renal disease is thought to be triggered after renal cortex concentrations exceed some threshold concentration, often late in life (Goyer and Clarkson 2001).

The potential for chronic exposure to cadmium from soil to significantly increase body burden and lead to increased incidence or earlier onset of disease depends partly on the bioavailability of cadmium in this matrix. The relative bioavailability of metals from soil depends on multiple factors, including what metal phases are present and the characteristics of the specific soil. Cadmium can occur in soil as a complex mixture of solid-phase compounds of varying particle size and morphology, including discrete mineral phases, coprecipitated and sorbed species associated with soil minerals or organic matter, and dissolved species that may be complexed by a variety of organic and inorganic ligands. These characteristics affect the solubility, and hence, the relative bioavailability of metals from soil. For example, cadmium carbonate in soil is highly soluble, whereas cadmium sulfate and cadmium sulfide complexes are less soluble

(Kelley et al.. 2002). The soil pH is another important factor, with cadmium solubility in soil decreasing as soil pH increases, due to cadmium adsorption to soil particles and formation of irreversibly insoluble complexes.

Studies that have demonstrated reduced relative bioavailability of cadmium from soil have been conducted in rats (Kelley et al. 2002) and in juvenile swine (Schroder et al. 2003). An *in vitro* bioaccessibility model has also been used (Schroder et al. 2003). Swine are useful in assessing bioavailability because of the similarity in gastrointestinal parameters between swine and humans. Feeding behavior, gastrointestinal anatomy, acid secretion, and the development of small-intestinal absorption mechanisms are quite similar between swine and humans (Weis and LaVelle 1991). For these reasons, swine have been used as a surrogate for humans in the fields of pharmaceutical research and nutrition (Dodds 1982; Miller and Ullrey 1987). Juvenile animals are preferred, because metal absorption is frequently greater in younger animals, and thus, this model predicts uptake in children, who may have greater exposure than adults. The juvenile swine model has been used to assess the oral bioavailability of both lead and arsenic in soil, and the results from these studies have been used to develop relative bioavailability adjustments (RBA) for human health risk assessment by the U.S. Environmental Protection Agency (Kelley et al. 2002; NRC 2003); however, the model has not been modified to reflect the toxicokinetic behavior of cadmium. As noted above, cadmium accumulates in the kidney and liver. Thus, cadmium concentrations in blood, kidney, and liver may be used to estimate relative bioavailability of test substrates. In addition to assessing the relative bioavailability of cadmium from soils, this study examined the effects of dose and time-since-administration on blood levels of cadmium following ingestion.

The materials and methods used to conduct the study and analyze the data are described in depth in the manuscript that was submitted to the journal *Environmental Science and Technology* (Schoof et al.) and in the project report by Casteel et al. (see Supplemental Materials for Section 4). The soils used in this study were surficial samples (0–3 in.) from sites with elevated levels of cadmium in the soil, including Point Mugu, California (sample PTMG), smelter sites in Colorado (sample CO-SCS) and Oklahoma (sample OK-SS), and Dugway Proving Ground in Utah (sample DPGC). Anhydrous cadmium chloride ($CdCl_2$, Sigma) was used as the soluble cadmium reference material. The samples were tested for pH; total organic carbon (TOC); total carbon, from which total inorganic carbon (TIC) was calculated; cation exchange capacity (CEC); cadmium concentration (in triplicate); and metals concentrations (arsenic, chromium, copper, iron, lead, manganese, mercury, nickel, phosphorus, and zinc).

4.2.2 Results

4.2.2.1 Soil Chemistry and Cadmium Mineralogy

Soil chemistry and metals concentrations in the soil samples are tabulated in the manuscript (Supplemental Materials for Section 4). The reported cadmium concentrations were used to prepare soil doses that would achieve the target dose levels. Cadmium concentrations were far higher in the PTMG soil (4,109 mg/kg) than in the other soils (47 to 452 mg/kg), and the PTMG soil was also high in chromium, nickel, and phosphorus. The OK-SS soil had exceptionally high zinc concentrations. Values of pH in three of the four test soils were near neutral (7.43 to

7.55), while the DPGC soil exhibited a more basic pH (9.06). TOC values ranged from 1.90% to 4.98%, while TIC ranged from less than 0.05% to 1.51%. CEC values did not range widely among the soils—values were 52.2 to 70.1 meq/100 g.

Each test soil exhibited distinct cadmium mineralogy, with only a few forms dominating the cadmium-bearing mineral assemblage. These mineralogic forms are cadmium oxide (PTMG), cadmium-calcium-metal oxide (PTMG and CO-SCS), cadmium-metal oxide (CO-SCS), cadmium-metal sulfate (DPGC), and cadmium-iron oxide (OK-SS). All other cadmium-bearing phases were found to account for less than 8% of cadmium mineral mass.

4.2.2.2 Cadmium in Blood

Blood cadmium concentrations were initially at or below the method detection limit of 0.1 µg/L in all groups and remained at or below detection limits in the control-group animals. In animals given repeated oral doses of cadmium chloride and PTMG soil at doses of 60 µg/kg/day or greater, blood levels began to rise within 1 to 2 days, and continued to rise until the end of the study, day 15. For the other three soils (CO-SCS, OK-SS, and DPGC), only animals in the 60-µg/kg/day dose groups were sampled, and even at this dose, blood levels of cadmium were predominately at or below the detection limit of 0.1 µg/L for the duration of the study.

Although the same trends in blood cadmium concentrations were evident in bleed I (collected prior to the first daily dose) and bleed II (collected 2 hours after the second daily dose), the results for bleed II were more variable. This result is consistent with the rapidly changing blood cadmium concentrations associated with the absorption of cadmium after the dose was given. Bleed II was included in this study to try to capture data on peak blood cadmium concentrations, and the bleed II values were indeed greater than the bleed I values for the dose groups that exhibited a response in bleed I. As would be expected, the steep slope of the concentration-vs.-time curve during this interval leads to greater variability in the blood cadmium concentrations. Because of this increased variability in bleed II data, RBA calculations were based on data from bleed I.

The measurement endpoint used to quantify the blood cadmium response was the area under the curve (AUC) for blood cadmium concentration vs. time for days 0 to 14. The AUC was selected because it is the standard pharmacokinetic index of chemical uptake into the blood compartment, and is relatively insensitive to small variations in daily blood cadmium levels. The AUC was calculated using the trapezoidal rule to estimate the AUC between each time point that a blood cadmium value was measured (days 0, 6, 8, 10, 12, and 14), and summing the areas across all time intervals in the study. The mean AUC for each pig was then plotted against the body-weight-adjusted dose for that pig by dosing material. The dose-response patterns appear to be linear for the soluble reference material (cadmium chloride) and for the PTMG soil for bleed I. It was not possible to prepare dose-response curves based on blood data for the CO-SCS, OK-SS, and DPGC soils, because blood cadmium results were at or below detection limits.

Liver and kidney data were subjected to the weighted simultaneous linear regression data analysis described above. The RBA values for each response endpoint (liver, kidney, and blood) for each soil are presented in Table 4-5. The RBAs for three of the soils (CO-SCS, OK-

SS, PTMG) were greater than 0.5, while the RBAs for the DPGC soil were substantially lower. For the CO-SCS, OK-SS, and DPGC soils, kidney and liver RBA estimates were in good agreement. For the PTMG soil, kidney and blood AUC RBA estimates were in good agreement, while liver estimates were much higher. The liver results for this soil appear to be an anomaly.

4.2.3 Discussion

In this study, the bioavailability of cadmium from four contaminated site soils was determined relative to soluble cadmium chloride in the blood, kidney, and liver of juvenile swine. All inorganic cadmium forms commonly present in soils induce toxicity by the same mechanism, so these forms can be considered together when assessing bioavailability. The oral toxicity reference values for cadmium are based on a number of chronic studies of renal disease in humans that formed the basis for a toxicokinetic model that was used to estimate the no-observed-adverse-effect level (NOAEL) from cumulative lifetime exposures (U.S. EPA 2004). Because the kidney is the primary target organ of toxicity for cadmium, RBA results for that tissue are considered most relevant for risk assessment.

Assuming that the kidney results should be given the greatest weight, the three soils with the greatest cadmium concentrations (PTMG, CO-SCS, and OK-SS) demonstrated modest reductions in bioavailability relative to cadmium chloride (RBA values of 0.60, 0.89, and 0.79, for each soil, respectively). In contrast, the DPGC soil yielded a considerably lower cadmium RBA of 0.18 based on kidney data. An examination of soil characteristics and cadmium mineralogy suggests that this outcome may be due to the more basic soil pH and high clay content of this soil, and the occurrence of a cadmium form not found in the other soils. Cadmium was present in the PTMG, CO-SCS, and OK-SS soils in a variety of cadmium oxide phases (cadmium-calcium-metal oxide, cadmium-metal oxide, cadmium oxide, and cadmium-iron oxide), and in the DPGC soil, as cadmium-metal sulfate. The basic pH and the high clay content in the DPGC soil would decrease metal solubility and may have contributed to the lower RBA values.

The cadmium-containing soils tested in the present study also had elevated concentrations of other metals that might induce the expression of metallothioneins (MTs), proteins that have been reported to affect cadmium toxicity or toxicokinetics (Klaassen et al. 1999; Miles et al. 2000; Pinot et al. 2000). Recently, renal and hepatic MT induction was shown to be increased in juvenile swine dosed with soils containing elevated zinc concentrations, while a similar induction was not observed in animals receiving cadmium chloride and no excess zinc (Turk et al. 2001). Based on the present study's finding that the soil with the much higher zinc level (OK-SS) has RBAs similar to the other two soils dominated by cadmium oxides, it does not appear that MT induction is a critical factor affecting cadmium bioavailability from soil in this model.

The relative bioavailability of soil cadmium has also been assessed in rats, resulting in RBA estimates somewhat lower than those from the juvenile swine model. A comparison of several studies to the results from this study revealed that studies in rats tend to produce lower RBA values than studies in swine. Different results are likely due to anatomical and physiological

differences between the two animal models, but the results among the several studies evaluated reflect sufficient consistency to lend confidence to our reported results.

This study provides further evidence of the value of the juvenile swine model in assessing the relative bioavailability of soil cadmium, as well as arsenic, lead, and perhaps other metals, and reinforces the importance of including soil characterization and mineralogical analyses in these studies. The three soils with similar chemical and physical characteristics yielded similar kidney RBA values, ranging from 0.60 to 0.89. Little difference was observed in RBAs for all the oxides of cadmium in these neutral-pH soils, regardless of mean particle size for the phase. In contrast, the alkaline soil with a cadmium sulfate phase had a much lower RBA, despite having the smallest mean particle size. This finding suggests that the solubility of the predominant cadmium phases may be a more significant factor in controlling relative bioavailability than is particle size.

4.2.4 References

Dodds, J.W. 1982. Fed. Proc. 41:247–256.

Goyer, R.A., and T.W. Clarkson. 2001. Toxic effects of metals. In: Klassen, C.D. (Ed.). Casarett & Doull's Toxicology: The Basic Science of Poisons, 6th ed. McGraw-Hill, New York, pp. 811–867.

Kelley, M.E., S.E. Brauning, R.A. Schoof, and M.V. Ruby. 2002. Assessing oral bioavailability of metals in soil. Battelle Press, Columbus, OH.

Klaassen, C.D., J. Liu, and S. Choudhuri. 1999. Annu. Rev. Pharmacol. Toxicol. 39:267–294.

Miles, A.T., G.M. Hawksworth, J.H. Beattie, and V. Rodilla. 2000. Crit. Rev. Biochem. Mol. Biol. 35:35–70.

Miller, E.R., and D.E. Ullrey. 1987. Annual Rev. Nutr. 7:381–382.

NRC. 2003. Bioavailability of contaminants in soils and sediments: Processes, tools, and applications. National Research Council, National Academy Press, Washington, DC.

Pinot, F., S.E. Kreps, M. Bachelet, et al. 2000. Rev. Environ. Health 15:229–323.

Satarug, S., M.R. Haswell-Elkins, and M.R. Moore. 2000. Br. J. Nutr. 84:791–802.

Schroder, J.L., N.T. Basta, J. Si, S.W. Casteel, T. Evans, and M. Payton. 2003. Environ. Sci. Technol. 37:1365–1370.

Turk, J.T., T.J. Evans, S.W. Casteel, and N.T. Basta. 2001. Toxicol. Sci. 60(Suppl.):414 (abstract).

U.S. EPA. 2004. Cadmium. Integrated Risk Information System (IRIS). U.S. Environmental Protection Agency. Available at http://www.epa.gov/iris/.

Weis, C.P., and J.M. LaVelle. 1991. Chem. Spec. Bioavail. 3:113–120.

Table 4-1. Soil arsenic mineralogy data (frequency % / arsenic mass distribution %)

	MTSS	WISS	FLCDV	CAMT	WAOS	NYOS	COSCS	CORS	COSS	FLCPS
Arsenopyrite	--		--	9.0/70.4	--	--			--	
As(metals) oxide	0.2/6.4		--	--	--	--			--	
Calcite	--		--	--	--	--			--	
Calcium arsenate ($CaAsO_4$)	--		--	--	--	--			--	
CrCuAs	--		--	--	--	--			--	
Clay	--		93.2/85.5	--	--	--			--	
Iron oxides (FeOOH)	29.3/68.2		5.3/14.4	68.2/27.2	18.6/1.1	9.5/6.9			32.7/22.2	
Iron sulfate ($FeSO_4$)	11.5/23.1		--	6.7/2.3	--	--			52.1/76.7	
Lead arsenate ($PbAsO_4$)	--		--	--	44.1/98.3	1.1/37.2			--	
Manganese oxides (MnOOH)	1.5/0.4		--	--	3.8/0.1	89/54.5			--	
Slag	57.5/1.9		--	--	--	--			--	
No. particles counted	*130*		*147*	*109*	*215*	*112*			*183*	
Arsenic concentration (mg/kg)	*650*		*189*	*300*	*301*	*125*			*1492*	

MTSS = Montana smelter soil
WISS = Western iron slag soil
FLCDV = Florida cattle dip vat soil
CAMT = California mine tailings
WAOS = Washington orchard soil
NYOS = New York orchard soil
COSCS= Colorado smelter composite soil
CORS = Colorado residential soil
COSS = Colorado smelter soil
FLCPS = Florida chemical plant soil

Table 4-2. Relative oral bioavailability of arsenic from 10 soil samples

		Alternative RBA Measurements		
Soil Sample	RBA[a]	RBA[b]	Animal Model	Ref.[c]
MTSS	0.13 ± 0.05 (36)	0.20	Cynomolgus monkey	1
WISS	0.13 ± 0.07 (52)	0.29	Swine	2
FLCDV	0.31 ± 0.04 (14)	0.25	*Cebus* monkey	3
CAMT	0.19 ± 0.02 (11)	--	--	--
WAOS	0.24 ± 0.09 (36)	--	--	--
NYOS	0.16 ± 0.08 (49)	--	--	--
COSCS	0.23 ± 0.13 (54)	--	--	--
CORS	0.17 ± 0.08 (49)	0.18–0.45	Swine	4
COSS	0.05 ± 0.04 (81)	--	--	--
FLCPS	0.08 ± 0.04 (50)	--	--	--

[a] Results expressed as mean ± SD (N=5); COV in parentheses. The RBA was calculated by dividing the percent of dose excreted in urine by the average percent of dose excreted in urine after administration of sodium arsenate in water by gavage for each animal.

[b] RBA was measured using urinary excretion data, although not necessarily using the same experimental approach as in the present study. RBAs for WISS and FLCDV were obtained in splits of the same soil sample. RBAs for MTSS and CORS were obtained from different soil samples from the same site.

[c] References: 1) Freeman et al. 1995; 2) Rodriguez et al. 1999; 3) Roberts et al. 2002; 4) Casteel et al. 2001.

MTSS = Montana smelter soil

WISS = Western iron slag soil

FLCDV = Florida cattle dip vat soil

CAMT = California mine tailings

WAOS = Washington orchard soil

NYOS = New York orchard soil

COSCS = Colorado smelter composite soil

CORS = Colorado residential soil

COSS = Colorado smelter soil

FLCPS = Florida chemical plant soil

Table 4-3. Arsenic recovery and RBA from a spiked soil sample[a]

Subject	% Dose in Urine	% Dose in Feces	% Total Recovery	RBA
7490	32.6	37.9	70.5	0.63
7630	34.0	35.7	69.7	0.97
7516	26.6	44.0	70.6	0.84
Mean ± SD	31.1 ± 3.9	39.2 ± 4.3	70.2 ± 0.5	0.81 ± 0.17

[a] Each animal received a single gavage dose of soil spiked with sodium arsenate (0.5 mg As per kg body weight, prepared three hours before dosing). The arsenic dose was calculated based on natural arsenic content in the soils (3.6 mg per kg soil) plus the arsenic added as sodium arsenate. The results reflect cumulative excretion in urine and feces over four days, expressed as a percent of administered dose. The RBA was calculated by dividing the percent of dose excreted in urine by the average percent of dose excreted in urine after administration of sodium arsenate in water by gavage for each animal.

Table 4-4. Urinary and fecal recovery of arsenic after a gavage dose of sodium arsenate

	Sodium Arsenate Dose (as As)[a]			
	0.25 mg/kg	0.50 mg/kg	1.0 mg/kg	Mean ± SD
% Dose in urine	35.6 ± 8.6	40.9 ± 6.0	45.3 ± 16.7	40.6 ± 10.1
% Dose in feces	45.9 ± 12.3	40.0 ± 9.2	40.5 ± 8.9	42.1 ± 9.1
% Total recovery	79.5 ± 5.1	80.9 ± 9.0	81.5 ± 6.2	80.7 ± 4.2

[a] Each animal (N=7) received, on separate experimental days, single doses of 0.25, 0.50, and 1.0 mg/kg As by gavage. The results reflect cumulative excretion in urine and feces over four days after the dose. There was no significant difference in the % of dose recovered in urine from the three sodium arsenate doses, nor was there a significant trend.

Table 4-5. Cadmium relative bioavailability estimates

	Pt. Mugu Soil (PTMG)	CO Smelter Soil (CO-SCS)	OK Smelter Soil (OK-SS)	Dugway Soil (DPGC)
Kidney				
RBA[a]	0.60	0.89	0.79	0.18
Lower bound[b]	0.52	0.61	0.53	0.07
Upper bound	0.69	1.19	1.07	0.30
Standard error	0.05	0.17	0.16	0.07
Liver				
RBA	0.96	0.66	0.76	0.09
Lower bound	0.80	0.33	0.40	–0.02
Upper bound	1.19	1.03	1.16	0.21
Standard error	0.11	0.21	0.22	0.07
Blood AUC (bleed1)				
RBA[c]	0.56	NA	NA	NA
Lower bound	0.40	--	--	--
Upper bound	0.89	--	--	--
Standard error	0.12	--	--	--

[a] RBA – Relative bioavailability adjustment

[b] The upper- and lower-bound values represent the upper and lower 95th percentile values on the RBA estimates (based on application of Fieller's formula).

[c] RBA based on blood area under the curve (AUC) was fit excluding the control (0 dose) data, because the response at 0 dose was non-detect.

NA – not analyzed

5 Dermal Absorption of Metals by Humans

As described above for oral exposures, analysis of the primary metals of concern for human receptors indicates that investigations should focus on arsenic and cadmium. Therefore, these two metals also served as the metals in soil that were investigated for potential dermal absorption.

5.1 Arsenic

Exponent coordinated with personnel at the University of California, San Francisco (UCSF) to conduct research on dermal absorption of arsenic from soils in Rhesus monkeys. The monkeys were trained at UCSF to accept a low-arsenic diet, and Exponent visited the labs to observe dosing procedures and make recommendations on how to improve the administration of test substances. Two "shared funding" sources were secured (through industry groups) that provided data on the urinary excretion fraction of an intravenous (i.v.) dose, as well as an additional trial for soluble arsenic.[2] Data from all research at UCSF that was coordinated by Exponent is available to SERDP researchers to facilitate interpretation of data regarding dermal absorption of arsenic from soils.

A study of dermal arsenic absorption from residue on wood treated with chromated copper arsenate (CCA) was also completed in this animal model under shared funding. This work yielded valuable insight into working with this dermal arsenic model. A manuscript presenting the initial results of the dermal arsenic study, using soluble arsenic and arsenic from CCA-treated wood, has been published (Wester et al. 2004). The manuscript, titled "*In Vivo* Percutaneous Absorption of Arsenic from Water and CCA-Treated Wood Residue," is included in the Supplemental Materials for Section 5. The results of this research indicated that the model produces reliable results. These findings support that absorption of soluble arsenic in solution is consistent with results from prior research using the more sensitive methods that required application of radiolabeled arsenic—a requirement that prevented testing of soils from existing sites. Additionally, the results from this research indicate that the absorption of arsenic from the complex CCA-wood matrix is negligible (urinary arsenic excretion is not elevated above background), indicating that arsenic may exist in the environment in stable forms that are not available for dermal absorption.

Under the SERDP funding, pilot work was conducted to develop a research model that would ensure good contact of the test substrate with the skin of the monkeys, and would provide the monkeys with a low-arsenic diet to allow detection of small amounts of absorbed arsenic. Subsequent to stabilizing the research model, four soil samples have been tested. Originally, the expectation was to evaluate soil samples from four distinct sites. However, based on critical review of early results, a professional reviewer suggested that the dermal absorption of arsenic

[2] Some of this funding was also directed toward assessing dermal absorption of arsenic from a CCA-treated wood matrix. This dosing was conducted during a delay in SERDP funding, and did not affect the schedule for SERDP samples. In fact, allowing use of the data from dosing of soluble arsenic under this funding expedited the SERDP-funded research.

may be controlled by the hydration level of the skin, with dissolution/absorption reactions allowed only from the aqueous phase. Therefore, rather than evaluating samples from four sites, the SERDP funding was used to evaluate two soils, each dosed dry and wet.

5.1.1 *In Vivo* Model for Percutaneous Absorption

Female Rhesus monkeys were selected for this research because of their ability to duplicate the biodynamics of percutaneous absorption in humans, and because previous studies of percutaneous arsenic absorption have used this same model. Prior research indicates that percutaneous absorption in the Rhesus monkey is similar to absorption in humans across a variety of chemicals and a range of dermal penetration characteristics (Wester and Maibach 1975). This research indicates that measurements from the monkey are just slightly higher than their counterparts in the human. Results from other species (pig, rat, rabbit) are not nearly as close to the values measured in man, and indicate that, of the species tested, absorption in the monkey is closest to that in the human.

The monkeys were approximately 20 years old, which is the same approximate age as the monkeys used in the previous dermal arsenic absorption research (Wester et al. 1993). The animals reside within the monkey colony maintained by UCSF, and had not been used in active research for 18 months. Prior to the beginning of the SERDP-related research, no topical doses had been applied to the skin of these animals for more than 4 years.

Each topical dose was applied to a pre-measured 100-cm^2 area of abdominal skin of three monkeys. The dosing area was demarked by "masking" the boundaries with a single layer of Tegaderm™ (a water-vapor-permeable adhesive membrane available from 3M)[3] and then was dosed by spreading the fluid (5 $\mu L/cm^2$) or residue (4 mg/cm^2) evenly across the 100-cm^2 dosing area. The dosing area was then covered with a layer of Tegaderm™ to ensure that the material remained in contact with the skin. The Tegaderm™ patch over the dosing area extended well beyond the boundaries of the exposure area. In addition to the Tegaderm™ patch, the abdomen of each monkey was wrapped with Spandage Instant Stretch Bandage[4] to ensure that the applied dose was kept in direct contact with the skin throughout the dosing period. This bandage is of a web construction; most of the Tegaderm™ was exposed to the open air for moisture and air exchange. Following application of the topical doses, the monkeys were placed in metabolic restraint chairs for the duration of the 8-hour dosing period. The 8-hour dosing period was selected to represent an upper bound of time that an individual might remain in contact with residues, and is also the upper limit of time that the monkey can remain in the metabolic restraint chair. During this time, the monkeys had free access to water, but were restricted from touching their abdominal area. Researchers remained in the room and interacted with the monkeys, and the monkeys were hand-fed bananas and liquid diet during this stage.

Urine was collected during the 8-hour dosing period in a pan under the metabolic chair. After 8 hours, the monkeys were removed from the chairs, the Spandage bandage and Tegaderm™ patch were removed, and the applied doses were removed using a soap and water wash

---

[3] 3M Tegaderm™ (1629) (3M Health Care, St. Paul, Minnesota 55144).

[4] Spandage Instant Stretch Bandage (MEDI-TECH International Corp. Brooklyn, New York 11242).

(50/50 v/v, soap and water, followed by water, soap, and two final water washes). The monkeys were then transferred to metabolic cages for continued urine collection over the following 7 days.

As with humans, significant exposure to arsenic occurs from the normal diet (Schoof 1999a,b; Yost 2003). Urinary excretion of total arsenic for Rhesus monkeys on the standard diet of Purina Monkey Chow falls in the range of 5 to 15 μg/day—levels that would obscure accurate detection of the arsenic that might be absorbed following topical application of arsenic. Therefore, the monkeys were provided a low-arsenic diet (Primate Liquidiet from BioServe, Inc.) for 7 days prior to each dose. The powdered Liquidiet formulation also was prepared into meal bars, which were provided *ad libitum* to the monkeys during the research period (7 days prior to dosing through 7 days after dosing). The diet was supplemented with pieces of banana and apple, which are both known to be low in total arsenic (Schoof et al. 1999a). De-ionized water was provided *ad libitum*. The liquid diet was provided as both liquid and solid forms. Preference was for the solid form. The monkeys maintained their body weight during the study.

The monkey urine samples were preserved with nitric acid (2%) at the time of collection, and shipped to Battelle Pacific Northwest Laboratories in Sequim, Washington, for analysis. At Battelle, the urine samples were acidified with an additional 2% (by volume) of concentrated nitric acid and analyzed for total arsenic by ICP/MS (Method 1638, U.S. EPA 2002). This method provides a method detection limit (MDL) of approximately 0.1 μg/L arsenic in monkey urine. Quality assurance and quality control (QA/QC) samples included a method blank, duplicates, matrix spikes, and a laboratory control sample at a 5% frequency of analysis.

5.1.2 Study Design

An open crossover design was used, in which each animal is dosed in each of the trials (soluble arsenic in solution applied to the skin, soil applied to the skin, and i.v. injection), with a washout period of at least 14 days between each dose. This design allows for each animal to serve as its own internal control.

The intravenous dose (1,060 μg arsenic/monkey) was administered as a solution of sodium arsenate heptahydrate in de-ionized water (2,120 mg/L arsenic). For the intravenous dose, each monkey received 0.5 mL of the dosing solution injected into the saphenous vein. The intravenous dose was given while the monkeys were in their metabolic cages, so the monkeys did not spend any time in the metabolic restraint chairs, as they did with the topical doses.

For the soluble arsenic dose, arsenic was administered in water onto the monkey's skin at an application rate of 5 μL/cm^2 evenly applied across 100 cm^2 of skin, to achieve a total dermal dose of 1,430 μg arsenic. The solution was prepared from sodium arsenate heptahydrate in de-ionized water, which was acidified with 1% nitric acid (trace-metal grade).

The soil samples selected for evaluation in the percutaneous absorption study were surficial soil samples, one collected adjacent to a pesticide production facility in Middleport, New York, that had historically produced arsenical pesticides, and the other collected from a residential area in Denver, Colorado, with a history of herbicide application. A very fine particle size fraction (<150 μm) was selected for study, because the fines are the soil fraction that would be expected

to adhere to the surface of the skin, and the smaller particle size has a larger surface area from which absorption may occur. For very fine soil (i.e., silty clay), a loading of 5.4 mg/cm^2 of skin results in a monolayer (U.S. EPA 2001). In order not to exceed a monolayer of application, a percutaneous soil loading rate of 4 mg/cm^2 was selected for the study. This soil application rate resulted in a total dose of 560 μg arsenic and 492 μg arsenic for the two soils, respectively.

5.1.3 Results

Table 5-1 provides a summary of the applied doses and associated results of the percutaneous absorption research. Displayed on the table is information identifying the dosing trial, arsenic concentration in the dosed substrate, the mass of arsenic applied to the skin, and the calculated absorption, expressed as the percent of the applied dose that was absorbed. This includes a summary of the data regarding percutaneous absorption of arsenic when applied as soluble arsenic in solution, as well as from dry and wet application of the two soils evaluated. For reference, the 1993 research conducted by Wester, which has served as the basis for regulatory default assumptions on dermal absorption of arsenic (using radiolabled arsenic), is provided in the Supplemental Materials for Section 5. For the Middleport soil, the wet application was tested in two separate dosing trials. This was done to evaluate the consistency (i.e., intra-animal variability) across dosing trials and to increase the number of doses for this particular soil, in order to add statistical power.

For the soluble dose, absorption rates ranged from 0.32 to 4.3% for the three monkeys in the study, with an average absorption rate of 2.9% for the group. These results are consistent with results from Wester et al. (1993), wherein absorption rates were relatively consistent (range of 2% to 6.4%) despite a five-orders-of-magnitude change in the dose levels applied. Converse to the results for soluble arsenic, data from dermal application of arsenic in soils indicate virtually no absorption. Results from statistical evaluation indicate that the urinary arsenic excretion levels in the animal exposed to arsenic in soil are not statistically different than background. This is also depicted in Figure 5-1, which shows urinary mass excretion in 24-hour increments following each dosing trial for each monkey. In this figure, the increased urinary arsenic excretion following exposure to soluble arsenic in solution is apparent in the first 24 hours post dosing, returning to baseline by 48 or 72 hours post dosing. Following application of arsenic in soils, no similar increase in urinary arsenic excretion can be observed.

5.1.4 Discussion

The results of this research indicate that the methodology developed under this component of the SERDP project can be used to evaluate dermally absorbed arsenic from environmental samples. The development of this method was challenging because of the high degree of background arsenic exposure from the diet and the potential for that background exposure to obscure any signal from a dermally applied dose. Use of the low-arsenic diet resulted in an approximately 4-fold decrease in urinary arsenic excretion relative to the standard primate diet, and allowed for detection above background of a dermally applied soluble arsenic dose, without use of radiolabel. Findings from this research also include:

- Repeated dosing of the same soil under the same conditions (i.e., two separate dosing trials of the Middleport soil applied wet) resulted in highly reproducible results.
- Percutaneous absorption of arsenic from soils did not result in urinary arsenic levels that were distinguishable from background for soils applied either wet or dry. The calculated range of absorption from the wet soils extends higher than calculated for dry soils, but remains so low that it is impossible to determine whether this indicates a true increase in absorption from wet soils or simply variability within the measurements.
- Calculated values of percutaneous absorption of arsenic from the two soils tested were not different: average absorption of 0.24% vs 0.18% for the Colorado residential soil and the New York soil applied dry, and corresponding averages of 0.50% and 0.39% when applied and wet.

Although the results indicate that the urinary arsenic levels following topical administration of arsenic in soils are not distinguishable from background, the non-zero values for background urinary arsenic excretion, and the variability of the measured background values, impose some limits regarding the sensitivity of the model to detect an absorbed dose. A statistical evaluation using a comparison of means (t-test) for our data indicates that the absorbed dose would need to be in the range of 0.10 to 0.14 of the applied dose from Middleport soils, or 0.12 to 0.16 of the applied dose for the Denver residential soils. Thus, while these data suggest that there may not be any dermal absorption of arsenic from the soils (no monkey demonstrated urinary arsenic excretion that was statistically different from background), the uncertainty associated with the research model tells us that dermal absorption of arsenic from soils is at least an order of magnitude lower than absorption from soluble arsenic in solution.

5.1.5 References

Schoof, R. 1999a. A market basket survey of inorganic arsenic in food. Food Chem. Toxicol. 37(8):839–846.

Schoof, R. 1999b. Dietary exposure to inorganic arsenic. In: W.R. Chappell, C.O. Abernathy, and R.L. Calderon (Eds.). Proc. Third International Conference on Arsenic Exposure and Health Effects, pp. 81–88. Elsevier Sciences Ltd.

U.S. EPA. 2001. Risk assessment guidance for Superfund. Volume I: Human health evaluation manual (Part E, Supplemental guidance for dermal risk assessment), Interim Draft, September. U.S. Environmental Protection Agency, Office of Emergency and Remedial Response, Washington, DC.

U.S. EPA. 2002. Analysis of multi-media, multi-concentration samples for metals by 200-series metals methods and 1600-series trace metals methods. U.S. Environmental Protection Agency, Office of Water, Washington, DC.

Wester, R.C., H.I. Maibach, L. Sedik, J. Melendres, and M. Wade. 1993. *In vivo* and *in vitro* percutaneous absorption and skin decontamination of arsenic from water and soil. Fund. Appl. Toxicol. 20:336–340.

Wester, R.C., and H.I. Maibach. 1975. Percutaneous absorption in the rhesus monkey compared to man. Tox. Appl. Pharmacol. 32:394–398.

Wester, R.C., L. Hui, S. Barbadillo, et al. 2004. *In vivo* percutaneous absorption of arsenic from water and CCA-treated wood residue. Toxicol. Sci. 79:287–295.

Yost, L.J., S.-H. Tao, S.K. Egan, L.M. Barraj, K.M. Smith, J.S. Tsuji, Y.W. Lowney, R.A. Schoof, and N.J. Rachman. 2004. Estimation of dietary intake of inorganic arsenic in U.S. children. Hum. Ecol. Risk Assess. 10:473–483.

5.2 Cadmium

Dermal absorption of cadmium in soil was studied in human cadaver skin at the Dermatology Department at UCSF. A study design was prepared, and the pilot study was conducted to ensure that cadmium was detectable in the receptor fluid using two aliquots of reference material (cadmium chloride mixed with Yolo County soil, <150-μm size fraction) prepared to contain 400 and 2000 mg cadmium/kg soil (dry weight). Each of these reference materials was evaluated for percutaneous absorption of cadmium in one cadaver skin type. The study design is provided in the Supplemental Materials for Section 5.

Following a considerable amount of pilot work conducted with UCSF, it became apparent that limitations of the research design would not permit generation of meaningful data on the dermal absorption rate for cadmium in soil. This occurred because of the amount and variability of background concentrations of cadmium in the cadaver skin, and candidate receptor fluids that are used in the test method. As a result, the values that this test system provided for dermally absorbed cadmium from soil are well above values that would be meaningful, given what is already known about absorption of soluble forms of cadmium (i.e., the method is not adequately sensitive).

Given these preliminary results, Exponent conferred with Battelle and SERDP, and it was agreed that discontinuation of the dermal absorption research on cadmium was appropriate.

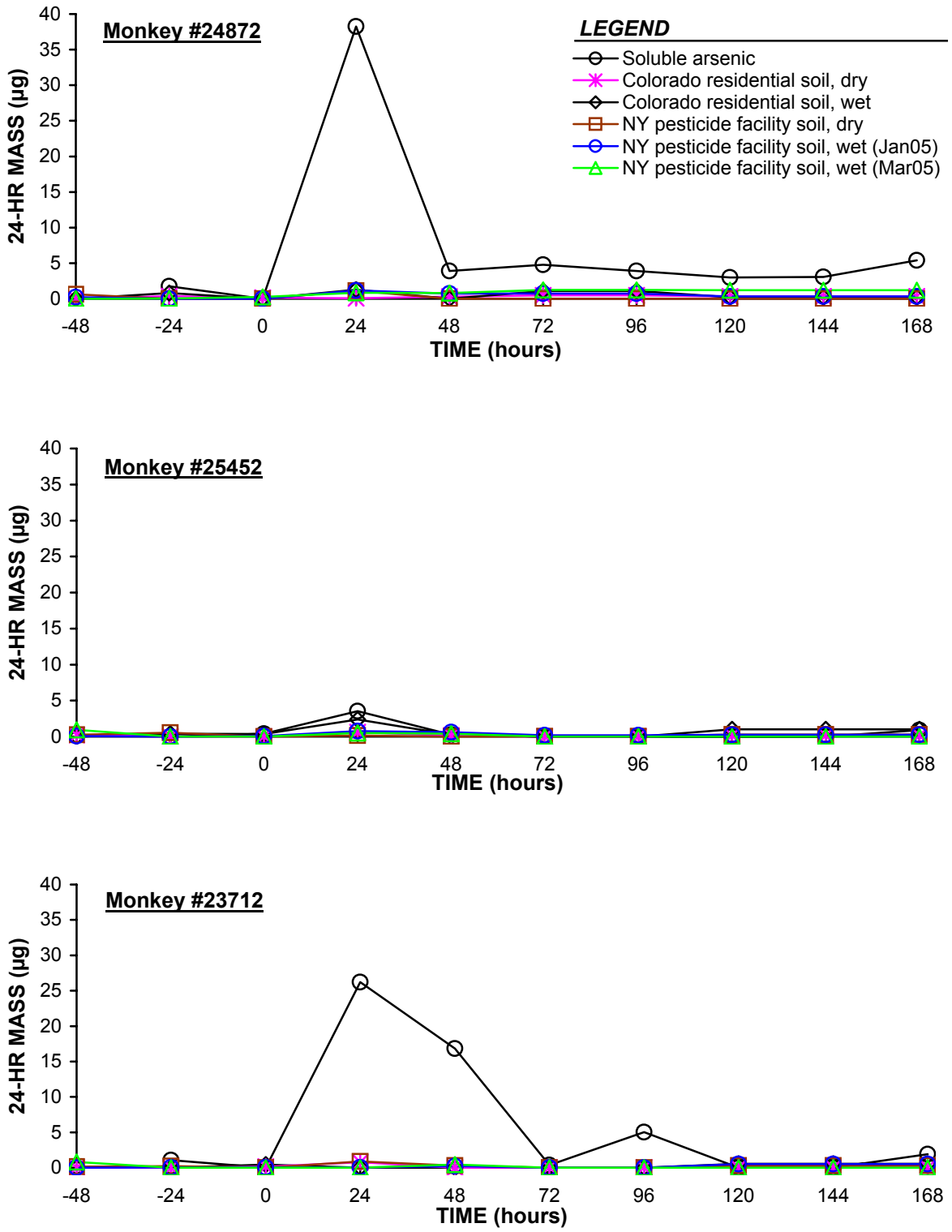


Figure 5-1. Urinary arsenic mass excretion (corrected) in 24-hour increments

Table 5-1. Summary of applied arsenic doses and percent absorption for dermal absorption studies

Study	Study Dates	Arsenic Concentration in Dosing Material	Volume of Dosing Material Administered	Arsenic Mass Dosed (µg)	Arsenic Mass per Unit Area (µg/cm^2)	Percent Absorption (0–96 hrs)	
						Average	Range
Intravenous	Jul. 2003	2,120 mg/L	0.5 mL	1,060	--	82%	80–84%
Soluble dose	Apr. 2003	2,860 mg/L	0.5 mL	1,430	14.3	2.9%	0.32–4.3%
Colorado residential soil (<150 µm)	Feb. 2005	1,230 µg/g (dry)	400 mg	492	4.9	0.24%	0.19–0.33%
	Mar. 2004	1,230 µg/g (wet)				0.50%	0–0.85%
New York pesticide facility soil (A1B21, <150 µm)	Nov. 2003	1,400 µg/g (dry)	400 mg	560	5.6	0.18%	0.04–0.25%
	Jan. 2005	1,400 µg/g (wet)				0.39%	0.05–0.74%
	Mar. 2005	1,400 µg/g (wet)				0.39%	0.09–0.90%

Note: -- – Not available or not applicable

6 Bioavailability of Metals to Wildlife

Only limited research has been conducted on the bioavailability of metals from soil to wildlife. Given this lack of information, ecological risk assessments generally assume that metals in soil are equally bioavailable as in the critical toxicity study, potentially resulting in overestimates of risk. Research was undertaken for SERDP to begin to address this data gap.

6.1 Relative Oral Bioavailability of Metals in Soil to the American Robin

6.1.1 Objectives

This summary section details how Exponent designed a research program to evaluate the bioavailability of metals in birds exposed to soil via the oral pathway, relative to the bioavailability of the same metals when dosed in soluble forms. The target metals for this research are lead, chromium, and cadmium in soil, and soluble spike solutions. The soil was dosed at two different concentrations, and two of three soluble spike dose groups were matched to each soil dose group.

6.1.2 Methods

6.1.2.1 Species Selection

The first portion of this research involved selecting an appropriate receptor species to study. As stated above, the American robin (*Turdus migratorius*) was determined to be an appropriate species for evaluating metals bioavailability. The American robin and American woodcock have the greatest potential for soil exposure based on behavior and diet.

Surrogate avian receptors, such as the quail, European starling, and house sparrow, were considered initially for the research. However, on close examination, none of these species appeared to be appropriate. Quails are considered herbivores, while robins and woodcocks are omnivores. The digestive systems of omnivores rely on particle retention, pH, intestinal surface area, transit time, and microbial digestion to aid with digestion, whereas herbivores do not rely on gastric acids, but rather, the gizzard. Therefore, it would not be appropriate to collect data on an herbivorous bird and extrapolate the data to an omnivorous species of interest for this study.

Similarly, the foraging methods, dietary needs, and physiology of the house sparrow are quite different from the robin and woodcock, and would likely not provide applicable information if used as a surrogate. European starlings also were not selected as surrogates, because they exhibit iron absorption and toxicity that are quite different from other birds, and therefore, may handle other metals differently as well.

Therefore, the American robin appeared to be the only acceptable avian receptor for this research (despite the fact that this causes the research to be more difficult and expensive to perform, especially because robins are not acclimated to a laboratory setting). A more thorough discussion of the technical issues associated with selection of the robin as the receptor of choice is presented in the memorandum included in the Supplemental Materials for Section 6.

6.1.2.2 Protocol Development

After determining that the appropriate avian receptor for SERDP bioavailability research would be the American robin, it was necessary to select a laboratory and develop a protocol by which the project could be carried out. The protocol was titled, "Evaluating the Oral Bioavailability of Metals from Soil and Earthworms to American Robins" and is included in the Supplemental Materials for Section 6. It was submitted to the DoD for review and approval.

The protocol describes in detail the experimental design and general procedures, the collection of robins, the care and treatment of robins in captivity, the test groups to which the robins would be assigned, and the types of dosing regimens that would be undertaken. The preparation of the dosing solutions/vehicles are also described. Additional information included in the protocol includes an examination and justification of the laboratory animals required for the research, and a summary of the quality of life to which the animals would be accustomed, as well as veterinary care that would be available and the qualifications of the researchers. Finally, the calculation of relative bioavailability and the statistics that would be used were outlined.

The protocol served as a long-term plan for SERDP, showing the type and extent of research that would be carried out by Exponent, and allowing for project evaluations that would result in meaningful data.

6.1.2.3 Research Undertaken

The first step of the research was to develop a pilot study that would help determine how to best assess the bioavailability of heavy metals to American robins prior to initiating the full-scale study. Specifically, the pilot study was conducted to determine how to effectively dose the robins in captivity, and to ensure that metal absorption could be measured in the tissues of the robins when dosed with DoD soils and associated metal spikes. The study was performed from May 14 through August 19, 2002, at the Genesis Laboratories facility in Wellington, Colorado. Sixteen American robins were wild-caught from Larimer and Weld Counties, Colorado, using mist nets and Potter traps, between May 13 and June 10, 2002. The methods used to capture the birds and maintain them in captivity are detailed in a memorandum included in the Supplemental Materials for Section 6. A spike solution consisting of six target metals and a DoD test soil was provided to Genesis Labs by Exponent, so that these test substances could be mixed with the food and served to the birds as feed balls.

As originally designed, the avian pilot study was going to incorporate the following three groups of birds.

Pilot Group #1 (Positive Control):

Six robins were to be dosed with food that was blended with a metals spike solution designed to deliver a mass of metals equivalent to the concentrations of the six metals of concern in one of three DoD test soils. The metals of concern were lead, zinc, chromium, cadmium, mercury, and selenium.

Pilot Group #2 (Treatment Group):

Six robins were to be dosed with food that was blended with DoD test soil. The metals of concern and concentrations were intended to be identical to those used in the spike solution for Pilot Group #1.

Pilot Group #3 (Negative Control):

Three robins were to be fed clean lab food throughout the study.

However, early on in the study, it was discovered that the robins did not readily accept the spike solutions in their diet, and therefore, the study shifted focus to determine the best way to dose the birds. Specifically, it was important to determine which metals were aversive to the birds, and/or how many metals the birds could tolerate in their dosed food. A full description of the methods that were used to encourage the birds to accept food containing spike solutions is included in the aforementioned memorandum.

The next phase of the research was completed in two phases. Phase 1 consisted of dosing three groups of robins (Dose Groups #1, #2, and #3), where each dose group consisted of six robins.

Dose Group #1

Dose Group #1 served as the positive control and was dosed with a multiple metal spike that consisted of lead, chromium, and cadmium.

Dose Group #2

Dose Group #2 consisted of six robins that were dosed with food that was blended with DoD test soil (Point Mugu #1b).

Dose Group #3

Dose Group #3 served as the negative control and consisted of six robins that were fed unamended food for the duration of the study.

All animals were acclimated to the laboratory setting and were dosed for 28 days, during the morning hours, and were fasted for two hours prior to dosing. Uneaten food was collected and recorded and stored in the freezer, so that the mass of uneaten food for each bird could be recorded to facilitate accurate evaluation of administered dose. Dose Group #1 served as the positive control for Dose Group #2. The spike solution was intended to deliver the same mass of metals to robins as the birds in Dose Group #2 received from the soil. Dose Group #3 served

as the negative control, and the food that was fed to these birds did not contain any additional metals other than those normally present in trace amounts in the regular bird diet. All feed balls were prepared using the same type of food.

Phase 2 research focused on the same three target metals as Phase 1 (lead, cadmium, and chromium) and used the same soil (Point Mugu #1b) that was used in Phase 1. Phase 2 research also consisted of three dose groups (Pilot Dose Groups #4, #5, #6), and each dose group consisted of six robins, similar to the Phase I research. However, the dosing concentrations were different from those used in Phase 1 research, and are described below.

Dose Group #4

Dose Group #4 served as the positive control for Dose Group #5, and was dosed with a multiple metal spike consisting of lead, chromium, and cadmium. The spike solution was administered at three times the amount administered to Dose Group #1.

Dose Group #5

Dose Group #5 consisted of six robins that were dosed with food that was blended with DoD test soil (Point Mugu #1b). The soil was added to the food so that robins consumed three times more soil than those in Dose Group #2.

Dose Group #6

Dose Group #6 consisted of six robins that were dosed with the same multiple metal spike as Dose Group #4, except the spike solution was administered at one-half the amount currently being administered to Dose Group #1.

6.1.2.4 Challenges

The pilot study, as it was initially designed, could not be carried out because the robins did not readily consume feed balls dosed with six-metal spike solution, regardless of the strength at which the spike solution was served. Therefore, the pilot study had to shift from mirroring the full-scale study that was outlined in the protocol (see Supplemental Materials for Section 6) to instead determining whether the robins had an aversion to any of the target metals. Additionally, the study metals had to be limited to lead, chromium, and cadmium.

6.1.3 Results

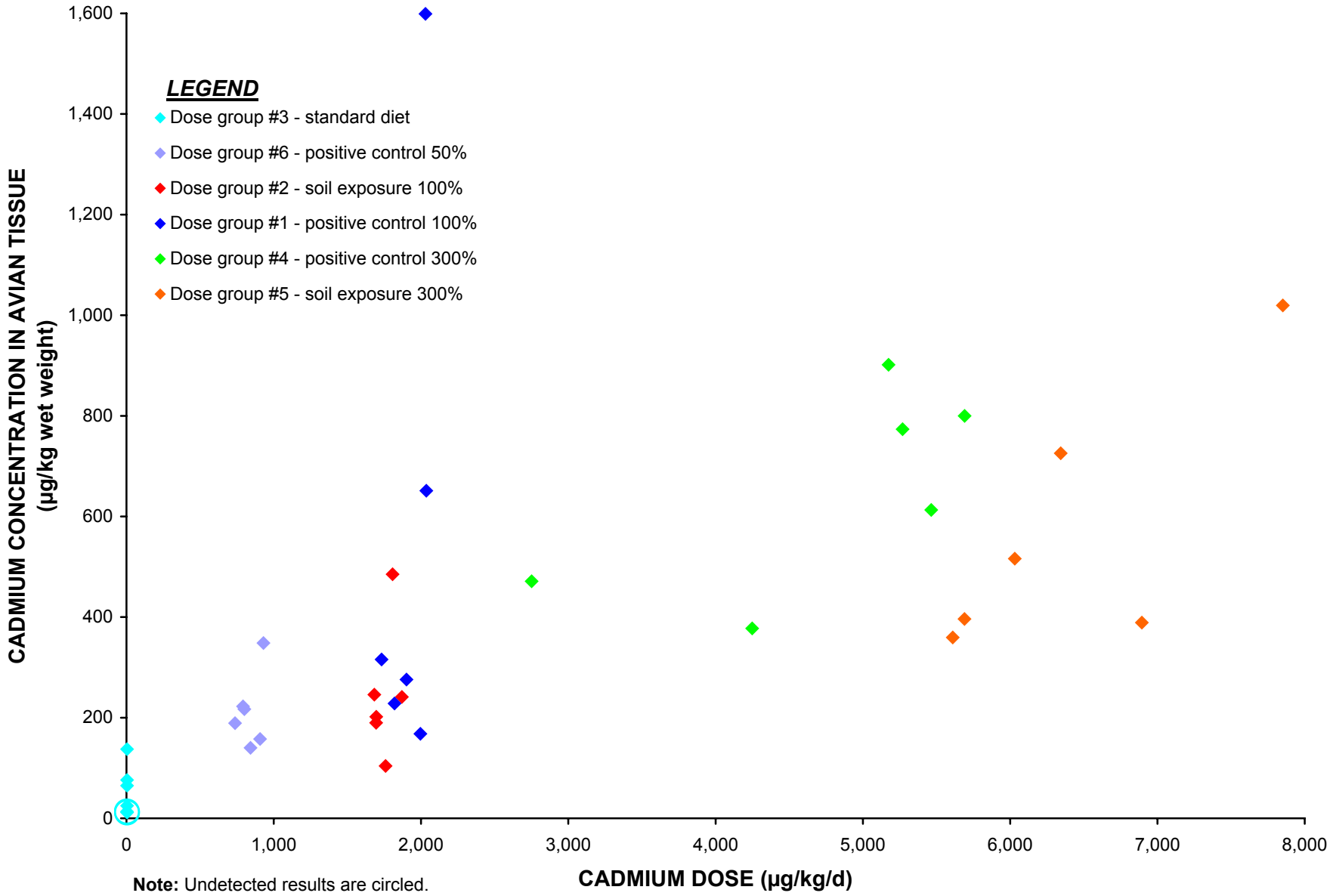
The results of the pilot study indicated that when four or more metals are mixed together in a spike solution at a concentration equal to $1/30^{th}$ the LD50, the food becomes unpalatable to the birds. It was discovered that individual metals could be dosed successfully in feed balls, but that feed balls would need to be prepared in an alternative manner to allow more thorough homogenization of the spike solutions with the food.

The animals in the six dose groups that were used in the Phase 1 and Phase 2 research were sacrificed at the end of the study, and the results from analyzing the carcasses were plotted for

each metal in Figures 6-1 through 6-3. The results from the avian research did not provide meaningful information due to a high degree of variability of food ingestion by the birds, and the corresponding variability in metal doses administered. Figure 6-1 presents the data for cadmium. As this figure depicts, there appears to be a dose-related increase in cadmium tissue concentrations. For the data on lead, depicted in Figure 6-2, there also appeared to be a dose-related increase in lead tissue concentrations; however, the variability in the negative control (Dose Group #3) and the low-dose groups (Dose Group #2) make it impossible to extract any meaningful information regarding the relative oral bioavailability of lead from soil. Figure 6-3 seems to indicate that chromium is not absorbed by the American robin, as there is no dose-related increase in absorption.

6.1.4 Overall Conclusions

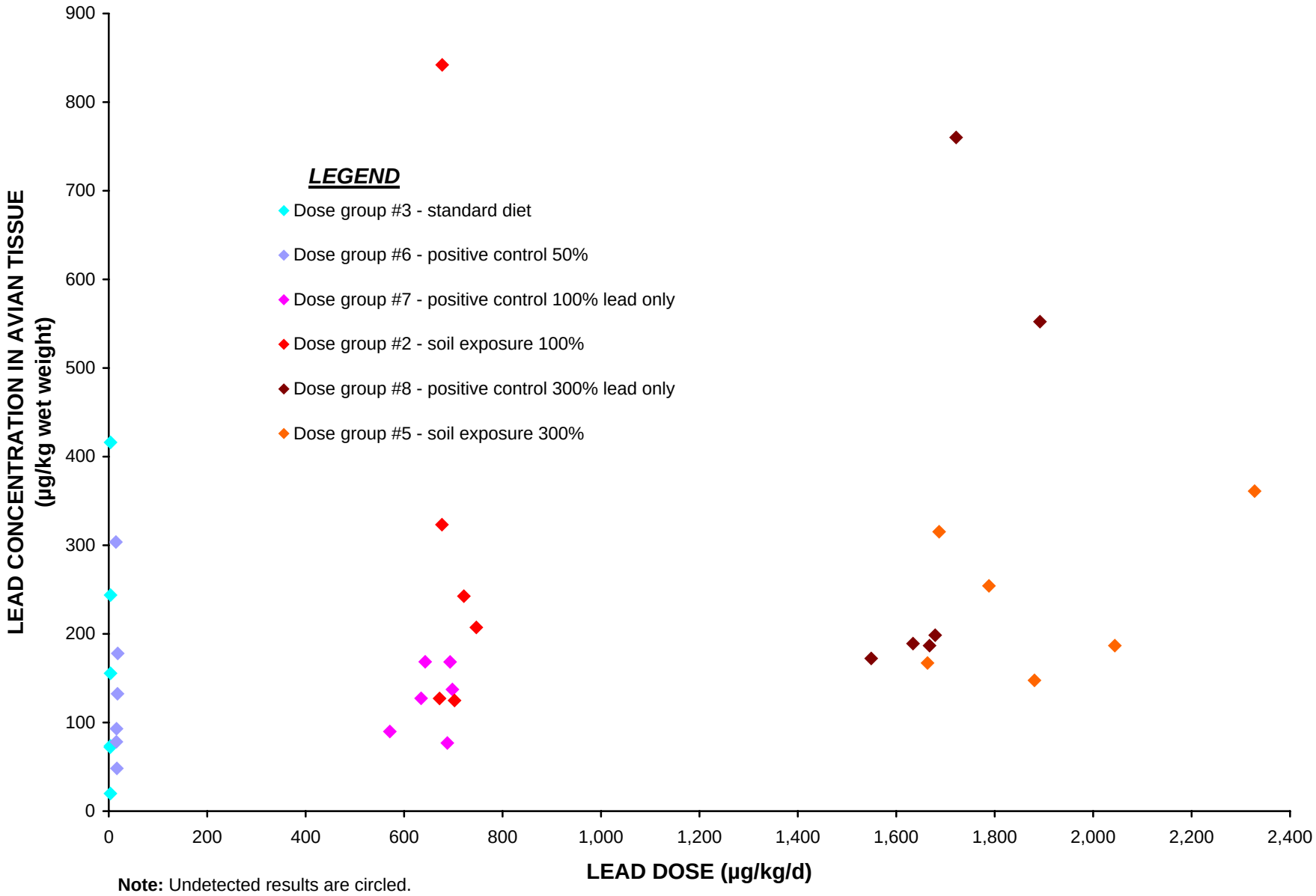
Although the pilot study did not answer the original questions as intended, it was successful in terms of working through issues and challenges that would have been encountered during the full-scale study. After carrying out the Phase 1 and Phase 2 research, which dealt with dosing the captive robins with actual soil and spike solutions, as well as having a negative control, it became apparent that wild-caught American robins are not the ideal receptor to use for avian bioavailability studies, due to their erratic eating behavior in captivity. For the type of investigation that was undertaken for the SERDP research, the rate of food intake needs to be well controlled and consistent across animals, in order to ensure administration of the target dose. To the extent that food consumption was erratic for these animals, the variability in dosing makes it impossible to derive meaningful conclusions from the resulting data. Perhaps better results could be obtained with this receptor by acclimating birds to the lab from the time they are hatched, so that they are accustomed to a dosing regimen and laboratory food.



Note: Undetected results are circled.

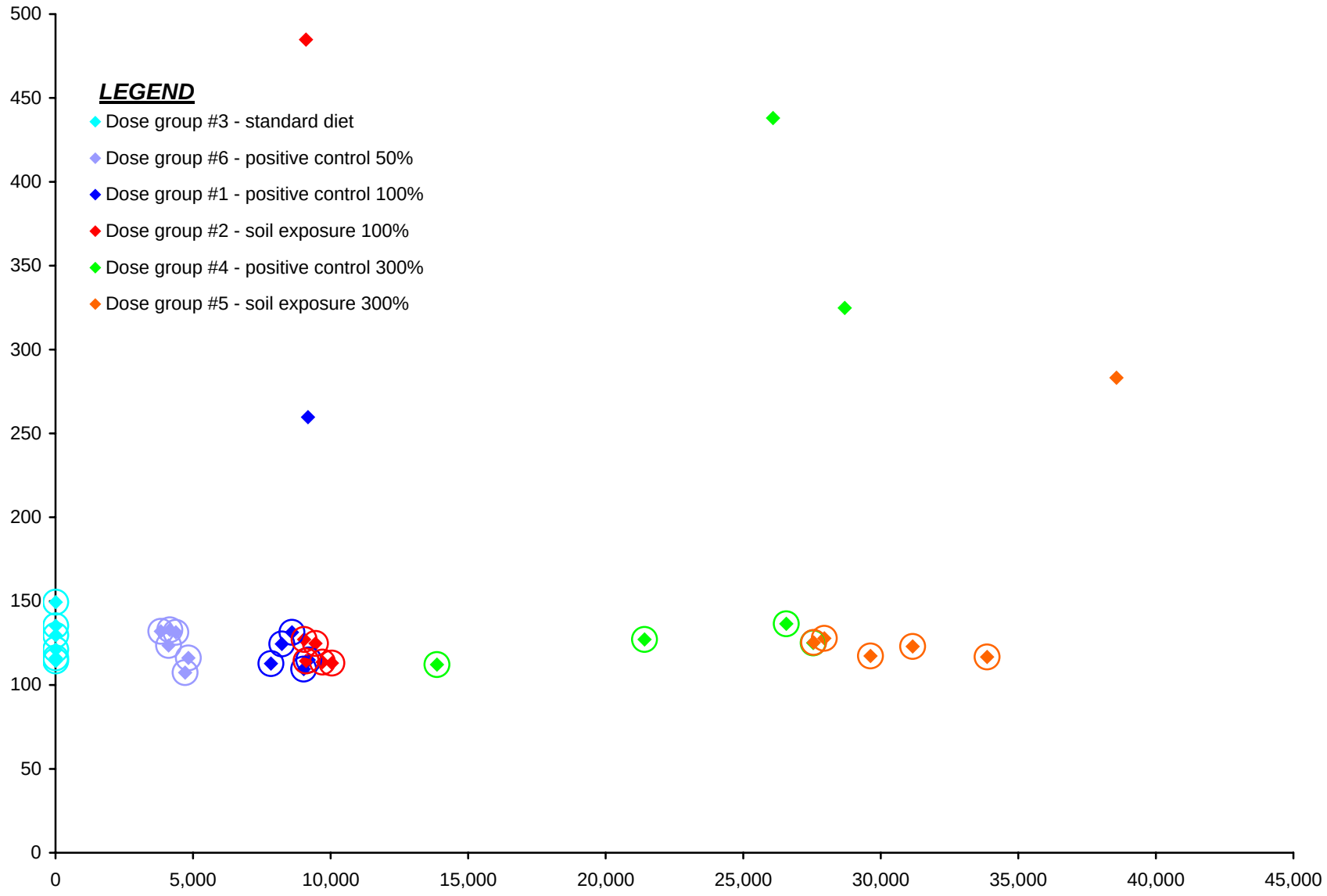
Figure 6-1. Cadmium concentration in avian tissue versus dose (individual bird data plotted).

\\boulder3\data\Groups\Data Management\1191_SERDP\Avian\Figures 1 2 3.xls\Figure 1 4/15/2005 3:54 PM



Note: Undetected results are circled.

Figure 6-2. Lead concentration in avian tissue versus dose (individual bird data plotted).



Note: Undetected results are circled.

Figure 6-3. Chromium concentration in avian tissue versus dose (individual bird data plotted).

6.2 Least Shrew Bioavailability Research

6.2.1 Introduction

Small mammals such as shrews are among the wildlife receptors for which ecological risk assessment models consistently indicate the greatest level of potential exposure to metals in soil. These small mammals receive much of their soil exposure from direct soil ingestion during foraging and preening activities, or from consumption of soil-laden earthworms. In assessing risks to these receptors from soil contamination, the standard method is to assume that contaminants have a relative bioavailability of 100% (i.e., the efficiency of metals absorption from ingested soil is equal to that which occurred in the laboratory tests conducted to determine toxicity thresholds). However, as discussed above for human bioavailability, a growing body of research indicates that many chemicals—including metals—are less bioavailable from ingested soil than from the soluble forms that are typically used in laboratory toxicity tests, when dosed in a similar manner. To evaluate this hypothesis, research was conducted to measure the absorption of metals by least shrew (*Cryptotis parva*) after soil ingestion, relative to the absorption of the soluble forms of these metals that have been used in toxicity studies. The purpose of this research was to develop an *in vivo* model for measuring the relative bioavailability of metals in soil to shrews, and to produce data that can be used in ecological risk assessments and to develop data that can be used to validate *in vitro* tests.

6.2.2 Study Design

6.2.2.1 Species Selection

EPA considers the short-tailed shrew to be a good sentinel receptor for the mammalian insectivore guild. This species is also the mammalian receptor that yields the lowest ecological soil screening levels (eco-SSLs) for most of the metals that have been evaluated in the eco-SSL process, and thus will be important in setting soil screening levels for ecological receptors. Short-tailed shrews are not generally used for laboratory research, because they must be wild-caught, may have diseases, and often do not adjust well to captivity. No established colonies of short-tailed shrew were available for this research. Therefore, the least shrew, which had already been shown to adapt to a laboratory environment, was selected as a surrogate. Only female shrews were used, because the oral bioavailability of lead in other small mammals (e.g., rats) has been observed to be dependent on the sex of the animals (Freeman et al. 1992), and because females are considered to be more ecologically sensitive than male shrews. Information on the care and handling of the least shrews during the course of the experiment is provided in the manuscript that presents this work and results. The manuscript are provided in the Supplemental Materials for Section 6.

6.2.2.2 Soil Characterization

Because this research was funded by SERDP, the focus was on metals that occur in soils at DoD facilities. As a precursor to this research, metal concentration data for a wide variety of DoD

facilities were screened against established regulatory criteria for ecological endpoints. The metals that most frequently exceeded ecological screening criteria, in order, are lead, cadmium, mercury, zinc, arsenic, and chromium. An attempt was made to identify and collect soils from sites that contained this suite of metals at concentrations that would yield measurable post-dosing concentrations in the shrew, but would not be toxic in the sub-acute dosing periods that were used in this study. No soils were found that contained concentrations of mercury that would allow for measurement of oral absorption. In addition, the cat food that constituted the basal diet of the least shrew colony used in this study was high in zinc concentration. As a result, the elevated background zinc concentrations in shrew precluded the ability to measure zinc absorption from soil in this study. Thus, mercury and zinc were eliminated from the list, and the resultant target metals for this study were lead, cadmium, arsenic, and chromium.

Four soils containing a mixture of target metals were used in this research. These included soils from the Naval Weapons Air Station located in Point Mugu, California (hereafter referred to as DoD-PM soil), a mixture of soils from the Dugway Proving Grounds and Picatinny Arsenal (referred to as the DoD-DP soil), an orchard soil from Washington State (Orchard soil), and a soil collected in the vicinity of a smelter in Colorado (Smelter soil). Because none of the test soils contained detectable quantities of Cr(VI), the Smelter soil, which contained only 19 mg/kg chromium, was spiked with soluble Cr(VI) to achieve a soil concentration of 1,835 mg/kg chromium [1,355 mg/kg measured as Cr(VI)]. Metals concentrations and soil characterization data for the test substrates are presented in Table 6-1. The test soils ranged in texture from sand to sandy loam, with pH and total organic carbon (TOC) ranging from 5.9 to 8.0 and 0.75% to 3.26%, respectively. Arsenic concentrations ranged over a five-fold difference (60 to 331 mg/kg), while cadmium (2.4 to 1,755 mg/kg), chromium (36 to 8,362 mg/kg), and lead (257 to 2,640 mg/kg) covered approximately 3, 2, and 1 order of magnitude in concentration ranges, respectively. Please refer to the manuscript (Supplemental Materials for Section 6) for information on the soil handling and analytical methods.

6.2.2.3 Dosing

In these experiments, the relative bioavailability from the test soils was assessed by comparing the absorption of each target metal after soil ingestion to the absorption of soluble forms of the metals. Both the test soils and the aqueous mixtures of soluble metal salts (or soluble spikes, referred to as “reference mixtures”) were mixed with basal diet and dosed to groups of shrews for 28 days. Feed preparation, feeding regimen, and preparation of the reference mixtures are described in the manuscript.

Three dose levels of each test soil or soluble spike reference material were given to assess whether any dose-response relationship existed for metal absorption. For a given test soil, the maximum daily mass that could be given safely (estimated from toxicity data and the metal concentrations in each soil) was established and is referred to as the “100% soil dose” (this ranged from 0.01 to 0.20 g soil/day for the four soils tested, depending on the metal concentrations they contained). The other two soil doses were given at one-half and one-fourth of the initial soil dose (referred to as the “50% and 25% soil doses”). For each of the different test soils, that were evaluated during separate dosing trials, a negative-control dose group was included and was fed standard shrew diet. Doses of the target metals given as the reference mixtures were matched, to the extent practicable, to those delivered in the three doses for each

soil. However, because each shrew consumed the dosed food *ad libitum*, doses received by individual shrews varied.

Twelve shrews was selected as the starting value for the number of individual animals in each dose group. As a result of variable shrew mortality rates in the different dose groups, the number of shrews varied from six to eleven animals after 28 days of dosing (Table 6-2). For all dose groups with eight or fewer animals surviving for the entire dosing period, all of the surviving animals were analyzed. For dose groups with more than eight surviving animals, eight animals were selected at random for analysis. After the 28-day dosing period, the shrews were terminated, and the body burden of the target metals was determined. (Analytical methods are described in the manuscript provided in the Supplemental Materials for Section 6). Using regression methods (described in the attached manuscript), the relative bioavailability was then calculated from the concentrations of a target metal in the soil-dosed animals, relative to the concentrations in the reference-dosed animals. The relative bioavailability for each metal in each soil was then estimated as the ratio of the slope of the regression for the soil versus that for the reference material**.**

Average doses delivered to each dose group of shrews are reported in Table 6-3 (calculated from metal concentrations measured in each batch of feed and average shrew body weight during the study). Actual doses delivered were consistent with the four-fold target difference between the low and high doses for each of the metals, in each of the four test soils, except in cases where the dosed feed concentrations were very close to the standard diet concentrations. The ratios of metal doses delivered as soil in feed, relative to doses delivered as the matched reference mixture in feed (Table 6-3), generally ranged from 0.85 to 1.15, with a few values outside this range (0.73 and 1.37 represented the absolute limits of the range). These results indicate that the dose ranges for metals in each soil and reference mixture were approximately four-fold, and that similar metal doses were delivered in both the soil-dosed feed and the matched reference materials.

6.2.3 Results

High shrew mortality was observed in the first dosing trial (43%), and decreased as the study progressed (8% during the final dosing trial, which is similar to that observed in the shrew colony as a whole on a monthly basis; Table 6-2). With the exception of the 100% exposure group for the Smelter soil, mortality rates were as high for the control groups fed standard diet alone as they were for dose groups fed soil or reference mixtures (Table 6-2), indicating that mortality was not due to metals exposure. It is believed that the elevated mortality rates early on were due to insufficient food and the effects of isolation.

During the first dosing trial (using DoD-PM soil), when mortality rates were at their highest, all of the dose groups experienced a decrease in body weight during the 28-day period (Table 6-4). This was followed by increases in body weight during the DoD-DP dosing trial, consistent with the decline in mortality rates. However, during the last (Smelter soil) dosing trial, body weights declined to an even greater extent than during the DoD-PM dosing trial, even though mortality rates had decreased to levels seen in the overall shrew colony. The Smelter soil delivered the greatest doses of arsenic, cadmium, and chromium, and the second-highest dose of lead,

suggesting that one, or some combination, of these metals may have been responsible for the decreases in body weight during the dosing trial with this soil. It should also be noted that shrew mortality was elevated in the 100% Smelter soil dose (36%; Table 6-2), consistent with the decreases in body weight (–25%; Table 6-4) in this dose group.

The shrews exhibited a clear dose-response for arsenic, cadmium, and lead in the test soils (insufficient data were available for chromium to make this determination). Figure 6-4 summarizes all of the dose vs. tissue concentration data for lead in the four test soils that were dosed to the shrews.

6.2.3.1 Relative Bioavailability

Arsenic — Relative bioavailability of arsenic from soil ranged from 7% to 49% for the three soils in which the regression model yielded significant results (Table 6-5)—dosing of the DoD-PM soil (82 mg/kg As) and associated spike yielded all non-detect values in the post-dosing shrew tissues. The 7% relative bioavailability value for arsenic in the DoD-DP soil undoubtedly has greater uncertainty associated with it than would be implied by the standard error associated with this value, because many of the tissue arsenic concentrations from the soil-dosed animals were non-detect values, and those that were detects were only slightly greater than the detection limit (Figure 6-5). As a result, the slope of the dose-response curve for the soil-dosed animals is less certain than that for the reference material–dosed animals. In contrast, arsenic concentrations in shrew tissues of animals dosed with the Orchard (Figure 6-6) and Smelter soils were well above detection limits, yielding more robust estimates of relative bioavailability. These results suggest that a dose of approximately 3,500 μg As/kg-day in soil (Table 6-3) should be considered the lower limit for arsenic in this shrew model.

Cadmium — Relative bioavailability of cadmium from soil ranged from 13% to 81% for the three soils for which the regression model yielded significant results (Table 6-5)—shrew tissue concentrations after dosing of the Orchard soil (2.4 mg/kg Cd) were all non-detect. Similarly, shrew tissue concentrations after dosing with the DoD-DP were largely nondetects (Figure 6-7) (analogous to the situation for arsenic in this soil). Thus, the 13% relative bioavailability value for cadmium in this soil is more uncertain than the values for the DoD-PM and Smelter soils, but certainly indicates low absorption of cadmium (and arsenic) from this soil, relative to exposure to soluble salts of these metals. Based on these data, a dose of at least 500 μg Cd/kg-day in soil (Table 6-3) is required for this shrew model.

Chromium — Chromium(III) does not appear to have been absorbed in the shrew, either from soil or the reference mixtures, regardless of dose level. Doses up to nearly 20,000 μg/kg-day were delivered in the DoD-PM soil and its associated reference mixture (the DoD-PM soil contained 8,362 mg/kg Cr, almost entirely as Cr(III) [Table 6-2]), and yet no evidence of chromium absorption was observed for either the test soil or its reference mixture (Figure 6-8). The same was observed with the DoD-DP and Orchard soils, which delivered smaller doses of Cr(III) than the DoD-PM soil. For the Smelter soil, which was spiked with Cr(VI) and delivered doses up to nearly 90,000 μg-kg/day of chromium (Table 6-3), uptake into shrew tissue was observed (Figure 6-9), and tissue response increased with increasing dose. At the intermediate dose of chromium from this soil (42.6 mg/kg-day, or a total dose of 1,192 mg Cr), only a very small fraction (approximately 7.4×10^{-6}) of the chromium dose mixed with soil was

found in shrew tissues (average of 0.0089 mg Cr/shrew). In the absence of a soluble reference dose, it is not possible to calculate a relative bioavailability value for Cr(VI) from the Smelter soil.

Lead — Relative lead bioavailability from soils ranged from 21% to 60% (Table 6-5), with detectable lead concentrations in tissue from all four soils tested. The analytical results from the Orchard soil were anomalous, in that tissue lead concentrations from the soil exceeded those from the associated reference mixture (initial relative bioavailability estimate of 129%), suggesting that soil lead was more readily absorbed than the lead acetate spike. Because it seemed very unlikely that this could occur, and because the dose-response from the lead reference doses associated with the other three soils tested yielded consistent results that contradicted results for lead from the Orchard reference mixture, the average dose-response from the lead acetate given during the DoD-PM, DoD-DP, and Smelter soil trials was used to calculate the relative bioavailability of lead from the Orchard soil. The anomalous behavior of lead in the reference material associated with the Orchard soil may have been due to formation of a sparingly soluble precipitate in the reference solution, because the lead concentration in the Orchard reference material was greater than in any of the others. This would explain how lead absorption from the Orchard soil could have exceeded that from the reference material. Based on these results, a minimum lead dose from soil of approximately 300 μg/kg-day is required for use of the shrew model.

6.2.3.2 Effect of Soil Parameters on Metal Bioavailability

The relative bioavailability values from this study (Table 6-5) were compared to the soil parameters and soil metal concentrations (Table 6-2) to assess whether a particular soil variable appeared to control the relative bioavailability of any target metal. This analysis was conducted both graphically and using Pearson's correlation at $\alpha = 0.05$. The only significant correlations were for the relative bioavailability of cadmium, which was inversely correlated with both CEC and DCB-extractable extractable iron concentrations of the test soils. In addition, the graphed data suggested that relative lead bioavailability increased with increasing soil lead concentration. However, with only a few data points per metal, it is not possible to have a high level of confidence in these correlations.

6.2.4 Conclusions

The research described herein involved the development of a novel animal model for assessing the relative bioavailability of metals from soil. The shrew model differs from existing animal models for estimating metals bioavailability from soil (e.g., rats, swine, and monkeys) in that shrews have very high metabolic and food consumption rates, and are quite fragile. As observed during this study, high mortality occurs with minor changes in diet or habitat. Despite the difficulties in working with this model, this study demonstrates that it is possible to obtain reliable estimates of relative metals bioavailability from the shrew. Results indicate that the relative bioavailability of arsenic, cadmium, and lead ranged from 7% to 49%, 13% to 81%, and 21% to 60%, respectively. Cr(III) was not absorbed from soil, even at very high doses, and Cr(VI) was absorbed to a slight extent from a soil that was spiked with a high concentration of Cr(VI).

Based on the study results, it is clear that arsenic, cadmium, and lead are absorbed to varying extents from different soils in this shrew model, and that site-specific (or soil-specific) factors affect the relative absorption of the metals. Cr(III) was not absorbed in a detectable manner, and soluble Cr(VI) spiked into soil was absorbed to a limited extent, the degree of which cannot be quantified from the study results.

The relative bioavailability values for arsenic, cadmium, and lead in soil are generally consistent with those observed in other animal models. For example, relative arsenic bioavailability from 13 soils has been evaluated in a juvenile swine model, resulting in values ranging from near 0 to 52% (Casteel et al. 1997). In addition, five Florida soils were evaluated in a *Cebus* monkey model, yielding relative bioavailability estimates for arsenic of 10% to 25% (Roberts et al. 2002). The range of relative arsenic bioavailability values observed in this study (7% to 49%) falls within the range observed in juvenile swine, and is somewhat greater than that observed in Cebus monkeys.

The DoD-PM soil used in this study was also evaluated for relative cadmium bioavailability in a juvenile swine model, yielding an average relative bioavailability estimate of 78%, based on measurement of both kidney and liver endpoints (Schoof et al. 2005). Given that the DoD-PM soil yielded a relative cadmium bioavailability of 81% in the shrew, these two animal models appear to be yielding similar results. The juvenile swine model was developed as a surrogate for absorption in human children, and has been used extensively to evaluate relative lead bioavailability in soil. Data from the swine model indicate a broad range of relative bioavailability results for lead in soil (19% to 90% [Ruby et al. 1993]). This is generally consistent with results observed in the shrew model, wherein relative lead bioavailability ranged from 21% to 67%. Given the consistent results obtained from this shrew model, and the fact that these results are comparable to results from established animal models, the shrew model appears to be a useful tool for assessing metals uptake from soil into shrew and other small mammals, and for improving the accuracy of ecological risk assessment.

6.2.5 References

Casteel, S.W., L.D. Brown, and M.E. Dunsmore. 1997. Relative bioavailability of arsenic in mining wastes. Document Control Number 4500-88-AORH. Veterinary Medical Diagnostic Laboratory, College of Veterinary Medicine, University of Missouri, Columbia, MO.

Freeman, G.B., J.D. Johnson, J.M Killinger, et al. 1992. Relative bioavailability of lead from mining waste soil in rats. Fund. Appl. Toxicol. 19:388–398.

Roberts, S.M., W.R. Weimar, J.R.T. Vinson, J.W. Munson, and R.J. Bergeron. 2002. Measurement of arsenic bioavailability in soil using a primate model. Toxicol. Sci. 67:303–310.

Ruby, M.V., A. Davis, T.E. Link, et al. 1993. Development of an *in vitro* screening test to evaluate the *in vivo* bioaccessibility of ingested mine-waste lead. Environ. Sci. Technol. 27(13):2870–2877.

Schoof, R.A., C.W. Casteel, T.J. Evans, et al. 2005. Soil cadmium bioavailability as a function of mineralogy and soil characteristics. Environ. Sci. Technol. (in press).

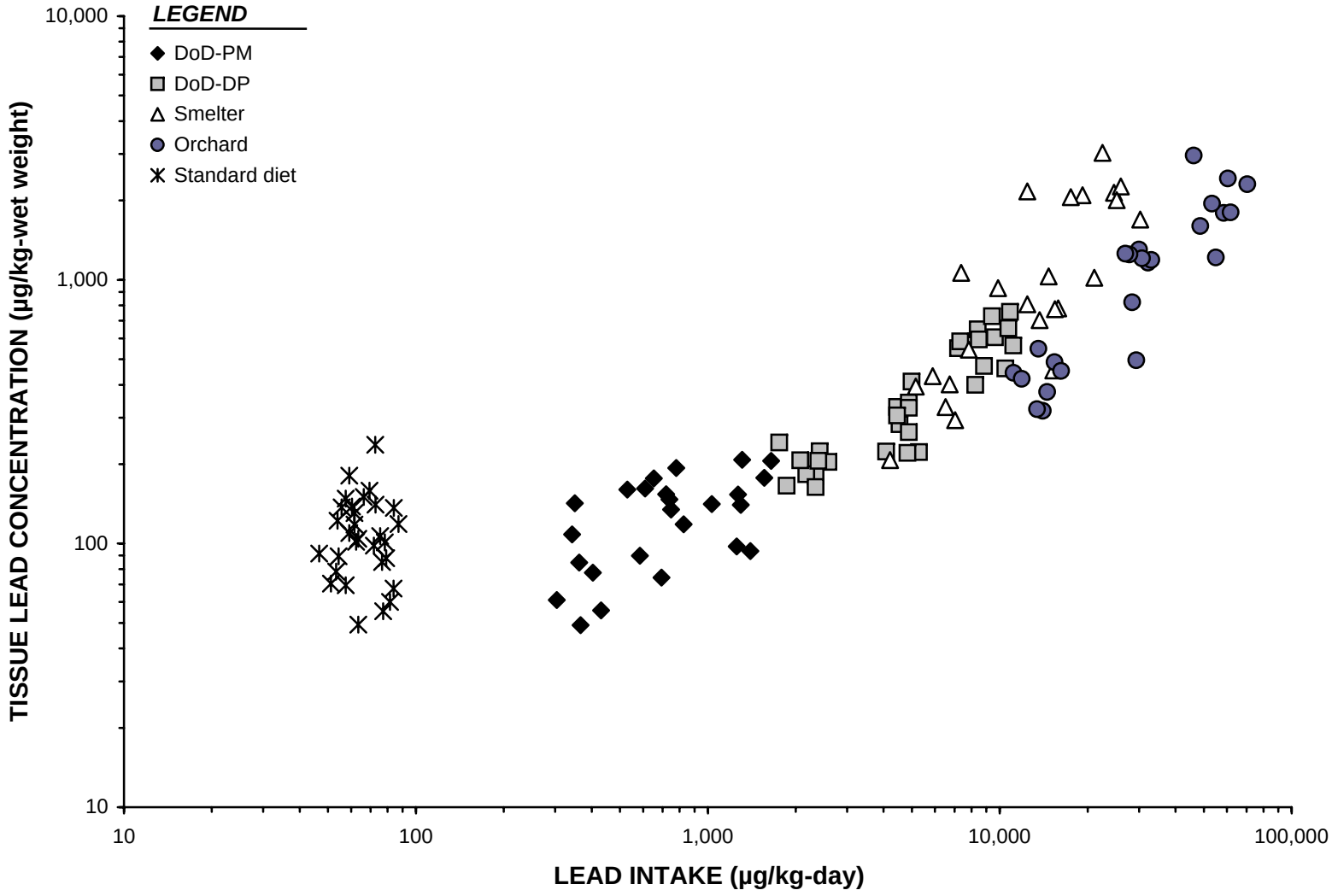


Figure 6-4. Dose-response for lead in all four test soils dosed to shrew

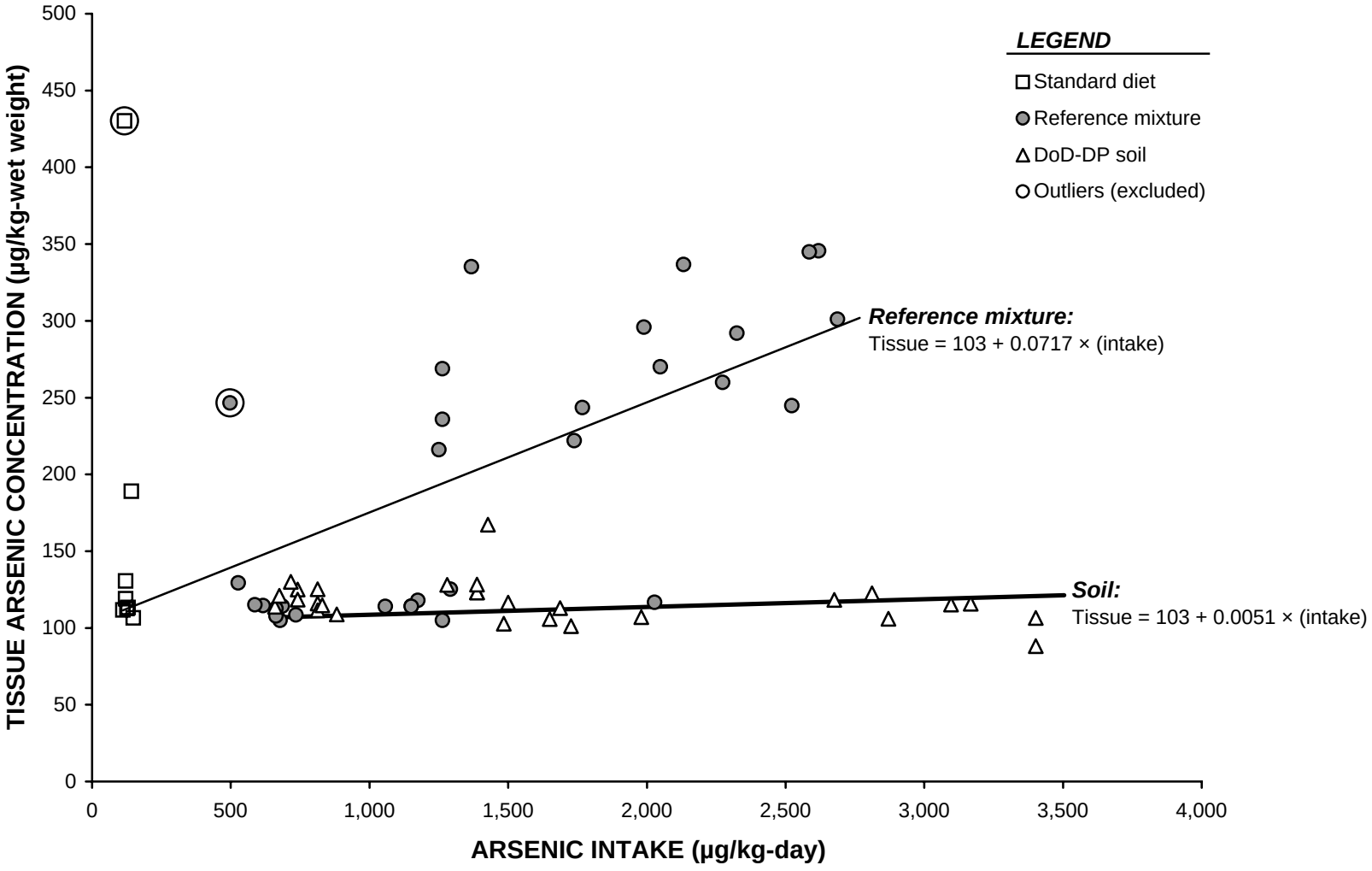


Figure 6-5. Dose-response graph for arsenic in the DoD-DP soil

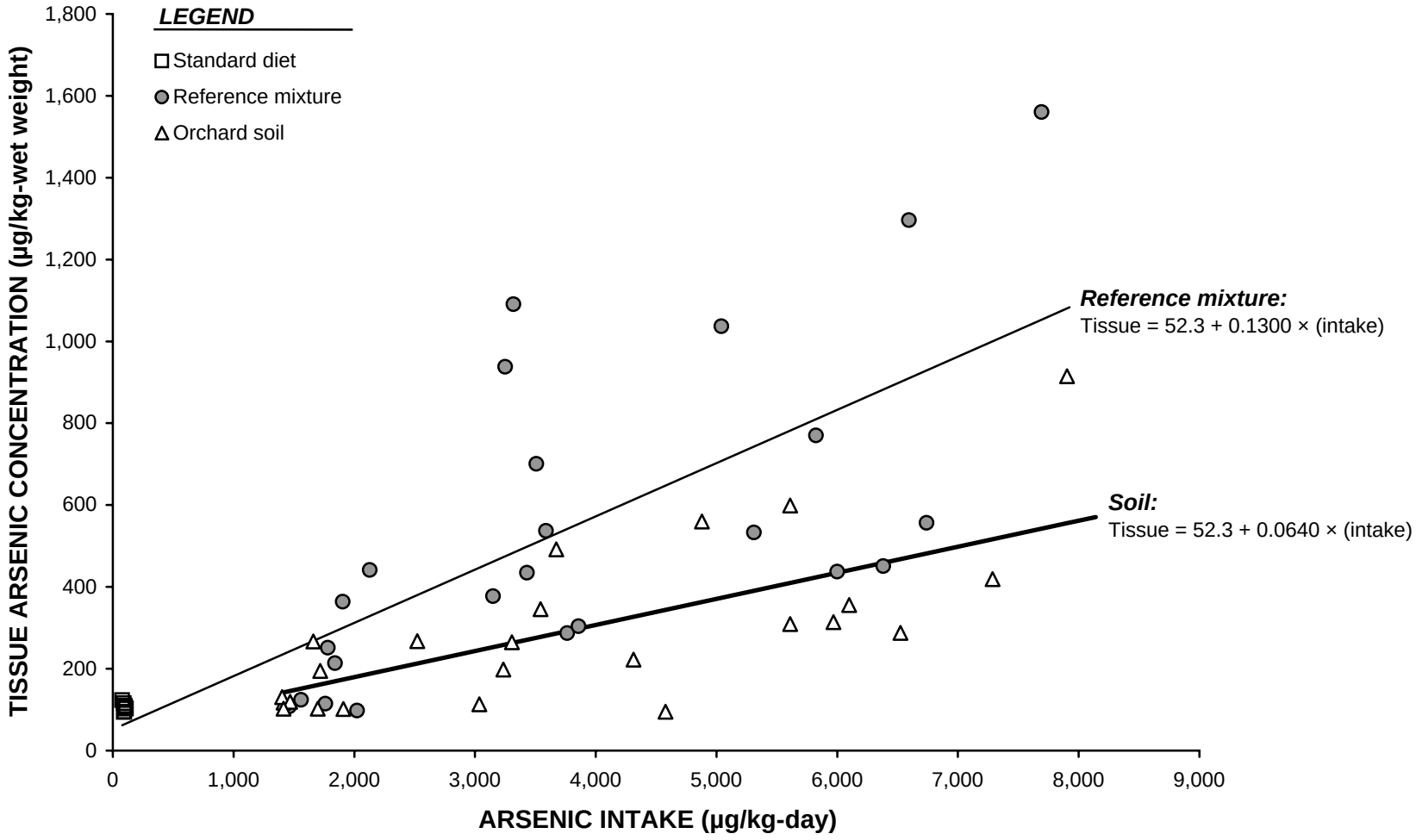


Figure 6-6. Dose-response graph for arsenic in the Orchard soil

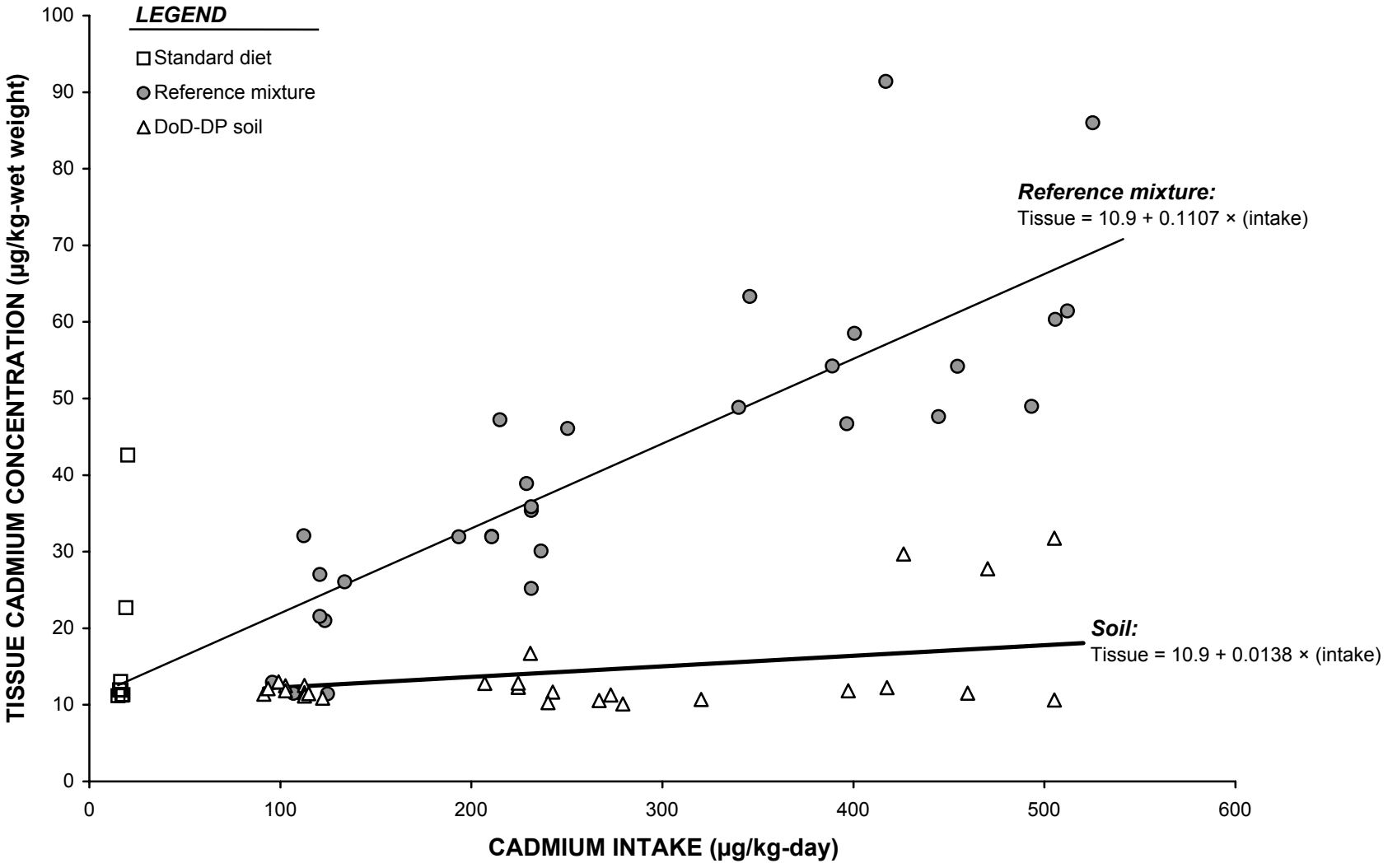


Figure 6-7. Dose-response graph for cadmium in the DoD-DP soil

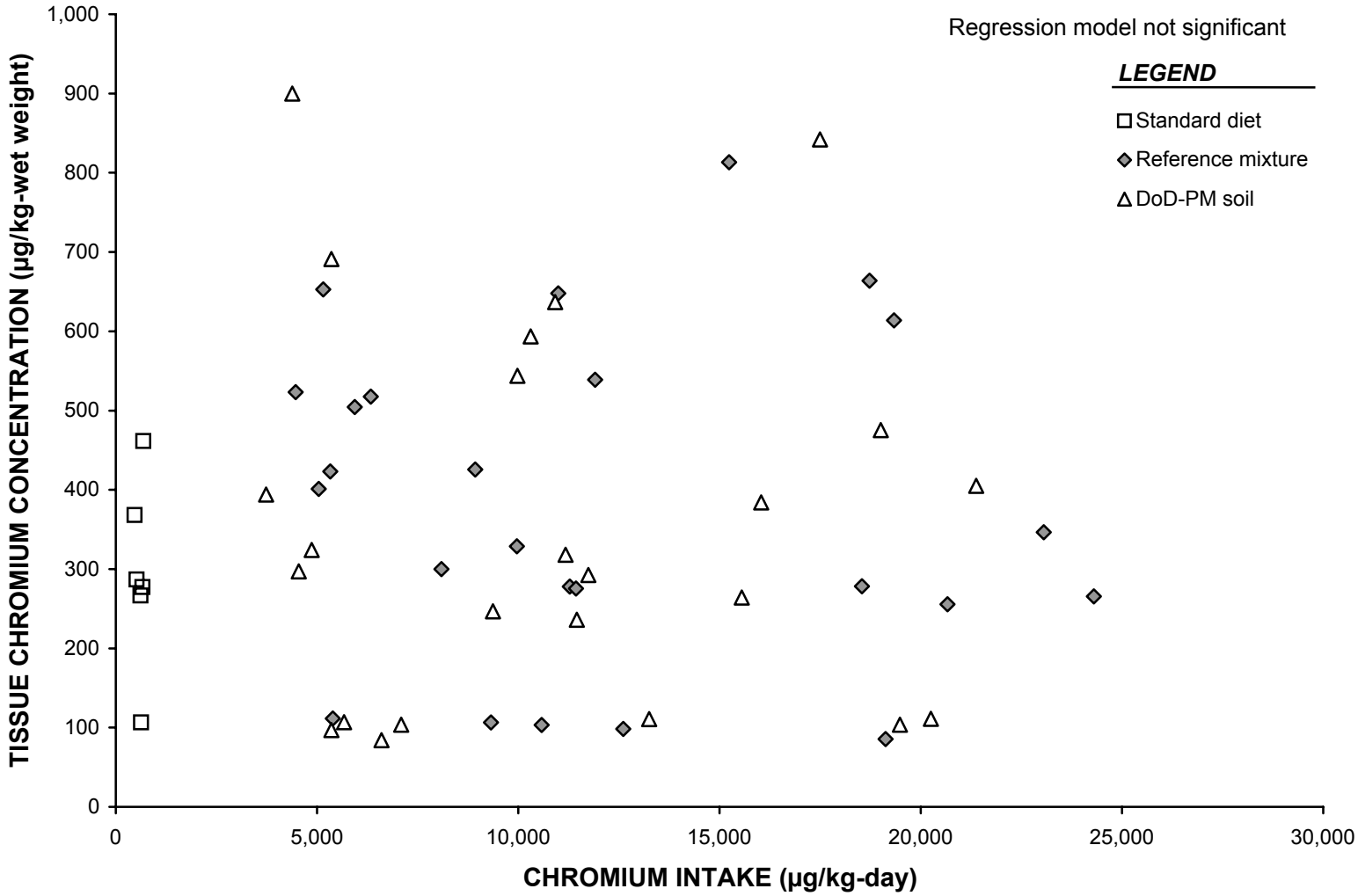


Figure 6-8. Dose-response for chromium in the DoD-PM soil

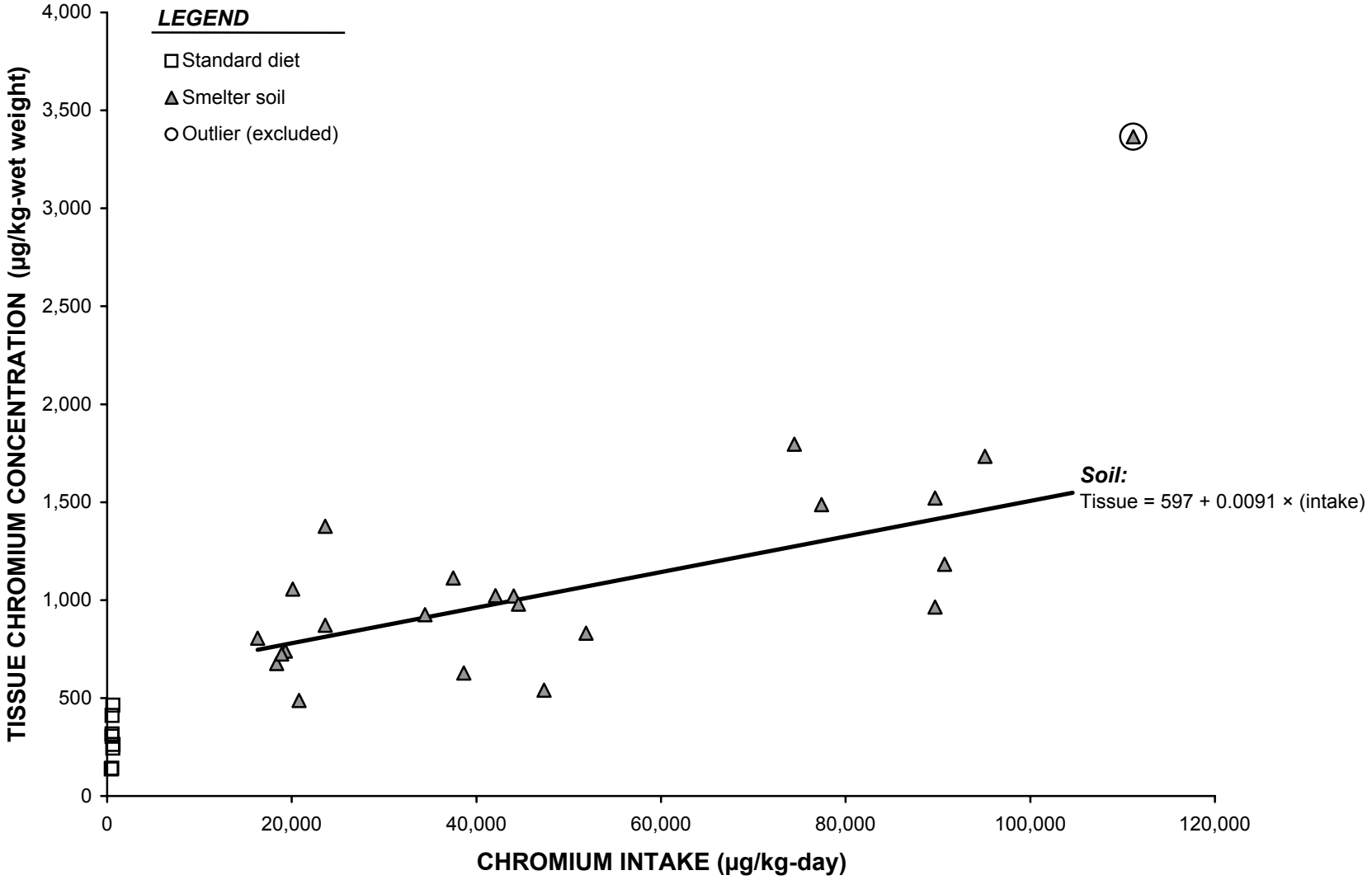


Figure 6-9. Dose-response for chromium in the Smelter soil

Table 6-1. Characterization data for soils dosed to shrews

Chemical	Units	DoD-PM	DoD-DP	Orchard	Smelter
Conventionals					
pH	s.u.	7.6	8.0	5.9	7.5
Total organic carbon	%	0.75	3.26	2.98	2.09
Total inorganic carbon	%	0.53	1.02	1.27	0.05 *U*
Cation exchange capacity	meq/100g	46.2	71.3	74.0	52.3
DCB extractable iron	mg/kg	3,830	12,240	5,630	5,110
Particle Size Distribution					
Coarse sand (425 – 2,000 µm)	%	42.3	43.1	28.0	19.8
Medium sand (250 – 425 µm)	%	30.3	12.1	17.9	13.8
Fine sand (75 – 250 µm)	%	21.9	28.3	25.2	35.7
Silt (4 – 75 µm)	%	2.2	15.4	26.9	27.5
Clay (< 4 µm)	%	3.3	1.1	2.0	3.2
Inorganics					
Arsenic	mg/kg	82	60	284	331
Cadmium	mg/kg	1,755	14	2.4	423
Chromium	mg/kg	8,362	79	36	1850
Chromium, hexavalent	mg/kg	1.1	0.5 *U*	0.5 *U*	1,355
Iron	mg/kg	9,330	20,900	22,300	15,800
Lead	mg/kg	569	257	2640	585
Manganese	mg/kg	78	498	394	479
Mercury	mg/kg	0.85	11.3	0.04	7.4
Nickel	mg/kg	1,870	41	16	13
Phosphorus	mg/kg	1,710	1,560	887	673
Zinc	mg/kg	706	356	286	1,200

Table 6-2. Shrew mortality rates

	DoD-PM			DoD-DP			Orchard			Smelter		
	Initial n	Final n	Mortality Rate	Initial n	Final n	Mortality Rate	Initial n	Final n	Mortality Rate	Initial n	Final n	Mortality Rate
Standard Diet	19	6	68%	16	8	50%	12	11	8%	11	11	0%
Soil Exposure 25%	12	9	25%	12	10	17%	12	9	25%	11	10	9%
Reference Material 25%	12	7	42%	12	9	25%	12	10	17%	11	11	0%
Soil Exposure 50%	13	8	38%	12	11	8%	12	9	25%	11	11	0%
Reference Material 50%	12	10	17%	12	10	17%	12	10	17%	11	11	0%
Soil Exposure 100%	17	7	59%	12	7	42%	12	11	8%	11	7	36%
Reference Material 100%	12	8	33%	12	12	0%	12	11	8%	11	10	9%
Total	97	55	43%	88	67	24%	84	71	15%	77	71	8%

Table 6-3. Average doses (µg/kg-day) received by each dose group

	Standard Diet	25% Doses		50% Doses		100% Doses		Ratio of Soil:Reference Material		
		Soil	Reference Material	Soil	Reference Material	Soil	Reference Material	25% Doses	50% Doses	100% Doses
DoD-PM										
Arsenic	150	169	212	214	228	308	367	0.80	0.93	0.84
Cadmium	20	1,003	994	1,949	1,865	3,856	3,671	1.01	1.04	1.05
Chromium	595	5,291	5,380	11,025	10,510	18,458	19,873	0.98	1.05	0.93
Lead	73	319	366	726	688	1,252	1,346	0.87	1.05	0.93
DoD-DP										
Arsenic	127	768	628	1,536	1,224	3,061	2,227	1.22	1.26	1.37
Cadmium	17	106	117	248	224	455	435	0.91	1.11	1.04
Chromium	506	1,375	1,272	2,371	1,947	3,965	3,363	1.08	1.22	1.18
Lead	62	2,390	2,218	4,979	4,731	10,266	9,224	1.08	1.05	1.11
Orchard										
Arsenic	97	1,586	1,808	3,525	3,484	6,236	6,199	0.88	1.01	1.01
Cadmium	16	17	36	20	60	20	87	-- [b]	-- [b]	-- [b]
Chromium	518	669	518	1,022	514	1,351	477	-- [b]	-- [b]	-- [b]
Lead	76	12,472	13,792	30,251	29,834	59,056	56,846	0.90	1.01	1.04
Smelter										
Arsenic	173	5,042	5,083	7,790	10,702	16,919	18,263	0.99	0.73	0.93
Cadmium	20	4,220	4,319	8,084	9,284	17,174	15,977	0.98	0.87	1.07
Chromium	564	20,127	603	42,556	627	89,738	504	-- [a]	-- [a]	-- [a]
Lead	56	5,817	6,344	11,898	13,702	25,077	23,288	0.92	0.87	1.08

[a] Ratio not relevant because the soil was spiked with 1,835 mg/kg Cr, but the reference material was not spiked with Cr because it caused a precipitate to form (possibly $PbCrO_4$) in the reference solution.

[b] Ratio not relevant because Cd and Cr were not added to the reference material matched to the Orchard soil, because there was insufficient Cd and Cr in the Orchard soil to be detectable post-dosing in shrew tissue.

Table 6-4. Shrew body-weight changes

	DoD-PM			DoD-DP			Orchard			Smelter		
	Initial (g)	Final (g)	Percent change	Initial (g)	Final (g)	Percent change	Initial (g)	Final (g)	Percent change	Initial (g)	Final (g)	Percent change
Standard Diet	4.5	4.0	-11%	4.5	5.3	18%	4.7	4.4	-6%	4.7	4.6	-2%
Soil Exposure 25%	5.0	4.4	-12%	5.1	5.9	16%	4.9	4.8	-2%	5.0	4.5	-10%
Reference Material 25%	4.6	4.2	-9%	4.6	5.4	17%	4.6	4.6	0%	5.2	4.9	-6%
Soil Exposure 50%	4.7	3.8	-19%	4.4	5.4	23%	4.4	4.3	-2%	4.8	4.2	-13%
Reference Material 50%	4.2	4.1	-2%	4.6	4.9	7%	4.4	4.5	2%	4.8	4.2	-13%
Soil Exposure 100%	4.3	4.1	-5%	4.4	4.7	7%	4.7	4.5	-4%	5.1	3.8	-25%
Reference Material 100%	4.7	4.5	-4%	4.5	5.0	11%	5.0	4.9	-2%	5.7	4.8	-16%
Average	4.6	4.2	-9%	4.6	5.2	14%	4.7	4.6	-2%	5.0	4.4	-12%

Table 6-5. Summary of relative bioavailability estimates

	DoD-PM	DoD-DP	Orchard	Smelter
Arsenic				
RBA	not significant	0.07	0.49	0.31
outliers	none	2	none	none
Lower bound	--	-0.10	0.33	0.20
Upper bound	--	0.21	0.65	0.45
Standard Error	--	0.09	0.10	0.08
p-value (adj. R-sq	0.76 (-2.7%)	<0.001 (62%)	<0.001 (58%)	<0.001 (59%)
Cadmium				
RBA	0.81	0.13	not significant	0.66
outliers	1	none	2	3
Lower bound	0.65	-0.003	--	0.57
Upper bound	0.99	0.24	--	0.76
Standard Error	0.10	0.07	--	0.05
p-value (adj. R-sq	<0.001 (78%)	<0.001 (80%)	0.34 (0.34%)	<0.001 (90%)
Chromium				
RBA	not significant	1.9	not significant	-0.02
outliers	none	1	none	1
Lower bound	--	--	--	--
Upper bound	--	--	--	--
Standard Error	--	--	--	--
p-value (adj. R-sq	0.76 (-1.7%)	0.028 (7.9%)	0.47 (-0.88%)	<0.001 (72%)
Lead				
RBA	0.21	0.34	0.60[a]	0.51
outliers	none	none	1	1
Lower bound	--	0.26	0.53	0.40
Upper bound	--	0.42	0.69	0.62
Standard Error	--	0.05	0.05	0.06
p-value (adj.R-sq.	<0.001 (35%)	<0.001 (85%)	<0.001 (80%)	<0.001 (79%)

-- Fieller's theorem does not apply or provides uncertain results so no standard error was calculated

[a] - As described in text, relative bioavailability of lead in Orchard soil was calculated using the average lead
acetate dose response from the other three dosing trials (I.e., those for DoD-PM, DoD-DP, and

7 Summary of *In Vitro* Research

The overall objective of the research undertaken under this SERDP funding is to use animal (*in vivo*) testing results, described in the prior sections of this report, as the basis to derive a benchtop (or *in vitro*) physiologically-based extraction test (PBET) for each route/receptor pair tested. Such tests have been proposed for several organic chemicals (Bondi et al. 2003; Ruby 2002) and inorganic chemicals (Kelley et al. 2002; Ruby et al. 1996; Rodriguez et al. 1999) in soil, and an *in vitro* test has been validated for lead to predict oral bioavailability in humans. In order to evaluate the potential for development of an *in vitro* method for each receptor/pathway combination investigated in the *in vivo* research component of this project, soils were tested *in vitro* under a variety of conditions, and the results were evaluated for correlation to the *in vivo* results. These results are discussed below, as the "*in vivo* to *in vitro*" (IV:IVT) correlations.

The testing and results discussed below are organized to parallel the discussion of *in vivo* research, above. Specifically, investigations relevant to human receptors are presented; first for oral exposures to arsenic (evaluating correlations with data from *in vivo* testing in cynomolgus monkeys), and then for oral exposures to cadmium (evaluating correlations with data from *in vivo* testing in swine). This is followed by discussion of the *in vitro* approaches to estimating dermal absorption of arsenic. Then, results relevant to the ecological receptor—the shrew—are presented.

7.1 Human Exposure – Oral

The sections that follow discuss research results regarding the development and assessment of PBETs, derived from *in vivo* studies, that would be predictive of relative oral bioavailability estimates for arsenic and cadmium.

7.1.1 Arsenic

Relative oral bioavailability values for arsenic in soil were derived using a cynomolgus monkey model that was developed and implemented at the University of Florida. Ten test soils, along with three reference doses of sodium arsenate in solution, were evaluated in this monkey model. The results from this *in vivo* study, and the resultant relative oral bioavailability research, are presented in Section 4.1 of this report, and additional details are included with the Supplemental Materials for Section 4. (It should be noted that this *in vivo* research continues under funding from an industrial source, and four more arsenic-bearing soils are slated to be tested for arsenic bioavailability in the cynomolgus monkey model. These new data will be added to the existing database, and a manuscript discussing all findings will be prepared during the first half of 2005.)

Prior to this study, several researchers have attempted to develop PBETs for arsenic that correlate with results from *in vivo* models, and these previous efforts have been considered in development of the research conducted for this project. For example, Rodriquez et al. (1999) reported that both a PBET consisting of a stomach-phase extraction (pH 1.8, 60 min. in a stirred beaker at 37 °C, maintained anaerobic with argon gas), or that stomach-phase extraction

followed by a small-intestinal phase (pH 5.5, addition of bile acids and pancreatic enzymes, 60 min. in a stirred beaker at 37 °C, maintained anaerobic with argon gas), correlated equally well with RBAs from the juvenile swine model for 13 mining-related samples (r^2 = 0.69 and 0.67, respectively, $p < 0.01$). These data suggest that the extent of arsenic dissolution during an acidic gastric-like extraction is predictive of RBAs in the juvenile swine model. Subsequent research using the PBET protocol developed by the Solubility/Bioavailability Research Consortium (SBRC) (published in Kelley et al. 2002) has confirmed that arsenic dissolution in an acidic gastric-like extraction is most likely to be predictive of arsenic bioavailability from an *in vivo* model, and the *in vitro* research reported herein was based on that premise.

As a starting point, the PBET protocol developed by the SBRC (see Supplemental Materials for Section 7) was run at pH values of 1.5 and 2.5 to evaluate arsenic bioaccessibility from the 10 test soils that had been evaluated for relative arsenic bioavailability in the cynomolgus monkey model. The results from this extraction testing are presented in Table 7-1, and the IV:IVT correlations are presented in Figure 7-1.

A trend of increasing arsenic bioaccessibility with increasing bioavailability is notable at both extraction pH values, with the exceptions of the Florida Cattle Dip Vat (FLCDV) and the California Mine Tailings (CAMT) soils (the *in vitro* results for these samples were confirmed by an independent laboratory). The arsenic mineralogy results (presented in Section 2 of this report) indicate that the FLCDV soil contains primarily arsenic adsorbed to clay, which would be expected to yield a high RBA value (as was seen in the cynomolgus monkeys). This suggests that the *in vitro* test, as specified under the SBRC method, is underestimating arsenic bioaccessibility from this particular soil type. The CAMT soil also appears anomalous, and in this case, the arsenic mineralogy is dominated by the relatively insoluble mineral arsenopyrite, suggesting either that the *in vivo* data are anomalously high (which would be supported by the mineralogy results) or that the *in vitro* test is underestimating arsenic bioaccessibility for this arsenic form as well. [Soils to be tested in the monkeys in upcoming weeks include a pure arsenopyrite-spiked sample, which will help resolve remaining questions regarding bioavailability of this form of arsenic]

Eliminating the FLCDV and CAMT samples from the IV:IVT correlation yields good correlations at both pH values (r^2 = 0.87 and 0.82 for pH 1.5 and 2.5, respectively; Figure 7-2). Given the strength of these correlations, it appears that the SBRC *in vitro* test method is predictive of arsenic bioavailability for 8 of the 10 samples tested.

Based on the arsenic forms in the two soils for which the SBRC *in vitro* method does not appear to provide a good "fit," additional research was undertaken to improve the correlation between the *in vivo* and *in vitro* data for the full set of studied soils. Two modifications were tested: first was to use the standard SBRC *in vitro* method, buffered to pH 2.5, but with the addition of 500 mg/L of phosphate. Secondly, a similar extraction solution was used, but replacing the glycine buffer with phosphate, included at the same molarity as the glycine that it replaced (0.4N). Because of the geochemical dynamics between arsenic and phosphate, the addition of the phosphate to the system should be effective in displacing adsorbed arsenic.

Figures 7-3 and 7-4 show the IV:IVT correlations following the addition of phosphate. The results of the phosphate-containing extractions indicated that addition of 500 mg/L of phosphate

caused only a minor alteration in the resulting bioaccessibility. Use of phosphate as a buffer in place of the glycine resulted in more dramatic effects and were evaluated further. Results for the phosphate-buffered extractions and the corresponding SBRC-based extractions are presented in Table 7-2. As anticipated, addition of phosphate to the *in vitro* extraction system results in a higher measured extraction efficiency for the FLCDV soil that contains the sorbed arsenic. The extraction efficiency of the *in vitro* method increases for this sample with increasing phosphate concentration, with the IV:IVT correlation improving for the highest phosphate system. A similar effect was seen for the CAMT soil, for which the addition of phosphate increased arsenic solubility from the soil and enhanced the IV:IVT correlation at the highest phosphate concentration tested. Surprisingly, however, addition of phosphate to the extraction fluid decreased arsenic solubility for other soil samples in the testing array (specifically for the Washington Orchard Soil [WAOS] and for the Western Iron Slag Sample [WISS]). Because of the shift for these two samples toward decreased solubility in the presence of the high-phosphate extraction fluid, the resulting IV:IVT correlation for the full set of soils tested was not improved by the addition of phosphate. However, if the maximum value achieved from either the original SBRC method at pH 2.5 or the phosphate-buffered extraction is selected for each sample tested, the IV:IVT correlation is quite good (r^2 value of 0.76) (Figure 7-5). Based on these findings, a preliminary protocol has been developed that specifies parallel extraction of soil samples in a glycine-buffered system as well as a phosphate-buffered system, and selection of the maximum percent extraction efficiency of the two systems for use in assessing the corresponding estimates of relative bioavailability. As shown in the upper graph in Figure 7-6, the equation for regression of the maximum *in vitro* results from the two extractions against the *in vivo* results indicate a good correlation ($r^2 = 0.745$)

$$RBA = 0.23(IV) + 0.058$$

where:

RBA represents the estimate of relative oral bioavailability *in vivo*

IV represents the maximum percent extraction found in either *in vitro* extraction procedure.

It is important to note in this approach that the slope of the line (0.23) is significantly lower than the value of 1, indicating that the extraction efficiency of the *in vitro* methodology is significantly higher (i.e., more aggressive) than that of the *in vivo* system using the cynomolgus monkey. This indicates that the regression equation is important to "convert" the *in vitro* results into the corresponding value for relative oral bioavailability. It also indicates that use of a simple extraction test (e.g., the standard SBRC protocol) generally provides an extreme upper-end prediction of oral bioavailability, and would overestimate relative bioavailability measured in the *in vivo* system for nearly all samples tested to date.

The r^2 value of 0.745 indicates that the regression equation provided by this IV:IVT correlation fits the data quite well (upper graph, Figure 7-6). For the two samples for which this equation would most underpredict relative oral bioavailability based on extraction data, the magnitude of the underprediction is approximately 6%; i.e., the "predicted" relative oral bioavailability would be 6% lower than the measured relative oral bioavailability value for that sample.

In addition to the simple regression modeling described above, a multivariate statistical evaluation of the full set of data available for these soils was evaluated in order to understand which factors most affect the relative oral bioavailability of arsenic from these samples. Factors included in the analysis include arsenic concentration, soil pH, DCB-extractable iron (proposed to be a predictor of arsenic solubility in biological systems [Yang et al. 2002]), mineralogy, all soil parameters listed in Table 2-2 of this report, and extraction efficiency in the glycine- and phosphate-buffered solutions evaluated above. This full suite of data was available for 9 of the 10 soils dosed to the cynomolgus monkeys.

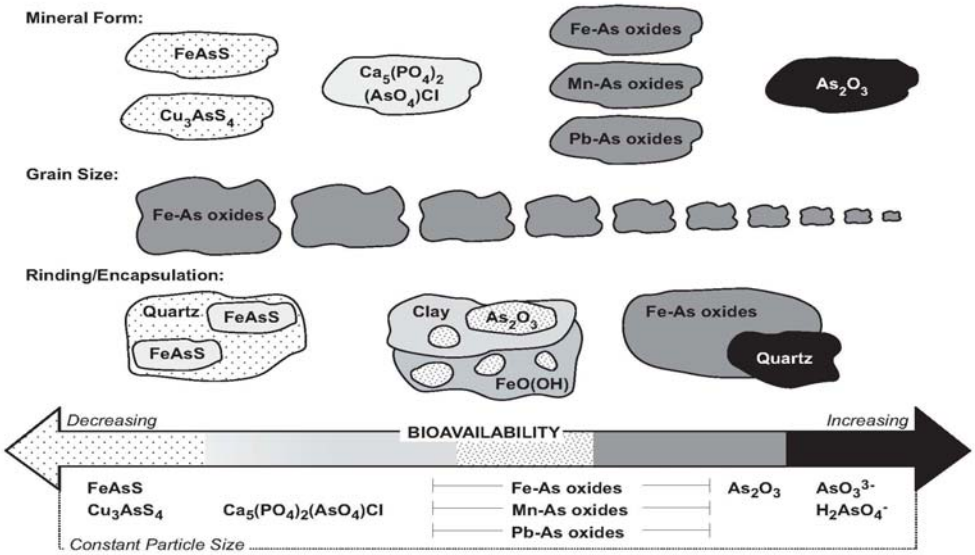
Results of this multivariate analysis indicate that factors including concentrations of TOC, extractable iron oxide, copper, and zinc significantly affected the goodness of fit of predictions to the RBA values from the *in vivo* study. When all these factors are included, a regression equation of

$$RBA = -0.045 \times TOC + 0.00002 \times (FeO) - 0.00009 \times Cu - 0.00003 \times Zn + 0.20$$

can be fitted to the data ($r^2 = 0.927$) (lower graph, Figure 7-6).

In this figure, the depicted diagonal line on the lower graph represents a perfect fit (i.e., one to one correlation) between the measured RBA and predictions of various models. The green diamonds indicate the measured RBA vs. the value predicted by the in vitro extraction testing specified above and in the attached protocol ($r^2 = 0.745$). The blue stars represent the predictions of the multivariate regression model ($r^2 = 0.927$). In addition to depicting the regressions derived from our research, this figure also shows the fit using the regression equation proposed by Yang et al. (2002). In their research they identified an equation that relied on soil pH and iron oxide content to predict arsenic bioaccessibility from soils. As demonstrated on Figure 7-6, while their model may predict bioaccessibility under the conditions they specify, the equation does not appear to serve as a meaningful predictor of relative oral bioavailability as measured in the cynomolgus monkey.

Generally, it appears that there are several factors that affect the relative oral bioavailability of arsenic. These are captured in the diagram below.



Schematic of how different arsenic species, particle sizes, and morphologies affect arsenic bioavailability

Finally, as discussed above, four additional soils are currently being tested for relative oral bioavailability in the cynomolgus monkey model developed under this SERDP funding. These include three samples (of differing arsenic concentrations and bioaccessibility) from a site in upstate New York, and one sample of crystalline arsenopyrite mixed with soil. Although the funding for testing these samples is being provided by an alternative source, the results will be made available to augment the database developed for SERDP, and will be included in publication of the *in vivo* research results. Once *in vivo* testing of those soils has been completed, the data will be added to the database, and the IV:IVT correlation will be re-examined. That fuller evaluation will be provided to SERDP when it is available. If the study is reported in the form of a manuscript for publication, that manuscript will be submitted to SERDP as soon as it is completed, which is anticipated to be in the summer of 2005.

7.1.2 Cadmium

Relative bioavailability values for cadmium in soil were derived using a juvenile swine model that was developed and applied at the University of Missouri–Columbia. Four test soils, along with three reference doses of cadmium chloride, were evaluated in this swine model. The results from this *in vivo* study, and the resultant relative oral bioavailability values, are described above in Section 4.2 and the associated Supplemental Materials for Section 4. In addition, a fifth soil (OK Smelter #2) that had been evaluated previously for cadmium bioavailability in the swine model at the University of Missouri was obtained from Dr. Nick Basta (Ohio State University), and was also subjected to the *in vitro* testing. This fifth soil was evaluated only for relative cadmium bioavailability based on a measurement endpoint in kidneys of the test animals, while the other four test soils studied in the SERDP project were evaluated for

endpoints in both kidney and liver. Because it was unclear whether the kidney- or the liver-based RBA values represented the superior measure of relative cadmium bioavailability from soil, the values from these two endpoints were averaged to provide a single comparison value for the *in vitro* testing results (because the OK Smelter #2 soil had only a kidney-based RBA value, this was used alone as the value for comparison against the *in vitro* results).

The only previous published research on PBETs that would correlate with relative oral bioavailability measurements for cadmium in soil was published during the course of the SERDP project work, by Schroder et al. (2003). In this research, the *in vitro* extraction method described above, from Rodriguez et al. (1999), was used for cadmium in soils. The best IV:IVT correlation reported by Schroder was obtained using a gastric-phase extraction (pH maintained at 1.8, 60 min. in a stirred beaker at 37 °C) that was maintained under anaerobic conditions by constantly diffusing argon gas through the solution. Based on an evaluation of 10 soils in this *in vitro* test, a linear IV:IVT correlation with $r^2 = 0.74$ was obtained.

Based on this previous research, the PBET developed by the SBRC was used as a starting point for evaluating cadmium bioaccessibility, and was run at pH values of 1.5 and 2.5. The major limitation to a strong IV:IVT correlation for cadmium in this study is the limited *in vivo* data set, and the fact that four of the five RBA values are clustered at high relative bioavailability values (78% to 94%; Figure 7-7). Despite this, the *in vitro* test results at both pH 1.5 and 2.5 yielded strong, and equivalent, IV:IVT correlations (r^2 values of 0.94 and 0.95, respectively). However, the IV:IVT correlation based on the PBET at a pH of 2.5 yields a slope closer to 1.0, suggesting that this pH value more closely resembles that of the gastric environment in the juvenile swine. In both cases, the Dugway composite soil, which yielded a much lower RBA value than any of the other soils, controls the slope of the IV:IVT correlation.

Thus, the SBRC *in vitro* test run at a pH of 2.5 provides a PBET that correlates strongly with results from the juvenile swine model, based on this limited *in vivo* data set. The r^2 value for the research reported herein and that reported by Shroeder (i.e., r^2 of 0.94 and 0.74, respectively), suggest that either extraction approach should provide in vitro bioaccessibility data that permit a reasonable estimation of the relative oral bioavailability of cadmium as measured in the swine. The *in vitro* approaches used by both are based on virtually identical chemical principals (acid extraction, agitated at physiological temperatures), and either approach appears to provide data that are predictive of relative oral bioavailability of cadmium, thereby each piece of research service to corroborate the other. The r^2 value reported herein is higher than that reported by Schroeder et al. (2003), but as discussed above, since it is derived from a smaller dataset and is largely controlled by one sample, our database is somewhat less robust, despite the larger correlation value. Conversely, because of the added complexity of the in vitro method used by Schroeder et al. over the method used herein (i.e., addition of food to the system, constant maintenance of pH, and requirement of continuous diffusion of argon into the system to maintain the anaerobic environment), the SBRC-based method may be preferred because it is a simpler extraction process (and therefore more amenable to use in a variety of analytical labs). An appropriate next step in determining the most simple yet effective extraction strategy for prediction of the relative oral bioavailability of cadmium would be to share soil samples between the labs (i.e., Shroeder and Exponent) to cross validate each datum, and to determine whether one model is more predictive than the other.

7.2 Human Exposure – Dermal

7.2.1 Arsenic

Dermal arsenic absorption from two soils was assessed using the Rhesus monkey model described in Wester et al. (2004). Test soils from the Colorado residential soil, and from a pesticide production facility in Middleport, New York (Middleport soil), were evaluated and yielded dermal absorption values of arsenic from these soils that were not distinguishable above background, with theoretical limits of dermal absorption of approximately 0.16% and 0.12% of the applied doses, for the two soils, respectively. Complete details of the dermal arsenic *in vivo* study can be found in Section 5.1 and the Supplemental Materials for Section 5.

These two soils were subjected to an extraction procedure using human sweat, which was based on the sweat extraction method developed by Horowitz and Finley (1993). The extraction involved leaching 1.0 g of soil (<150-μm size fraction) in 25 mL of human sweat at 30 °C for 8 hours, with mixing on a shaker table. The Colorado residential soil and Middleport soil yielded 11% and 1.8% extractable arsenic, respectively. The results from the sweat extraction indicate that the amount of arsenic extractable in human sweat is more than an order of magnitude higher than the average fraction of arsenic that is actually dermally absorbed. This is not surprising, because the conditions of the sweat extraction are considerably more aggressive and conducive to arsenic dissolution than conditions at the skin surface. In addition to the two site soils tested for dermal absorption in the *in vivo* research, arsenic extraction in sweat was also evaluated for a Yolo County soil that was spiked with soluble arsenic just prior to the extraction. This soil was tested because it represents the same type of soil and arsenic conditions that were reported in Wester's earlier research (1993). In that research, they investigated dermal absorption of radiolabeled arsenic that had been freshly mixed with Yolo County soil. For this soil, the *in vivo* results indicate dermal absorption of 3.2% to 4.5% of the applied dose. In comparison, the sweat extraction of a similarly-prepared sample (without radiolabel) yielded a 72% extraction efficiency. This finding is consistent with the results for the site soils, in that the sweat extraction was more than an order of magnitude higher than the dermal absorption efficiency.

These results indicate that a reasonable estimate of dermal arsenic absorption from soil can be obtained by applying the sweat extraction procedure developed for this project, and then dividing the fraction of sweat-extractable arsenic by 15 to 20. Documentation of the extraction procedure for using sweat is provided in the Supplemental Materials for Section 7. It should be noted that an estimate of dermal arsenic absorption developed in this manner is based on a limited *in vivo* data set.

Possibly more important than providing the basis for evaluation of a predictive *in vitro* method, the results of the *in vivo* testing of dermal absorption of arsenic suggest that absorption from "field-derived" arsenic-containing soils does not result in urinary arsenic levels that are distinguishable from background, and establishes that dermal absorption of arsenic from environmental soils is significantly lower than the default assumption recommended by EPA (3% based on testing of soluble arsenic freshly mixed with soil). These results suggest that percutaneous absorption of arsenic from environmental soils does not contribute significantly to

total arsenic exposures, and can be appropriately excluded from exposure evaluations. Other risk evaluations conducted recently have come to similar conclusions (Kwon 2004; CPSC 2003; U.S. EPA 1996). Additionally, evidence from biomonitoring studies indicates that the standard exposure assessment assumptions for soil ingestion conservatively account for measured soil arsenic exposure, and that any additional exposure via percutaneous absorption is negligible. Two EPA studies (Glass and SAIC 1992; Walker and Griffin 1998) compared arsenic dose estimates via soil ingestion to measured urinary arsenic levels in human populations. In both studies, the calculated exposures either matched or overestimated the actual levels compared to the biomonitoring data. In summary, these studies found no contribution of arsenic dose due to percutaneous absorption. In other words, this indicates that the relative contribution of arsenic from the percutaneous pathway is negligible in comparison to the amount of exposure via oral intake. This is a more important conclusion that can be substantiated by the *in vivo* research than simply forming the basis for *in vitro* simulation methodologies.

7.3 Wildlife Exposure

Oral bioavailability to the shrew of arsenic, cadmium, chromium, and lead from four test soils was studied; this study and the resultant relative bioavailability values are presented in a manuscript by Ruby et al. (see Supplemental Materials for Section 6). For the *in vitro* research, the PBET developed by the SBRC (see Supplemental Materials for Section 7) was used as a starting point for evaluating the bioaccessibility of target metals in soil, and was run at pH values of 1.5, 2.5, 3.5, and 4.5. The *in vitro* results, and associated IV:IVT correlations for each metal, are described below. Specific extraction procedures that provided the best correlation to the in vivo data for each metal (arsenic, cadmium, chromium, and lead) are provided in the Supplemental Materials for Section 7.

7.3.1 Arsenic

The shrew research yielded RBA values for arsenic for three soils (see Table 5 of Ruby et al. included in Supplemental Materials for Section 6), so the *in vivo* data set on which to base an IV:IVT correlation is a limited one. Comparison of the *in vivo* RBA results against the *in vitro* values at the four different pH values (Figure 7-8) indicates that the SBRC *in vitro* test run at a pH value of 2.5 yields the best IV:IVT correlation ($r^2 = 0.83$). The slope of the pH 2.5 IV:IVT correlation of close to 0.7 indicates that this *in vitro* test slightly overestimates the shrew-based RBA values for arsenic at this pH value. The fact that the slope of the line for the IV:IVT correlation does not equal one does not mean that the model is not predictive, but rather only that the *in vitro* results cannot be used directly without adjustment to account for the slope of the line. This is consistent with the IV:IVT correlation evaluation conducted by EPA for lead. In their assessment, the linear relation is provided, and the results of the *in vitro* testing are used to estimate *in vivo* results.

These results indicate that, based on a limited *in vivo* data set, the SBRC *in vitro* test run at a pH of 2.5 is capable of predicting the shrew-based RBA values for arsenic in soil.

7.3.2 Cadmium

The shrew research yielded three RBA values for cadmium (see Table 5 of Ruby et al., provided in the Supplemental Materials for Section 6), so the *in vivo* data set on which to base an IV:IVT correlation is limited. Comparison of the *in vivo* RBA results against the *in vitro* values at the four different pH values (Figure 7-9) indicates that the SBRC *in vitro* test run at a pH of 4.5 yields the best IV:IVT correlation ($r^2 = 0.998$), while the extraction at pH 3.5 also yielded a reasonable correlation ($r^2 = 0.88$). The slope of the pH 4.5 IV:IVT correlation of 3.2 indicates that the PBET underestimates the shrew-based RBA values for cadmium by about a factor of about three (see Section 7.3.1 above regarding the meaning of the slope as it pertains to predictiveness).

Based on this data set, the SBRC *in vitro* test run at a pH of 4.5 is recommended for estimating the oral bioavailability of cadmium in soil to shrew.

7.3.3 Chromium

There was not sufficient *in vivo* data from the shrew study to develop an IV:IVT correlation for chromium (see discussion in Ruby et al., in Supplemental Materials for Section 6). However, the limited data set that is available suggests that there is little, if any, dose-response relation between the administered dose of chromium and the absorbed dose (or body burden) of chromium in the exposed animal. This suggests that some factor is regulating the uptake of chromium into the tissues, and that relative oral bioavailability may not be a concern for this metal in soil.

7.3.4 Lead

The shrew research yielded four RBA values for lead (see Table 5 of Ruby et al. in Supplemental Materials for Section 6). As described in Ruby et al., the RBA value for lead from soil DoD-PM likely has more uncertainty associated with it than do the other three soils. Comparison of the *in vivo* RBA results against the *in vitro* values at the four different pH values (Figure 7-10), indicates a lack of IV:IVT correlation when all four data points are included. This is odd because the SBRC *in vitro* test correlates strongly with oral lead bioavailability in the juvenile swine model at both pH 1.5 and 2.5 (r^2 values of 0.87 and 0.85, respectively, with 15 soils tested both *in vivo* and *in vitro*). Given the uncertainty associated with the *in vivo* data for soil DoD-PM, this soil was eliminated from the IV:IVT analysis. When this change was made, the IV:IVT correlation improved for both pH 1.5 and 2.5 (r^2 values of 0.69 and 0.66, respectively; Figure 7-10). Thus, it appears that the SBRC *in vitro* test, which is highly effective for predicting the oral bioavailability of lead to juvenile swine, is also useful for predicting this endpoint in shrew. Based on these results, the SBRC *in vitro* test, run at a pH value of 2.5, is recommended for estimating the oral bioavailability of soil lead to shrew.

7.3.5 Summary of *In Vitro* Testing for Wildlife Receptors

Overall, for the research focused on wildlife receptors, acceptable IV:IVT correlations were established for all of the metals evaluated except chromium, for which there appears to be little dose-related absorption into tissues. Standardized methods for conducting these extractions are provided in the Supplemental Materials for Section 7. The standard methods provide adequate documentation so that extractions could be replicated in any lab. As mentioned in each of the preceding sections on wildlife results, the primary limitation to developing robust IV:IVT correlations for these metals is the limited number of soils that have been tested in the *in vivo* system. The research conducted under the SERDP funding does appear to have resulted in the development of a meaningful animal model for assessing the relative oral absorption of metals from soils, as well as a protocol for *in vitro* estimation of relative oral absorption. It would be useful to expand upon the database that has been started under this project work, to provide a stronger database.

7.4 References

Bondi, G. K. Jones, K. Semple, A. Craven, P. Burauel. 2003. Nonextractable pesticide residues in soil. Environ. Sci. Technol. 37 (7):139-144.

CPSC Consumer Products Safety Commission (CPSC) 2003. Briefing Package: Petition to ban chromated copper arsenate-treated wood in playground equipment. Available at http://www.cpsc.gov/phth/ccafact.html.

Glass, G.L., and SAIC. 1992. Baseline risk assessment, Ruston/North Tacoma Operable Unit, Commencement Bay Nearshore/Tideflats Superfund site, Tacoma, Washington. Prepared for U.S. EPA Region 10. Science Applications International Corporation, Bothell, WA. January.

Horowitz, S.B., and B.L. Finley. 1993. Using human sweat to extract chromium from chromite ore processing residue: Applications to setting health-based cleanup levels. J. Toxicol. Environ. Health 40:585–599.

Kelley, M.E., S.E. Brauning, R.A. Schoof, and M.V. Ruby. 2002. Assessing oral bioavailability of metals in soil. Battelle Press, Columbus, OH.

Kwon, H. H. Zhang, Z. Wang, GS Jhangri, X. Lu, N. Fok, S. Gabos, XF Li, XC Li. 2004. Arsenic on the hands of children after playing in playgrounds. Environ. Health. Perspect. 112(14):1375-1380.

Rodriguez, R.R., N.T. Basta, S.W. Casteel, and L.W. Pace. 1999. An in vitro gastrointestinal method to estimate bioavailable arsenic in contaminated soils and solid media. Environ. Sci. Technol. 33(4):642–649.

Ruby, M.V., A. Davis, R. Schoof, S. Eberle, and C.M. Sellstone. 1996. Estimation of lead and arsenic bioavailability using a physiologically based extraction test. Environ. Sci. Technol. 30(2):422–430.

Ruby, M.V., K.A. Fehling, D.J. Paustenbach, B.D. Landenberger and M.P. Hosapple. 2002. Oral bioaccessibility of Dioxins/Furans at low concentrations in soil. Environ. Sci. Technol. 36(22):4905-4911.

Schroder, J.L, N.T. Basta, J. Si, S.W. Casteel, T. Evans, and M. Payton. 2003. In vitro gastrointestinal method to estimate relative bioavailable cadmium in contaminated soil. Environ. Sci. Technol. 37(7):1365–1370.

U.S. EPA. 1996. Butte Priority Soils Operable Unit baseline human health risk assessment for arsenic. Silver Bow Creek/Butte area NPL Site. Prepared for U.S. Environmental Protection Agency, Region VIII, Montana Office. CDM Federal Programs Corporation, Golden, CO.

Walker, S., and S. Griffin. 1998. Site-specific data confirm arsenic exposure predicted by the U.S. Environmental Protection Agency. Environ. Health Persp. 106(3):133–139.

Wester, R.C., L. Hui, S. Barbadillo, et al. 2004. *In vivo* percutaneous absorption of arsenic from water and CCA-treated wood residue. Toxicol. Sci. 79:287–295.

Yang, JK, MO Barnette, PM Jardine, NT Basta, and SW Casteel. 2002. Adsorption, sequestration, and bioaccessibility of As(V) in soils. Environ. Sci. Technol. 36(21):4562-4569.

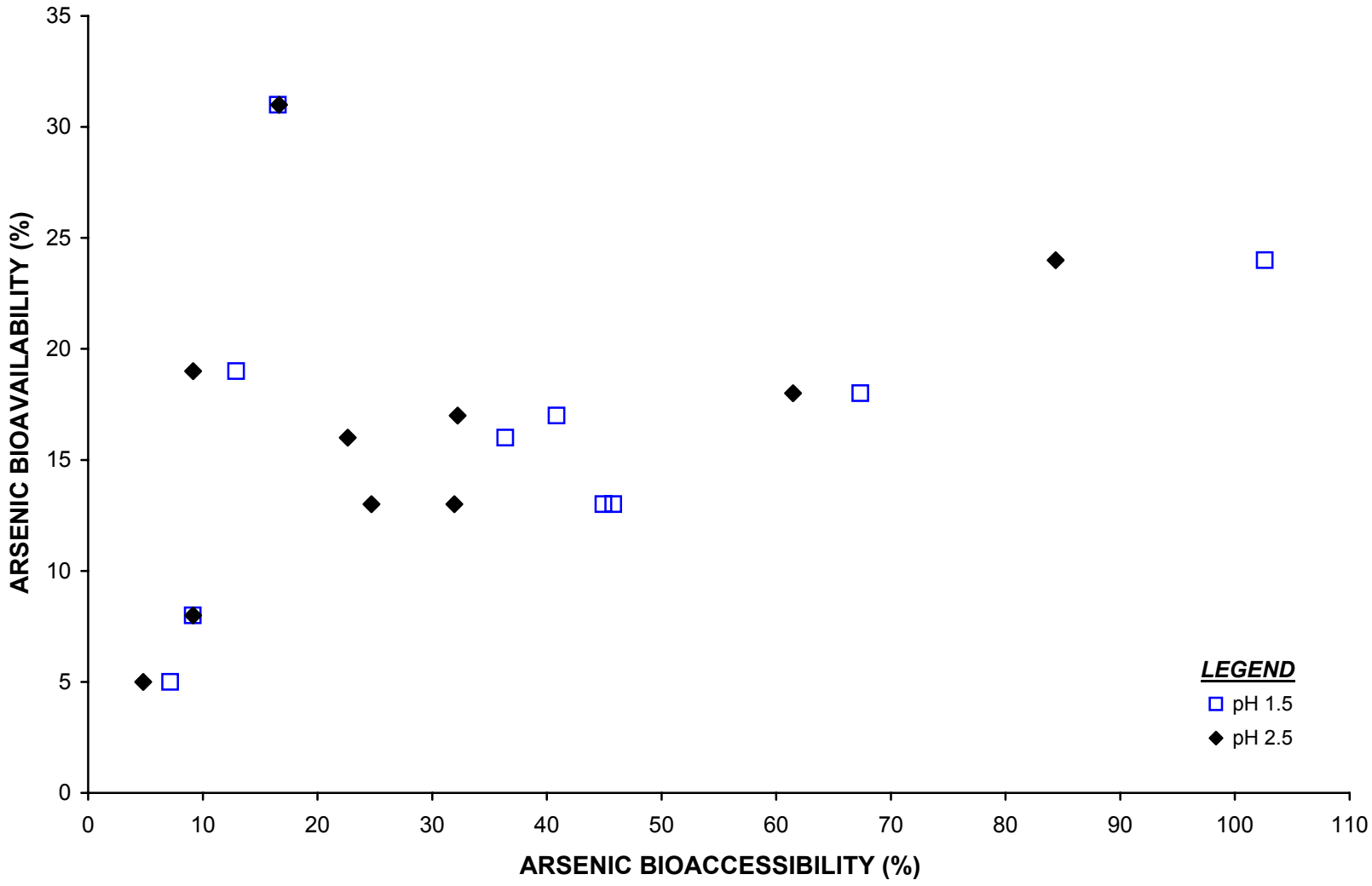


Figure 7-1. IV:IVT correlation for relative bioavailability of arsenic in cynomolgus monkeys: SBRC method at pH 1.5 and 2.5

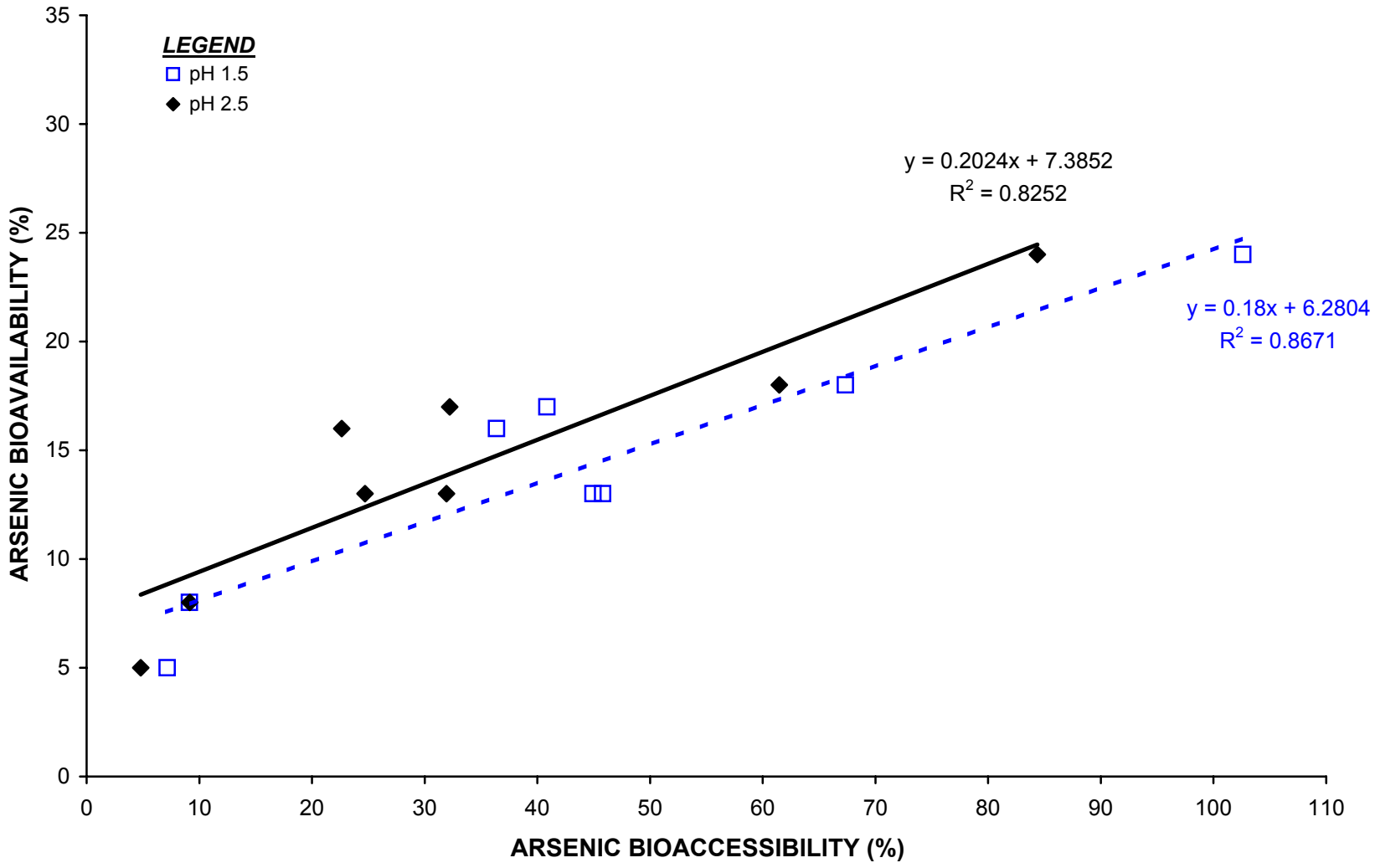


Figure 7-2. IV:IVT correlation for relative bioavailability of arsenic in cynomolgus monkeys: SBRC method at pH 1.5 and 2.5 without soil samples CDV and CAMT

\\Boulder3\Data\Projects\1191_SERDP\final_SERDP_report\In_vitro\
Tables from MVR AprOct2004_in-vitro_v4.xls Fig MVR-2 4/22/2005 (10:56 AM)

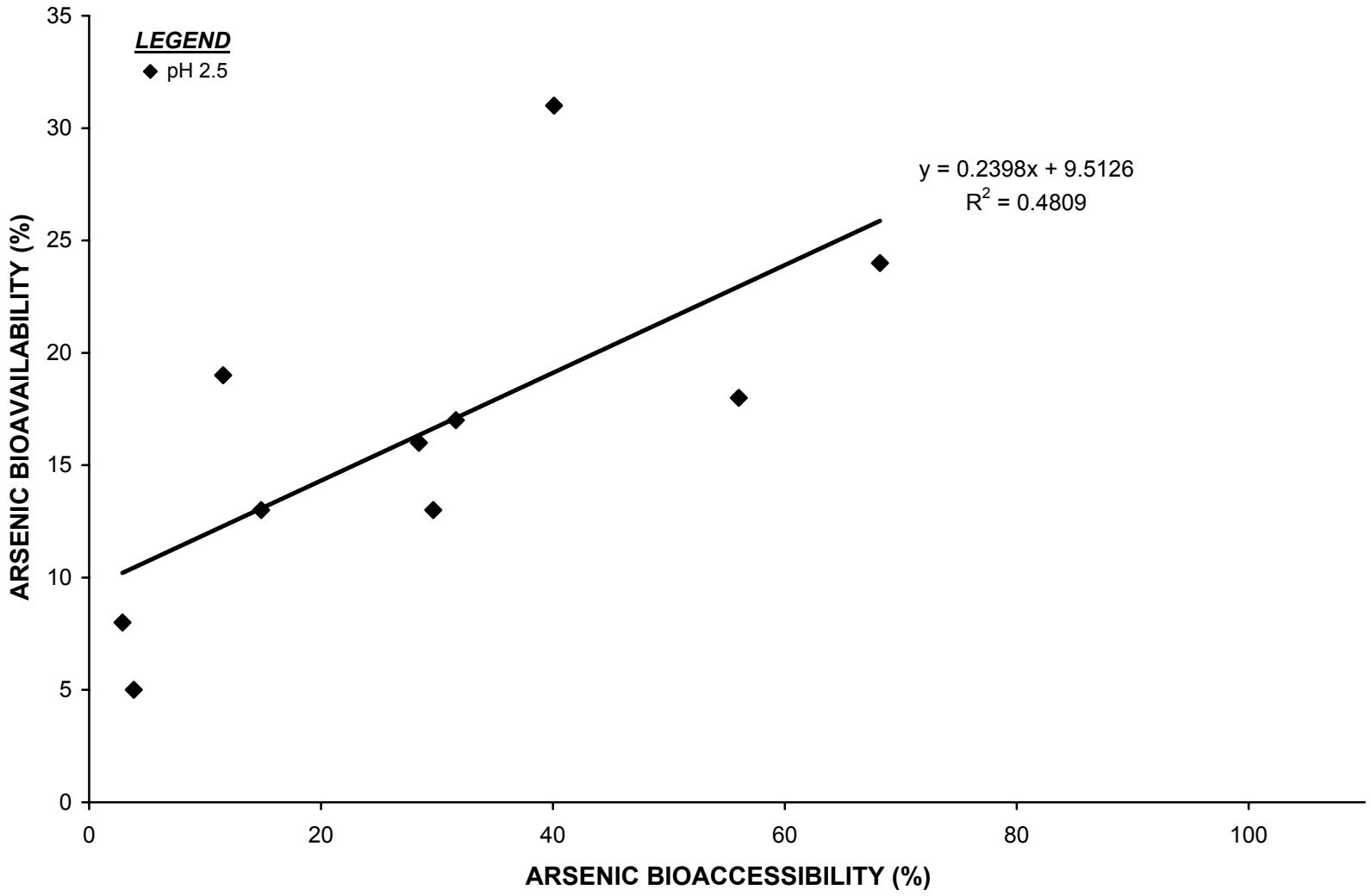


Figure 7-3. IV:IVT correlation of relative oral bioavailability of arsenic in cynomolgus monkeys: SBRC + 500 mg/L PO_4

\\Boulder3\Data\Projects\1191_SERDP\final_SERDP_report\In_vitro\
FROM LARRY Apr2005_in-vitro_ARSENIC_v1.xls Fig Cyno_Gly+500 ppm PO4 4/22/2005 (10:59 AM)

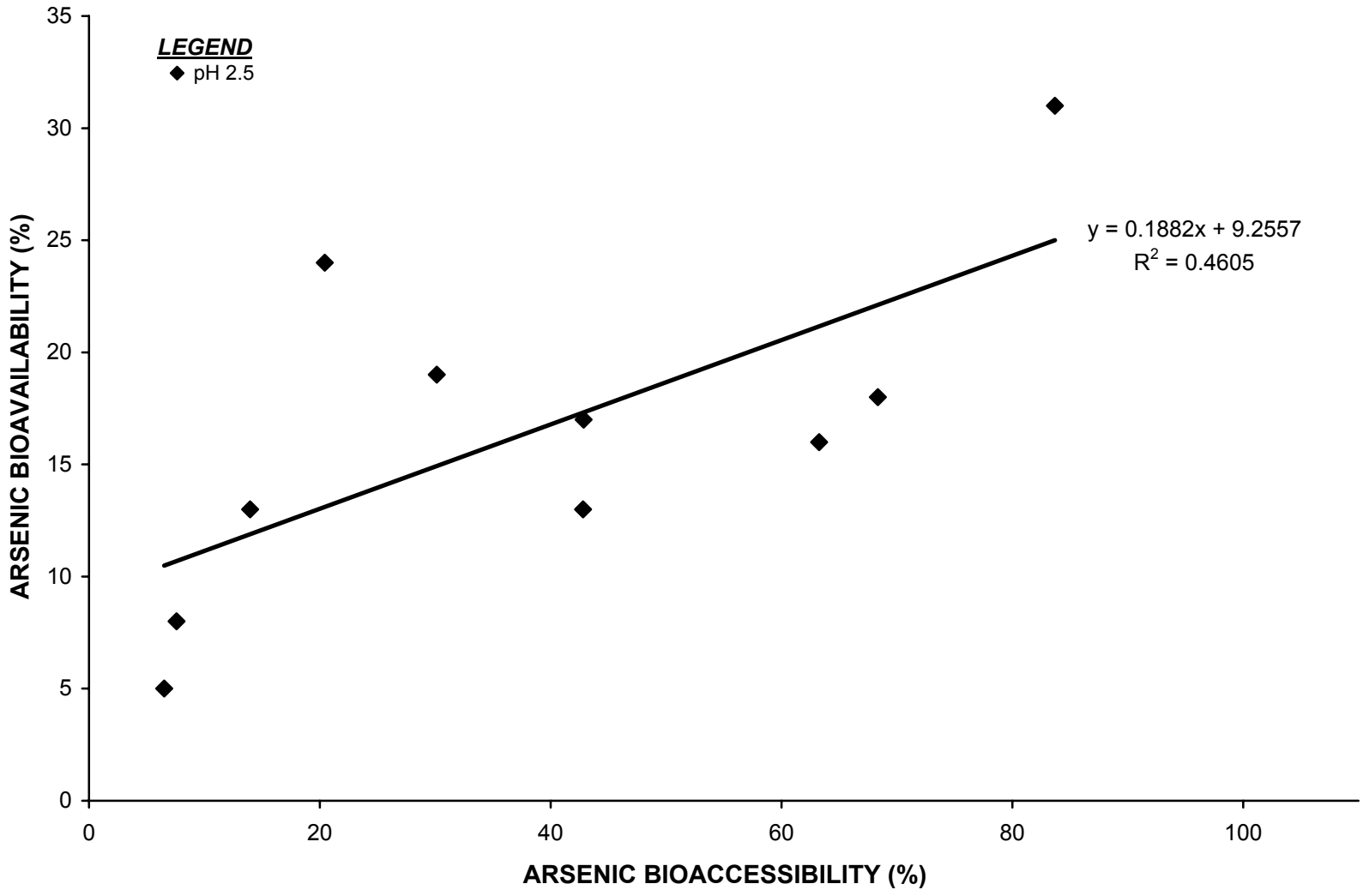


Figure 7-4. IV:IVT correlation of relative oral bioavailability of arsenic in cynomolgus monkeys: 0.4 M KH_2PO_4 in place of glycine buffer

\\Boulder3\Data\Projects\1191_SERDP\final_SERDP_report\In_vitro\
FROM LARRY Apr2005_in-vitro_ARSENIC_v1.xls Fig Cyno_0.4M KH2PO4 4/22/2005 (11:00 AM)

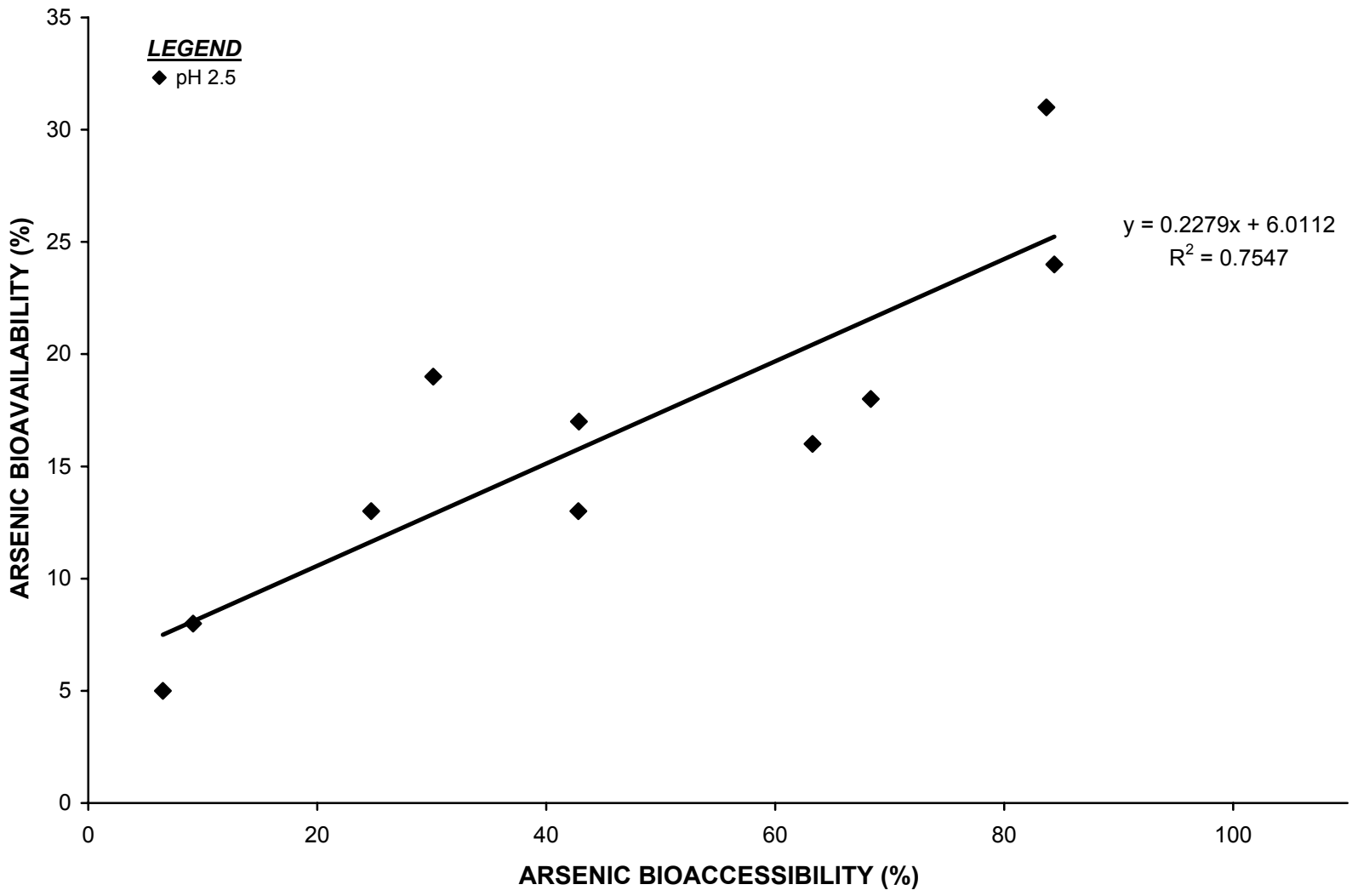
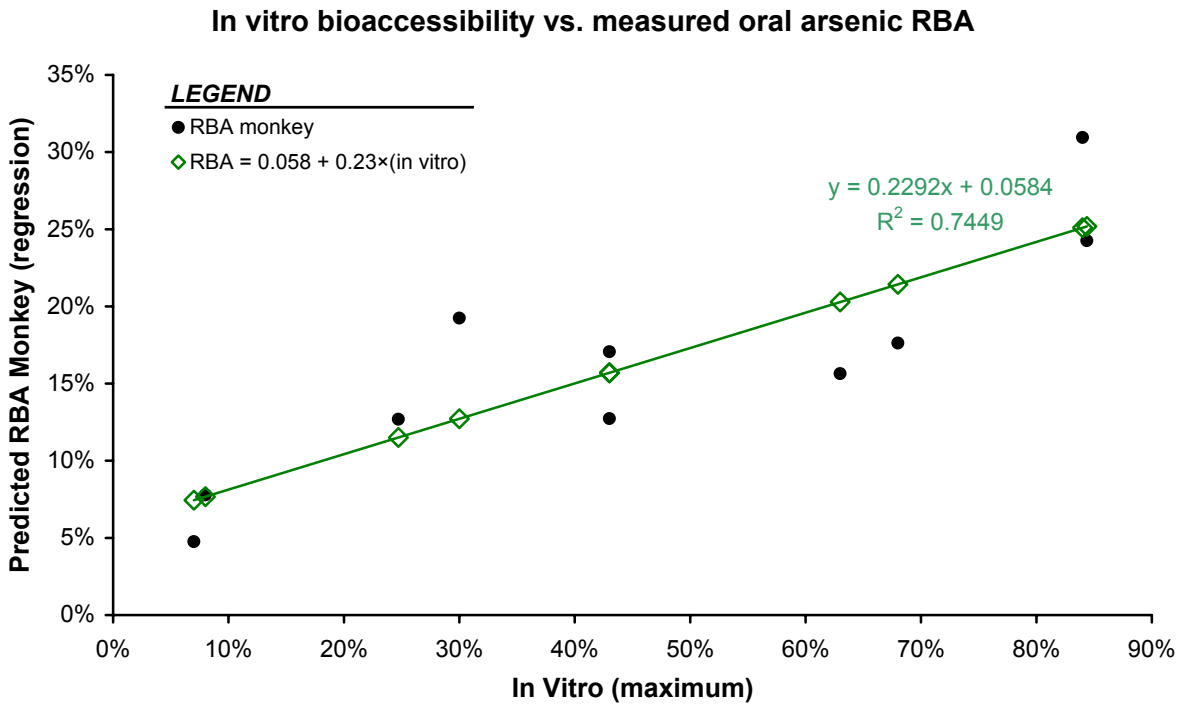
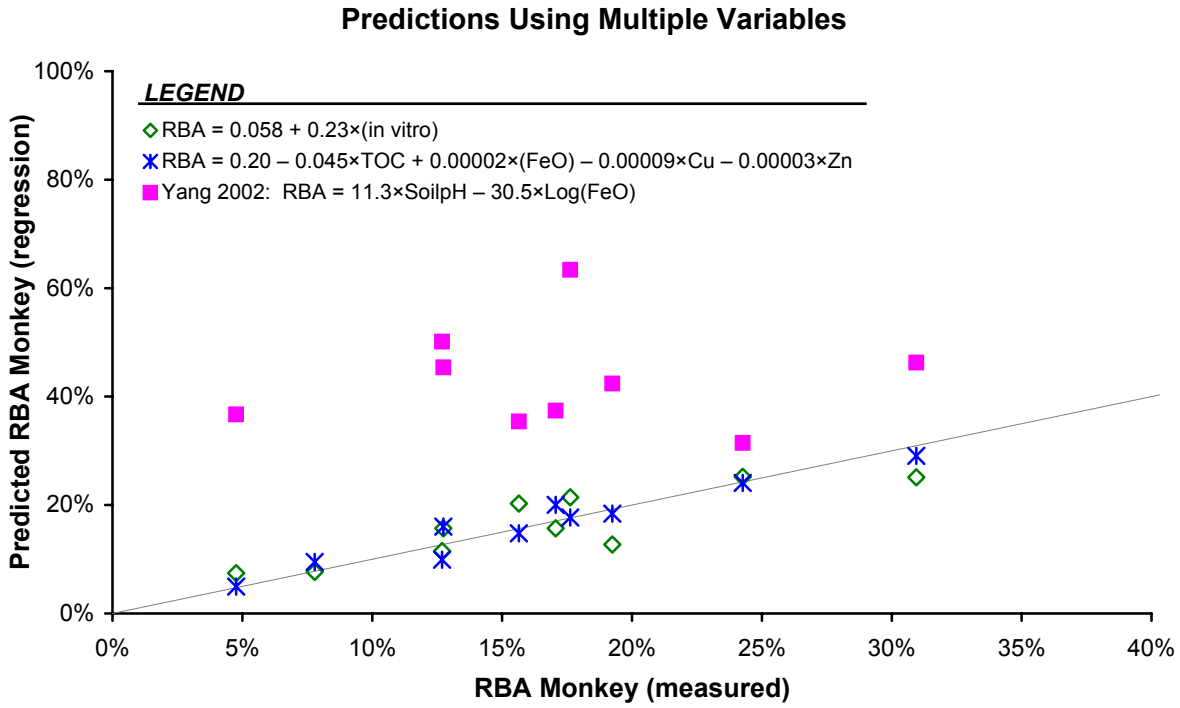


Figure 7-5. IV:IVT correlation of relative oral bioavailability of arsenic in cynomolgus monkeys: Highest bioaccessibility of pH 2.5 SBRC method or 0.4 M KH_2PO_4

\\Boulder3\Data\Projects\1191_SERDP\final_SERDP_report\In_vitro\
FROM LARRY Apr2005_in-vitro_ARSENIC_v1.xls Fig Cyno_High Combined As(2.5) 4/22/2005 (11:00 AM)





Note: FL iron slag soil excluded from multi-variable regression because no iron oxide data is presently available.

Figure 7-6. Regression of predicted vs. measured oral arsenic RBAs

\\Boulder3\Data\Projects\1191_SERDP\final_SERDP_report\In_vitro\
PredictedRBA2_FriApr15-2005.xls Fig 4/15/2005 (5:53 PM)

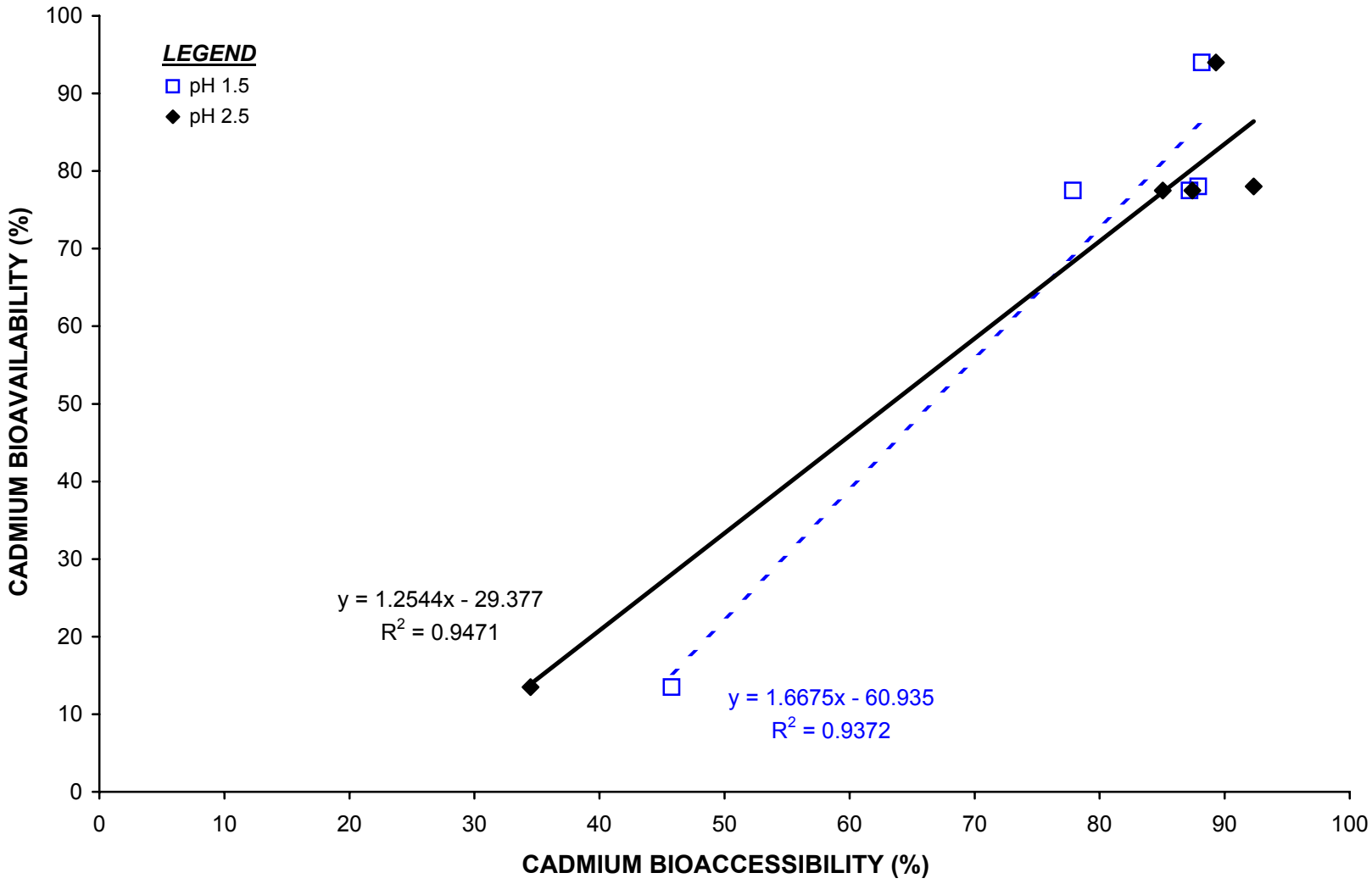


Figure 7-7. IV:IVT correlation for relative bioavailability of cadmium in swine: SBRC method at pH 1.5 and 2.5

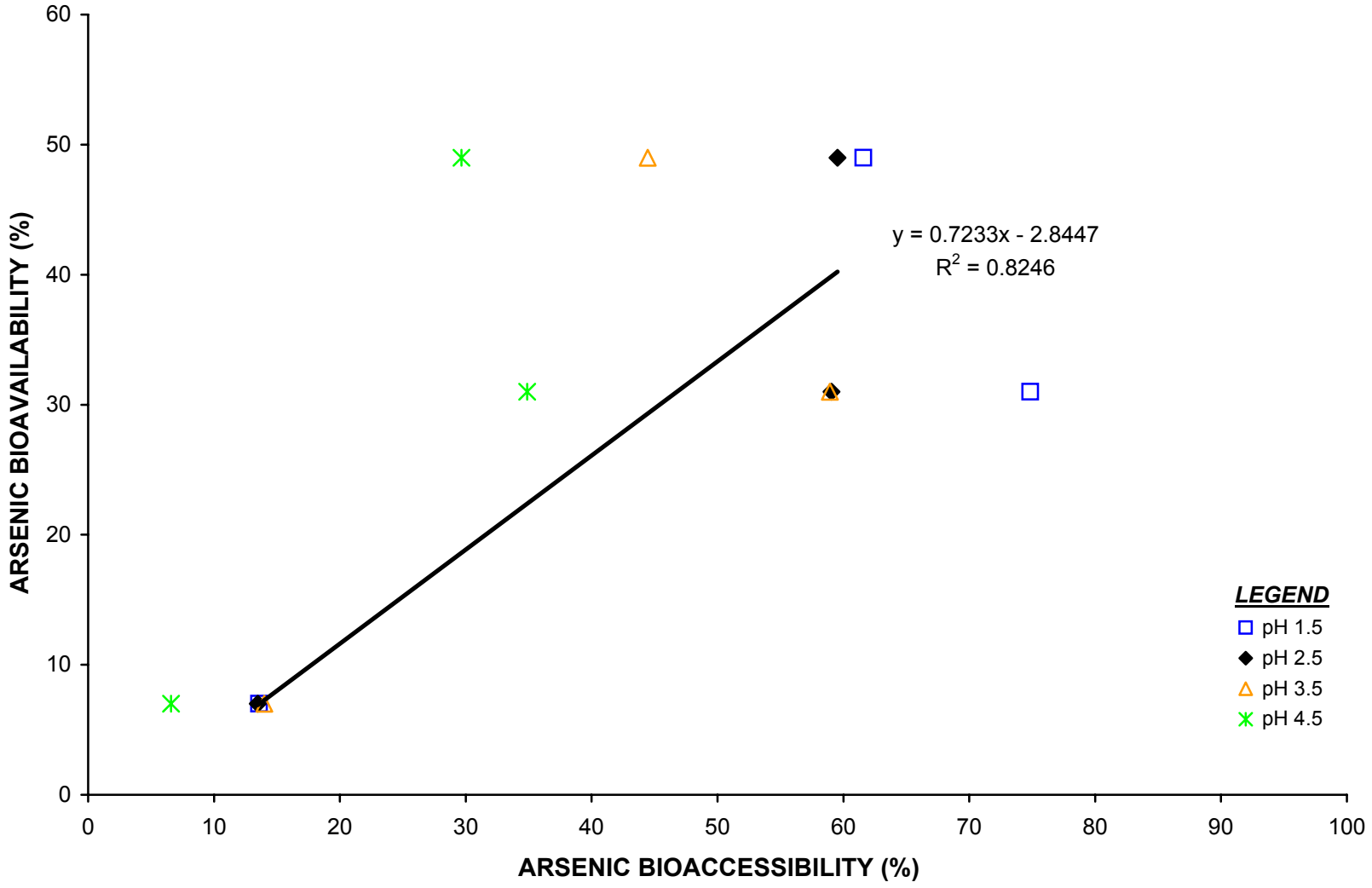


Figure 7-8. IV:IVT correlation for relative bioavailability of arsenic in shrews: SBRC method at pH 1.5, 2.5, 3.5, and 4.5

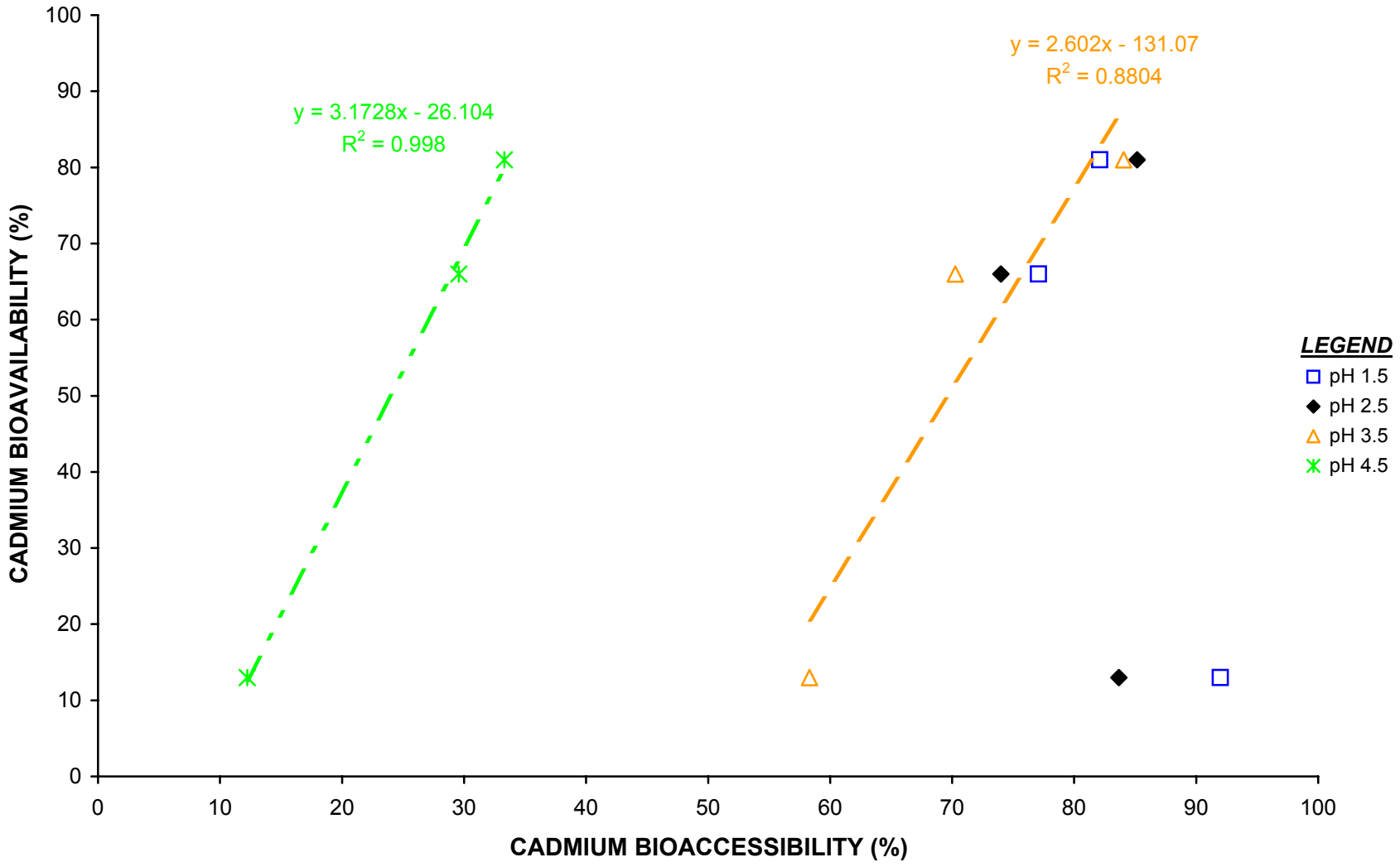


Figure 7-9. IV:IVT correlation for relative bioavailability of cadmium in shrews: SBRC method at pH 1.5, 2.5, 3.5, and 4.5

\\Boulder3\Data\Projects\1191_SERDP\final_SERDP_report\In_vitro\
Tables from MVR AprOct2004_in-vitro_v4.xls Fig MVR-5 4/22/2005 (10:58 AM)

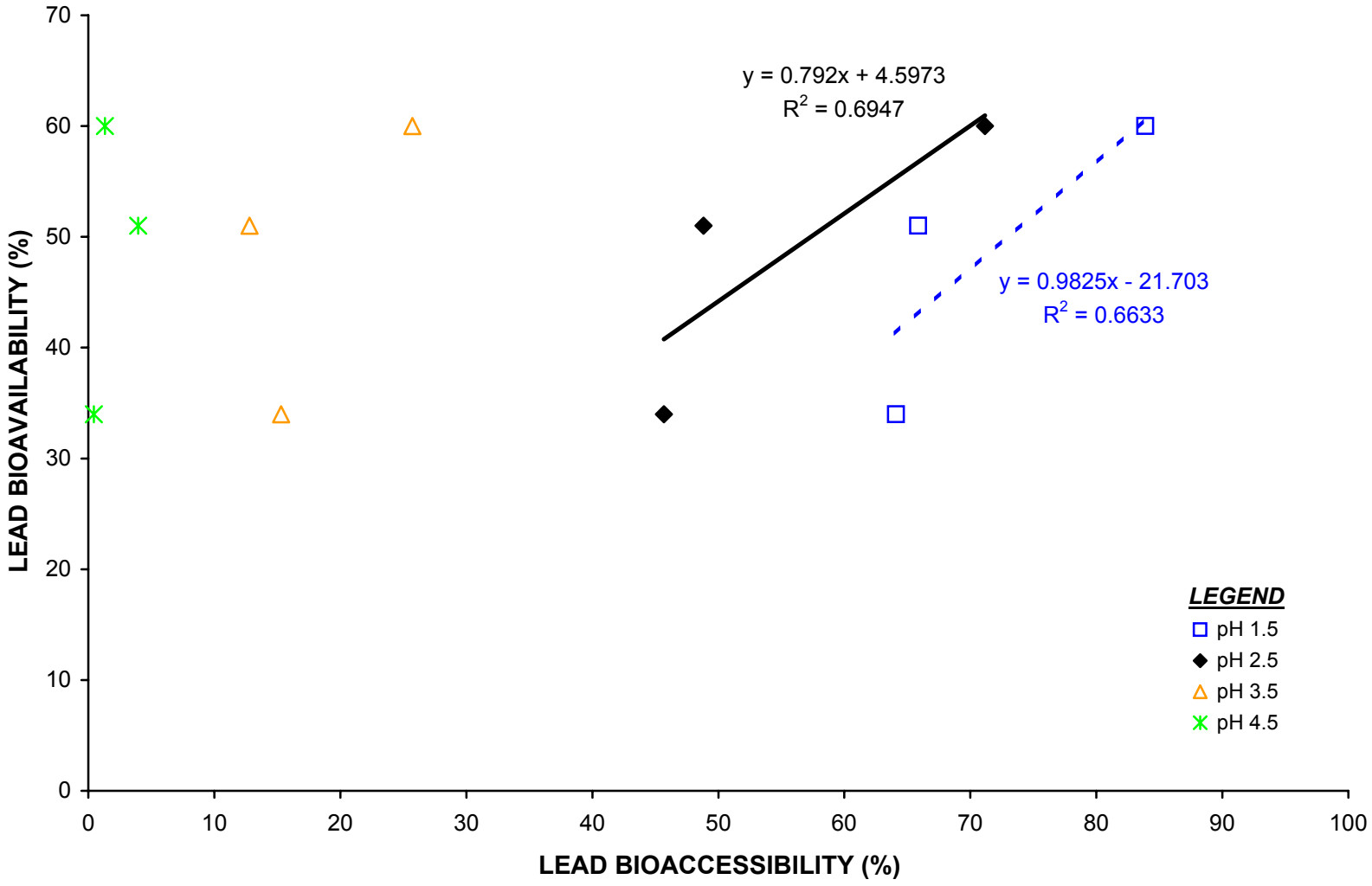


Figure 7-10. IV:IVT correlation for relative bioavailability of lead in shrews: SBRC method at pH 1.5, 2.5, 3.5, and 4.5 without soil sample DoD-PM

Table 7-1. *In vitro* arsenic extraction of SERDP soil research substrates

Extraction ID	Soil	Grain Size	Arsenic Conc. in Substrate (mg/kg)	Mass of Soil Tested (g)	Mass of Arsenic in Soil Extracted (mg)	Post-Test pH (s.u.)	Arsenic Conc. in Extract (mg/L)	Volume of Extract (L)	Mass of Arsenic in Extract (mg)	Arsenic Bioaccessibility (%)	Arsenic Bioavailability[a] (%)
Extraction Fluid pH = 1.5											
Shrew											
IES013	DoD-PM	<500 µm	81.9	1.0438	0.086	1.67	0.217	0.100	0.022	25	
IES014	DoD-DP	<500 µm	58.2	1.0123	0.059	1.74	0.080 *U*	0.100	0.008	14	7
IES015	CO Smelter Composite	<500 µm	331	1.0054	0.333	1.65	2.491	0.100	0.249	75	31
IES016-17	WA Orchard	<500 µm	282	1.0369 [b]	0.294 [b]	1.66 [b]	1.814 [b]	0.100 [b]	0.181 [b]	62 [b]	49
Monkey - Cebus											
IES040	Florida Cattle Dip Vat	<250 µm	150	1.0093	0.152	1.64	0.544	0.100	0.054	36	24.7
IES041-42	Florida Power Co. #1	<250 µm	230	1.0028 [b]	0.230 [b]	1.66 [b]	0.897 [b]	0.100 [b]	0.090 [b]	39 [b]	14.6
IES043, S02002	Florida Pesticide #1	<250 µm	273	1.0057 [b]	0.275 [b]	1.82 [b]	2.139 [b]	0.100 [b]	0.214 [b]	78	10.7
IES039	Florida CCA	<250 µm	86.3	1.0093	0.087	1.65	0.595	0.100	0.060	68	16.3
IES044, S02003	Florida Pesticide #2	<250 µm	653	0.9978 [b]	0.652 [b]	1.76 [b]	6.540 [b]	0.100 [b]	0.654 [b]	100	17
Monkey - Cynos											
IES040	Florida Cattle Dip Vat	<250 µm	150	1.0093	0.152	1.64	0.251	0.100	0.025	17	31
IES049-50	California Mine Tailings	<250 µm	300	1.0078 [b]	0.302 [b]	1.71 [b]	0.390 [b]	0.100 [b]	0.039 [b]	13 [b]	19
IES051	Montana Smelter	<250 µm	647	1.0065	0.651	1.75	2.926	0.100	0.293	45	13
IES052	Rodriguez#8	<250 µm	1,412	1.0038	1.417	1.84	6.488	0.100	0.649	46	13
IES053	WA Orchard	<250 µm	301	1.0039	0.302	1.66	3.099	0.100	0.310	103	24
IES054	NY Orchard Soil	<250 µm	123	1.0040	0.124	1.67	0.450	0.100	0.045	36	16
IES019	CO Smelter Composite	<250 µm	398	1.0209	0.406	1.65	2.735	0.100	0.274	67	18
IES057	VB/I-70	<250 µm	869	1.0046	0.873	1.66	3.567	0.100	0.357	41	17
IES055	Colorado Smelter	<250 µm	1,492	1.0042	1.498	1.71	1.071	0.100	0.107	7	5
IES045	Fl Inglis	<250 µm	273	1.0058	0.275	1.68	0.251 *U*	0.100	0.025	9	8
Extras											
IES018	DoD-PM	<250 µm	165	1.0251	0.169	1.73	0.622	0.100	0.062	37	
IES056	CO Smelter Composite	<250 µm	398	1.0015	0.399	1.69	3.072	0.100	0.307	77	
IES020	OK Smelter	<250 µm	77.2	1.0656	0.082	1.77	0.294	0.100	0.029	36	
IES021	Dugway Composite	<250 µm	8.50	1.0100	0.009	1.89	0.088	0.100	0.009	103	

Table 7-1. (cont.)

Extraction ID	Soil	Grain Size	Arsenic Conc. in Substrate (mg/kg)	Mass of Soil Tested (g)	Mass of Arsenic in Soil Extracted (mg)	Post-Test pH (s.u.)	Arsenic Conc. in Extract (mg/L)	Volume of Extract (L)	Mass of Arsenic in Extract (mg)	Arsenic Bioaccessibility (%)	Arsenic Bioavailability[a] (%)
Extraction Fluid pH = 2.5											
Shrew											
IES026	DoD-PM	<500 µm	81.9	1.0023	0.082	2.57	0.106	0.100	0.011	13	
IES027	DoD-DP	<500 µm	58	1.0196	0.059	2.61	0.080 *U*	0.100	0.008	13	7
IES028	CO Smelter Composite	<500 µm	331	1.0022	0.332	2.54	1.959	0.100	0.196	59	31
IES029	WA Orchard	<500 µm	282	1.0042	0.283	2.52	1.686	0.100	0.169	60	49
Monkey - Cebus											
IES061	Florida Cattle Dip Vat	<250 µm	150	1.0036	0.151	2.57	0.263	0.100	0.026	17	24.7
IES062	Florida Power Co. #1	<250 µm	230	1.0072	0.231	2.56	0.706	0.100	0.071	31	14.6
IES063	Florida Pesticide #1	<250 µm	273	1.0038	0.274	2.63	1.731	0.100	0.173	63	10.7
IES060	Florida CCA	<250 µm	86.3	1.0037	0.087	2.63	0.552	0.100	0.055	64	16.3
IES064-65	Florida Pesticide #2	<250 µm	653	1.0018 [b]	0.654 [b]	3.00 [b]	6.537 [b]	0.100 [b]	0.654 [b]	100 [b]	17
Monkey - Cynos											
IES061	Florida Cattle Dip Vat	<250 µm	150	1.0036	0.151	2.57	0.251	0.100	0.025	17	31
IES070	California Mine Tailings	<250 µm	300	1.0028	0.301	2.63	0.275	0.100	0.028	9	19
IES071-72	Montana Smelter	<250 µm	647	1.0042 [b]	0.650 [b]	2.66 [b]	2.075 [b]	0.100 [b]	0.207 [b]	32 [b]	13
IES073	Rodriguez#8	<250 µm	1,412	1.0084	1.424	2.64	3.518	0.100	0.352	25	13
IES074	WA Orchard	<250 µm	301	1.0038	0.302	2.56	2.548	0.100	0.255	84	24
IES075	NY Orchard Soil	<250 µm	123	1.0074	0.124	2.56	0.281	0.100	0.028	23	16
IES077	CO Smelter Composite	<250 µm	398	1.0009	0.398	2.57	2.449	0.100	0.245	61	18
IES078	VB/I-70	<250 µm	869	1.0043	0.873	2.57	2.813	0.100	0.281	32	17
IES076	Colorado Smelter	<250 µm	1,492	1.0061	1.501	2.59	0.720	0.100	0.072	5	5
IES066	Fl Inglis	<250 µm	273	1.0047	0.274	2.60	0.251 *U*	0.100	0.025 *U*	9	8
Extras											
IES030	DoD-PM	<250 µm	165	1.0033	0.166	2.64	0.273	0.100	0.027	16	
IES031	CO Smelter Composite	<250 µm	398	1.0129	0.403	2.54	2.410	0.100	0.241	60	
IES032-33	OK Smelter	<250 µm	77.2	1.0379 [b]	0.080 [b]	2.62 [b]	0.182 [b]	0.100 [b]	0.018 [b]	23 [b]	
IES034	Dugway Composite	<250 µm	8.50	1.0052	0.009	2.70	0.080 *U*	0.100	0.008	94	

Table 7-1. (cont.)

Extraction ID	Soil	Grain Size	Arsenic Conc. in Substrate (mg/kg)	Mass of Soil Tested (g)	Mass of Arsenic in Soil Extracted (mg)	Post-Test pH (s.u.)	Arsenic Conc. in Extract (mg/L)	Volume of Extract (L)	Mass of Arsenic in Extract (mg)	Arsenic Bioaccessibility (%)	Arsenic Bioavailability[a] (%)
Extraction Fluid pH = 3.5											
Shrew											
IES088-89	DoD-PM	<500 µm	81.9	1.0009 [b]	0.082 [b]	3.47 [b]	0.118 [b,c]	0.100 [b]	0.012 [b]	14 [b]	
IES090	CO Smelter Composite	<500 µm	331	1.0034	0.332	3.49	1.957	0.100	0.196	59	31
S02000-3.5	WA Orchard	<500 µm	282	1.0044	0.283	3.24	1.259 [c]	0.100	0.126	44	49
S02001-3.5	DoD-DP	<500 µm	58	1.0005	0.058	3.51	0.081 [c]	0.100	0.008	14	7
Extraction Fluid pH = 4.5											
Shrew											
IES080	DoD-PM	<500 µm	81.9	1.0047	0.082	5.10	0.045 [c]	0.100	0.005	5	
IES081	CO Smelter Composite	<500 µm	331	1.0049	0.333	4.98	1.160	0.100	0.116	35	31
S02000-4.5	WA Orchard	<500 µm	282	1.0025	0.283	4.28	0.838 [c]	0.100	0.084	30	49
S02001-4.5	DoD-DP	<500 µm	58	1.0021	0.058	5.90	0.038 [c]	0.100	0.004	7	7

Notes: *U* – Undetected; value represents detection limit

[a] Bioavailability results from in vivo studies.
[b] Average of duplicate extractions.
[c] Analyzed by ICP/MS

Table 7-2. Impact of phosphate buffering on *in vitro* bioaccessibility of arsenic from soils

Extraction ID	Soil	Grain Size	Arsenic Conc. in Substrate (mg/kg)	Mass of Soil Tested (g)	Mass of Arsenic in Soil Extracted (mg)	Post-Test pH (s.u.)	Arsenic Conc. in Extract (mg/L)	Volume of Extract (L)	Mass of Arsenic in Extract (mg)	Arsenic Bioaccessibility (%)	Arsenic Bioavailability[a] (%)
0.4 M Glycine Extraction Fluid pH = 1.5											
Monkey - Cynos											
IES040	Florida Cattle Dip Vat	<250 µm	150	1.0093	0.152	1.64	0.251	0.100	0.025	17	31
IES049-50	California Mine Tailings	<250 µm	300	1.0078 [b]	0.302 [b]	1.71 [b]	0.390 [b]	0.100 [b]	0.039 [b]	13 [b]	19
IES051	Montana Smelter	<250 µm	647	1.0065	0.651	1.75	2.926	0.100	0.293	45	13
IES052	Rodriguez#8	<250 µm	1,412	1.0038	1.417	1.84	6.488	0.100	0.649	46	13
IES053	WA Orchard	<250 µm	301	1.0039	0.302	1.66	3.099	0.100	0.310	103	24
IES054	NY Orchard Soil	<250 µm	123	1.0040	0.124	1.67	0.450	0.100	0.045	36	16
IES019	CO Smelter Composite	<250 µm	398	1.0209	0.406	1.65	2.735	0.100	0.274	67	18
IES057	CO Residential Soil	<250 µm	869	1.0046	0.873	1.66	3.567	0.100	0.357	41	17
IES055	Colorado Smelter	<250 µm	1,492	1.0042	1.498	1.71	1.071	0.100	0.107	7	5
IES045	Fl Inglis	<250 µm	273	1.0058	0.275	1.68	0.251 *U*	0.100	0.025	9	8
0.4 M Glycine Extraction Fluid pH = 2.5											
Monkey - Cynos											
IES061	Florida Cattle Dip Vat	<250 µm	150	1.0036	0.151	2.57	0.251	0.100	0.025	17	31
IES070	California Mine Tailings	<250 µm	300	1.0028	0.301	2.63	0.275	0.100	0.028	9	19
IES071-72	Montana Smelter	<250 µm	647	1.0042 [b]	0.650 [b]	2.66 [b]	2.075 [b]	0.100 [b]	0.207 [b]	32 [b]	13
IES073	Rodriguez#8	<250 µm	1,412	1.0084	1.424	2.64	3.518	0.100	0.352	25	13
IES074	WA Orchard	<250 µm	301	1.0038	0.302	2.56	2.548	0.100	0.255	84	24
IES075	NY Orchard Soil	<250 µm	123	1.0074	0.124	2.56	0.281	0.100	0.028	23	16
IES077	CO Smelter Composite	<250 µm	398	1.0009	0.398	2.57	2.449	0.100	0.245	61	18
IES078	CO Residential Soil	<250 µm	869	1.0043	0.873	2.57	2.813	0.100	0.281	32	17
IES076	Colorado Smelter	<250 µm	1,492	1.0061	1.501	2.59	0.720	0.100	0.072	5	5
IES066	Fl Inglis	<250 µm	273	1.0047	0.274	2.60	0.251 *U*	0.100	0.025 *U*	9	8
0.4 M KH_2PO_4 Extraction Fluid pH = 2.5											
Monkey - Cynos											
IMPO24-26	Florida Cattle Dip Vat	<250 µm	150	1.0128 [c]	0.152 [c]	2.59 [c]	1.274 [c]	0.100 [c]	0.127 [c]	84	31
IMPO27	California Mine Tailings	<250 µm	300	1.0089	0.302	2.63	0.912	0.100	0.091	30	19
IMPO28	Montana Smelter	<250 µm	647	1.0009	0.647	2.66	2.772	0.100	0.277	43	13
IMPO29	Rodriguez#8	<250 µm	1,412	1.0001	1.412	2.64	1.972	0.100	0.197	14	13
IMPO30	WA Orchard	<250 µm	301	1.0036	0.302	2.56	0.617	0.100	0.062	20	24

Table 7-2. (cont.)

Extraction ID	Soil	Grain Size	Arsenic Conc. in Substrate (mg/kg)	Mass of Soil Tested (g)	Mass of Arsenic in Soil Extracted (mg)	Post-Test pH (s.u.)	Arsenic Conc. in Extract (mg/L)	Volume of Extract (L)	Mass of Arsenic in Extract (mg)	Arsenic Bioaccessibility (%)	Arsenic Bioavailability[a] (%)
0.4 M KH_2PO_4 Extraction Fluid pH = 2.5 (cont.)											
Monkey - Cynos (cont.)											
IMPO31	NY Orchard Soil	<250 µm	123	1.0041	0.124	2.56	0.782	0.100	0.078	63	16
IMPO32	CO Smelter Composite	<250 µm	398	1.0345	0.412	2.57	2.814	0.100	0.281	68	18
IMPO33	CO Residential Soil	<250 µm	869	1.0027	0.871	2.57	3.734	0.100	0.373	43	17
IMPO34	Colorado Smelter	<250 µm	1,492	1.0068	1.502	2.59	0.980	0.100	0.098	7	5
IMPO35	Fl Inglis	<250 µm	273	1.0085	0.275	2.60	0.209	0.100	0.021	8	8
IMPO36	NY pesticide facility, A1B20 (S0005B)	<250 µm	1,000	1.0099	1.010	2.60	5.609	0.100	0.561	56	
IMPO37	NY pesticide facility, T5E3 (S0008B)	<250 µm	339	1.0014	0.339	2.60	2.446	0.100	0.245	72	
IMPO38	NY pesticide facility, T15-E4 (S0013B)	<250 µm	549	1.0053	0.552	2.60	2.274 [d]	0.100	0.227	41	
Highest %BIOACCESS of 0.4 M Glycine/0.4 M KH2PO4 Extraction Fluid (pH = 2.5)											
IMPO24-26	Florida Cattle Dip Vat	<250 µm	150	1.0128 [c]	0.152 [c]	2.59 [c]	1.274 [c]	0.100 [c]	0.127 [c]	84	31
IMPO27	California Mine Tailings	<250 µm	300	1.0089	0.302	2.63	0.912	0.100	0.091	30	19
IMPO28	Montana Smelter	<250 µm	647	1.0009	0.647	2.66	2.772	0.100	0.277	43	13
IES073	Rodriguez#8	<250 µm	1,412	1.0084	1.424	2.64	3.518	0.100	0.352	25	13
IES074	WA Orchard	<250 µm	301	1.0038	0.302	2.56	2.548	0.100	0.255	84	24
IMPO31	NY Orchard Soil	<250 µm	123	1.0041	0.124	2.56	0.782	0.100	0.078	63	16
IMPO32	CO Smelter Composite	<250 µm	398	1.0345	0.412	2.57	2.814	0.100	0.281	68	18
IMPO33	CO Residential Soil	<250 µm	869	1.0027	0.871	2.57	3.734	0.100	0.373	43	17
IMPO34	Colorado Smelter	<250 µm	1,492	1.0068	1.502	2.59	0.980	0.100	0.098	7	5
IES066	Fl Inglis	<250 µm	273	1.0047	0.274	2.60	0.251 *U*	0.100	0.025 *U*	9	8

Notes: *U* – Undetected; value represents detection limit

[a] Bioavailability as measured in cyno monkeys.
[b] Average of duplicate extractions.
[c] Average of triplicate extractions.
[d] Average of laboratory duplicates

Table 7-3. Extraction of arsenic from soils using human sweat

Soil	Grain Size	Arsenic Soil Concentration (mg/kg)	Arsenic in Sweat: Extract Concentration (mg/L)	Arsenic in Sweat: Extract Concentration (µmol/L)	Arsenic in Sweat: Percent Extracted (%)
NY pesticide facility soil	<150 µm	1,610	1.15	15.3	1.8%
CO residential soil	<150 µm	1,230	5.33	71.1	11%
Yolo County soil[a]	180–300 µm	3,633	106	1,414	72%

[a] Soil was spiked with approximately 3,500 mg/kg arsenic 1 hour before the extraction test was performed.

Supplemental Materials for Section 3

White Paper

Evaluation of the Metals that Drive Risk-Based Remedial Decisions at DoD Sites

Prepared for

Strategic Environmental Research and Development Program

Prepared by

Exponent
Boulder, Colorado

Evaluation of the Metals that Drive Risk-Based Remedial Decisions at DoD Sites

Introduction

The Department of Defense (DoD) has undertaken the task of cleaning up wastes that have resulted from industrial, commercial, training, and weapons testing activities, as well as cleaning up and closing military bases so that properties can be transferred to local communities for economic revitalization (U.S. EPA 1997). Among the challenges in this effort is the process of prioritizing sites for clean up, and determining what needs to be cleaned up and to what extent. For properties on which soils are contaminated with metals, the amount of the metal in soil that could actually be absorbed by human or ecological receptors (i.e., the bioavailability of the metal) can be an important factor in determining the degree to which the contaminated soils need to be remediated. This occurs because the bioavailability of metals from soil is generally less than that assumed by the default values used in human health and ecological risk assessment.

Frequently, the factors that determine bioavailability are highly site specific. Because standard assays for bioavailability (*in vivo* [animal] studies) are costly and require three to six months to complete, the Exponent team has undertaken a course of research on behalf of the Strategic Environmental Research and Development Program (SERDP) to develop simple extraction tests that predict human and ecological exposures to metals in soils. To maximize the applicability of this research to DoD sites, the first step has involved determining which metals drive risk-based remedial decisions for soils at DoD sites. In this white paper, we evaluate which metals that occur in soil at DoD sites are primarily responsible for driving risk-based remediation decisions, and we identify the receptors (i.e., human or ecological) that drive these decisions.

Exponent used several approaches in conducting this analysis. Information for this analysis was solicited from:

- Various branches of the military (Army, Navy, Air Force)
- EPA regional toxicologists
- Coordinators within the Federal Facilities Restoration and Reuse Office (FFRRO)
- Comprehensive Environmental Response, Compensation and Liability Information System (CERCLIS)
- EPA Records of Decision (RODs)
- DoD Environmental Cleanup Office.

The goal of this exercise was to answer the following three questions:

1. What metals drive risk-based remedial decisions at DoD facilities?
2. For facilities where more than one metal poses risk, what are the metals of concern and how do they compare in perceived importance?
3. For the metals that pose the highest risk, what is the receptor of greatest concern (human or ecological)?

This document describes the avenues that were pursued to locate useful information, presents the information obtained, describes the manner in which these data were assessed, and finally, discusses the conclusions that can be drawn regarding the metals and exposure pathways that are important determinants for remediation of metals in soils at DoD facilities.

Sources of Information

Various sources within the DoD and EPA were contacted to identify sources of information on metal concentrations at DoD sites, their potential for health effects on human and ecological receptors, and their influence in remedial decisions for soil. Figure 1 provides a schematic of the different agencies and groups that were contacted. Our goal in contacting these individuals was to identify and gain access to databases that would provide answers to the three questions posed in the Introduction, above. Overall, we found that few compiled databases exist that contain the entirety of the information we were looking for. Therefore, partial information from several sources was tapped, as well as the subjective opinions of professionals involved in the assessment and remediation of federal facilities.

Databases

Ultimately, we identified five databases that could be queried to provide information relevant to our task. Three were military databases: the Environmental Restoration Program Information Management System (ERPIMS) database from the Air Force, an unnamed database containing metals data from Army sites (provided by Mark Barnett of Auburn University), and the Normalization of Environmental Data System (NORM) database from the Navy. We also analyzed the data contained in the Restoration Management Information System (RMIS) maintained by the Environmental Cleanup Office of the DoD, and the Comprehensive Environmental Response, Compensation and Liability Information System (CERCLIS) database maintained by the EPA. In addition, the Superfund Hazardous Waste Site website (located at http://www.epa.gov/superfund/) includes a Resource Center in which databases can be searched by the general public. Using the advanced query option, we extracted information pertaining to Site Names, CERCLIS ID, Site ID, City, Metal Contaminants of Concern, and Contaminated Media (we selected soil); however, no concentration data were available on this website.

At the outset of our effort to collect data, we also attempted to obtain information from the database on Records of Decision (RODs INFO) maintained by the EPA. The RODs INFO

database provides a compilation of the information that is part of Records Of Decision for sites that have been addressed under the Comprehensive Environmental Response, Compensation and Liability Act of 1980 (CERCLA, generally referred to as "Superfund"). While this information may have proved quite useful, extracting appropriate data from this database was extremely cumbersome. In addition, the database includes information only for sites where RODs have been issued, thus excluding sites where data exist but a remedial approach has not been selected and a ROD has therefore not been issued. Because of these obstacles, the RODs INFO search was aborted, and subsequent efforts were focused on the other available databases.

In each instance, we contacted the database manager to determine the best manner of extracting the relevant information. Five specific questions were posed to each contact:

- Which DoD facilities present risks from potential exposures to metals in soils?
- Which specific metals are of concern?
- Which receptors (human or ecological) are of concern for metals in soils?
- Which human and ecological exposure pathways are potentially of concern for metals in soils?
- Which human exposure scenarios (e.g., workers, residents, trespassers) are potentially of concern for exposure to metals in soils?

Ultimately, we received from each of the databases a download of information, hereafter referred to as "data sets," regarding the soil concentration data for sites that are known to have metals in soil. These concentration data were then compared to a consistent set of risk-based screening criteria (described below) to determine which metals most frequently exceeded these criteria.

It is important to note that the Exponent team generally relied on the information in the data sets as supplied by the various sources. Aside from very minor modifications required to conduct the screening (e.g., standardizing concentrations to be expressed in units of mg/kg), few changes were made to the data sets. It was assumed that the information provided in the data sets was technically accurate, and no outside verification of the data was performed. However, in the process of preparing this report, the Exponent team did identify what appear to be errors within several of the databases. It is beyond the scope of this evaluation to verify and/or correct the data included in the various databases, and any flaws intrinsic to the databases may affect the conclusions drawn from the risk-based screening of the data. These issues, and some specifics regarding the flaws identified in the databases, are discussed further in the conclusions section of this document.

Agency Interviews

In addition to the objective information provided by the data sets, we queried individuals who are involved with the risk assessment of federal facilities regarding their opinions on the

questions posed in the Introduction. Specifically, within each of the 10 EPA regions, we attempted to contact a Regional Toxicologist and the Regional Contact in the Federal Facilities Restoration and Reuse Office (FFRRO). We were not able to interview both of these persons in every region, but we persisted until we had made contact with at least one individual in each region. These individuals were asked the questions listed in the Introduction, and their responses were tabulated. These responses are discussed below.

Screening Criteria

As described above, the Army, NORM, ERPIMS, RMIS, and CERCLIS databases were successfully queried by their database managers, and query results from each database were provided to Exponent. The data sets that were provided to Exponent included soil metal concentration data for sites where metals had been detected. The concentration data in each data set were then compared to health-based screening criteria to determine which sites contained metals in soil at concentrations that might present a health risk. To conduct this screening, Exponent compiled health-based screening concentrations for several endpoints. Because we are interested in potential risks to both human and wildlife receptors, we screened the site concentration data against criteria based on human health (for both industrial and residential exposure scenarios) and ecological receptors (mammalian and avian receptors). This section describes the selection of the criteria that were used to screen the data.

Human Health Criteria

Human health criteria were obtained from EPA's Soil Screening Guidance (U.S. EPA 2001), which is a tool developed by the EPA to help standardize and accelerate the evaluation and cleanup of contaminated soils at sites on the National Priorities List (NPL) with future residential land use. Criteria adopted from the generic Soil Screening Levels (SSLs) that were developed for combined ingestion and dermal exposure in a residential scenario were used to assess risk to human receptors in a residential setting (U.S. EPA 2001). Screening criteria that would be protective under industrial land use were selected from the generic SSLs provided for an outdoor worker scenario (U.S. EPA 2001). The specifics of the criteria derivation can be found in U.S. EPA (2001).

All values were used exactly as they were reported by EPA, with the exception of arsenic. For arsenic, the health-based screening criteria reported by EPA are 0.4 mg/kg and 2 mg/kg for the residential and industrial land use scenario, respectively. While these values are consistent with the specified method for setting screening levels equal to a cancer risk of one in a million, these specific values fall below background concentrations for arsenic in soil throughout much of the United States (Dragun and Chiasson 1991). Therefore, both the residential and industrial criteria listed by EPA were multiplied by a factor of 10, effectively raising the target cancer risk associated with each to one in one hundred thousand, the middle of the acceptable risk range specified by EPA. This was done to ensure that the importance of arsenic in risk-based decisions was not artificially elevated due to the unreasonably low screening values for this metal.

If the EPA guidance did not contain values for the metals that were measured at DoD sites, health-based screening values from EPA Region IX Preliminary Remediation Goals (PRGs) were incorporated as surrogates (U.S. EPA 2000a). Table 1 lists the human health-based screening criteria, and denotes whether the values were selected from the list of SSLs (U.S. EPA 2001) or PRGs (U.S. EPA 2000a).

Ecological Criteria

Specific screening values for avian and mammalian receptors were selected for each metal. Ecological Soil Screening Levels (EcoSSLs; U.S. EPA 2000b) were used, if they were available. If EcoSSLs for avian and mammalian receptors were not available for a particular metal, we used the Preliminary Remediation Goals for Ecological Endpoints (Efroymson et al. 1997)—specifically, American woodcock goals—as a surrogate for avian screening values, and short-tail shrew goals as a surrogate for mammalian screening values. Table 1 lists the specific values and the source for each of the ecological screening criteria that were used in this evaluation. Although it is beyond the scope of the current effort to review the technical basis and merits of the screening value for each metal, it is important to mention that the screening concentration for mercury is highly conservative for use in most contexts. This is because the current screening value (from Efroymson et al. 1997) is based on the assumption that 100% of the mercury is present in soils as methyl mercury. In aerobic soil environments, which are the soils of interest for evaluating ingestion by wildlife, mercury exists almost entirely in the inorganic form, which is substantially less toxic than the organic form. Therefore, the SSL based on organic mercury is extremely conservative for most sites.

Data Analysis

Each data set that was used for this project was subjected to minor modifications to simplify data interpretation. These modifications, primarily name changes, are described in detail in Appendix A. These manipulations were cosmetic and only helped to streamline the data analysis. The integrity of each data set was not compromised in any way.

Each specific concentration within each of the five data sets was compared to human and ecological screening criteria, and the ratios of the concentrations to the criteria were calculated. If the calculated ratio was less than or equal to unity (i.e., ≤ 1), then it was assumed that the concentration did not present a potential human or ecological health hazard. If the ratio of a concentration to the screening criterion did exceed unity (i.e., value >1), then this was assumed to indicate the potential for adverse effects. Determining the actual risk of adverse effects would require further evaluation, on a site-specific basis. This approach served as the backbone of the data-set queries that were conducted by Exponent.

Figure 2 is a flow chart that depicts how the data were handled in each data set to generate answers to the questions of interest regarding metals of potential concern at DoD sites. Three lines of inquiry were pursued with each data set.

The first set of analyses was aimed at determining, for each data set, which metal exceeded the health-based screening criteria most frequently. Graphs were constructed to present the

percentage of sites in each data set at which any of the four sets of criteria (residential, industrial, avian, mammalian) were exceeded at least once. Each site was counted only once. Figures 3a through 3f provide the results of those queries for the Army, ERPIMS, NORM, RMIS, CERCLIS, and Superfund data sets, respectively. These figures show the percentage of metal-contaminated sites that exceed the health-based criteria for any of the receptors of concern, and they are sorted by metal, from the metal with the lowest percentage of exceedances to that with the highest. This analysis was further refined to examine which metals present risk for human receptors versus ecological receptors. For this analysis, all the data sets were combined and screened against either human or ecological criteria. If data exceeded criteria more than once for a particular site, the site was counted only once. The human health screening results are displayed in Figure 3g, and the ecological screening results are displayed in Figure 3h.

In the second set of analyses, the five sites with the highest "risk" (i.e., the highest ratio of site average metal concentrations to screening criteria when averaged across all metals for each site) were selected from each data set. Exponent then determined, for those five sites, what metals were present at concentrations above screening values. The goal of this effort was to determine, for facilities where more than one metal poses risk, what the metals are and how they compare. This analysis was conducted separately for each potential receptor (human and ecological) and each data set. Figures 4a through 4e depict the results based on residential screening criteria across the different data sets. Figures 5a through 5e depict the results based on industrial screening criteria. The results based on the ecological endpoints are also presented—Figures 6a through 6e present the analysis based on avian screening criteria, and 7a through 7e, the mammalian screening criteria.

The final issue that Exponent attempted to address in this evaluation was to determine, for the metals in each data set that pose the highest risk, what receptor is of primary concern. To accomplish this, we provide a table for each data set that lists the metals that exceed criteria, and show the percentage of sites within that data set at which specific criteria are exceeded. Tables 2a through 2e present these results for the Army, ERPIMS, NORM, RMIS, and CERCLIS data sets, respectively. Within each table, highlighted values show the specific receptor for which the highest percentage of sites exceed the screening criterion. This was done to allow a quick, visual comparison between the different receptor groups (human vs. ecological), to determine which receptor results in the highest percentage of exceedances for each metal.

Interviews with Agency Staff

In addition to requesting database queries, Exponent also interviewed professional staff within each EPA region regarding their knowledge or impressions of which metals are driving risk-based remedial decisions at DoD sites. The individuals contacted were either regional toxicologists, or the Regional Contact for the FFRRO. One individual within the California EPA was also included in the interviews. The interviewees were asked the same questions as were asked of the database managers (see above).

Table 3 lists the individuals contacted, along with the information that they provided regarding the primary risk drivers at DoD sites, including the metals of concern, receptors, exposure scenarios, and exposure pathways of concern.

The information provided by these individuals was generally anecdotal. None of the EPA personnel indicated that they had compiled information from the DoD sites within the region. For some regions (e.g., Region VIII), it appears that metals are not driving risks at the DoD facilities, but rather, organic compounds are the primary concern. In nearly all instances, the interviewees indicated that human receptors were driving remedial decisions, and that ingestion of soils was the exposure pathway of concern. Only occasionally were ecological receptors or other exposure pathways mentioned.

Because of the requirement to evaluate human exposures under the scenario of potential future residential development, residential receptors were the primary receptors of concern, but interviewees indicated that worker, trespasser, and recreational exposure scenarios were also risk drivers. In general, the metals of concern coincide with the historical land use of the site. For example, lead is of concern for former firing ranges. Chromium showed up near former plating shops, and arsenic appears to be a problem from historical use of pesticides. Several individuals suggested that the frequent concern regarding chromium may be an artifact of the screening process, which incorporates the assumption that all chromium occurs in the more toxic hexavalent form, rather than the comparatively benign, but much more environmentally common, trivalent form.

Compilation of the interview results indicates that, overall, lead and arsenic are the metals that most frequently present health threats at DoD facilities. Cadmium and chromium followed next, and then beryllium. No other metals were mentioned consistently during the interviews.

Results

What metals drive risk-based remedial cleanup decisions at DoD facilities?

The data used to answer this question are presented in Figures 3a–f. These figures display the percentage of sites that exceed at least one set of criteria (residential, industrial, avian, or mammalian) for each of the six data sets. The top five metals from each data set were then entered into either a human or ecological matrix (Tables 4a and 4b, respectively) to summarize the overall results.

Using the Table 4a and 4b matrices, it is evident that lead is the metal that most commonly exceeds risk-based criteria in all the data sets. For human health risks (Table 4a), lead is followed by arsenic, cadmium, chromium, and antimony as metals that present risk at the most DoD sites. To more closely examine the metals that present risk for human and ecological receptors, Figures 3g and 3h have been included. The results suggest that for human receptors (Figure 3g), lead, arsenic, chromium, cadmium, and antimony most commonly exceed residential and industrial human health criteria. Lead, zinc, mercury, chromium, selenium, and cadmium most commonly exceed avian and mammalian ecological criteria (Figure 3h). These

metals are similarly indicated as those that most commonly exceed ecological risk-based criteria in Table 4b.

These results provide a general overview of the metals that may be causing the most concern at DoD sites; however, it is also of interest to compare the metals to each other in terms of risk, and to define which receptors are of the most concern, to more effectively determine which metals are likely driving remedial decisions at DoD sites.

For DoD facilities where more than one metal poses risk, what are the metals of concern and how do they compare?

Using one set of figures for each type of screening criteria (residential: Figures 4a–e; industrial: Figures 5a–e; avian: Figures 6a–e; mammalian: Figures 7a–e), we intended to determine which metals drive remedial decisions at the top five sites with the highest overall risk in each data set. Four graphs, one for each set of screening criteria (residential, industrial, avian, mammalian), were constructed for each data set. The residential-based graphs (Figures 4a–e) and industrial-based graphs (Figures 5a–e) were examined as one set, reflecting human receptors. These graphs indicate that lead poses the highest risk at most DoD sites for human exposures, followed by arsenic. Other metals also appear to pose risk, albeit less frequently, including antimony, iron, chromium, and mercury. Zinc and cadmium did not show particularly high concentrations at the five sites with the highest overall risk, although these metals often exceeded criteria, as discussed in the previous section.

The top five sites that exceeded the avian criteria are shown in Figures 6a–e. Mercury, selenium, zinc, and lead appear to have risks associated with them more often than the other metals when compared to avian criteria. Avian criteria for mercury were exceeded often because of the highly conservative value used in the screening (0.00051 mg/kg; Table 1).

For the mammalian screening (Figures 7a–e), a highly conservative screening criterion was also used for mercury. These screening values were adopted from the work of Sample and Efroymson at Oak Ridge National Laboratories, and were used because no other mercury screening values for birds or small mammals exist. The use of these values will tend to exaggerate the importance of mercury. Aside from mercury, arsenic, zinc and lead appear to have risks associated with them more often than the other metals when compared to mammalian criteria.

Using this information, it appears that lead and arsenic are the major players when human health criteria are involved, while for ecological receptors, selenium, zinc, lead, arsenic, and possibly mercury, are more apt to drive risk-based remedial decisions. Arsenic concentrations primarily exceeded mammalian screening values, while selenium selenium concentrations mostly exceeded avian screening criteria.

For the five metals in each data set that pose the highest average risk, what is the receptor of concern?

Based on information from Tables 2a–e, it is evident that the criteria for ecological receptors, represented by the screenings performed using the avian and mammalian criteria, were exceeded at more sites than those for human receptors, as represented by the residential and industrial criteria screenings. These results could indicate that ecological receptors are at greater risk from metals present at DoD sites than are human health receptors, but more likely reflect the conservative nature and uncertainty associated with the ecological screening criteria.

Conclusions

As discussed above, the screening conducted under this effort relied on data that were supplied to Exponent by various sources. Global verification of the values reported in each data set was beyond the scope of the current effort. However, during the screening of the various data sets, the Exponent team concluded that the databases that were queried to provide us with the relevant information are not completely accurate.

For example, the CERCLIS data set that was initially provided by EPA for this analysis reported cancer risk for metals that are not carcinogenic. (After discussions with EPA staff, the original CERCLIS data set was replaced with a data set expressing only concentration information rather than risks. The concentration data were then used in the screening, as described above.) Another flaw that appeared in the RMIS and Navy data sets involved the reporting of impossible metal concentrations in soil media. This took the form of reporting concentrations greater than one million parts per million. For example, the Navy database contains a single entry for iron at 30,300,300 ppm. This error may be due to incorrect data entry or incorrect reporting of units. After examining the entire RMIS data set that was provided to us, Exponent found that approximately two percent of all the data entries exceed one million parts per million, and that this error occurs for 14 separate metals. These errors most likely originated from incorrect reporting of units in the RMIS data set, but confirming the source of the errors is not possible.

The database managers for the various institutions were contacted and informed of the errors that were reported in the data sets we received from their respective institutions. Unfortunately, the contacts were unable to offer a solution to the flawed data sets, so the Exponent team used the data exactly as reported, because there was no feasible manner in which to correct them. The purpose of this project was to determine which metals are driving risk-based remedial decisions at DoD sites. To subjectively delete various data points or modify the existing data sets arbitrarily would have compromised the approach used in determining which metals drive remedial decisions. Knowing the limitations of these data sets prompted a need for other avenues of information gathering, such as the agency interviews that are discussed below. Therefore, the agency interviews were used to either corroborate or reject the results garnered from the data-set analysis. As a result, the Exponent team is obligated to offer the following disclaimer:

The results and interpretations based on the data sets are no more accurate than the accuracy of the data sets that were provided to the Exponent project team.

According to EPA's analysis of the RMIS database (U.S. EPA 1997), lead was the most frequent soil contaminant associated with DoD sites that require cleanup. Following lead were nickel, zinc, barium, cadmium, copper, and beryllium. In our analysis of the various databases, the metals that most frequently were associated with human health risk or remedial action at DoD facilities are lead, arsenic, cadmium, chromium, and antimony. This is depicted in Table 4a, which ranks the top five metals of human health concern in each of the five data sets. Similar results were obtained with the agency staff interviews (Table 3), which indicated an order of lead, arsenic, chromium, cadmium, and beryllium for the top five metals of concern for human health. Table 4b ranks the top five metals that most frequently exceeded ecological (avian and mammalian) screening criteria. The metals that were most frequently associated with ecological risk at DoD facilities are lead, cadmium, mercury, zinc, arsenic, chromium, and selenium.

Although contaminants other than metals may be driving remedial decisions at DoD sites, the purpose of this work was to examine metals only and determine which metals are of the most concern. The potential risk posed by exposure to organic contaminants was not examined as part of this effort. As indicated in the interviews with agency staff, there are many sites at which significant contamination exists, but at which metals are not believed to be of concern.

In evaluating these results, it is important to keep in mind that our analysis relied on data only from sites with metals detected in soils. We did not assess the percentage of sites where metals are considered potential contaminants of concern. Several professionals mentioned that volatile or semi-volatile organic compounds (VOCs or SVOCs) or radioactive components are more important at DoD sites than metals in soils. However, EPA indicates that for DoD sites that need cleanup, and that have identified soil contamination, the majority (>70%) are contaminated with metals (U.S. EPA 1997). Additionally, the EPA report indicates that for sites with soil contamination, metals occur more frequently than any other type of contamination (e.g., VOCs, SVOCs, fuels, explosives).

Based on interviews with Agency staff, it appears that different metals are associated with different operations or applications. For example, as stated above, lead contamination occurs at former firing ranges, arsenic in areas of historical pesticide use, and chromium at locations of former or current plating shops. This association results in significant heterogeneity regarding what metals are of concern, and suggests that contamination by some metals may be relatively localized (e.g., chromium), while others may be dispersed (e.g., arsenic). These interviews also indicate that human health considerations usually drive remedial actions for metals in soils, and that ecological receptors typically become an issue only if wetlands and sediments are part of the assessment. However, at sites where more sophisticated assessments have been conducted, ecological receptors (e.g., American robin, or burrowing animals) can drive risk for metals in soils. This information from interviews is in contrast to our screening of data against different criteria, which indicates that exceedances of risk "thresholds" based on ecological receptors occur more frequently than exceedances of human health standards. The focus on human health considerations may simply reflect the interest or technical background of the individuals interviewed (more were human health toxicologists than ecologists/ ecotoxicologists), or the prioritization of human over ecological health as a general societal trend.

According to Agency staff interviews, ingestion exposures typically are of greatest concern, while dermal exposure is the second most important pathway, followed by inhalation. Dermal absorption was considered an issue only for arsenic and cadmium in soils. However, agency staff did report that dermal exposures would be more important if point-of-contact symptoms (e.g., rashes) were "taken more seriously" in the risk assessment process.

The primary goals of this research were to identify and prioritize metals for bioavailability research, and to identify which metals were relevant to human versus ecological receptors. Combined evaluation of the results from the data-set screening and the Agency interviews indicates that bioavailability studies for human receptors should be focused on lead, arsenic, cadmium, chromium, and beryllium. However, because both *in vivo* (young swine model) and *in vitro* (extraction test) assays already exist for determining lead bioavailability in soil, the Exponent project team will focus their human bioavailability research on arsenic, cadmium, chromium, and beryllium. A similar evaluation for ecological receptors indicates that bioavailability research should focus on lead, cadmium, mercury, zinc, chromium, arsenic (mammalian only), and selenium (avian only).

References Cited

Dragun, J., and A. Chiasson. 1991. Elements in North American soils. Hazardous Materials Control Resources Institute.

Efroymson, R.A., G.W. Suter II, B.E. Sample, and D.S. Jones. 1997. Preliminary remediation goals for ecological endpoints, August 1997. U.S. Department of Energy, Office of Environmental Management, Oak Ridge National Laboratory. ES/ER/TM-162/R2.

U.S. EPA. 1997. Cleaning up the nation's waste sites: Markets and technology trends, 1996 edition. U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response, Washington, DC. PB96-178041.

U.S. EPA. 2000a. Region IX preliminary remediation goals. Region IX website. http://www.epa.gov/Region9/waste/sfund/prg/index.htm, updated November 22, 2000.

U.S. EPA. 2000b. Ecological soil screening level guidance, draft. U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response, Washington, DC. July 10, 2000.

U.S. EPA. 2001. Supplemental guidance for developing soil screening levels for Superfund sites, peer review draft. U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response, Washington, DC. OSWER 9355.4-24.

Figures

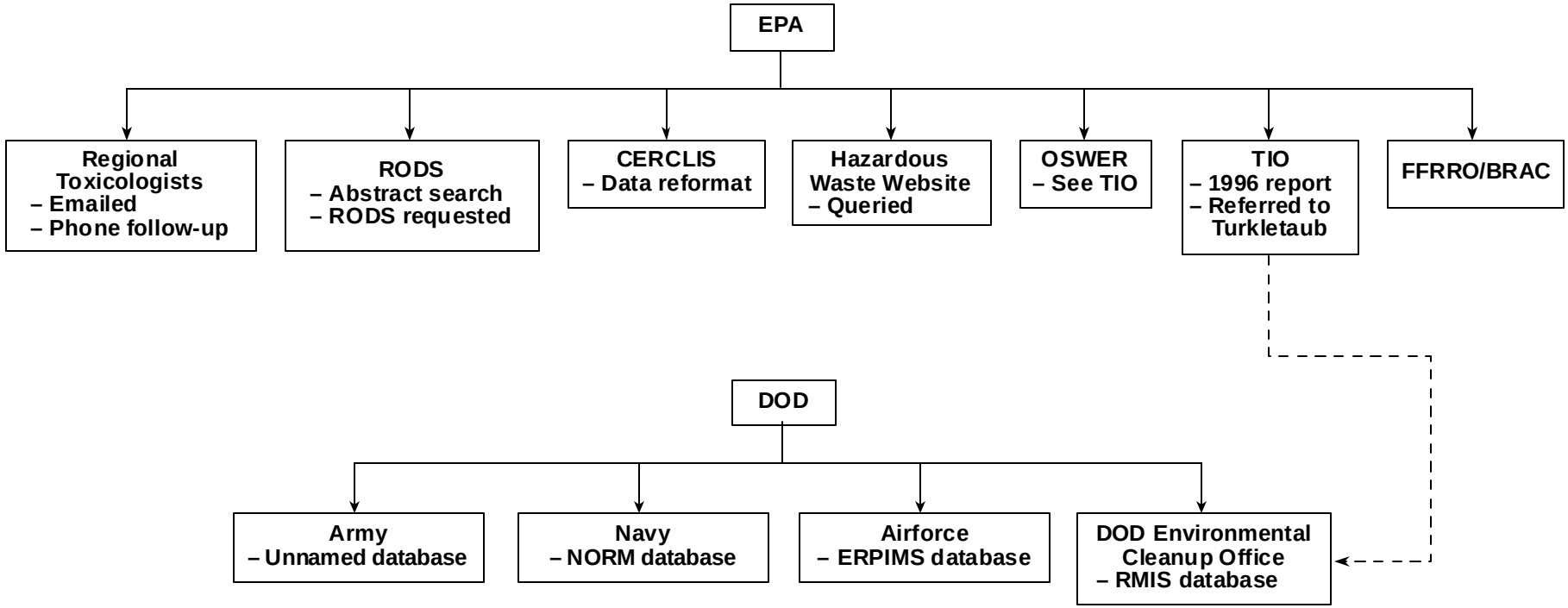


Figure 1. Information sources for the SERDP white paper.

\\Boulder1\Data\Groups\Graphics\Projects\1191.001\Serdp-accessing information.fh8 08/04/01 CO

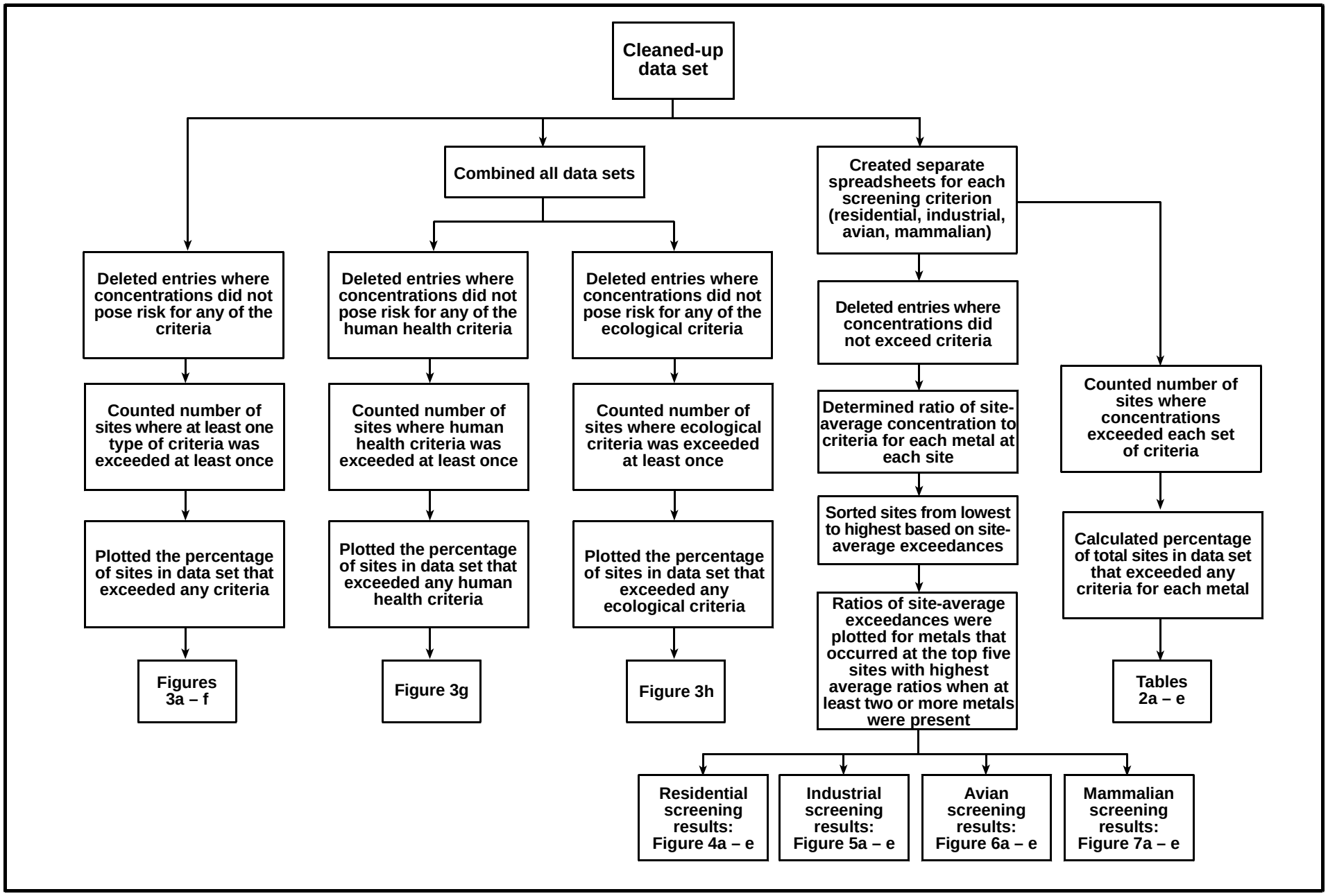


Figure 2. Data handling methods.

\\Boulder1\Data\Groups\Graphics\Projects\1191\Cleaned-up dataset.fh8 08/10/01 CO

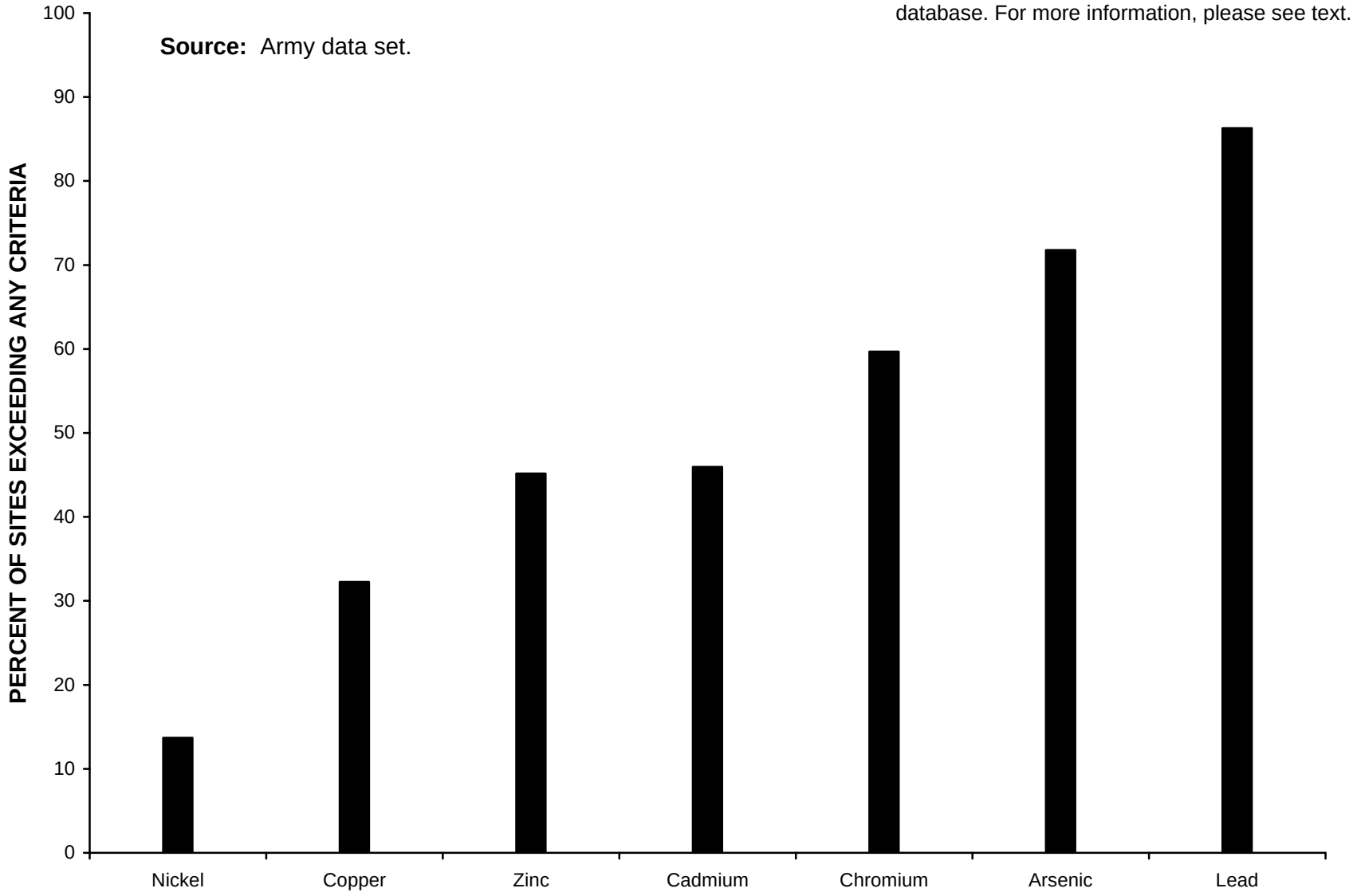


Figure 3a. Percent of metal-contaminated sites exceeding any criteria at least once (124 sites total).

Boulder1\data\projects\Xfer_Salatas\Figure 3a EQD_METALS_CR1 10/16/01 CO

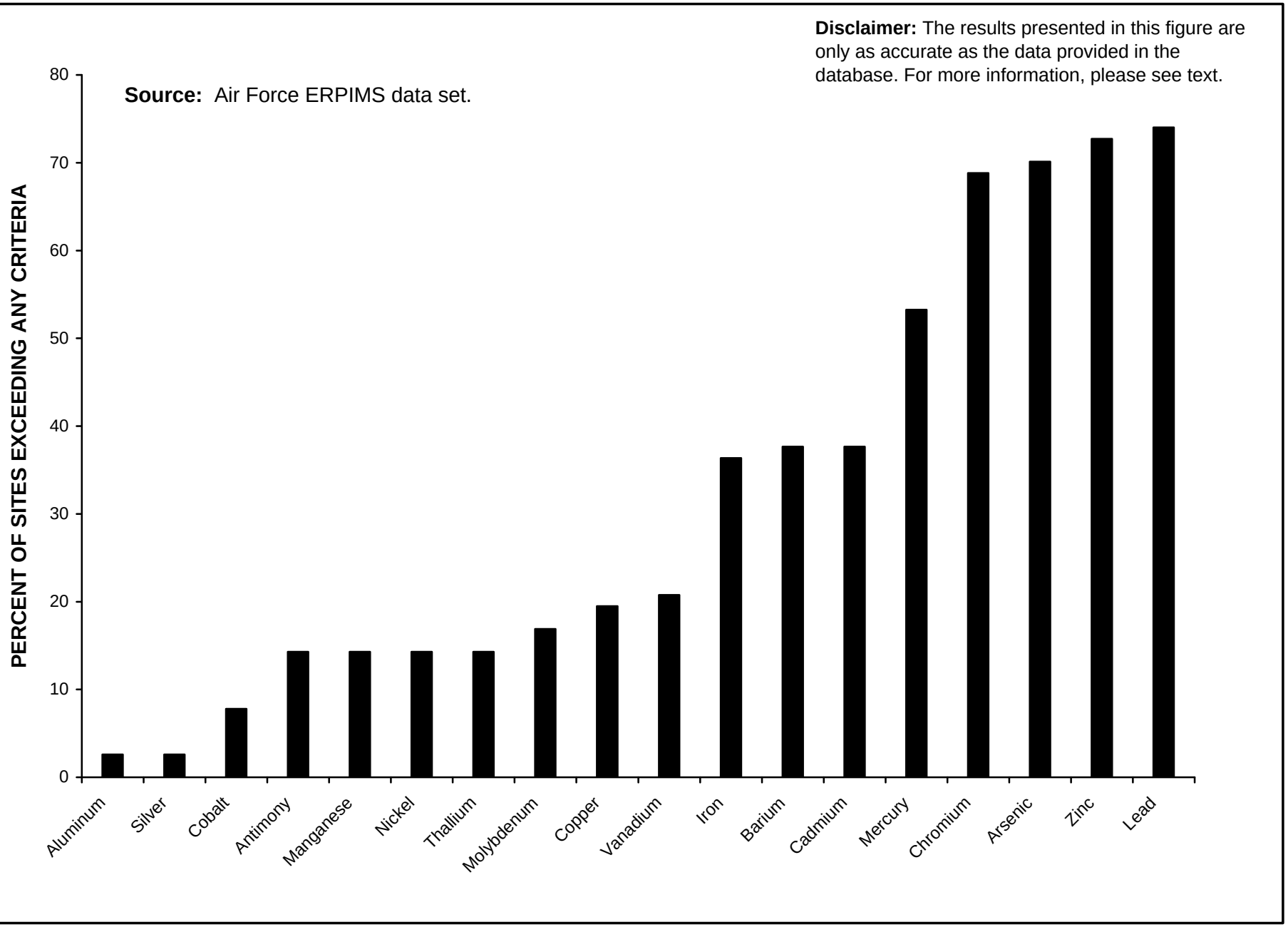


Figure 3b. Percent of metal-contaminated sites exceeding any criteria at least once (77sites total).

Boulder1\data\projects\Xfer_Salatas\Figure 3b ERPIMS-formatted 10/16/01 CO

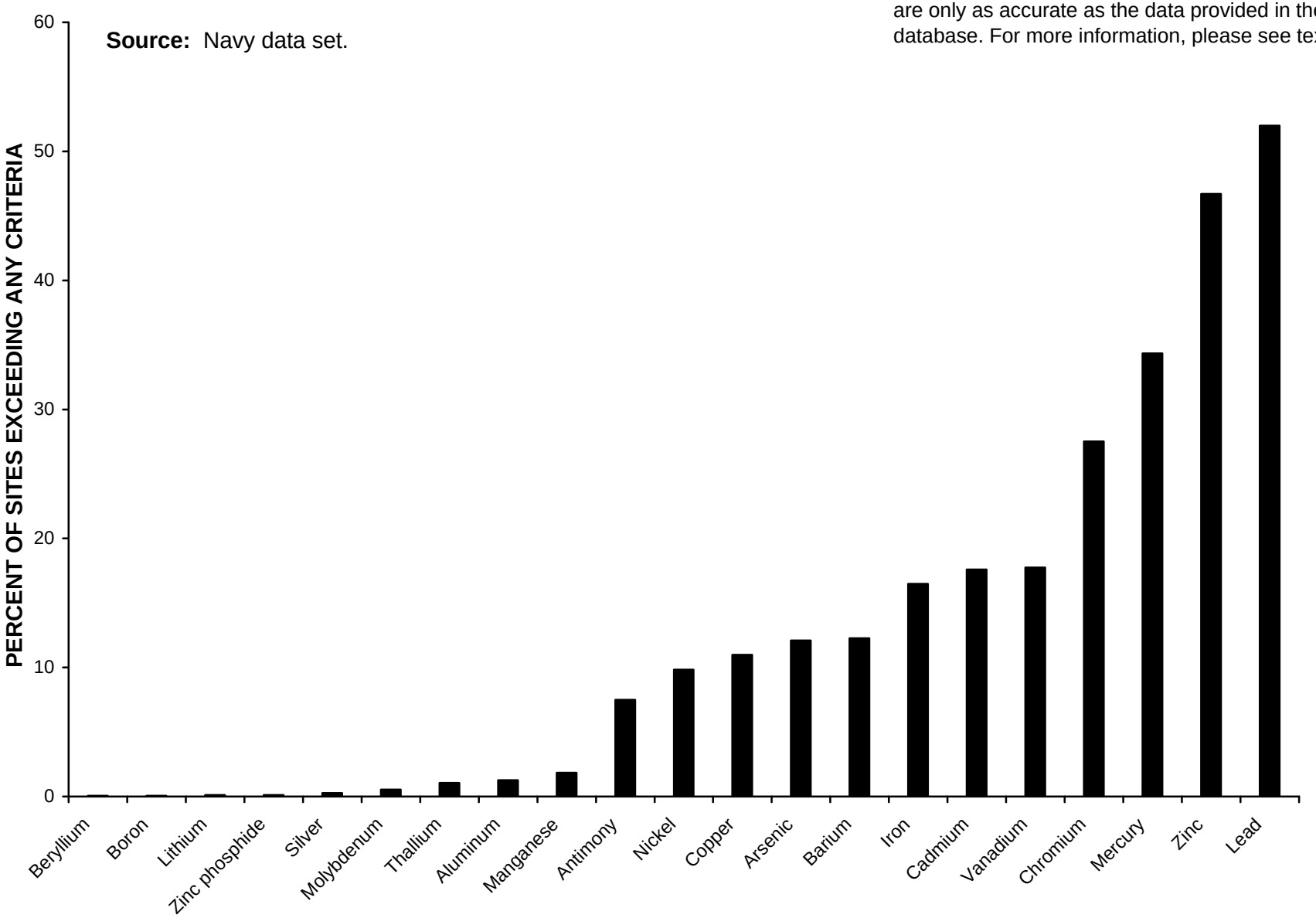


Figure 3c. Percent of metal-contaminated sites exceeding any criteria at least once (1893 sites total).

Boulder1\data\projects\Xfer_Salatas\Figure 3c RO-Soil-Metals Concentrations1-formatted 10/16/01 CO

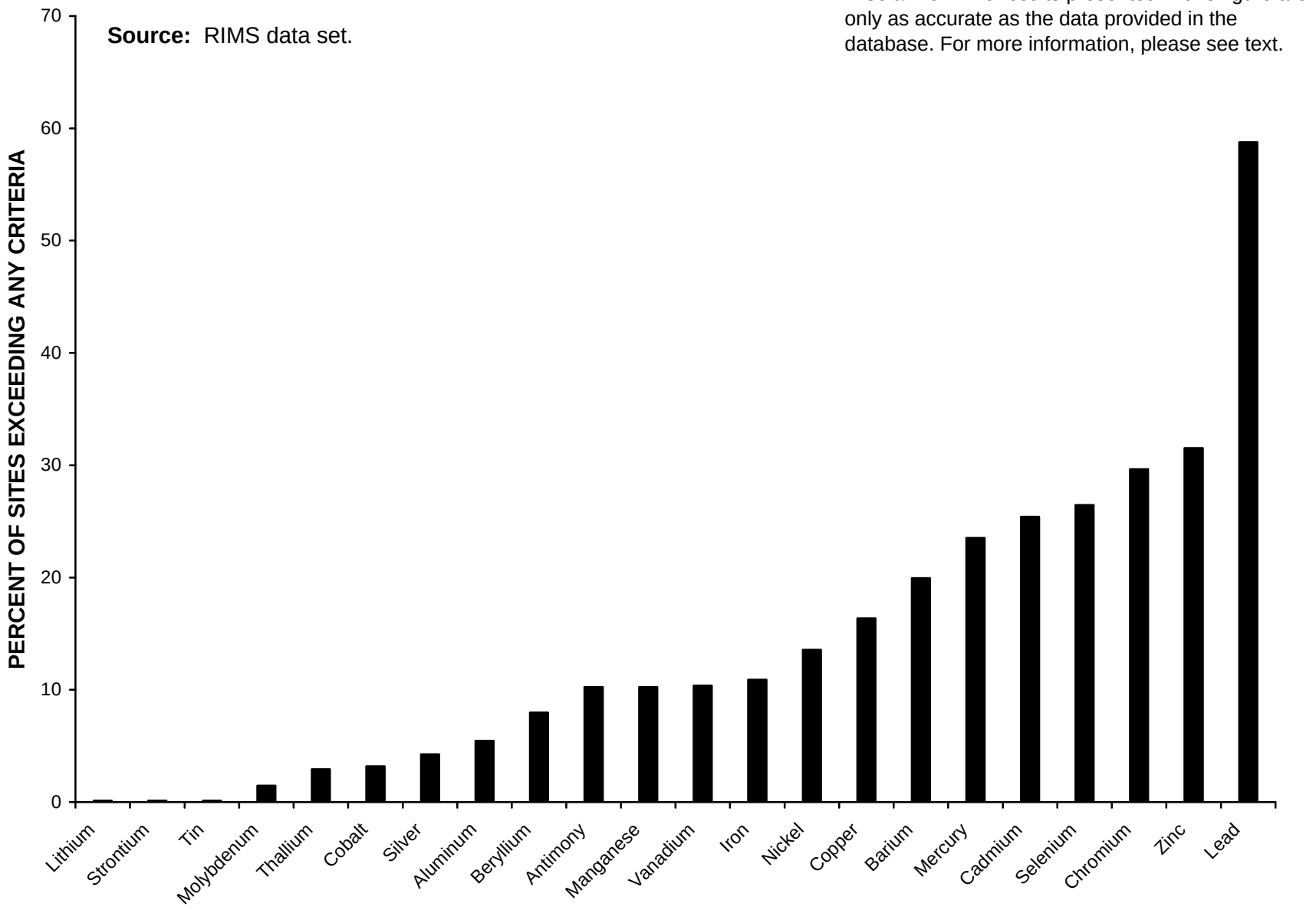


Figure 3d. Percent of metal-contaminated sites exceeding any criteria at least once (752 sites total).

Boulder1\data\projects\Xfer_Salatas\FIG 3d RMIS-formatted 10/16/01 CO

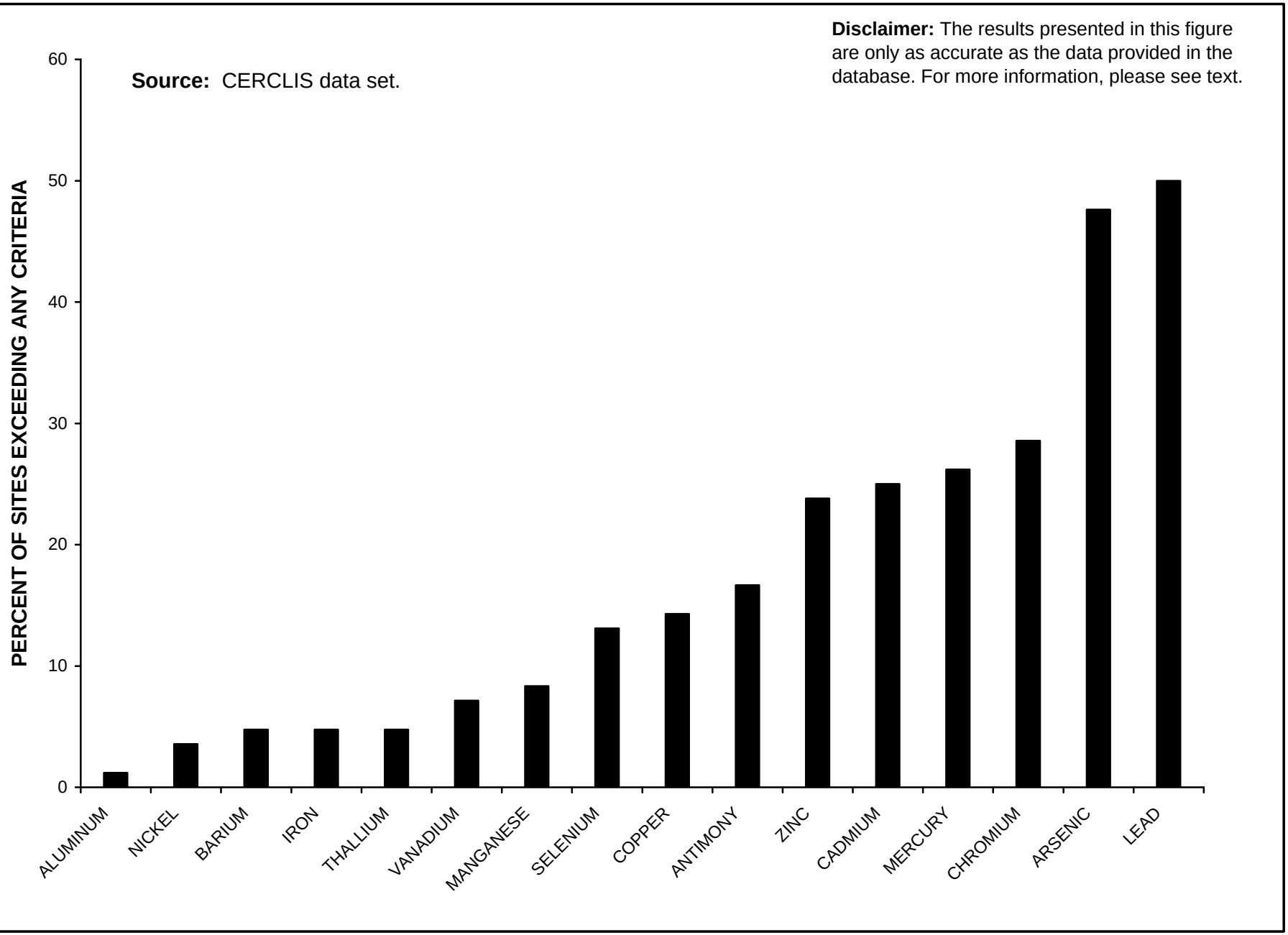


Figure 3e. Percent of metal-contaminated sites exceeding any criteria (84 sites total).

Boulder1\data\projects\Xfer_Salatas\Figure 3e 2nd CERCLIS DATABASE FROM HEATHER 10/16/01 CO

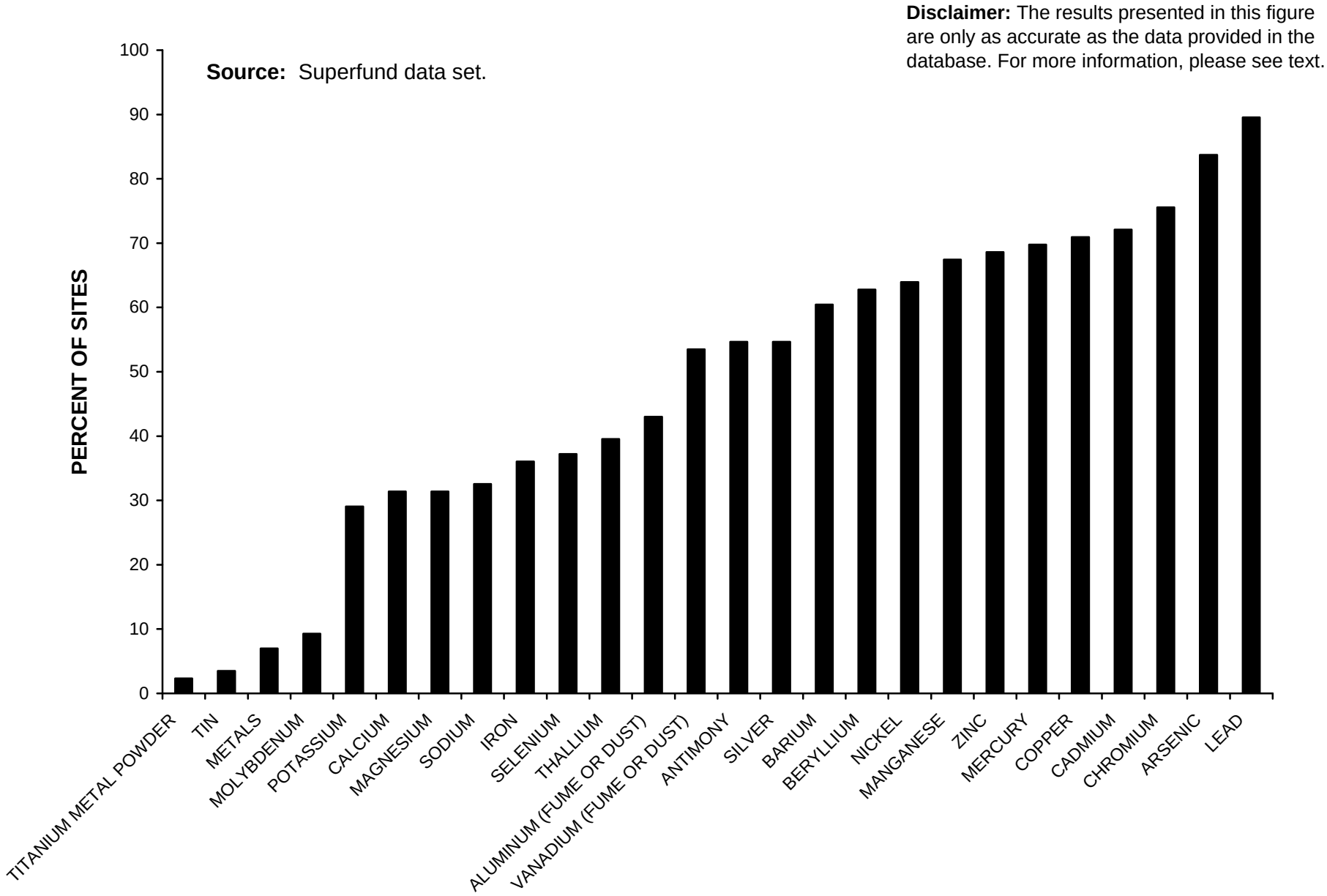


Figure 3f. Percent of metal-contaminated sites (86 sites total) where each metal is considered a contaminant of concern in the Superfund Hazardous Waste Site Advanced Query website.

Boulder1\data\projects\Xfer_Salatas\Figure 3f Advanced Query Data 10/16/01 CO

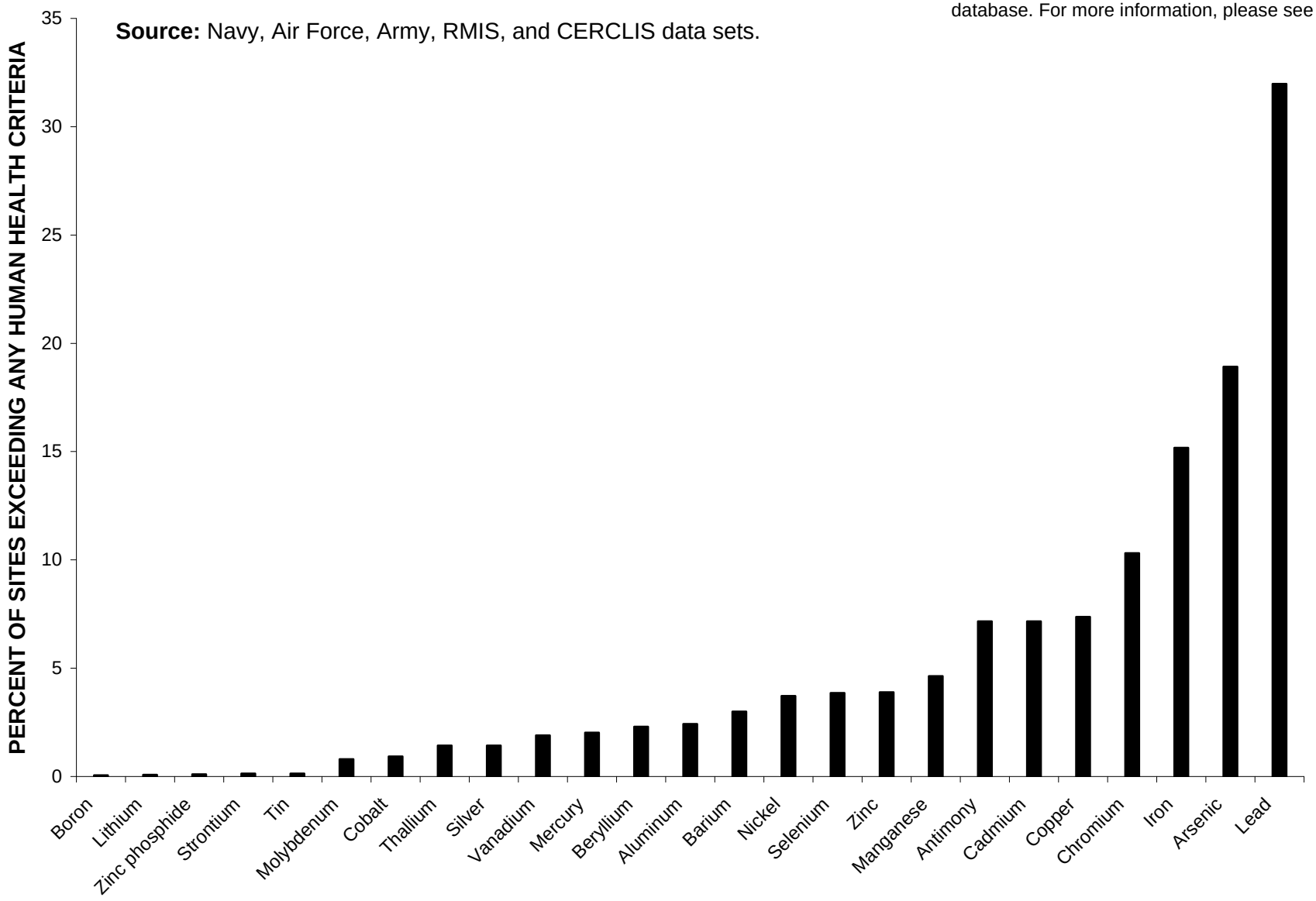


Figure 3g. Percent of metal contamined sites exceeding any human health criteria (industrial or residential) at least once for all data sets combined.

Boulder1\data\projects\Xfer_Salatas\Figure 3g BIG GRAPH 10/16/01 CO

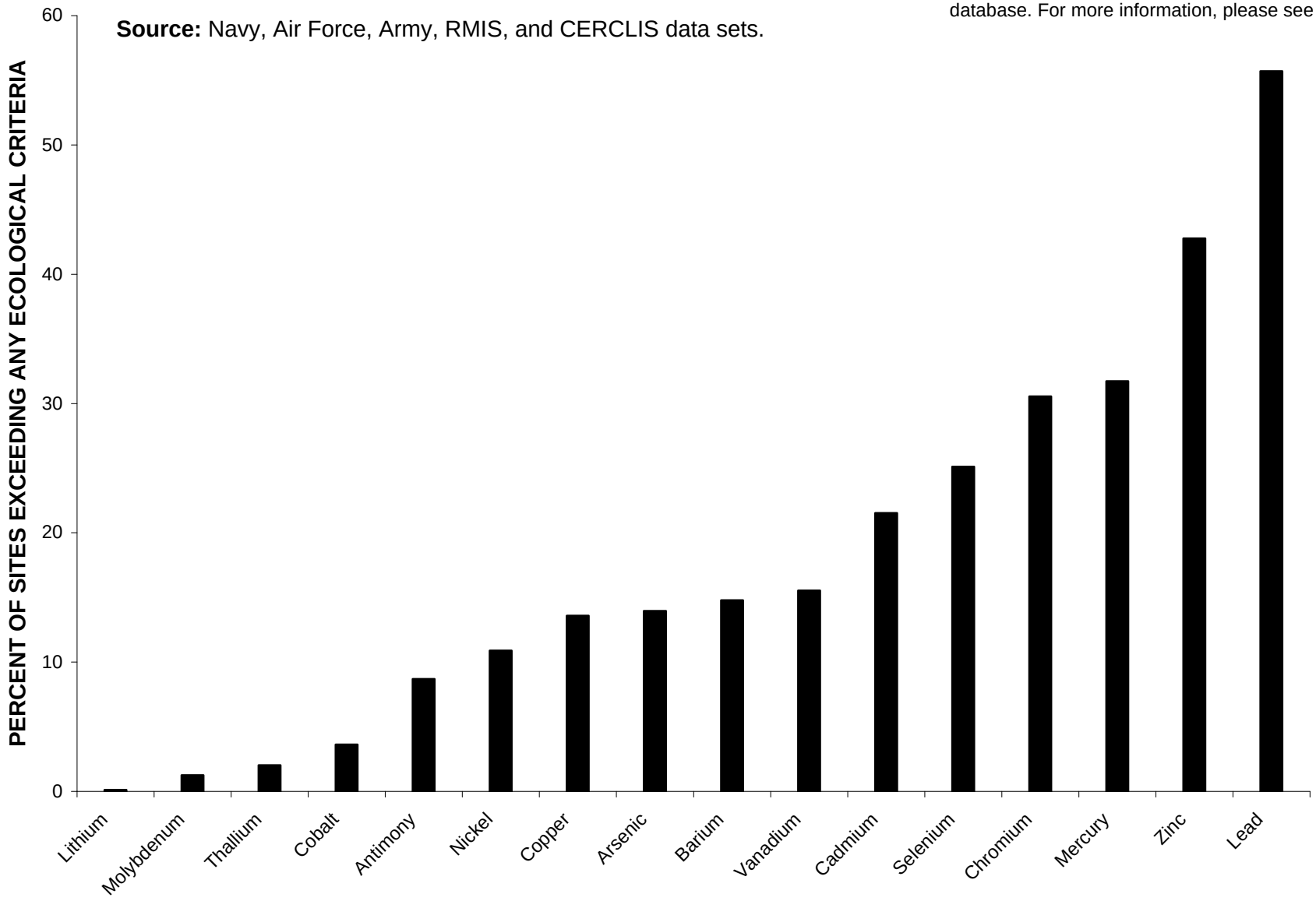


Figure 3h. Percent of metal contaminated sites exceeding any ecological criteria (avian or mammalian) at least once for all data sets combined.

Boulder1\data\projects\Xfer_Salatas\Figure 3h BIG GRAPH 10/16/01 CO

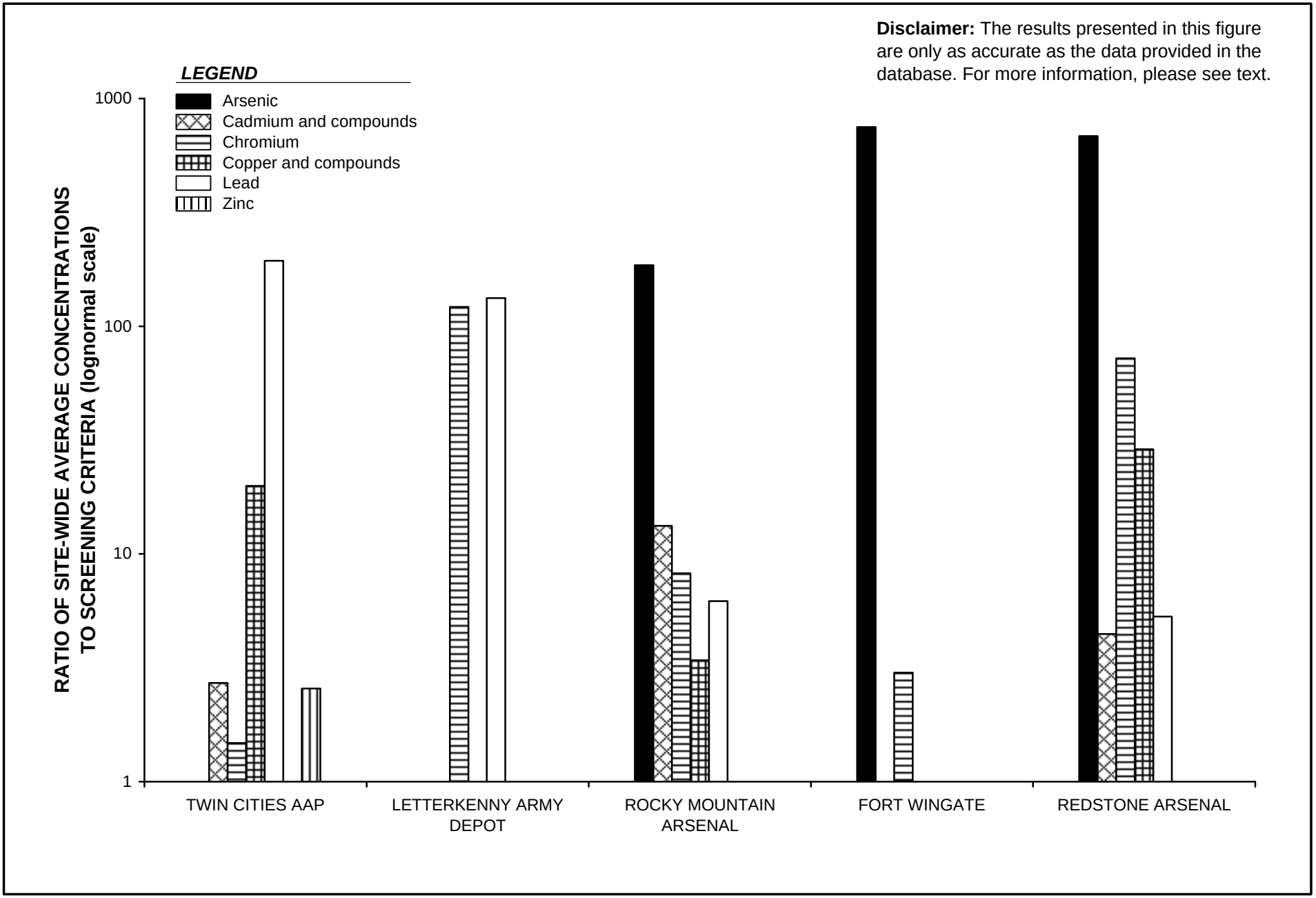


Figure 4a. Ratio of site-wide average concentrations to Residential Screening Criteria for the five sites with the highest total risk across all metals. Source: Army data set.

Boulder1\data\projects\xfer_salatas\Residential Fig 4a EQD_METALS_CR1 10/16/01 CO

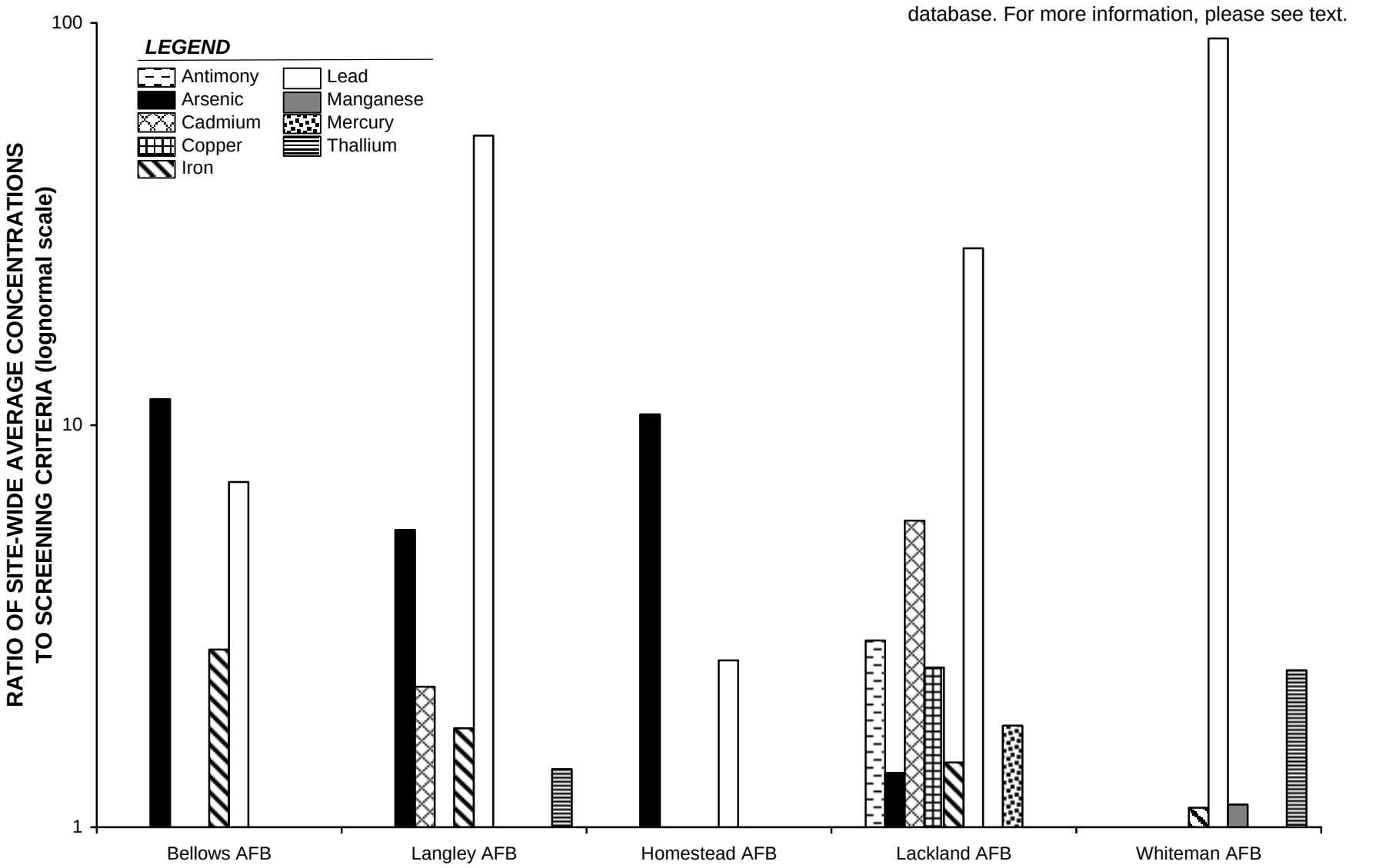


Figure 4b. Ratio of site-wide average concentrations to Residential Screening Criteria for the five sites with the highest total risk across all metals. Source: Air Force ERPIMS data set.

Boulder1\data\projects\Xfer_Salatas\Res Fig 4b ERPIMS-formatted 10/16/01 CO

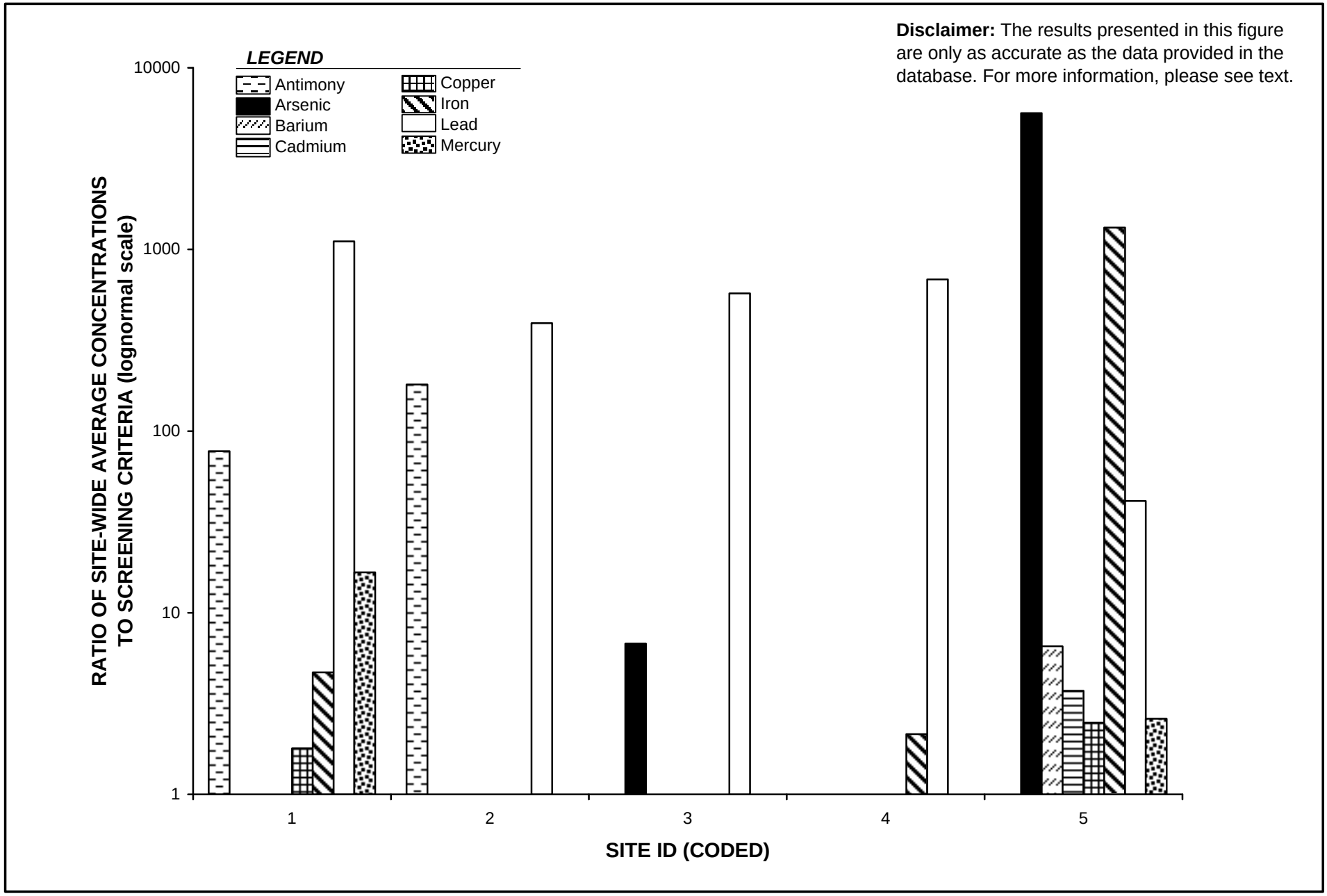


Figure 4c. Ratio of site-wide average concentrations to Residential Screening Criteria for the five sites with the highest total risk across all metals. Source: Navy data set.

Boulder1\data\projects\Xfer_Salatas\Res fig 4c RO-Soil-Metals Concentrations1-formatted 10/16/01 CO

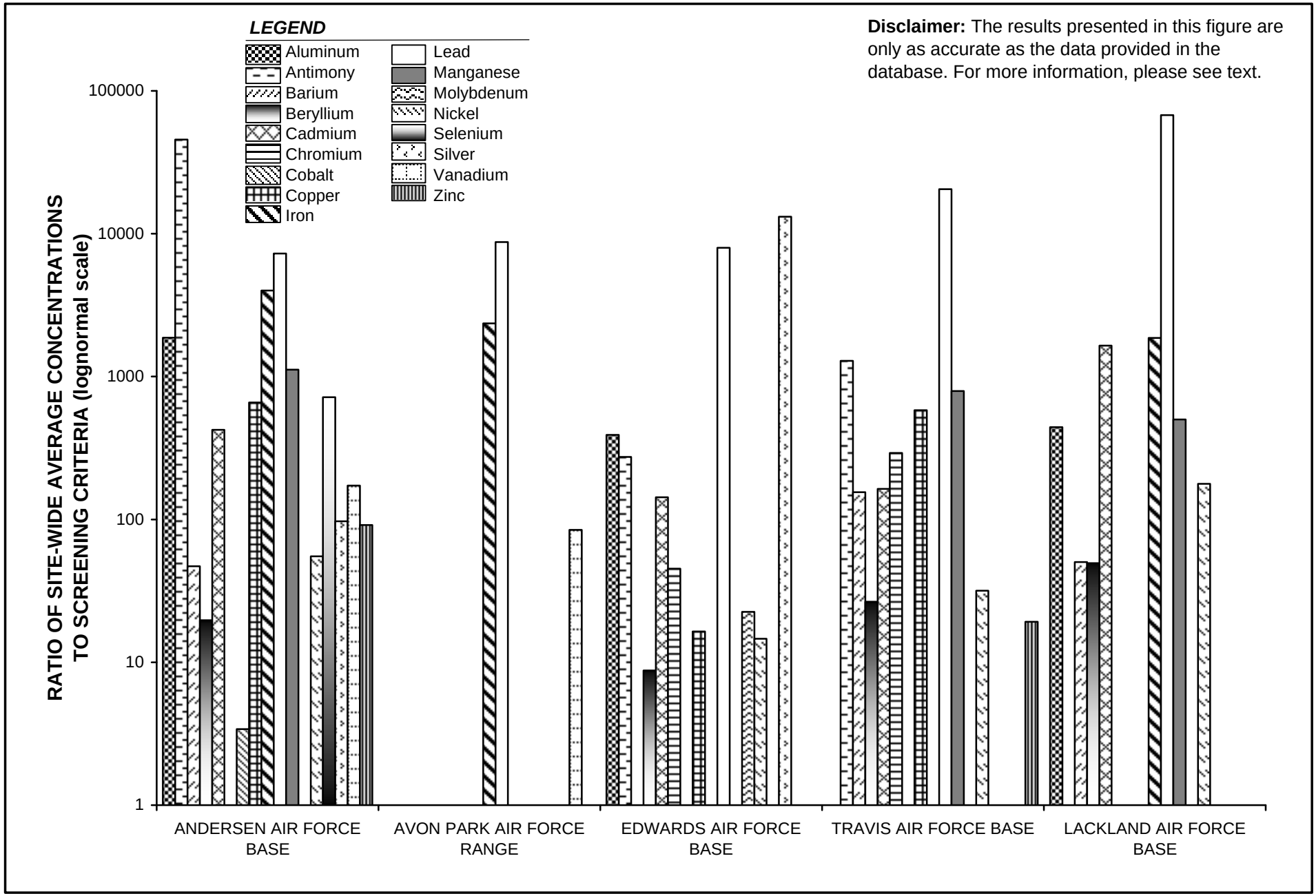


Figure 4d. Ratio of site-wide average concentrations to Residential Screening Criteria for the five sites with the highest total risk across all metals. Source: RMIS data set.

Boulder1\data\projects\Xfer_Salatas\Res Fig 4d RMIS-formatted 10/16/01 CO

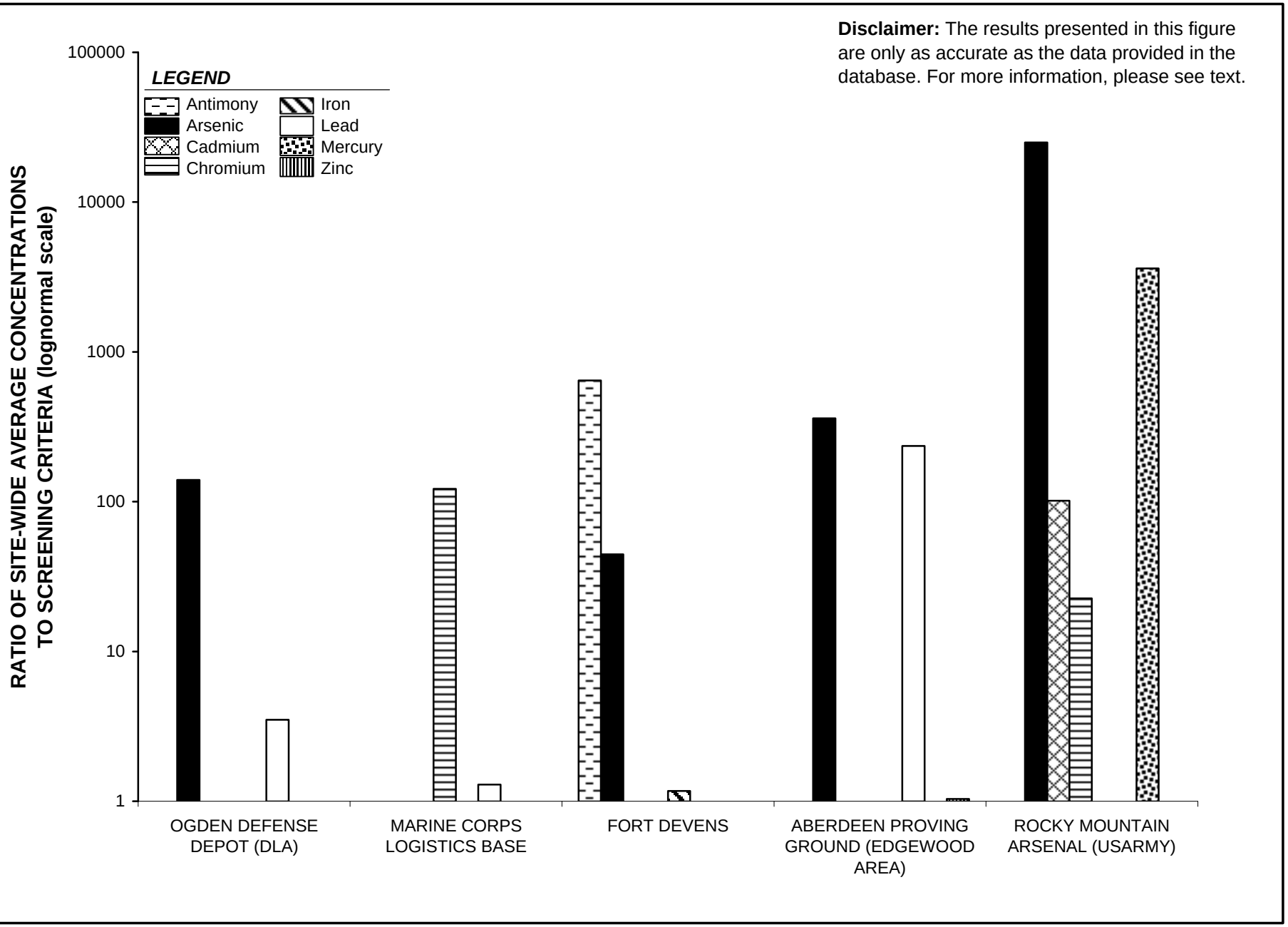


Figure 4e. Ratio of site-wide average concentrations to Residential Screening Criteria for the five sites with the highest total risk across all metals. Source: CERCLIS data set.

Boulder1\data\projects\Xfer_Salatas\Res fig 4e 2nd CERCLIS DATABASE FROM HEATHER 10/16/01 CO

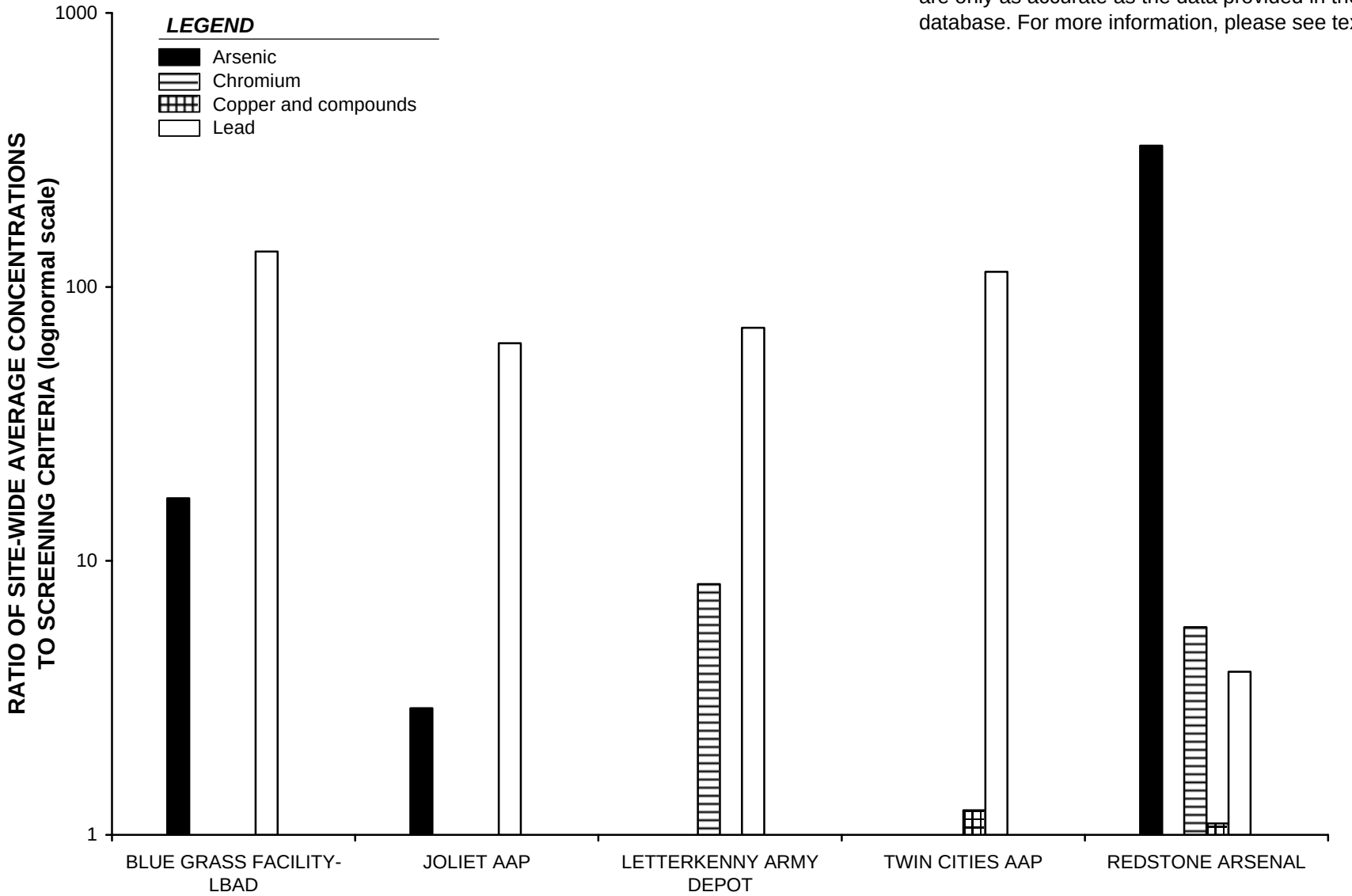


Figure 5a. Ratio of site-wide average concentrations to Industrial Screening Criteria for the five sites with the highest total risk across all metals. Source: Army data set.

Boulder1\data\projects\xfer_salatas\Industrial Fig 5a EQD_METALS_CR1 10/16/01 CO

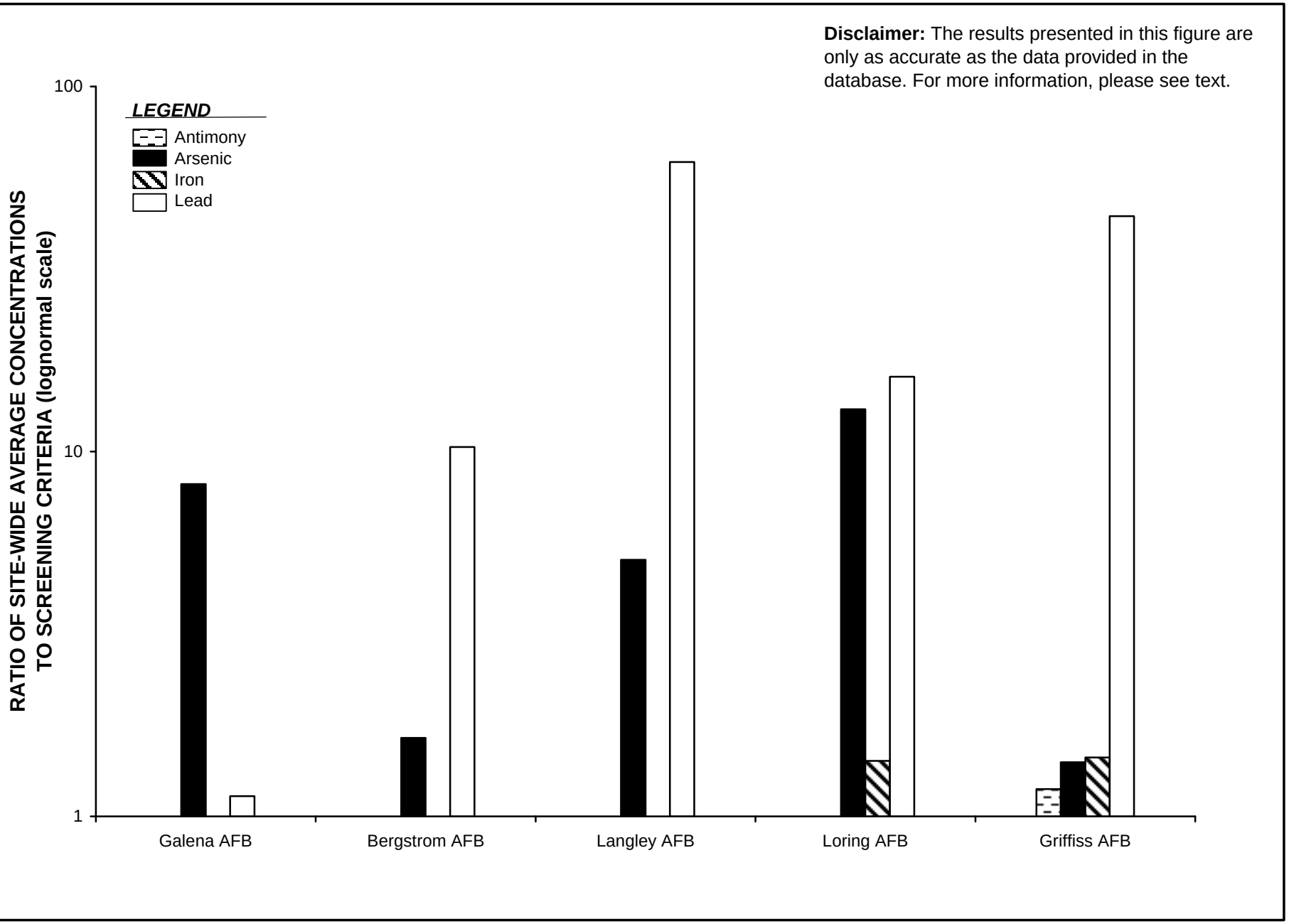
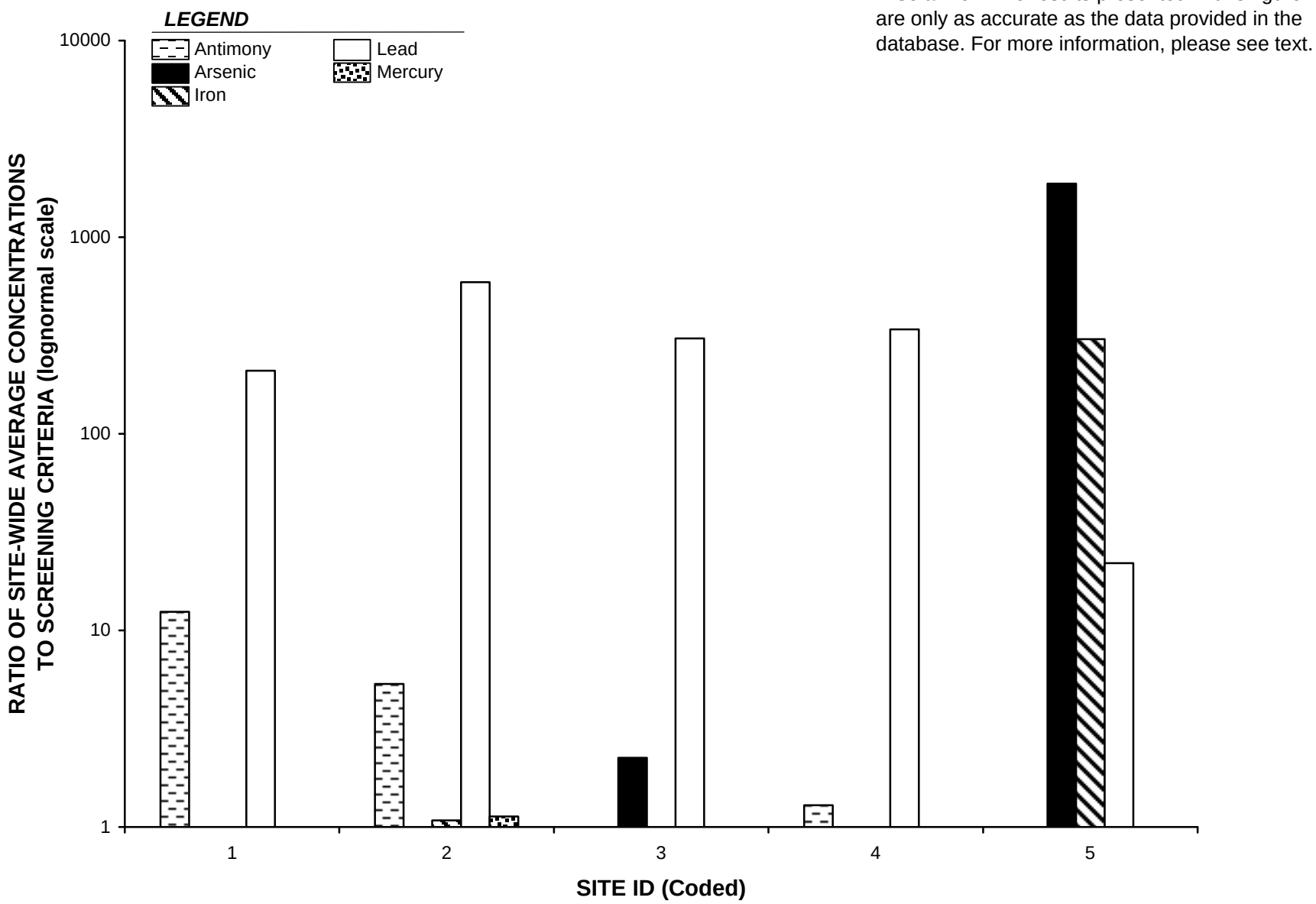


Figure 5b. Ratio of site-wide average concentrations to Industrial Screening Criteria for the five sites with the highest total risk across all metals. Source: Air Force ERPIMS data set.

Boulder1\data\projects\Xfer_Salatas\Ind fig 5b ERPIMS-formatted 10/16/01 CO



Disclaimer: The results presented in this figure are only as accurate as the data provided in the database. For more information, please see text.

Figure 5c. Ratio of site-wide average concentrations to Industrial Screening Criteria for the five sites with the highest total risk across all metals. Source: Navy data set.

Boulder1\data\projects\Xfer_Salatas\Indust Fig 5c RO-Soil-Metals Concentrations1-formatted 10/16/01 CO

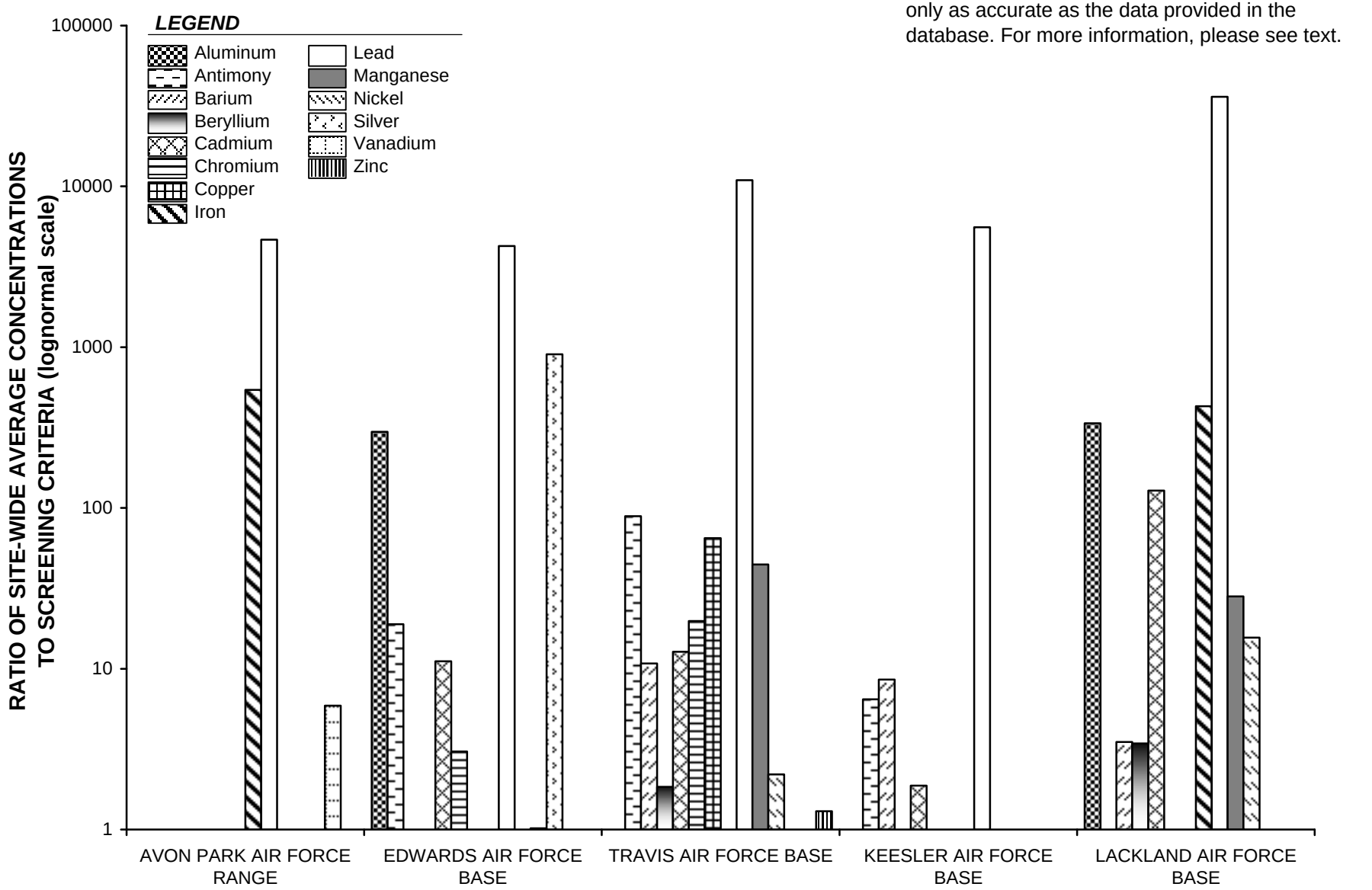


Figure 5d. Ratio of site-wide average concentrations to Industrial Screening Criteria for the five sites with the highest total risk across all metals. Source: RMIS data set.

Boulder1\data\projects\Xfer_Salatas\Indus Fig 5d RMIS-formatted 10/16/01 CO

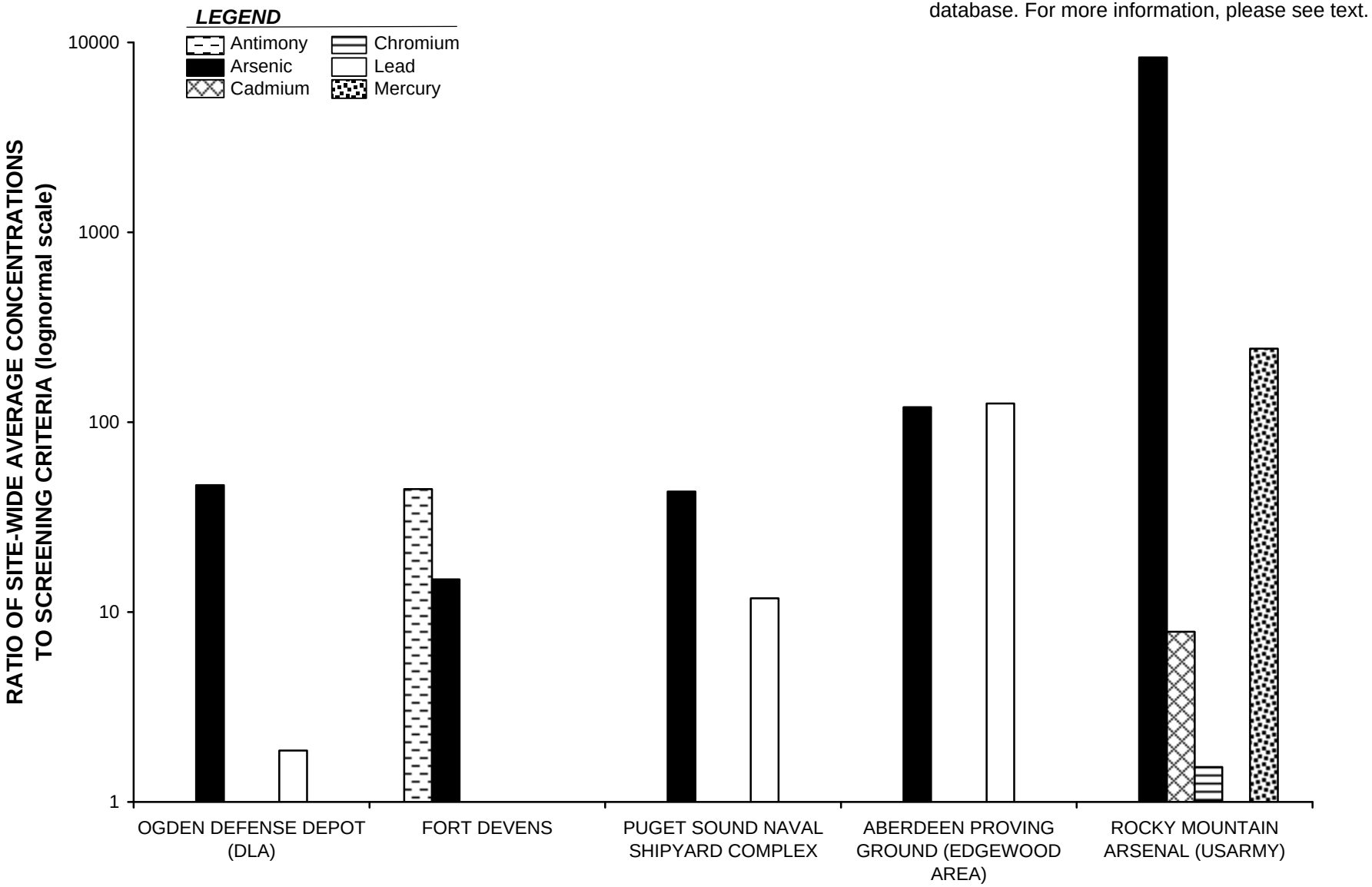


Figure 5e. Ratio of site-wide average concentrations to Industrial Screening Criteria for the five sites with the highest total risk across all metals. Source: CERCLIS data set.

Boulder1\data\projects\Xfer_Salatas\Indus fig 5e 2nd CERCLIS DATABASE FROM HEATHER 10/16/01 CO

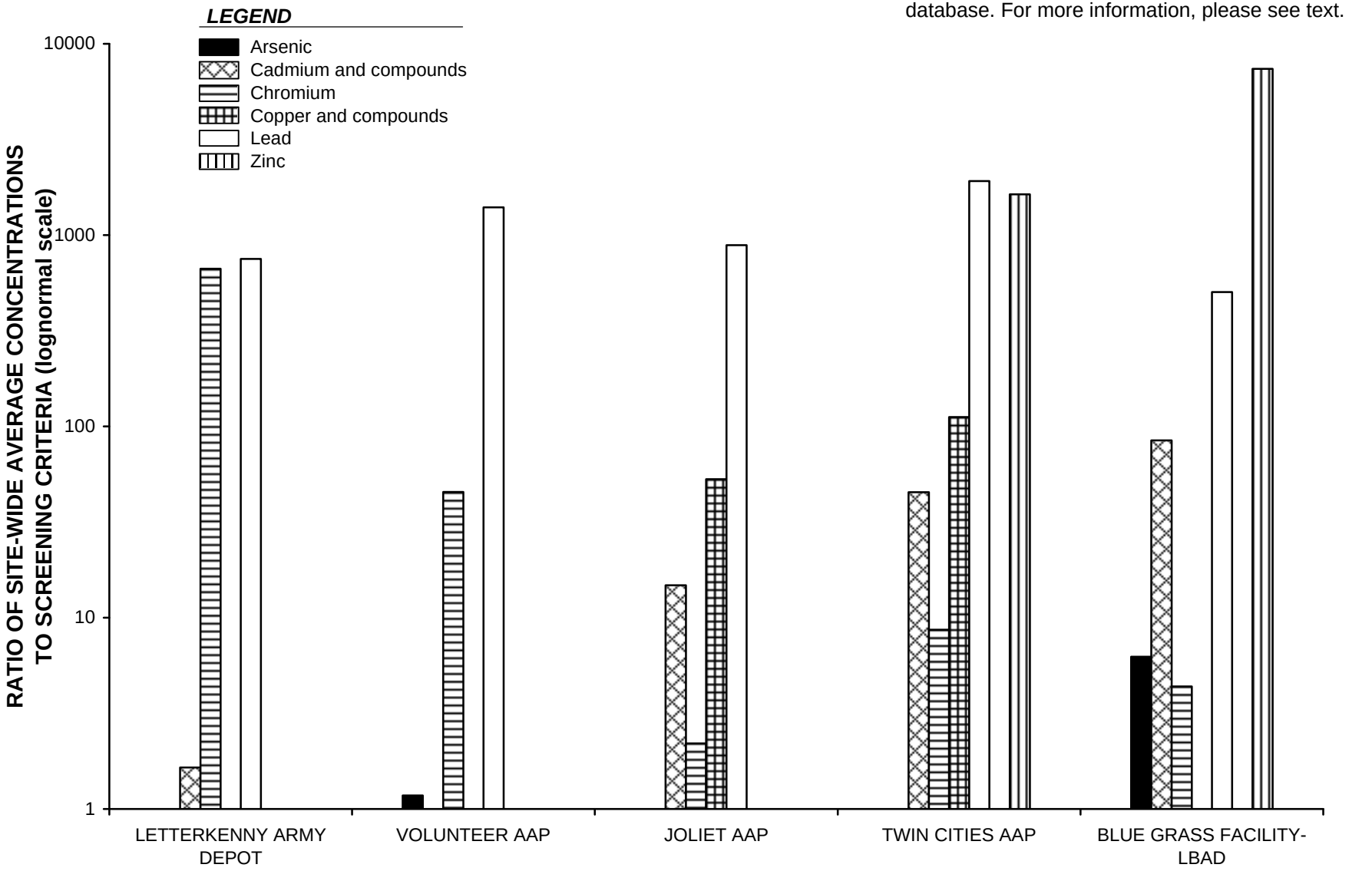


Figure 6a. Ratio of site-wide average concentrations to Avian Screening Criteria for the five sites with the highest total risk across all metals. Source: Army data set.

Boulder1\data\projects\xfer_salatas\Avian Fig 6a EQD_METALS_CR1 10/16/01 CO

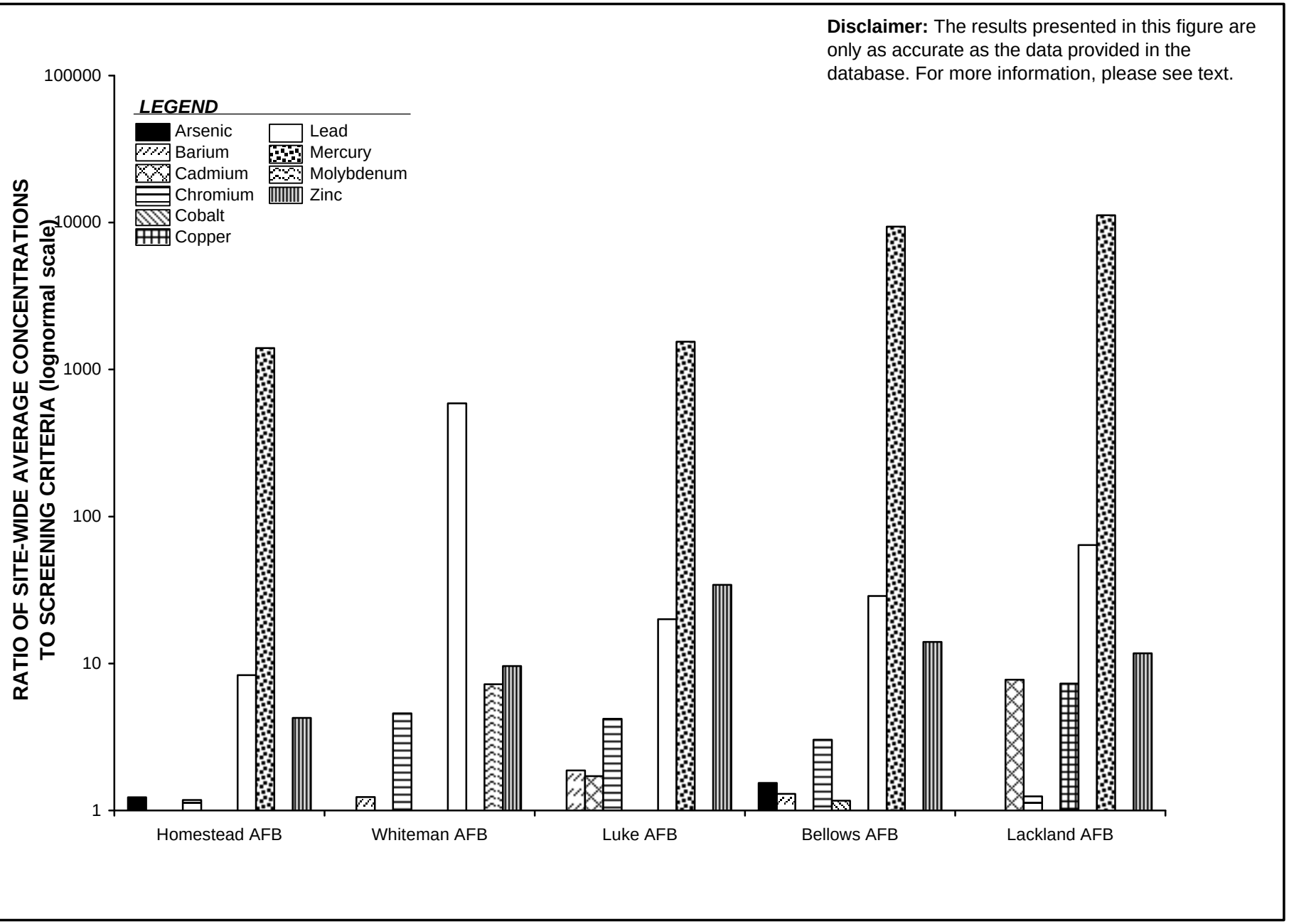


Figure 6b. Ratio of site-wide average concentrations to Avian Screening Criteria for the five sites with the highest total risk across all metals. Source: Air Force ERPIMS data set.

Boulder1\data\projects\Xfer_Salatas\Avian Fig 6b ERPIMS-formatted 10/16/01 CO

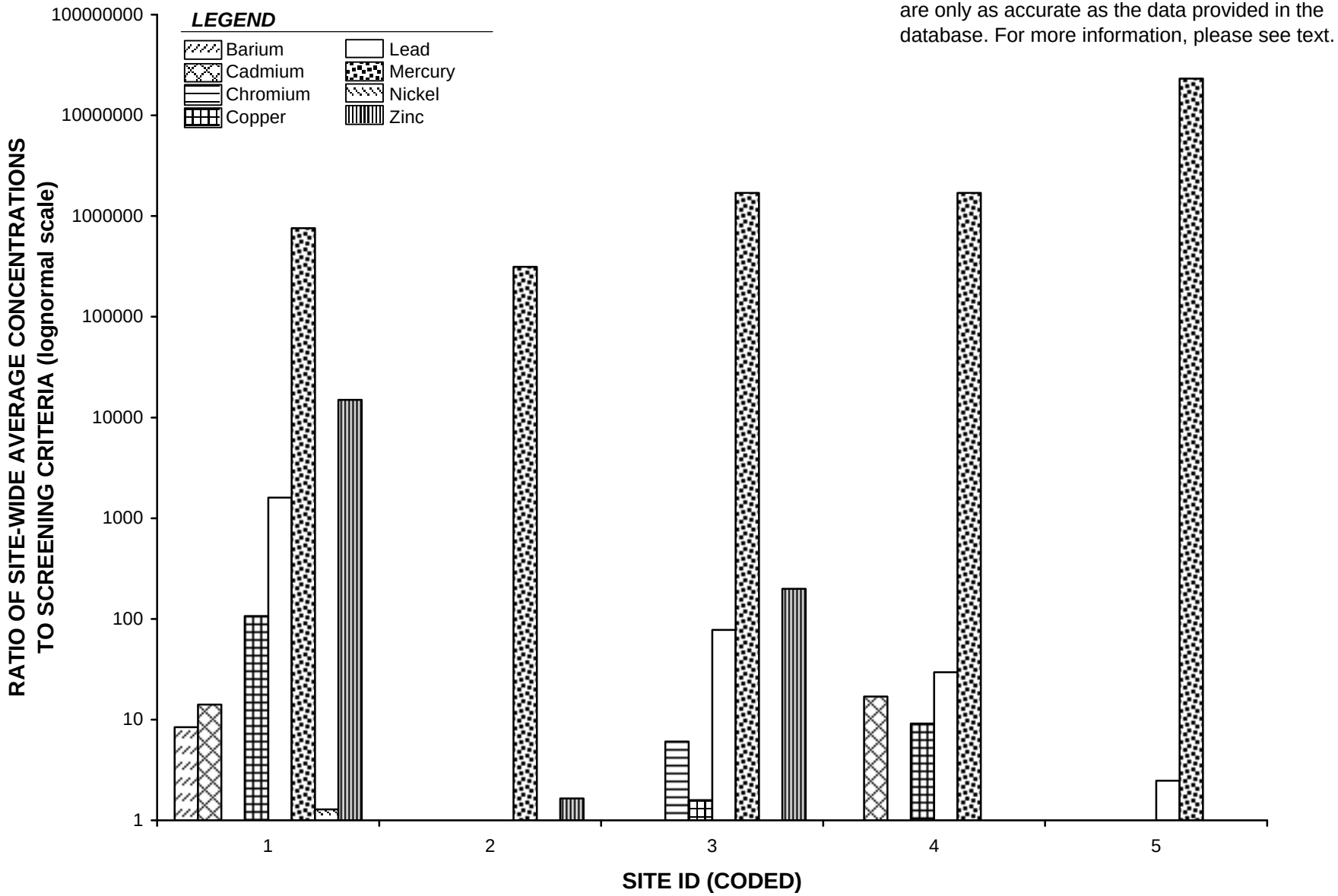


Figure 6c. Ratio of site-wide average concentrations to Avian Screening Criteria for the five sites with the highest total risk across all metals. Source: Navy data set.

Boulder1\data\projects\Xfer_Salatas\Avian fig 6c RO-Soil-Metals Concentrations1-formatted 10/16/01 CO

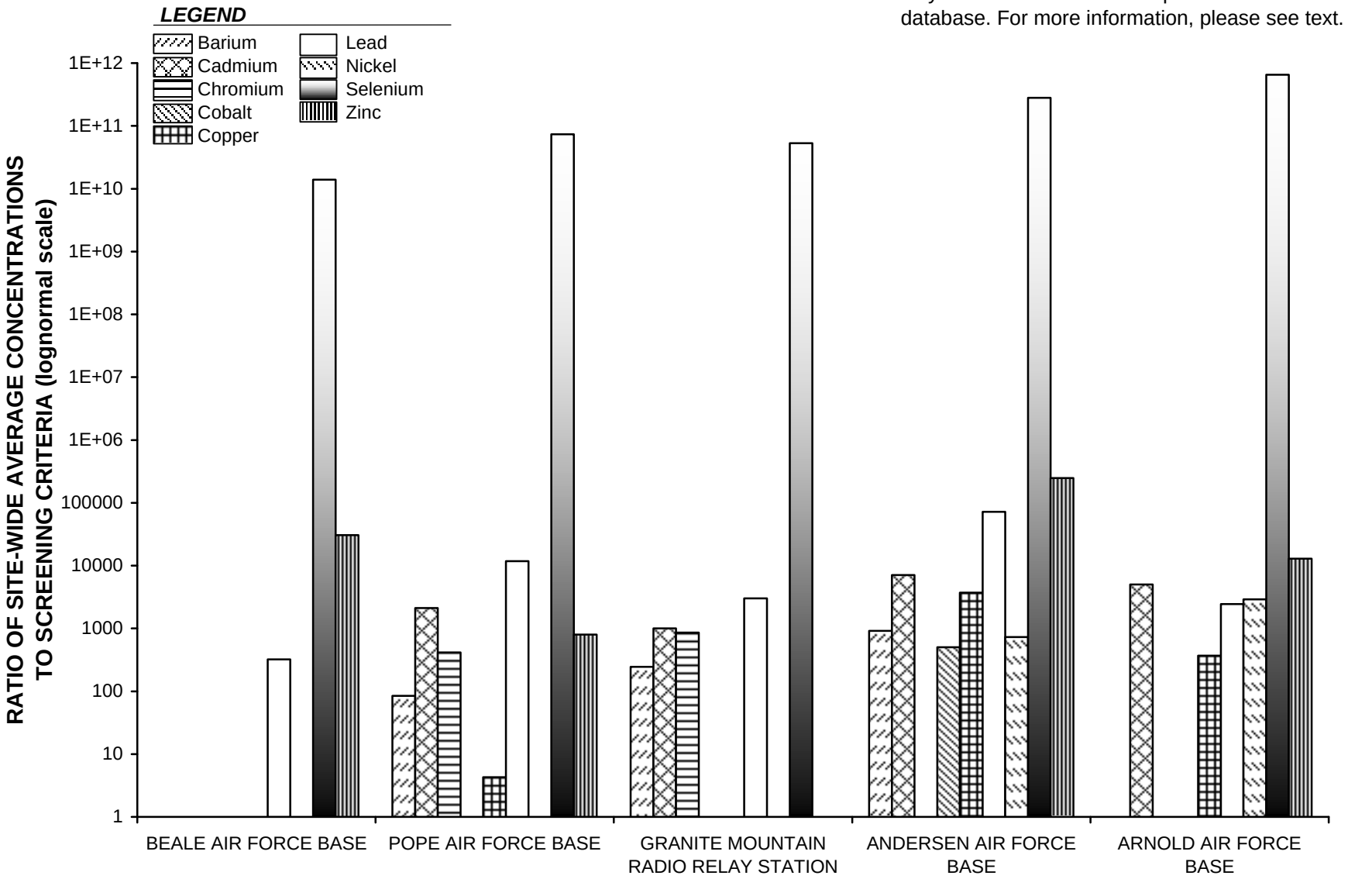


Figure 6d. Ratio of site-wide average concentrations to Avian Screening Criteria for the five sites with the highest total risk across all metals. Source: RMIS data set.

Boulder1\data\projects\Xfer_Salatas\Avian Fig 6d RMIS-formatted 10/16/01 CO

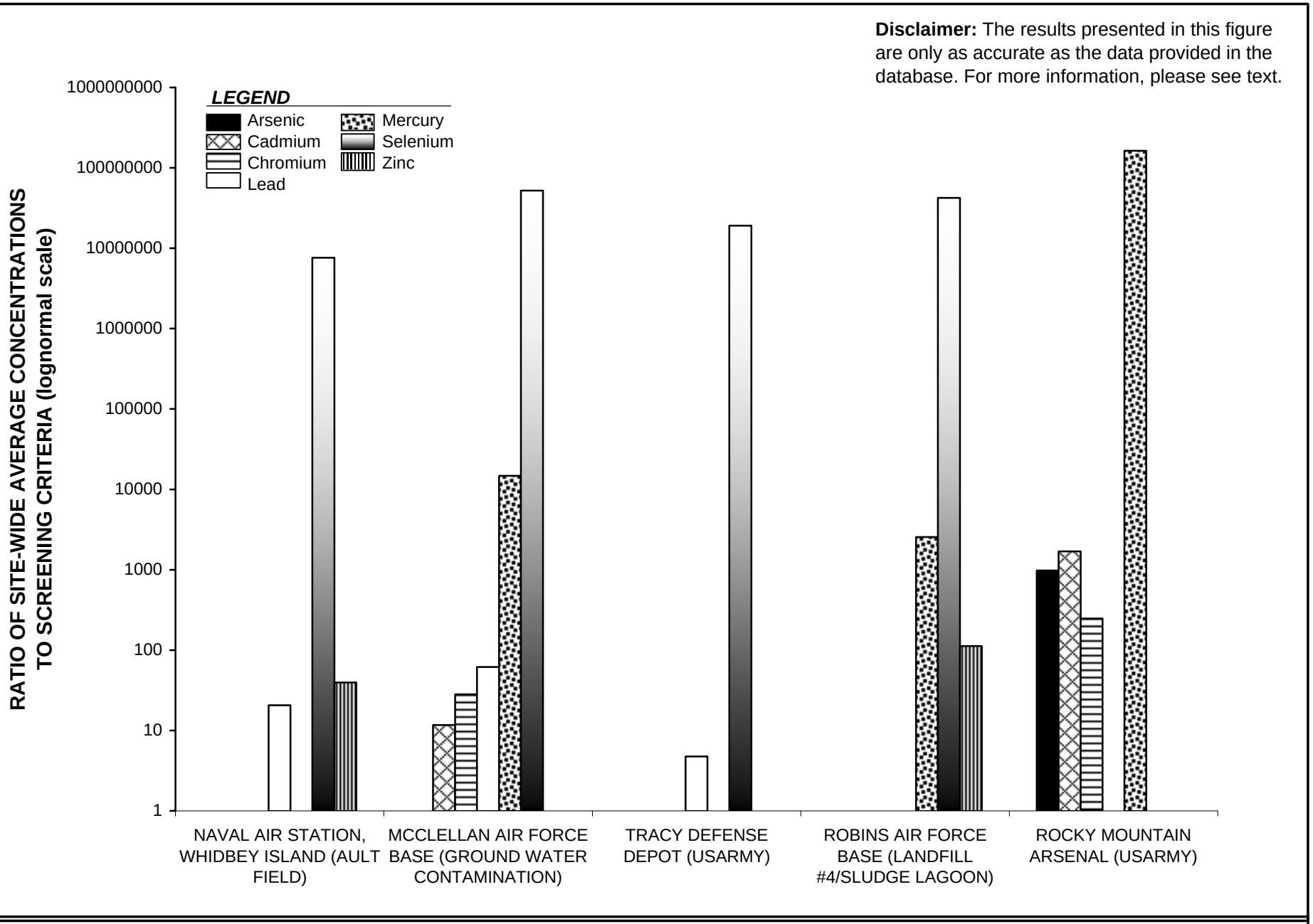


Figure 6e. Ratio of site-wide average concentrations to Avian Screening Criteria for the five sites with the highest total risk across all metals. Source: CERCLIS data set.

Boulder1\data\projects\Xfer_Salatas\Avian fig 6e 2nd CERCLIS DATABASE FROM HEATHER 10/16/01 CO

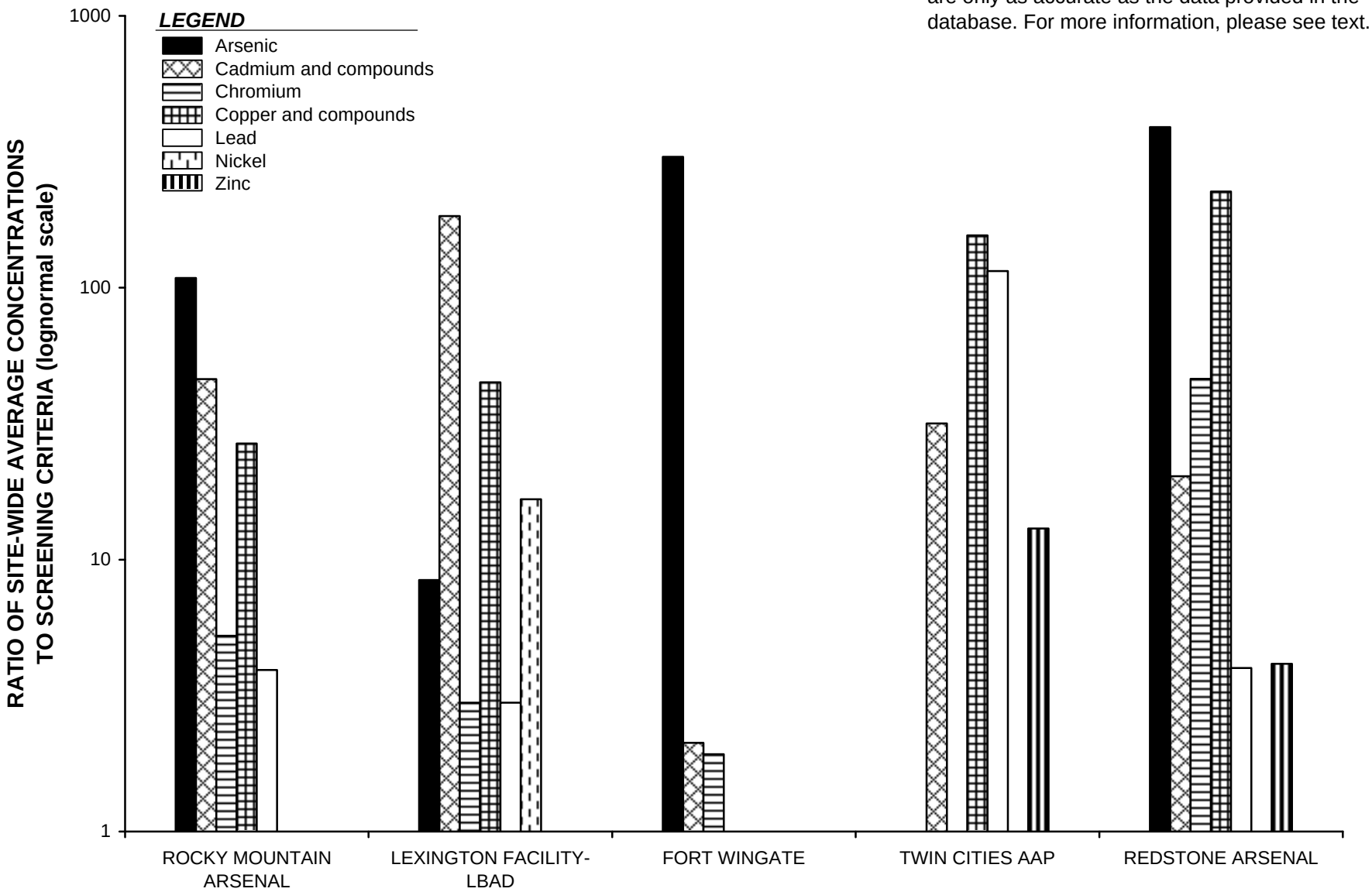


Figure 7a. Ratio of site-wide average concentrations to Mammalian Screening Criteria for the five sites with the highest total risk across all metals. Source: Army data set.

Boulder1\data\projects\xfer_salatas\Mammalian Fig 7a EQD_METALS_CR1 10/16/01 CO

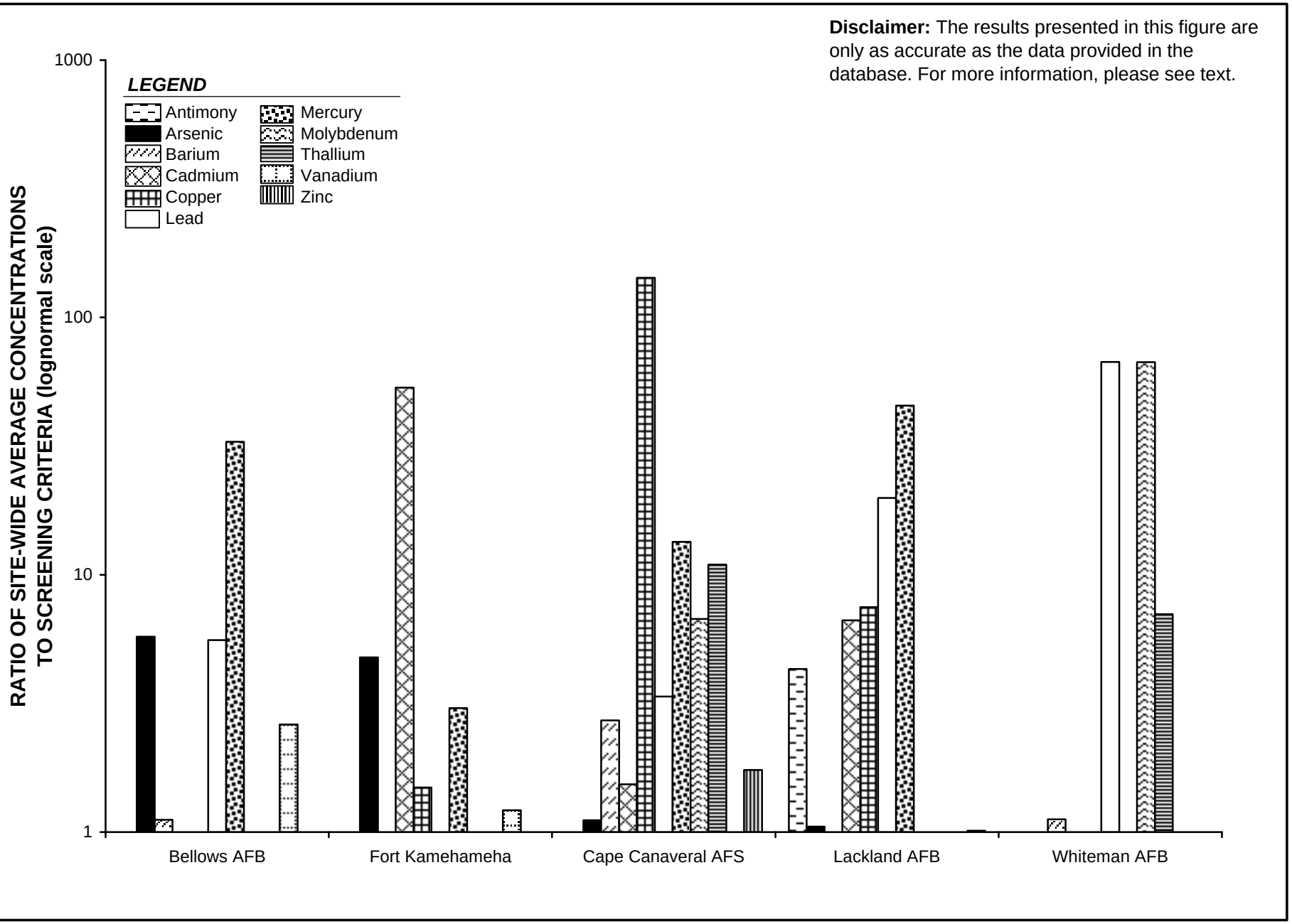
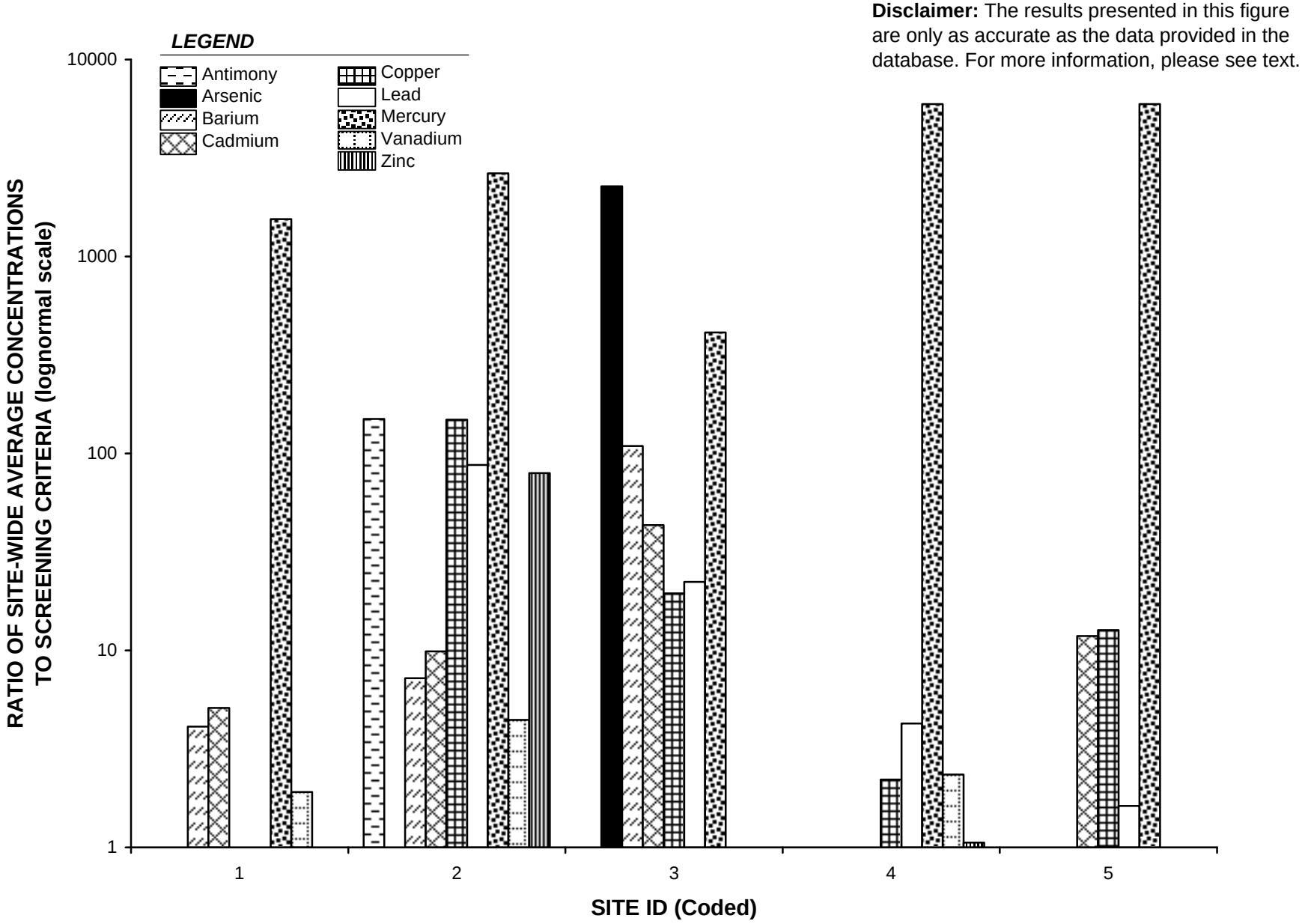


Figure 7b. Ratio of site-wide average concentrations to Mammalian Screening Criteria for the five sites with the highest total risk across all metals. Source: Air Force ERPIMS data set.

Boulder1\data\projects\Xfer_Salatas\Mammal Fig 7b ERPIMS-formatted 10/16/01 CO



Disclaimer: The results presented in this figure are only as accurate as the data provided in the database. For more information, please see text.

Figure 7c. Ratio of site-wide average concentrations to Mammalian Screening Criteria for the five sites with the highest total risk across all metals. Source: Navy data set.

Boulder1\data\projects\Xfer_Salatas\Mammal fig 7c RO-Soil-Metals Concentrations1-formatted 10/16/01 CO

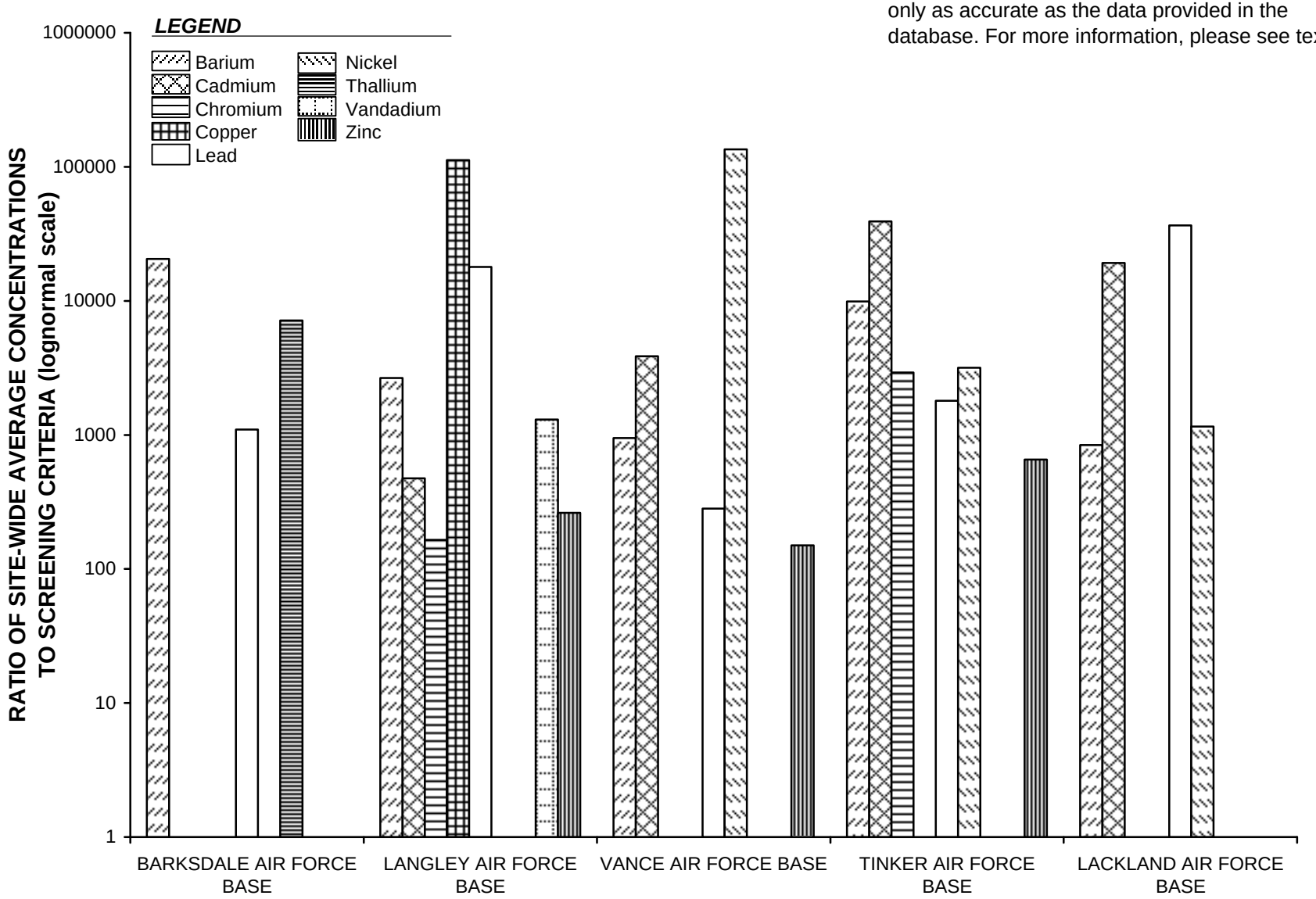


Figure 7d. Ratio of site-wide average concentrations to Mammalian Screening Criteria for the five sites with the highest total risk across all metals. Source: RMIS data set.

Boulder1\data\projects\Xfer_Salatas\Mammal Fig 7d RMIS-formatted 10/16/01 CO

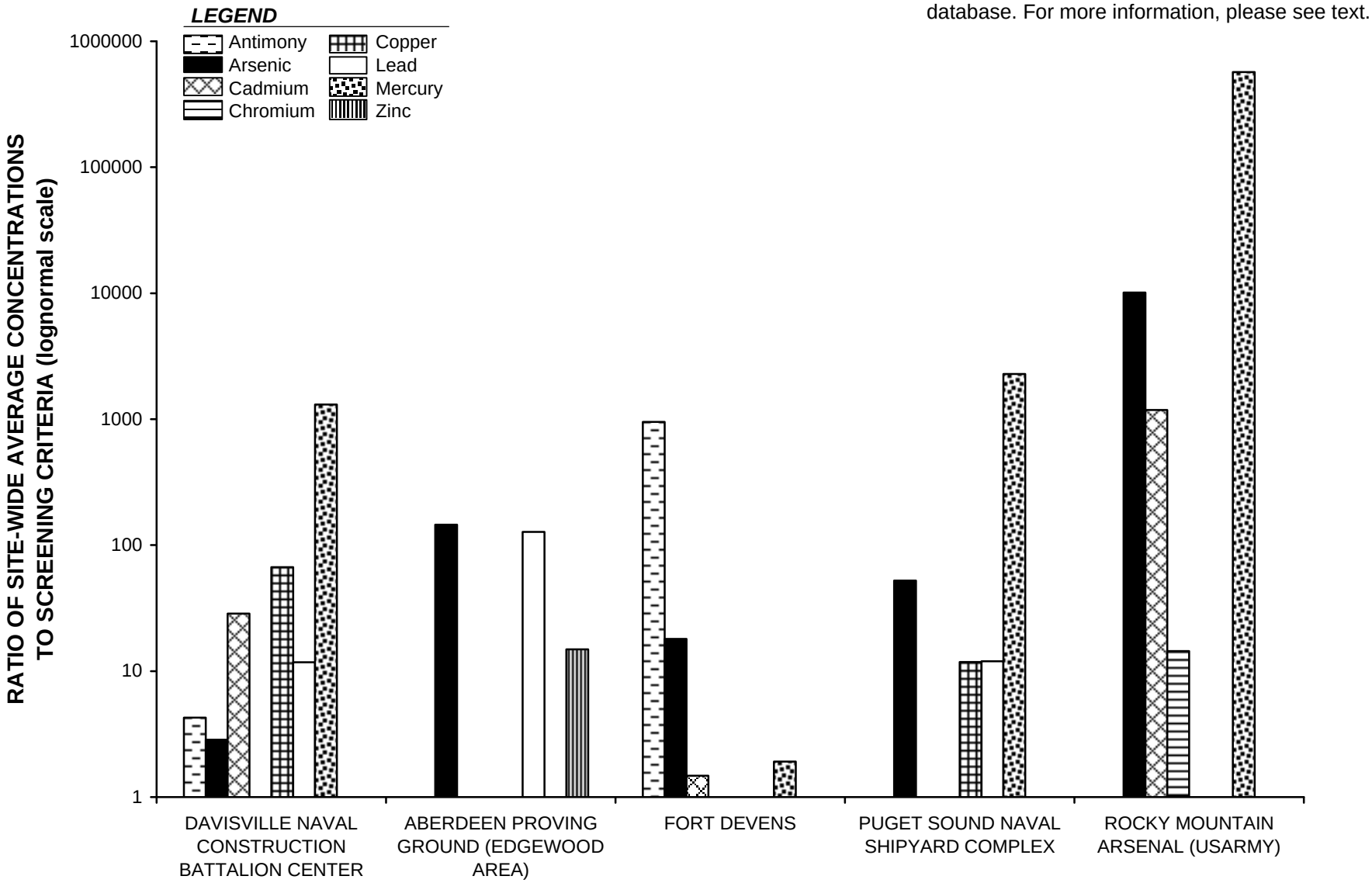


Figure 7e. Ratio of site-wide average concentrations to Mammalian Screening Criteria for the five sites with the highest total risk across all metals. Source: CERCLIS data set.

Boulder1\data\projects\Xfer_Salatas\Mammal fig 7e 2nd CERCLIS DATABASE FROM HEATHER 10/16/01 CO

Tables

Table 1. Screening criteria proposed for the SERDP screening exercise

	Human Health Criteria		Ecological Receptor Criteria	
Metal	Residential	Industrial	Avian	Mammalian
Arsenic	4 [a,b]	20 [a,b]	102 [c]	9.9 [c]
Lead	400 [b]	750 [b]	40.5 [c]	740 [c]
Cadmium	70 [b]	900 [b]	4.2 [c]	6 [c]
Copper	2900 [d]	76000 [d]	515 [c]	370 [c]
Chromium	230 [b]	3,400 [b]	21 [e]	360 [e]
Nickel	1,600 [b]	23,000 [b]	121 [c]	246 [c]
Zinc	23,000 [b]	340,000 [b]	8.5 [c]	1,600 [c]
Mercury	23 [b]	340 [b]	0.00051 [c]	0.146 [c]
Aluminum	76,000.00 [d]	100,000 [d]	--	--
Antimony	31 [b]	450 [b]	--	21 [e]
Barium	5,500 [b]	79,000 [b]	283 [c]	329 [c]
Beryllium	160 [b]	2,300 [b]	--	--
Boron	5,500 [d]	79,000 [d]	--	--
Cobalt	4,700 [d]	100,000 [d]	32 [e]	340 [e]
Iron	23,000 [d]	100,000 [d]	--	--
Lithium	1,600 [d]	41,000 [d]	--	390
Manganese	1,800 [d]	32,000 [d]	--	--
Molybdenum	390 [d]	10,000 [d]	44 [c]	4.75 [c]
Selenium	390 [b]	5,700 [b]	0.000001 [c]	--
Silver	390 [b]	5,700 [b]	--	--
Strontium	47,000 [d]	100,000 [d]	--	--
Thallium	6 [b]	91 [b]	--	2.1 [c]
Tin	47,000 [d]	100,000 [d]	--	--
Vanadium	550 [b]	7,900 [b]	--	55 [c]
Zinc phosphide	23 [d]	610 [d]	--	--

Notes: Chromium(VI) values were used to screen chromium. However, since Cr(VI) criterion was not available for avian receptors, we used Cr(III) criterion instead.

-- - data not available

[a] Increased original value by one order of magnitude.

[b] U.S. EPA 2001

[c] Efroymson et al. 1997

[d] Region IX PRG (U.S. EPA 2001)

[e] U.S. EPA 2000b

Table 2a. Percentage of Army sites exceeding specific criteria, in descending order[a]

Metal	Residential	Industrial	Avian	Mammalian
Lead	67	59	**84**	59
Arsenic	**70**	46	23	59
Chromium	27	5	**58**	21
Cadmium	21	6	**45**	43
Zinc	9	0	**44**	21
Copper	17	3	30	**31**
Nickel	3	1	**13**	9

Source: Army data set
Note: For each metal, the receptor with the greatest percentage of sites exceeding criteria is bolded.
[a] For example, arsenic concentrations exceeded the residential screening criterion 70% more than any other screening criterion.

Table 2b. Percentage of Air Force sites exceeding specific criteria, in descending order[a]

Metal	Residential	Industrial	Avian	Mammalian
Lead	38	34	**74**	34
Zinc	--	0	**73**	10
Arsenic	**70**	44	9	51
Chromium	16	0	**69**	10
Mercury	1	0	**53**	42
Cadmium	8	0	**38**	30
Barium	1	0	**38**	**38**
Iron	**36**	8	--	--
Vanadium	--	0	--	**21**
Copper	5	1	17	**19**
Molybdenum	--	0	4	**17**
Thallium	10	0	--	**14**
Nickel	--	0	**14**	8
Manganese	**14**	0	--	--
Antimony	12	3	--	**14**
Cobalt	--	0	**8**	0
Silver	**3**	0	--	--
Aluminum	**3**	1	--	--

Source: Air Force ERPIMS data set
Notes: For each metal, the receptor with the greatest percentage of sites exceeding criteria is bolded.
-- - no criteria
[a] For example, lead concentrations exceeded the avian screening criterion at 74% of all sites in the Air Force data set.

Table 2c. Percentage of Navy sites exceeding specific criteria, in descending order[a]

Metal	Residential	Industrial	Avian	Mammalian
Lead	25	18	**52**	18
Zinc	1	0	**47**	7
Mercury	2	0.32	**34**	27
Chromium	6	1	**28**	4
Vanadium	1	0.11	--	**18**
Cadmium	3	0.16	**18**	15
Iron	**16**	2	--	--
Barium	1	0.05	**12**	11
Arsenic	**12**	8	1	8
Copper	5	0	9	**11**
Nickel	2	0	**10**	6
Antimony	6	1	--	**8**
Manganese	**2**	0.05	--	--
Aluminum	**1**	1	--	--
Thallium	0.42	0	--	**1**
Molybdenum	0	0	0.11	**1**
Silver	0.26	0	--	--
Zinc Phosphide	0.11	0.05	--	--
Lithium	0.05	0	--	0.11
Boron	0.05	0	--	--
Beryllium	0.05	0.05	--	--

Source: Navy data set
Notes: For each metal, the receptor with the greatest percentage of sites exceeding criteria is bolded.
-- - no criteria
[a] For example, lead concentrations exceeded the avian screening criterion at 52% of all sites in the Navy data set.

Table 2d. Percentage of RMIS sites exceeding specific criteria, in descending order[a]

Metal	Residential	Industrial	Avian	Mammalian
Lead	43	39	**59**	39
Zinc	11	4	**32**	16
Chromium	16	9	**30**	14
Selenium	4	2	**26**	--
Cadmium	16	10	**25**	24
Mercury	1	0.13	**24**	10
Barium	9	5	**20**	19
Copper	12	4	16	**16**
Nickel	9	5	**14**	12
Iron	**11**	5	--	--
Vanadium	5	5	--	**10**
Manganese	**10**	6	--	--
Antimony	9	5	--	**10**
Beryllium	**8**	2	--	--
Aluminum	**5**	**5**	--	--
Silver	**4**	2	--	--
Cobalt	1	0	**3**	1
Thallium	3	1	--	**3**
Molybdenum	1	0.27	1	**1**
Tin	0.13	0.13	--	--
Strontium	0.13	0.13	--	--
Lithium	0.13	0	--	0.13

Source: RMIS data set.
Notes: For each metal, the receptor with the greatest percentage of sites exceeding criteria is bolded.
-- - no criteria

[a] For example, lead concentrations exceeded the avian screening critierion at 59% of the sites in the RMIS data set.

Table 2e. Percentage of CERCLIS sites exceeding specific criteria, in descending order[a]

Metal	Residential	Industrial	Avian	Mammalian
Lead	36	31	**50**	31
Arsenic	**48**	33	8	38
Chromium	13	5	**29**	13
Mercury	6	1	**26**	24
Cadmium	8	2	**25**	**25**
Zinc	4	0	**24**	10
Antimony	14	5	--	**17**
Copper	7	1	**14**	**14**
Selenium	0	0	**13**	--
Manganese	**8**	0	--	--
Vanadium	1	0	--	**7**
Thallium	4	1	--	**5**
Iron	**5**	0	--	--
Barium	1	0	**5**	4
Nickel	1	0	**4**	1
Aluminum	**1**	0	--	--

Source: CERCLIS data set
Notes: For each metal, the receptor with the greatest percentage of sites exceeding criteria is bolded.
-- - no criteria
[a] For example, lead concentrations exceeded the avian screening criterion at 50% of all sites in the CERLIS data set.

Table 3. Information provided by contacts within EPA regional offices

		Primary Risk Drivers			
EPA Region	Contact	Metals	Receptors	Scenario	Pathway
Region 1	Sarah Levinson	Mn, As, Tl, Cr, Be, Cd		residential, recreational, worker	ingestion
Region 2	Mark Maddaloni	Pb, As, Hg, Cd, Be	Human	worker, resident, trespassers	ingestion
	Bob Wing	Pb, As, Hg, Cd, Be	Human, eco	workers, residents, children	ingestion
Region 3	Jennifer Hubbard	Al, As, Cr, Pb, Mn, Fe	Human	various	
Region 4	Ted Simon	As, Pb, Cr	Human and Eco (shrew)	recreational (golf courses), occupational (shooting ranges)	ingestion
Region 5	Patricia VanLeeuwen				
	Mark Johnson	Pb, As, Cr, Tl, Be	Human and Eco	future recreational, worker, trespasser	
Region 6	Michael Overbay	Pb, Cr	Human	future worker, some residential	ingestion, dermal, inhalation
		Ba	Eco		
Region 7	Scott Marquess	Pb, some Cd, Cr, but not driving risks	Human	future industrial, current industrial	ingestion
Region 8	Susan Griffin	metals not driving risks		residential (required)	ingestion, homegrown produce (concern of public)
Region 9	Dan Stralka	Pb, Be, As, Al, Cr, Cu, Cd, Hg	Human (unless estuary)		
	Michael Work	Pb	Eco (American robin)		ingestion/food chain
Region 10	Nancy Harney	Pb	Human		
California EPA	Michael Anderson	Pb	Human		ingestion
		Pb, As, Cd, Ni, Se, Cr	Eco		food chain

Table 3. (cont.)

EPA Region	Contact	Comments	Sites from this Region to Include	As	Cd	Cr	Mn	Tl	Pb	Be	Hg	Fe	Al	Ba	Cu	Ni	Se
Region 1	Sarah Levinson	Thallium artifact of analytical, all Cr considered Cr(VI)	Portsmouth Shipyard, Kittering Maine, New London Sub Base,	x	x	x	x	x		x							
Region 2	Mark Maddaloni			x	x				x	x	x						
	Bob Wing		Seneca, Ft. Dix, Plattsburgh	x	x				x	x	x						
Region 3	Jennifer Hubbard	Metals rarely drivers.		x		x	x		x			x	x				
Region 4	Ted Simon	Pb at shooting ranges, arsenic pesticides on golf courses, chromium (at plating shops)		x		x			x								
Region 5	Patricia VanLeeuwen	If firing range, testing facility, or plating shop, then metals a concern. Thallium from rodenticide along fencelines.		x	x			x	x	x							
	Mark Johnson																
Region 6	Michael Overbay		Bergstrom AFB, Austin, TX; England AFB, Alexandria LA; Kelly AFB, San Antonio, TX; Eaker AFB, Blytheville,			x			x								
														x			
Region 7	Scott Marquess	Lead from shooting ranges and lead-based initiating compounds. Cd, Cr on occasion, but not driving cleanup. Bioavailability of explosives in soil would be very interesting.	Lake City, Iowa.						x								
Region 8	Susan Griffin																
Region 9	Dan Stralka	Low PRGs make drivers. Lead at shooting ranges, operational beryllium, arsenic from rodenticide use, aluminum from fumigant, chromium from Navy paints, copper and cadmium from Navy repair facilities. Mercury at Air Force bases.		x	x	x			x	x	x		x		x		
	Michael Work	Presidio -- cleanup 2 years ago.							x								
Region 10	Nancy Harney	Metals in soil rarely risk drivers.							x								
California EPA	Michael Anderson								x								
				x	x	x			x							x	x
				As	Cd	Cr	Mn	Tl	Pb	Be	Hg	Fe	Al	Ba	Cu	Ni	Se
			Totals:	8	6	6	2	2	12	5	3	1	2	1	1	1	1

Table 4a. Matrix summarizing top five metals from each data set screened for human health receptors

Data Set	Most Common Metal	Second Most Common Metal	Third Most Common Metal	Fourth Most Common Metal	Fifth Most Common Metal
ARMY	Lead	Arsenic	Chromium	Cadmium	Copper
AIR FORCE	Arsenic	Lead	Iron	Chromium	Antimony
NAVY	Lead	Arsenic	Iron	Chromium, Antimony	Copper
RMIS	Lead	Cadmium	Chromium	Copper, Iron, Manganese	Barium, Nickel, Antimony
CERCLIS	Arsenic	Lead	Antimony	Chromium	Cadmium

Table 4b. Matrix summarizing top five metals from each data set screened for ecological receptors

Data Set	Most Common Metal	Second Most Common Metal	Third Most Common Metal	Fourth Most Common Metal	Fifth Most Common Metal
ARMY	Lead	Cadmium	Arsenic	Chromium	Zinc
AIR FORCE	Lead	Mercury	Zinc	Chromium	Barium
NAVY	Lead	Mercury	Zinc	Cadmium	Chromium
RMIS	Lead	Cadmium	Zinc	Chromium	Barium
CERCLIS	Lead	Cadmium, Mercury	Arsenic	Chromium	Zinc

Appendix A

Modifications to the DoD Data Sets

Modifications to the DoD Data Sets

Each data set acquired by Exponent was subjected to minor modifications to simplify data interpretation. These slight revisions to some of the data sets did not compromise the integrity of any dataset in any way. The exact modifications made to each data set are described below.

ROD Search

Record of Decision (ROD) abstracts were obtained from the Superfund website. All of the abstracts were examined to determine which DoD sites mentioned metals of potential concern. If metals were mentioned in the abstract, an effort was made to obtain the full text of the ROD. After obtaining a few RODs, the length of each document and lack of any easily located concentration data for metals at the DoD sites were impediments to this search. Meanwhile, it was discovered that the electronic databases that were being queried provided the type of information we needed, and therefore, the ROD search was no longer vital. Hence, the ROD search was suspended, and efforts were refocused on the database analyses.

Air Force ERPIMS Data Set

Concentration data were provided in the ERPIMS data set. We compared the residential, industrial, avian, mammalian, and soil invertebrate criteria to the metal concentrations provided, except for the elements calcium, potassium, magnesium, sodium, titanium, and zirconium. Criteria were not available for these metals, so they were omitted from the data set.

Army Data Set

The Army data set includes site names, site IDs, brief site descriptions, contact names and phone numbers, Chemical Abstracts (CAS) ID numbers, maximum concentrations, concentration units, and brief remedial action descriptions. Additional fields are provided, but their meaning and significance were not clear. Nickel, lead, chromium, arsenic, cadmium, copper, and zinc were the only metals included in the data set. No modifications were needed.

CERCLIS Data Set

Initially, the CERCLIS data set that was provided to us reported on 84 sites; however, not all sites fell under the jurisdiction of the DoD. Therefore, facilities governed by the Federal Aviation Facility, Small Business Administration, Department of Transportation, Department of Energy, and Department of the Interior were eliminated, reducing the data set to 68 DoD sites. The remaining sites were limited to the jurisdiction of the Department of Logistic Affairs, Department of Defense, National Guard, U.S. Air Force, U.S. Navy, and U.S. Army. We also

eliminated any data entries associated with subsurface soils, but this did not result in any further reduction of the number of relevant DoD sites.

The data set provided detailed information, including EPA identification codes, site names, National Priorities List (NPL) status, federal agency, media, contaminant, scenario, land use, and exposure route. Human health-related hazard indices and cancer risks were listed for some entries in the data set, but no ecological receptor information was included. However, the cancer risk data provided by CERCLIS was perplexing, because risks greater than 0.000001 were reported for non-carcinogenic metals, such as silver, mercury, iron, vanadium, aluminum, and chromium. Inquiries were made to determine whether there was a potential error in the database, but apparently no errors were made, and site managers enter the data into the CERCLIS database. Other information was included in the data set provided to us (for example, cross media, operable unit, sign date, after-cleanup cancer risk, and hazard index), but was not deemed relevant for this stage of data analysis.

In the original database, chromium was usually reported generically as “chromium.” Only occasionally was chromium(III) or chromium(VI) specified, but due to the lack of consistent terminology, all chromium-related cells in the data set were changed to “chromium.” Similarly, lead occasionally was reported as “lead, inorganic,” but these cells were changed to “lead.”

Because we believed that the cancer risk and hazard index data provided by the CERCLIS data set were questionable, we applied our own set of industrial, residential, avian, mammalian, and soil invertebrate criteria to the limited concentration data that were provided. We first converted all concentration data to units of milligrams per kilogram, and then divided each concentration by its metal-specific criterion. If the units were not specified, or if the concentrations were reported in liquid units, the records were not used.

Navy NORM Data Set

The managers of the Navy database were not able to provide us with actual site names, so site names are coded numerically. The data set initially contained 36 compounds; however, some of the compound names were repetitive, so the names were changed to streamline the data and simplify analyses. For example, arsenic was described as arsenic (cancer), arsenic (III), arsenic (non-cancer), and arsenic. All four descriptive terms were changed to ”arsenic and compounds.” Similarly, “barium” and “barium and compounds” were changed to “barium and compounds.” These changes aided in sorting the numerical data and simplifying the graphs, but did not compromise or distort the results in any manner. The only compound removed completely from the data set was the radionuclide uranium 238. For a list of other descriptive name changes that were made to compounds in the NORM data set, please see Table A-1. After making the name changes, the data set contained 22 individual compounds.

Most of the numerical concentration data reported in the original database were reported in units of ppm; however, for some data, the units were missing. We assumed that all concentrations were reported in ppm; this assumption was validated by Martha Midgette of the Navy Headquarters (pers. comm. [e-mail]).

RMIS

The RMIS data set initially contained 60 analytes; however, not all of these analytes were relevant, so some entries were deleted. The list of metals, radionuclides, and compounds that were eliminated is provided below:

Plutonium 239

Potassium cyanide

Radium 226

Thorium 228, 229, 232, 234

Uranium 238

Copper cyanide

Zinc cyanide

Cyanide of sodium

Hydrocyanic acid, potassium salt

Barium cyanide.

The other small technical change we made to the data set was to change "chromium," "chromium (total)," and "chromium VI" to simply "chromium and compounds."

Superfund Hazardous Waste Site Advanced Query Form

The Superfund Hazardous Waste Site website (located at http://www.epa.gov/superfund/) includes a Resource Center section that contains databases that can be searched by the general public. Using the advanced query option, we extracted information pertaining to: Site Names, CERCLIS ID, Site ID, City, Metal Contaminants of Concern, and Contaminated Media (we selected soil). After retrieving the data, descriptions such as "hexavalent chromium" and "chromium (III)" were both changed to "chromium." Similarly, lead (inorganic) was changed to simply "lead."

Table A-1. List of descriptive name changes applied to the NORM data set

Original Names of Compounds from Navy Database	Final Compound Names
Antimony, antimony and compounds	Antimony
Arsenic, arsenic (cancer), arsenic (non-cancer), arsenic(III)	Arsenic
Barium, barium and compounds	Barium
Beryllium, beryllium and compounds	Beryllium
Cadmium, cadmium and compounds	Cadmium
Chromium, chromium(III), chromium total, chromium(VI), and compounds	Chromium
Copper, copper and compounds	Copper
Manganese, manganese and compounds	Manganese
Mercury, mercury (inorganic), mercury and compounds (inorganic)	Mercury
Nickel (soluble salts), nickel and compounds	Nickel
Silver and compounds	Silver
Strontium, stable	Strontium
Vanadium, vanadium oxide, vanadium sulfate	Vanadium

Acronym List

Acronyms and Abbreviations

BRAC	Base Realignment and Closure
CAS	Chemical Abstracts
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act of 1980
CERCLIS	Comprehensive Environmental Response, Compensation and Liability Information System
DoD	Department of Defense
EPA	U.S. Environmental Protection Agency
ERPIMS	Environmental Restoration Program Information Management System
FFRRO	Federal Facilities Restoration and Reuse Office
NORM	Normalization of Environmental Data System
OSWER	Office of Solid Waste and Emergency Response
PRG	Preliminary Remediation Goal
RMIS	Restoration Management Information System
ROD	Records of Decision
SERDP	Strategic Environmental Research and Development Program
TIO	Technology Innovation Office
VOC	volatile organic compound
SVOC	semivolatile organic compound

Human and Ecological Risk Assessment, 10: 983–997, 2004
Copyright © ASP
ISSN: 1080-7039 print / 1549-7680 online
DOI: 10.1080/10807030490887113

Metals that Drive Health-Based Remedial Decisions for Soils at U.S. Department of Defense Sites

Johanna H. Salatas,[1] Yvette W. Lowney,[2] Robert A. Pastorok,[1] Richard R. Nelson,[2] and Michael V. Ruby[2]
[1]Exponent, Bellevue, Washington, USA; [2]Exponent, Boulder, Colorado, USA

ABSTRACT

This study was undertaken to establish which metals are most likely to drive the risk-based remedial decision-making process at those U.S. Department of Defense (DoD) sites that are affected by metals in site soils. Our approach combined queries of various databases, interviews with U.S. Environmental Protection Agency (USEPA) experts in each Region, and communication with database administrators and DoD personnel. The databases that were used were comprehensive for DoD sites, yet sometimes contained inaccuracies. Metal concentration data for various DoD facilities were screened against established regulatory criteria for both human health and ecological endpoints. Results from this analysis were compared against the information gleaned from the interviews. This preliminary analysis indicates that the five metals that most frequently exceeded risk-based screening criteria for potential human health concerns at DoD sites, in descending order of frequency, are lead, arsenic, cadmium, chromium, and antimony. The metals that most frequently exceeded ecological screening criteria, in order, are lead, cadmium, mercury, zinc, arsenic, chromium, and selenium. Although the majority of USEPA personnel interviewed indicated that human health risk, rather than ecological endpoints, generally drives remedial decision-making, the data indicated that ecological screening thresholds were exceeded more often than human health standards.

Key Words: metals in soil, Department of Defense, human health risk, ecological risk, remedial decision-making.

INTRODUCTION

The U.S. Department of Defense (DoD) has undertaken the task of cleaning up wastes that have resulted from industrial, commercial, training, and weapons testing activities, as well as cleaning up and closing military bases so that properties can be transferred to local communities for economic revitalization (USEPA 1997a). It is estimated that DoD is responsible for remediation of approximately 8000 sites in

Received 17 September 2003; revised manuscript accepted 14 May 2004.
Address correspondence to Michael V. Ruby, Exponent, 4940 Pearl East Circle, Suite 300, Boulder, Colorado 80301. E-mail: rubym@exponent.com

the United States, a majority of which (67%) contain metal contamination in soils (USEPA 1997a). Among the challenges in this effort is the process of prioritizing sites for clean up, and determining what needs to be cleaned up and to what extent.

For properties on which soils are contaminated with metals, the amount of the metal in soil that could actually be absorbed by human or ecological receptors (*i.e.*, the bioavailability of the metal) can be an important factor in determining the degree to which the contaminated soils need to be remediated. This occurs because the bioavailability of metals from soil is generally less than that assumed by the default values used in human health and ecological risk assessment.

Frequently, the factors that determine bioavailability are highly site specific. Thus, to guide research on bioavailability of metals from soil, the research reported herein was undertaken to determine which metals potentially drive risk-based remedial decisions for soils at DoD sites. The research was structured to answer the following three questions:

1. What metals potentially drive risk-based remedial decisions at DoD facilities?
2. For facilities where more than one metal exceeds risk-based screening criteria, what are the metals of concern, and how do they compare in perceived importance?
3. For the metals that most often exceed the screening criteria, what is the receptor of greatest concern (human or ecological)?

To accomplish this, information was solicited from:

- Various branches of the military (Army, Navy, Air Force)
- U.S. Environmental Protection Agency (USEPA) regional toxicologists
- Coordinators within the Federal Facilities Restoration and Reuse Office (FFRRO)
- Comprehensive Environmental Response, Compensation and Liability Information System (CERCLIS)
- USEPA Records of Decision (RODs)
- DoD Environmental Cleanup Office.

This article describes the avenues that were pursued to locate useful information, presents the data obtained, describes the manner in which these data were assessed, and discusses the conclusions that can be drawn regarding the metals and exposure pathways that are important determinants for remediation of metals in soils at DoD facilities.

METHODS

Various individuals within the DoD and USEPA were contacted to identify sources of information on metal concentrations at DoD sites, their potential for health effects on human and ecological receptors, and their influence on remedial decisions for soil. Our goal in contacting these individuals was to identify and gain access to databases that would provide answers to the three questions posed in the Introduction. Overall, we found that no single database exists that contains the entirety of the information we sought. Therefore, we extracted information from several sources,

and augmented the data with subjective opinions of professionals involved in the assessment and remediation of federal facilities. Selection of individuals who provided opinions and information is described in the section titled "USEPA Interviews."

Databases

Ultimately, we identified five databases that could be queried to provide information relevant to our task. Three were military databases: the Environmental Restoration Program Information Management System (ERPIMS) database from the Air Force, an unnamed database containing metals data from Army sites, and the Normalization of Environmental Data System (NORM) database from the Navy. We also analyzed the data contained in the Restoration Management Information System (RMIS) maintained by the Environmental Cleanup Office of the DoD, and the CERCLIS database maintained by the USEPA. In addition, the Superfund Hazardous Waste Site website (available at http://www.epa.gov/superfund/) includes a Resource Center in which databases can be searched by the general public. Using the advanced query option, we extracted information pertaining to Site Names, CERCLIS ID, Site ID, City, Metal Contaminants of Concern, and Contaminated Media (we selected soil); however, no concentration data were available on this website.

At the outset of our effort to collect data, we also attempted to obtain information from the database on Records of Decision (RODs INFO) maintained by the USEPA. The RODs INFO database provides a compilation of the information that is part of Records of Decision for sites that have been addressed under the Comprehensive Environmental Response, Compensation and Liability Act of 1980 (CERCLA, generally referred to as "Superfund"). While this information may have proved quite useful, extracting appropriate data from this database was extremely cumbersome. In addition, the database includes information only for sites where RODs have been issued, thus excluding sites where data exist but a remedial approach has not been selected, and a ROD has therefore not been issued. Because of these obstacles, the RODs INFO search was aborted, and subsequent efforts focused on the other available databases.

Ultimately, we received from each of the databases a download of information, hereafter referred to as "data sets," regarding the soil concentration data for sites that are known to have metals in soil. These concentration data were then compared to a consistent set of risk-based screening criteria (described later) to determine which metals most frequently exceeded these criteria.

It is important to note that we generally relied on the information in the data sets as supplied by the various sources. Aside from very minor modifications required to streamline the screening (*e.g.*, standardization of units and spelling of metal names), few changes were made to the data sets, and the integrity of each data set was not compromised. It was assumed that the information provided in the data sets was technically accurate, and no outside verification of the data was performed. However, we did identify what appeared to be errors within several of the databases. It was beyond the scope of this evaluation to verify and/or correct the data included in the various databases; however, specifics regarding the flaws identified in the databases, and their implications, are discussed further in the Conclusions.

USEPA Interviews

In addition to the objective information provided by the data sets, we queried individuals who are involved with the risk assessment of federal facilities regarding their opinions on the questions posed in the Introduction. Specifically, within each of the 10 USEPA regions, we attempted to contact a Regional Toxicologist and the Regional Contact in the FFRRO. We were not able to interview both of these persons in every region, but we persisted until we had made contact with at least one individual in each region. These individuals were asked the questions listed in the Introduction, and their responses are discussed later.

Screening Criteria

As described earlier, the Army, NORM, ERPIMS, RMIS, and CERCLIS databases were queried by their database managers, and query results from each database were provided to the authors. The data sets included soil metal concentrations for sites where metals had been detected. The concentrations in each data set were then compared to health-based screening criteria to determine which sites contained metals in soil at concentrations that exceed screening criteria and might, therefore, suggest a further need for investigative consideration in health risk assessments.

Screening criteria are used during Step 2 of the Superfund Ecological Risk Assessment process, the screening-level risk calculation (USEPA 1997b, 1998, 1999). These criteria are intentionally conservative and are tools used to facilitate prompt identification of contaminants and exposure areas of concern during both remedial actions and some removal actions under CERCLA (USEPA 1996). The screening values are risk-based (*i.e.*, derived from toxicity information and assumptions regarding potential exposure levels) and are used to determine whether additional study is warranted, but do not necessarily eliminate the need to conduct site-specific risk assessments. If environmental concentrations of chemicals are below the screening criteria, then it is reasonable to assume that the chemicals present no significant potential for adverse health effects. Exceeding the screening levels suggests the potential need for further evaluation. Additionally, these levels can be used as Preliminary Remediation Goals (PRGs), provided appropriate conditions are met (USEPA 1999). Therefore, given the importance of health-based screening levels in the decision-making process, these criteria were compared to the soil concentrations provided in the data sets to draw preliminary conclusions regarding which metals may warrant additional study and potential remediation at DoD sites.

To conduct this screening, we compiled health-based screening concentrations for several endpoints. Because we were interested in determining what metals require further risk investigation for both human and wildlife receptors, we screened the site concentration data against criteria based on human health (for both industrial and residential exposure scenarios) and ecological receptors (mammalian and avian receptors).

Human Health Criteria

Human health criteria were obtained from USEPA's Soil Screening Guidance (USEPA 2001), which is a tool developed by USEPA to help standardize and

accelerate the evaluation and cleanup of contaminated soils at sites on the National Priorities List (NPL) for which future land use may be residential. Criteria adopted from the generic Soil Screening Levels (SSLs) that were developed for combined ingestion and dermal exposure in a residential scenario were used to assess human receptors in a residential setting (USEPA 2001). Screening criteria that would be protective under industrial land use were selected from the generic SSLs for an outdoor worker scenario (USEPA 2001).

All values were used as reported by USEPA, with the exception of arsenic. For arsenic, the health-based screening criteria reported by USEPA are 0.4 mg/kg and 2 mg/kg for the residential and industrial land use scenario, respectively. Although these values are consistent with the specified method for setting screening levels equal to a cancer risk of one in a million, these specific values fall below background concentrations for arsenic in soil throughout much of the United States (Dragun and Chiasson 1991). Regulatory agencies have acknowledged this complicating issue with the standard screening values for arsenic, and they recommend use of alternative risk-based screening values for arsenic that fall within USEPA's "acceptable cancer risk range of 10E-6 to 10E-4" (USEPA 2000a; Washington State Dept. of Ecology 2001). Therefore, both the residential and industrial criteria for arsenic listed by USEPA were multiplied by a factor of 10, effectively raising the target cancer risk associated with each to one in one hundred thousand, the middle of the acceptable risk range specified by USEPA. This was done to ensure that the importance of arsenic in risk-based decisions was not artificially elevated due to the natural background concentrations for this metal.

If the USEPA guidance did not contain values for the metals that were measured at DoD sites, health-based screening values from USEPA Region IX Preliminary Remediation Goals (PRGs) were incorporated as surrogates (USEPA 2000a). These values were selected as surrogates because of their common use by regulatory agencies outside of Region IX, and because their derivation is similar to the SSLs and incorporates several routes of potential exposure, thereby resulting in more health-protective screening values. Table 1 lists the human health-based screening criteria, and denotes whether the values were selected from the list of SSLs (USEPA 2001) or PRGs (USEPA 2000a).

Ecological Criteria

Specific screening values for avian and mammalian receptors were selected for each metal. Ecological Soil Screening Levels (EcoSSLs; USEPA 2000b) were used, if they were available. If EcoSSLs for avian and mammalian receptors were not available for a particular metal, we used the Preliminary Remediation Goals for Ecological Endpoints (Efroymson *et al.* 1997)—specifically, American woodcock goals—as a surrogate for avian screening values, and short-tail shrew goals as a surrogate for mammalian screening values. Table 1 lists the specific values and the source for each of the ecological screening criteria that were used in this evaluation.

Although it is beyond the scope of this report to review the technical basis and merits of the screening value for each metal, it is important to mention that the screening concentration for mercury is highly conservative for use in most contexts. This is because the current screening value (from Efroymson *et al.* 1997) is based on

Table 1. Human health and ecological screening criteria.

	Human health criteria		Ecological receptor criteria	
Metal	Residential	Industrial	Mammalian	Avian
Arsenic	4[a,b]	20[a,b]	9.9[c]	102[c]
Lead	400[b]	750[b]	740[c]	40.5[c]
Cadmium	70[b]	900[b]	6[c]	4.2[c]
Copper	2900[d]	76000[d]	370[c]	515[c]
Chromium	230[b]	3,400[b]	360[e]	21[e]
Nickel	1,600[b]	23,000[b]	246[c]	121[c]
Zinc	23,000[b]	340,000[b]	1,600[c]	8.5[c]
Mercury	23[b]	340[b]	0.146[c]	0.00051[c]
Aluminum	76,000[d]	100,000[d]	—	—
Antimony	31[b]	450[b]	21[e]	—
Barium	5,500[b]	79,000[b]	329[c]	283[c]
Beryllium	160[b]	2,300[b]	—	—
Boron	5,500[d]	79,000[d]	—	—
Cobalt	4,700[d]	100,000[d]	340[e]	32[e]
Iron	23,000[d]	100,000[d]	—	—
Lithium	1,600[d]	41,000[d]	390[c]	—
Manganese	1,800[d]	32,000[d]	—	—
Molybdenum	390[d]	10,000[d]	4.75[c]	44[c]
Selenium	390[b]	5,700[b]	—	0.000001[c]
Silver	390[b]	5,700[b]	—	—
Strontium	47,000[d]	100,000[d]	—	—
Thallium	6[b]	91[b]	2.1[c]	—
Tin	47,000[d]	100,000[d]	—	—
Vanadium	550[b]	7,900[b]	55[c]	—
Zinc phosphide	23[d]	610[d]	—	—

Chromium(VI) values were used to screen chromium. However, because Cr(VI) criterion was not available for avian receptors, we used Cr(III) criterion instead.
—, data not available.
[a]Increased original value by one order of magnitude; [b]USEPA (2001); [c]Efroymson *et al.* (1997); [d]Region IX PRG (USEPA 2000a); [e]U.S. EPA (2000b).

the assumption that 100% of the mercury present in soils exists as methyl mercury. In aerobic soil environments, which are the soils of interest for evaluating ingestion by wildlife, mercury exists almost entirely in the inorganic form, which is substantially less toxic than the organic form. Therefore, the SSL based on organic mercury is highly conservative for most sites.

DATA ANALYSIS AND RESULTS

Each specific concentration within each of the five data sets was compared to human and ecological screening criteria, and the ratios of the concentrations to the criteria were calculated. If the calculated ratio was less than or equal to unity (*i.e.*, ≤ 1), then it was assumed that the concentration did not present a potential human or ecological health hazard. If the ratio of a concentration to the screening criterion

did exceed unity (*i.e.*, value >1), then this was assumed to indicate the potential for affecting risk-based remedial decisions at the site. Fully characterizing the potential risk of adverse effects would require further evaluation on a site-specific basis, but this was beyond the scope of the present project. Therefore, this screening-level approach served as the backbone of the data-set queries that we conducted.

The first set of analyses was aimed at determining, for all data sets combined, which metals most frequently exceeded the health-based screening criteria. For human health screening, the metal concentrations were compared to residential and industrial criteria (Table 1), and for the ecological screening, the metal concentrations were compared to mammalian and avian criteria (Table 1). If data exceeded criteria more than once for a particular site, the site was counted only once. The results denoting the percentage of sites that exceeded human health criteria for each metal are presented in Figure 1, and those that exceeded ecological criteria are displayed in Figure 2.

In the second set of analyses, the five sites presenting the highest potential concern (*i.e.*, the highest ratio of site average metal concentrations to screening criteria when averaged across all metals for each site) were selected from all data sets combined. For those five sites with the highest overall ratio of screening level to site soil concentrations, we determined what metals were present at concentrations above screening values. The goal of this effort was to determine the relative contribution from metals in soil at facilities where more than one metal exceeds screening criteria. This analysis was conducted separately for each potential receptor (human—residential [Figure 3], human—industrial [Figure 4], mammalian [Figure 5], and avian [Figure 6]).

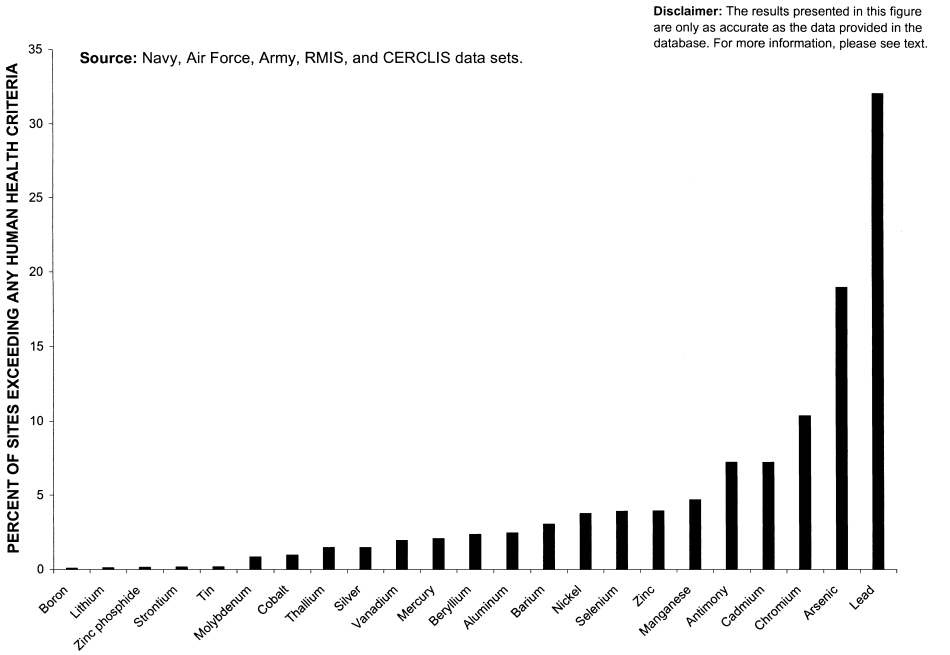


Figure 1. Percent of metal-contaminated sites that exceed any human health criteria (industrial or residential) at least once for all data sets combined.

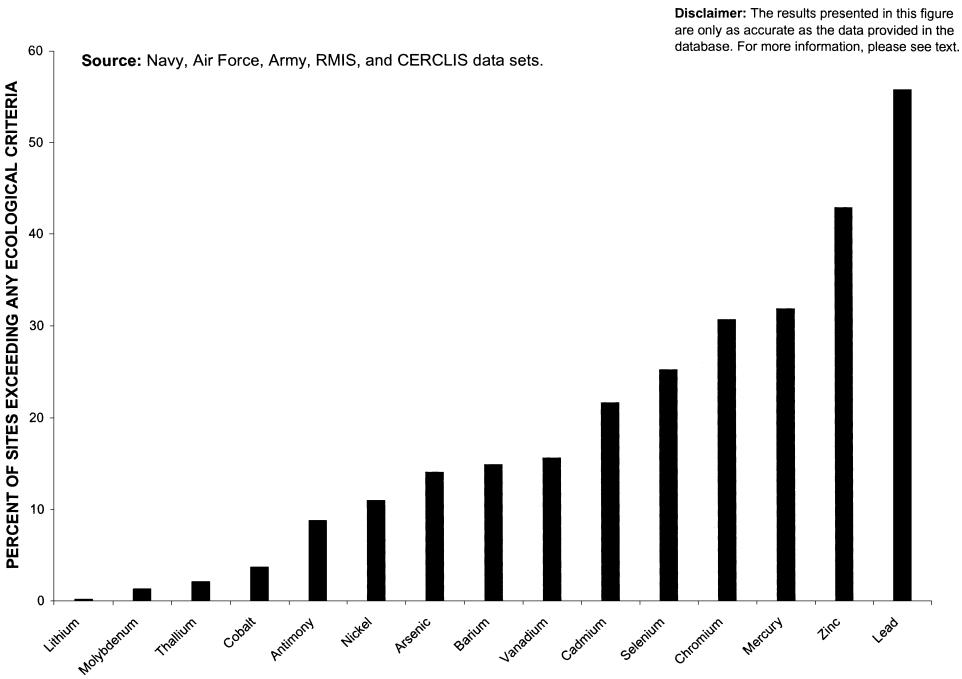


Figure 2. Percent of metal-contaminated sites that exceed any ecological criteria (avian or mammalian) at least once for all data sets combined.

The final analysis was designed to determine what receptor (human—residential, human—industrial, ecological—mammalian, or ecological—avian) is of primary importance for the metals associated with the highest exceedance of screening criteria, across all DoD sites evaluated. To accomplish this, we constructed a table (Table 2) of the metals that exceeded criteria in all data sets combined and indicated the percentage of sites at which specific criteria were exceeded. For each metal, boldface values show the specific receptor for which the highest percentage of sites exceeded the screening criterion.

Interviews With USEPA Staff

Professional staff within each USEPA region were queried regarding their knowledge or impressions of which metals are driving risk-based remedial decisions at DoD sites. The individuals contacted were either regional toxicologists or the Regional Contact for the FFRRO. One individual with the California EPA was also included in the interviews. Five specific questions were posed to each contact:

- Which DoD facilities present risks from potential exposures to metals in soils?
- Which specific metals are of concern?
- Which receptors (human or ecological) are of concern for metals in soils?
- Which human and ecological exposure pathways are potentially of concern for metals in soils?

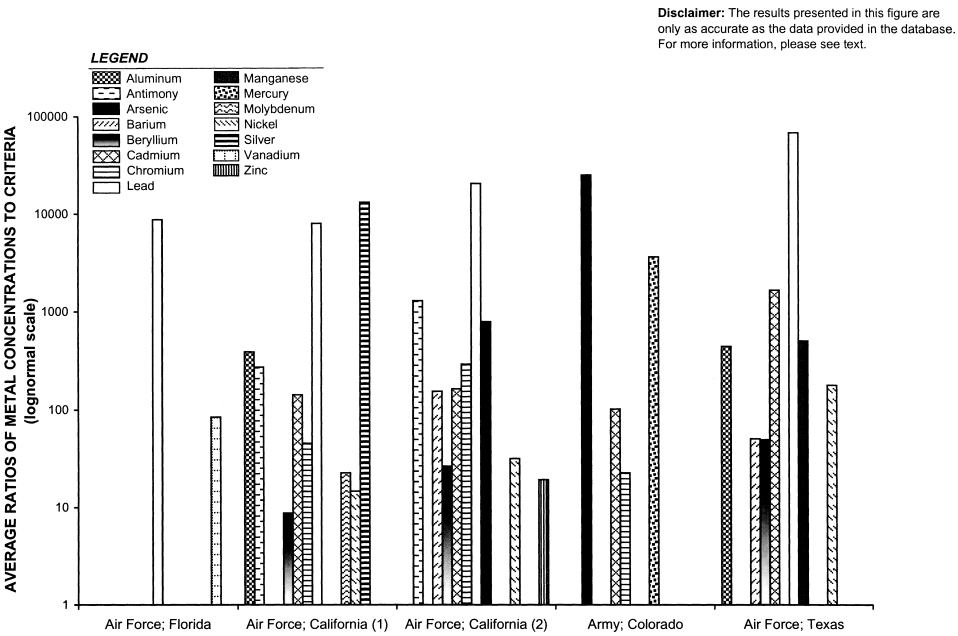


Figure 3. Average ratios of metal concentrations to residential screening criteria for the five sites with the highest screening criteria exceedances across all metals.

- Which human exposure scenarios (*e.g.*, workers, residents, trespassers) are potentially of concern for exposure to metals in soils?

The information provided by these individuals was generally anecdotal. None of the USEPA personnel indicated that they had compiled information from the DoD sites within the region. For some regions (*e.g.*, Region VIII), it appears that metals are not driving risks at the DoD facilities, but rather, organic compounds are the primary concern. In nearly all instances, the interviewees indicated that human receptors were driving remedial decisions, and that ingestion of soils was the exposure pathway of concern. Only occasionally were ecological receptors or other exposure pathways mentioned.

Because of the requirement to evaluate human exposures under the scenario of potential future residential development, residential receptors were the primary receptors of concern, but interviewees indicated that worker, trespasser, and recreational exposure scenarios could also be risk drivers. In general, the metals of concern coincide with the historical land use of the site. For example, lead is of concern for former firing ranges, arsenic appears to be a problem from historical use of pesticides, and chromium occurs near former plating shops. Several individuals suggested that frequent concern about chromium may be an artifact of the screening process, which incorporates the assumption that all chromium occurs in the more toxic hexavalent form, rather than the comparatively benign, but much more environmentally common, trivalent form.

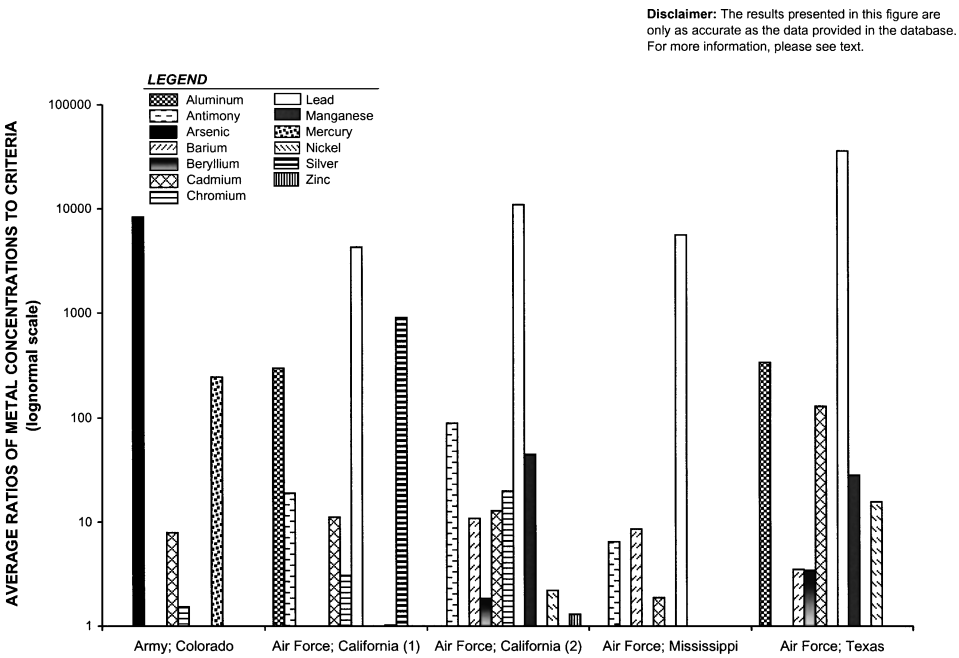


Figure 4. Average ratios of metal concentrations to industrial screening criteria for the five sites with the highest screening criteria exceedances across all metals.

Compilation of the interview results indicates that, overall, lead and arsenic are the metals that most frequently present health threats at DoD facilities. Cadmium and chromium follow next, and then beryllium. No other metals were mentioned consistently during the interviews.

DISCUSSION

What Metals Potentially Drive Risk-Based Remedial Cleanup Decisions at DoD Facilities?

Results presented in Figure 1 suggest that, for human receptors, lead, arsenic, chromium, cadmium, and antimony most commonly exceed residential and industrial human health screening criteria. Figure 2 results suggest that lead, zinc, mercury, chromium, selenium, and cadmium most commonly exceed avian and mammalian ecological screening criteria.

For DoD Facilities Where More Than One Metal Exceeds Screening Criteria, What are the Metals of Concern and How Do They Compare in Perceived Importance?

The answer to this question is depicted for five DoD sites that consistently exceed screening criteria in Figures 3 through 6. These graphs indicate that at the five sites with the highest overall screening criteria exceedances, none of the metals

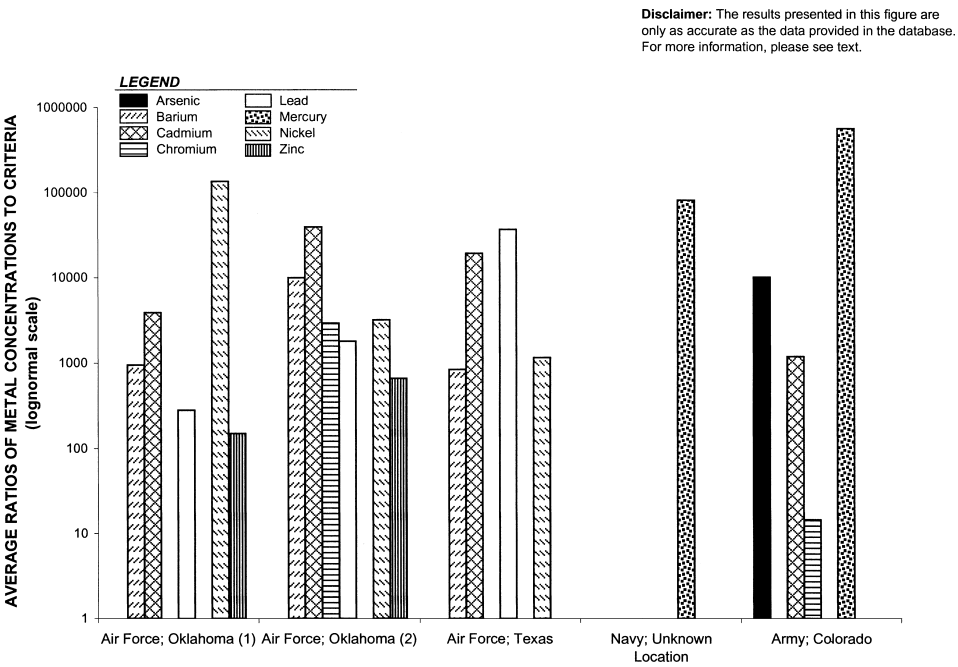


Figure 5. Average ratios of metal concentrations to mammalian screening criteria for the five sites with the highest screening criteria exceedances across all metals.

consistently show an ordered pattern in terms of driving exceedances, with the exception of lead. Lead consistently appears as a metal that exceeds screening criteria in both residential and industrial human receptors (Figures 3 and 4).

For mammalian receptors, none of the metals display an ordered pattern of importance when compared to mammalian screening criteria (Figure 5). However, selenium consistently appeared as the metal that exceeded screening criteria when avian receptors are the focus of screening assessments (Figure 6).

For the Metals with the Highest Exceedance of Screening Criteria, What is the Receptor of Greatest Concern (Human or Ecological)?

Based on the information provided in Table 2, it is evident that screening criteria for ecological receptors (mammalian and avian) were exceeded at more sites than those for human receptors (residential and industrial). This can be seen by scanning the rows for boldface numbers in Table 2, which indicate the receptor that exceeded criteria for the greatest percentage of sites.

These results could be interpreted to indicate that ecological receptors are at greater risk from metals present in soil at DoD sites than are humans, but these results more likely reflect the conservative nature and uncertainty associated with the ecological screening criteria. For example, screening levels for wildlife are typically developed for relatively small species with higher metabolic rates, smaller home ranges, and a clear direct or indirect exposure pathway link to soil. Therefore,

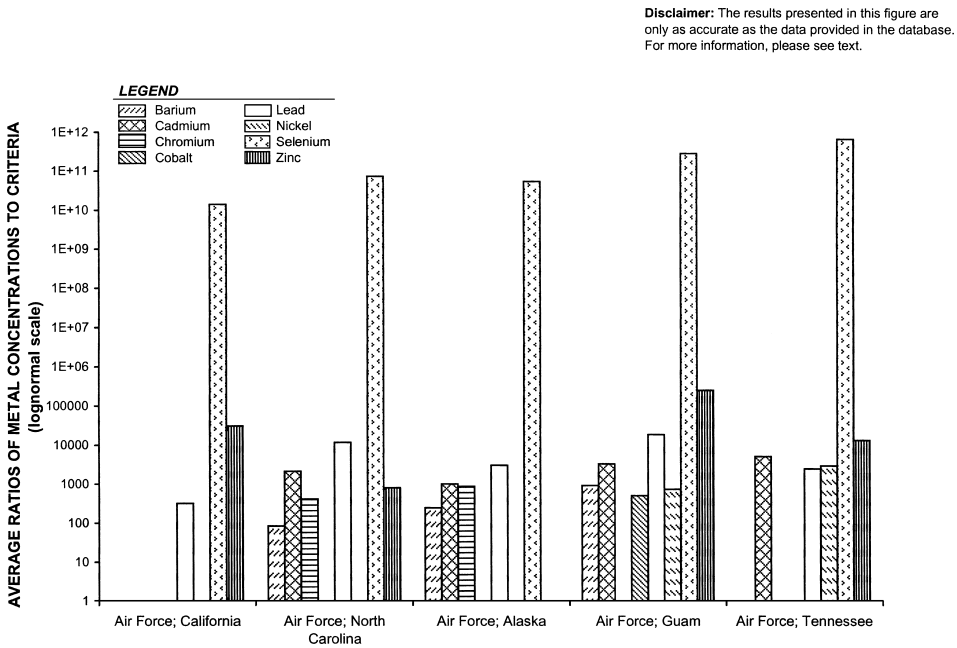


Figure 6. Average ratios of metal concentrations to avian screening criteria for the five sites with the highest screening criteria exceedances across all metals.

exposure could be assumed to be high, providing conservative screening values for various trophic groups (USEPA 2000b). Additionally, soil-screening levels are developed to be protective of rare, endangered, and threatened species that may not be present in the vicinity of a particular site. Also, uncertainty plays a significant role in setting the screening criteria. For ecological receptors, the available database for many metals regarding the toxicity or exposure levels is quite limited. In the face of such uncertainty, conservative (*i.e.*, health-protective) assumptions are incorporated into the calculations, thereby forcing the screening values lower.

Uncertainties Associated with the Data Sets

As discussed earlier, the screening conducted under this effort relied on data supplied by various sources. Global verification of the values reported in each data set was beyond the scope of the study. However, during the screening of the various data sets, we concluded that the databases that were queried to provide us with the relevant information, although comprehensive for DoD sites, are not completely accurate.

For example, the RMIS and Navy data sets occasionally reported impossible metal concentrations in soil media. This took the form of reporting concentrations greater than one million parts per million. After examining the entire RMIS data set that was provided to us, we found that approximately 2% of all the data entries exceed one million parts per million, and that this error occurs for 14 separate metals. These errors may be due to incorrect data entry or incorrect reporting of units. In

Table 2. Percentage of sites exceeding specific criteria.[a]

	Residential	Industrial	Mammalian	Avian
Lead	32.0	25.9	25.9	**55.6**
Zinc	3.8	1.0	10.4	**42.8**
Mercury	1.9	0.27	22.1	**30.4**
Chromium	10.3	3.3	7.9	**30.5**
Cadmium	7.2	3.1	19.1	**21.5**
Arsenic	**14.1**	8.9	10.4	2.2
Copper	7.4	1.3	**13.6**	12.3
Barium	2.9	1.4	12.8	**14.2**
Nickel	3.6	1.4	7.6	**10.9**
Vanadium	1.8	1.3	**14.9**	—
Iron	**14.5**	2.8	—	—
Antimony	6.9	2.1	**8.3**	—
Selenium	1.0	0.44	—	**7.2**
Manganese	**4.4**	1.5	—	—
Aluminum	**2.3**	1.8	—	—
Thallium	1.4	0.20	**1.9**	—
Beryllium	**2.1**	0.55	—	—
Molybdenum	0.20	0.068	**1.2**	0.41
Silver	**1.3**	0.48	—	—
Cobalt	0.24	0	0.38	**1.0**
Lithium	0.068	0	**0.10**	—
Zinc Phosphide	**0.068**	0.034	—	—
Strontium	0.034	0.034	—	—
Tin	0.034	0.034	—	—
Boron	0.034	0	—	—

Source: All data sets. *Note:* — no criteria.
[a]For example, lead concentrations exceeded residential screening crtierion at 32% of the sites. For each metal, the receptor with the greatest percentage of sites exceeding criteria is bolded.

evaluating this data set, caution was used to ensure that these incorrect data did not unduly influence the study results.

CONCLUSIONS

According to USEPA's analysis of the RMIS database (USEPA 1997a), lead was the most frequent soil contaminant associated with DoD sites that exceeded screening criteria. Following lead were nickel, zinc, barium, cadmium, copper, and beryllium. In our analysis of the various databases, the metals that most frequently were associated with exceeding human health screening criteria or remedial action criteria at DoD facilities were lead, arsenic, chromium, cadmium, and antimony (Figure 1). Similar results were obtained from the USEPA staff interviews, which indicated an order of lead, arsenic, chromium, cadmium, and beryllium for the top five metals of concern for human health. The metals at DoD facilities that most frequently exceeded ecologically based screening criteria were lead, zinc, mercury, chromium, and selenium for birds, and arsenic for mammals (Figure 2).

In evaluating these results, it is important to keep in mind that our analysis relied on data only from sites with metals detected in soils. We did not assess the percentage of sites where other compounds are considered potential contaminants of concern. Several of those contacted mentioned that volatile or semi-volatile organic compounds (VOCs or SVOCs) or radioactive components are more important at DoD sites than are metals in soils. However, USEPA indicated that for DoD sites that need cleanup, and that have identified soil contamination, the majority (>70%) are contaminated with metals (USEPA 1997a).

As would be expected, different metals are associated with different site operations. For example, as stated earlier, lead contamination occurs at former firing ranges, arsenic in areas of historical pesticide use, and chromium at locations of former or current plating shops. This association results in significant heterogeneity regarding what metals are of concern, and suggests that contamination by some metals may be relatively localized (*e.g.*, chromium), whereas others may be dispersed (*e.g.*, arsenic). These interviews also indicated that human health considerations usually drive remedial actions for metals in soils, and that ecological receptors typically become an issue only if wetlands and sediments are part of the assessment. This information provided to us from interviews contrasted with our screening of data against different criteria, which indicated that exceedances of screening criteria for ecological receptors occur more frequently than exceedances of human health criteria. Similarly, at sites where more complete risk assessments have been conducted, ecological receptors (*e.g.*, American robin or burrowing animals) can drive risk for metals in soils. The focus on human health considerations may simply reflect the interest or technical background of the individuals interviewed (*e.g.*, more interviewees were human health toxicologists, as opposed to ecologists or ecotoxicologists), or the prioritization of human over ecological health as a general societal trend.

According to USEPA staff interviews, ingestion exposures typically are of greatest concern, whereas dermal exposure is the second most important pathway, followed by inhalation. Dermal absorption was considered an issue only for arsenic and cadmium in soils. However, USEPA staff did report that dermal exposures would be more important if point-of-contact symptoms (*e.g.*, rashes) were "taken more seriously" in the risk assessment process.

The primary goals of this research were to identify and prioritize metals for bioavailability research, and to identify which metals were most relevant to human and ecological receptors. Combined evaluation of the results from the data set screening and the USEPA interviews indicated that bioavailability studies for human receptors should be focused on lead, arsenic, cadmium, chromium, and antimony. A similar evaluation for ecological receptors indicated that bioavailability research should focus on lead, cadmium, mercury, zinc, chromium, arsenic (for mammals), and selenium (for birds).

ACKNOWLEDGMENTS

The Strategic Environmental Research and Development Program (SERDP) is gratefully acknowledged for funding this research. We also thank the contacts at USEPA for providing information on risk assessment activities at DoD sites, the

database managers at the various DoD branches for sharing their databases, and the anonymous reviewers for providing helpful comments.

REFERENCES

Dragun J and Chiasson A. 1991. Elements in North American Soils. Hazardous Materials Control Resources Institute, Greenbelt, MD, USA

Efroymson RA, Suter GW II, Sample BE, *et al.* 1997. Preliminary Remediation Goals for Ecological Endpoints, August 1997. ES/ER/TM-162/R2. U.S. Department of Energy, Office of Environmental Management, Oak Ridge National Laboratory, Oak Ridge, TN, USA

USEPA (US Environmental Protection Agency). 1996. Soil Screening Guidance: User's Guide. EPA/540/R-96/018. Publication 9355.4-23. Office of Solid Waste and Emergency Response, Washington, DC, USA

USEPA (US Environmental Protection Agency). 1997a. Cleaning up the Nation's Waste Sites: Markets and Technology Trends, 1996 Edition. PB96-178041. Office of Solid Waste and Emergency Response, Washington, DC, USA

USEPA (US Environmental Protection Agency). 1997b. Ecological Risk Assessment Guidance for Superfund: Process for Designing and Conducting Ecological Risk Assessments. Interim Final. June 5

USEPA (US Environmental Protection Agency). 1998. Guidelines for Ecological Risk Assessment. Risk Assessment Forum. U.S. Environmental Protection Agency, Washington, DC, USA. EPA/630/R-95-002F. May 14, 1998 Federal Register 63(93):26846–924

USEPA (US Environmental Protection Agency). 1999. Ecological Risk Assessment and Risk Management Principles for Superfund Sites. OSWER Directive 9285.7-28.P. Office of Emergency and Remedial Response, Washington, DC, USA

USEPA (US Environmental Protection Agency). 2000a. Region IX Preliminary Remediation Goals. Available at http://www.epa.gov/Region9/waste/sfund/prg/index.htm, updated November 22, 2000

USEPA (US Environmental Protection Agency). 2000b. Ecological Soil Screening Level Guidance, Draft. July 10, 2000. Office of Solid Waste and Emergency Response, Washington, DC, USA

USEPA (US Environmental Protection Agency). 2001. Supplemental Guidance for Developing Soil Screening Levels for Superfund Sites, Peer Review Draft. OSWER 9355.4-24. Office of Solid Waste and Emergency Response, Washington, DC, USA

Washington State Department of Ecology. 2001. Model Toxics Control Act Cleanup Regulation. Available at http://www.ecy.wa.gov/biblio/9406.html (searched April, 2004)

Supplemental Materials for Section 4

PROTOCOL TITLE: **Oral Bioavailability of Arsenic from Soil in Cynomolgus Monkeys**

PRINCIPAL INVESTIGATOR:

(Dr. Stephen M. Roberts)

SCIENTIFIC REVIEW:

(Michael V. Ruby)

ATTENDING/CONSULTING VETERINARIAN:

(Dr. August Battles)

STATISTICAL REVIEW: A person knowledgeable in statistics has reviewed the experimental design.

PROTOCOL TITLE: Oral Bioavailability of Arsenic from Soil in Cynomolgus Monkeys

PRINCIPAL INVESTIGATOR: Dr. Stephen M. Roberts

I. NON-TECHNICAL SYNOPSIS: The purpose of this study is to evaluate the oral bioavailability of arsenic in soils following a single oral administration to male Cynomolgus monkeys. The bioavailability of arsenic from test soils will be determined relative to orally administered sodium arsenate dissolved in water. This study builds upon previous research (see Roberts et al. 2002) that used Cebus monkeys to evaluate the oral bioavailability of arsenic from five Florida soils, and observed a range of 11 to 25% arsenic bioavailability. Seven adult male Cynomolgus monkeys will be available for the study, with five monkeys making up each dose group. Each monkey assigned to a specific trial will receive, on separate occasions, doses of test soils and sodium arsenate in water. Prior to dosing with soils, a minimum of three distinct dose groups of sodium arsenate dissolved in water (reference material) will be studied in the monkey model. Subsequently, representative samples of the test soils (<250-μm size fraction) will be dosed by gavage to the Cynomolgus monkeys. Urine samples will be collected prior to dosing (i.e., background) and for four days after dosing, and the samples will be analyzed for total arsenic concentration by inductively coupled plasma/mass spectrometry (ICP/MS) at Battelle Pacific Northwest Labs. Urinary arsenic concentrations for each dosed monkey will be corrected for background arsenic concentration in that animal. The urinary excretion fraction (UEF) for arsenic in soil-dosed animals will then be calculated as the mass of arsenic (over background) in urine divided by the mass administered, and the UEF for sodium arsenate–dosed animals will be calculated in a similar manner. Relative arsenic bioavailability will be calculated from the UEF for soil-dosed animals relative to the UEF for sodium arsenate–dosed animals.

II. BACKGROUND:

Background: Metals, such as arsenic, occur in soil as a complex mixture of solid-phase compounds of varying particle size and morphology. These compounds include discrete mineral phases, coprecipitated and sorbed species associated with soil minerals, and dissolved species that may be complexed by a variety of organic and inorganic ligands. The occurrence and relative distribution of an element among these various phases, and the physical relation between the phases and the soil, control an element's solubility and, hence, its bioavailability. To date, there have been a number of arsenic bioavailability studies from soil; these have been conducted in rabbits (Freeman et al. 1993), juvenile swine (Casteel et al. 1997), and non-human primates (Freeman et al. 1995; Roberts et al. 2002). However, a formal evaluation of the arsenic forms in soil that control arsenic bioavailability has not been conducted. The *in vivo* studies to be conducted under this protocol will provide estimates of oral arsenic bioavailability from six soils of varying composition. These data will be used to assess the factors that may be controlling arsenic bioavailability, and to begin to develop a simple *in vitro* extraction test that will be predictive of this endpoint.

B. Literature Search:

1. Literature Source(s) Searched: Biomedical Research Database (BRD), Federal Research in Progress (FEDRIP), Medline, and Toxline.

2. Date and Number of Search: On January 6, 2003, the above databases were searched for 1963–present.

3. Key Words of Search: Key words used in the searches included arsenic, animal use, oral administration, bioavailability, and animal alternatives.

4. Results of Search: A number of previous studies of oral arsenic bioavailability from soil were identified. These included studies conducted in rabbits (Griffin and Turck 1991; Freeman et al. 1993), dogs (Groen et al. 1994), juvenile swine (U.S. EPA 1996; Casteel et al. 1997), microswine (Battelle 1996), and non-human primates (Freeman et al. 1995; Roberts et al. 2002). In aggregate, these studies demonstrate that relative arsenic bioavailability from soil is site specific and can be highly variable (a range of <1%–50% has been observed). Most of these studies were conducted on soils from mining and smelting sites, which may not be relevant to DoD sites. In addition, these studies represent a number of different animal models, with a few soils dosed to each one. As a result, no comprehensive *in vivo* database in a single animal model exists against which to validate an *in vitro* extraction test. Thus, the proposed study is necessary to provide this database.

III. OBJECTIVE/HYPOTHESIS:

This study will measure the oral bioavailability of arsenic from six soils in a monkey model. Based on similar research with other metals, it is believed that the forms of arsenic in soil will control the extent of oral arsenic bioavailability from soil.

IV. MILITARY RELEVANCE:

The proposed research will yield estimates of the relative bioavailability of arsenic in soil, which can be used in human health risk assessments at DoD sites. In addition, this study will form the basis for subsequent work to develop a simple extraction test that is predictive of arsenic bioavailability from soil to humans. This tool would then be available to DoD personnel for site-specific evaluation of arsenic bioavailability from soil at contaminated sites, resulting in more accurate exposure and risk estimates.

V. MATERIALS AND METHODS:

A. Experimental Design and General Procedures:

Seven adult male Cynomolgus monkeys will be available for the project, with five monkeys making up each dose group during the soil testing. The study will be performed

using a round-robin design, such that each monkey serves as its own control. Each monkey assigned to a specific trial will receive, on separate occasions, doses of test soils and sodium arsenate in water. Prior to dosing with soils, a minimum of three distinct dose groups of sodium arsenate dissolved in water (positive control) will be studied in the monkey model. Sodium arsenate will be dosed at 1.0, 0.5, and 0.1 mg As/kg body weight (Table 1) to all seven monkeys. Previous experience with a dose of 1.0 mg As/kg bw in Cebus monkeys indicated no adverse effects. Once characterization of the soluble arsenic dose has been completed, representative samples of each of six soils will be dosed by gavage to the Cynomolgus monkeys (five monkeys dosed with each soil). Target soil doses for each of the test substrates will range from 0.3 to 1.0 mg As/kg bw (see Table 1). Testing of one of the soil substrates will be performed in duplicate.

The monkeys will be housed in standard cages and will receive food (solid monkey diet) and water *ad libitum* between experiments. Prior to dosing with arsenic-containing soil or sodium arsenate in solution, the monkeys will be transferred to metal-free metabolism cages and fed a low-arsenic diet for 3-5 days. The monkeys will remain on the low-arsenic diet for the duration of the experiment. Baseline urine and fecal samples will be collected before each dose. Sixteen hours prior to the dose, the animal will be fasted, but will continue to have free access to water. Just prior to dosing, the animal will be transferred via pole and collar from the metabolism cage to a restraint chair. While in the restraint chair, an IV line will be placed, and the animal will be given intravenous fluids (sterile saline or lactated ringers solution; approximate rate of 5 mL/kg/hr) to ensure hydration and urine production. A gastric tube (8mm diameter lubricated Tygon tube) will be placed through a bite-bar and into the stomach (the tube is initially measured outside the body from the bottom of the rib cage to the mouth and indelibly marked at that point to denote the length that must be inserted to insure proper placement in the stomach). Once the tube is placed, the dose (arsenic in solution or a soil slurry) is delivered, and the tube is withdrawn. The animal will remain in the restraint chair for up to three hours after the dose. While in the restraint chair, the animal will be able to move its arms freely (except during dosing) out and above its mid-torso. A Lucite L-shaped shield will prevent contact with the lower torso and legs, which is important to prevent the animal from removing the IV line in the leg. The legs will be lightly tethered to allow free movement, except for the legs to cross (again, preventing removing of the IV line in the leg). Urine and feces produced during this period will be collected in a metabolism pan that is part of the restraint chair. Should the monkey become agitated during the experimental period, it will be sedated with an IM or IV dose of diazepam or midazolam (0.5 to 1.0 mg/kg bw). At the end of the initial three-hour urine/feces collection period, the animal will be returned via pole and collar to its metabolism cage, where it will remain for four days. During this time period, the monkeys will have free access to the low arsenic diet and to water. Urine and feces will be collected (separately) from the cage. The fecal samples will be archived for future analysis, if necessary. After four days, the animal will be transferred to its home cage via pole and collar, where it will receive standard monkey chow. There will be a wash-out period of at least two weeks in the home cage between experiments.

Urine samples will be shippedto Battelle Pacific Northwest Labs, where they will be analyzed for total arsenic concentration by inductively coupled plasma/mass spectrometry (ICP/MS).

Table 1. Soil sample dosing specifications for the *in vivo* monkey study

Sample ID	Sample No.	Arsenic Conc. in Soil (mg/kg)	Target Soil Dose (g soil/kg body wt.)	Target Arsenic Mass in Dose[a] (mg As/kg body wt.)
Positive control (Na_2HAsO_4)	PC-1	NA	NA	1.00[b]
Positive control (Na_2HAsO_4)	PC-2	NA	NA	0.50[b]
Positive control (Na_2HAsO_4)	PC-3	NA	NA	0.10[b]
California mine tailings/soil	CAMT	300	1.00	0.30
Washington orchard soil	WAOS	525	1.00	0.52
Massachusetts orchard soil	MAOS	1,000	1.00	1.00
New York orchard soil	NYOS	175	1.90	0.33
Montana mine waste/soil	MTMW	320	1.00	0.32
Montana smelter soil	MTSS	650	1.00	0.65

NA = not applicable

[a] A minimum arsenic dose of approximately 0.20 mg As/kg body wt. must be ingested in soil to obtain detectable arsenic in urine at all time points (assuming an RAF of 0.15).

[b] Sodium arsenate (positive control) doses are designed to bracket the arsenic doses from test soils.

B. Laboratory Animals Required and Justification:

1. Non-Animal Alternatives Considered:

At present, there are no non-animal alternatives that could accomplish the research objectives, because the measurement of metals bioavailability from soil is an inherently biological measure. Non-animal alternatives, such as computer modeling or leachability testing, would not be acceptable, because insufficient information is available to establish whether they would accurately predict the biological uptake of arsenic from soil in a mammalian receptor.

2. Animal Model and Species Justification:

The results of these studies may be used by regulatory agencies to determine safe human exposure limits for arsenic in soils. As such, confidence that the animal model used for these experiments is a reliable predictor for humans is extremely important. The Cynomolgus monkey has been used previously for measuring arsenic bioavailability

from soil (Freeman et al. 1995), and the results have been used by the U.S. EPA for human health risk assessment.

3. **Laboratory Animals:**

a. **Genus & Species:** *Macaca fascicularis*

b. **Strain/Stock:** N/A

c. **Source/Vendor:** Animals were ordered from Primate Products, Miami, Florida. USDA license # 93-R-369.

d. **Age:** 3 years

e. **Weight:** 4–5 kg

f. **Sex:** Male

g. **Special Considerations:** When purchased, the animals were certified seronegative for HerpesB/SRV/SIV/STLV.

h. **Other:**

4. **Total Number of Animals Required:**

a. **Species A:** 7 animals required

5. **Refinement, Reduction, Replacement:**

a. **Refinement:** Compared with earlier experimental designs, this protocol has been refined to reduce stress to the animals by eliminating the need for multiple blood samples, and for general anesthesia. Bioavailability will be assessed non-invasively by collection and measurement of arsenic in excreta.

b. **Reduction:** In the round-robin study design, each animal serves as its own control, which reduces the number of animals required. Also, a single set of reference materials (e.g., sodium arsenate in water) will provide the comparison data for multiple soil-arsenic measurements. This approach minimizes the number of experiments per animal.

c. **Replacement:** At this time, no non-animal model exists that can mimic or approximate oral arsenic bioavailability in humans.

C. Technical Methods:

1. Pain:

a. USDA (Form 18-3) Pain category:

(1) **No Pain**

____7_____(#)___100___% (Column C)

(2) **Alleviated Pain**

_____(#)_____% (Column D)

(3) **Unalleviated Pain or Distress**

_____(#)_____% (Column E)

b. Pain Alleviation:

(1) **Anesthesia/Analgesia/Tranquilization:**
Should the monkey become anxious during the experiment period, it will be sedated with a dose of diazepam or midazolam (0.5 to 1.0 mg/kg bw), as needed, administered by IM or IV in the saphenous vein (mid calf, 22 ga needle). When animals are weighed and transferred from their normal housing to the metabolism cage, a 10-mg/kg bw, IM injection of ketamine will be given (22 ga needle). All anesthetics and sedatives will be administered by John Munson (see Section V.F.)

(2) **Paralytics:** N/A

c. Alternatives to Painful Procedures: N/A

(1) **Source(s) Searched:** N/A

(2) **Date of Search:** N/A

(3) **Key Words of Search:** N/A

(4) **Results of Search:** N/A

d. **Painful Procedure Justification:** N/A

2. **Prolonged Restraint:** N/A

3. **Surgery:** N/A

   a. **Procedure:** N/A

   b. **Pre- and Postoperative Provisions:** N/A

   c. **Location:** N/A

   d. **Multiple Survival Surgery Procedures:** N/A

      (1) **Procedures:** N/A

      (2) **Scientific Justification:** N/A

4. **Animal Manipulations:**

   a. **Injections:** Animals will have an IV placed in the saphenous vein (located midline over the left or right calf) using a 22 ga catheter. The catheter will be placed so that it terminates below the bend in the knee. It will be held in place with 2 pieces of adhesive tape and a self adhesive wrap (Vetwrap). Intravenous fluids (sterile saline or lactated ringers solution; approximate rate of 5 mL/kg/hr) will be given to ensure hydration and urine production.

   b. **Biosamples:** N/A. Only passive collection of excreta (urine and feces) will be performed.

   c. **Animal Identification:** Tattoo located on the chest

   d. **Behavioral Studies:** N/A

   e. **Other procedures:** N/A

5. **Adjuvants:** N/A

6. **Study Endpoint:** The endpoint is the appearance of arsenic in excreta. This is known to be complete within 4 days of an oral dose. No adverse effects of treatment on the subjects are expected.

7. **Euthanasia:** At the completion of the study, animals will be placed in other studies or at another USDA-approved facility. No euthanasia will be required.

D. Veterinary Care:

1. Husbandry Considerations:

Animals are housed individually in primate cages appropriate for size and species in room CB48 at the University of Florida's (UF's) Animal Resources. A diet of Purina monkey chow is supplemented daily with fresh fruit. Drinking water is available *ad libitum*. Cages are cleaned daily. Toys and other enrichment apparatus are provided. Trained UF Animal Care Services (ACS) staff provide care for the animals outside of the experimental period.

During the experimental period, which is conducted in room CB47, the research staff provides animal care. At the beginning of each experiment, the Cynomolgus monkeys are weighed and transferred to metal-free metabolism cages. During the experiment, the monkeys are fed a nutritionally complete low-arsenic diet (Bio-Serv, Frenchtown, NJ, Catalog # F0550SP). While the monkeys are housed in their metal-free metabolism cages, they are provided with a modified enrichment program. A VCR is provided, with a number of tapes for the animals to watch. Also, modified metal-free toys are hung outside of the cages for the animals to manipulate.

Animals are assessed daily for any signs of pain or discomfort, unthriftiness, loss of appetite, or behavioral abnormalities. If any of the above occur, the veterinary staff will be contacted immediately. Animals will be weighed before each experiment. Any significant (>10%) loss of weight will signal the need for further health assessment.

a. Study Room:

Normal housing will be at the UF Animal Resources facility, room CB48. All procedures in metabolism cages will be performed in room CB47.

b. Special Husbandry Provisions:

Animals will be placed in metabolism cages during the 7-day experimental period.

2. Attending Veterinary Care:

Animals will be observed daily by UF ACS staff during normal housing. The research staff (see Section V.F.) will provide daily husbandry services during the experimental period. If an animal becomes ill or debilitated, it will be placed in the care of the attending veterinarian.

3. **Enrichment Strategy:** Enrichment will be altered, but not restricted. See section b. below for details.

a. **Dogs:** N/A

b. **Nonhuman Primates:**
Due to the nature of the experiments, it is imperative that the animals not ingest or otherwise contaminate the interior of the cages with metal from any source. Therefore, when the monkeys are housed in the metabolic cages, they will be provided with a modified enrichment program. A VCR and a number of children's video tapes are provided for the animals to watch. Special metal-free toys are hung outside of the cages for the animals to manipulate. During procedures, videos or soft music are played, depending on the temperament of the animal. While animals are housed in their metabolism cages in the procedure room, the experimental staff provide and monitor enrichment throughout the day. The room is quiet at night.

E. Data Analysis:

For each soil sample, arsenic bioavailability data will be obtained from five animals. Data from previous experiments have provided information on the variance expected in results among subjects within a treatment group (i.e., given the same soil sample). Based on a power of 0.8 and an alpha of 0.05, five observations per group should provide the ability to identify meaningful differences in bioavailability among samples. For each experiment, five subjects will be drawn from a pool of seven animals. In the event that data are lost from one of the original five experimental subjects (e.g., from incomplete urine), one of the reserve animals will be added to the experiment.

For each dose administered to the monkeys, the urinary excretion fraction (UEF; total μg excreted above background/total μg administered) will be calculated for each monkey. Assuming that no obvious outliers are observed, the individual UEFs will be used to calculate an average UEF for each dose. The UEFs for the three sodium arsenate doses will be averaged (if appropriate) to yield an average UEF for sodium arsenate in water. The relative absorption fraction (RAF) for arsenic from each soil will then be calculated as the UEF for the test soil divided by the UEF for sodium arsenate:

$$RAF_{(soil)} = UEF_{(soil)}/UEF_{(sodium\ arsenate)}$$

F. Investigator & Technician Qualifications/Training:

Dr. Stephen Roberts; Principal Investigator. Experienced with animal handling, restraint, training methods, and dosing techniques. PI on numerous projects involving various animal species. Completed the basic and primate online Laboratory Animal Training Association (LATA) module.

John Munson; Study Director. Former rodent facility manager. Experienced with primate handling, restraint, training methods, and dosing techniques. Completed the

basic and primate online LATA modules. John will be responsible for animal handling and dosing, and for administration of all anesthetic and sedatives.

Laura Camerden; Study Technician. Extensive experience working with primates, including handling and assisting with surgical procedures at Wyeth-Ayerst Pharmaceutical Co. Has completed all applicable online LATA modules. Laura will assist with animal handling and dosing, and with sample collection.

All persons listed above have completed the UF Health Risk Assessment for the EH&S Animal Contact Program, and have tested negative for tuberculosis (tested annually).

VI. Biohazard/Safety: N/A

VIII. ASSURANCES:

As the Primary Investigator on this protocol, I acknowledge my responsibilities and provide assurances for the following:

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

B. Duplication of Effort: I have made a reasonable, good-faith effort to ensure that this protocol is not an unnecessary duplication of previous experiments.

C. Statistical Assurance: I assure that I have consulted with an individual who is qualified to evaluate the statistical design or strategy of this proposal, and that the "minimum number of animals needed for scientific validity are used."

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

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

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

_____

(Dr. Stephen M. Roberts)

IX. Enclosures:

A. Literature Searches: N/A

B. Pathology Addendum: N/A

C. Pain Scoring Guidelines: N/A

D. Adjuvant Policy: N/A

References:

Battelle. 1996. Determination of the bioavailability of soluble arsenic and arsenic in slag following oral administration in microswine. Prepared for Dames and Moore, Sacramento, CA. Prepared by Battelle Columbus Operations, Columbus, Ohio.

Casteel, S.W., L.D. Brown, and M.E. Dunsmore. 1997. Relative bioavailability of arsenic in mining wastes. Document Control Number 4500-88-AORH. Veterinary Medical Diagnostic Laboratory, College of Veterinary Medicine, University of Missouri, Columbia, MO.

Freeman, G.B., J.D. Johnson, J.M. Killinger, S.C. Liao, A.O. Davis, M.V. Ruby, R.L. Chaney, S.C. Lovre, P.D. Bergstrom. 1993. Bioavailability of arsenic in soil impacted by smelter activities following oral administration in rabbits. Fund. Appl. Toxicol. 21:83–88.

Freeman, G.B., R.A. Schoof, M.V. Ruby, A.O. Davis, J.A. Dill, S.C. Liao, C.A. Lapin, and P.D. Bergstrom. 1995. Bioavailability of arsenic in soil and house dust impacted by smelter activities following oral administration in Cynomolgus monkeys. Fund. Appl. Toxicol. 28:215–222.

Griffin, S., and P. Turck. 1991. Bioavailability in rabbits of sodium arsenite adsorbed to soils. The Toxicologist 11(1):19.

Groen, K., H. Vaessen, J.J.G. Kliest, J.L.M. deBoer, T.V. Ooik, A. Timmerman, and R.F. Vlug. 1994. Bioavailability of inorganic arsenic from bog ore-containing soil in the dog. Environ. Health Persp. 102(2):182–184.

Roberts, S.M., W.R. Weimar, J.R.T. Vinson, J.W. Munson, and R.J. Bergeron. 2002. Measurement of arsenic bioavailability in soil using a primate model. Toxicol. Sci. 67:303–310.

U.S. EPA. 1996. Bioavailability of arsenic and lead in environmental substrates. 1. Results of an oral dosing study of immature swine. U.S. Environmental Protection Agency, Region 10, Seattle, WA.

PROGRESS REPORT

Initial experiments were conducted to assess the gastrointestinal absorption of sodium arsenate in solution in cynomolgus monkeys. This information is necessary to serve as a benchmark when measuring the relative bioavailability of arsenic from soil samples. In the first set of experiments, seven male monkeys were each administered 1.0 mg/kg arsenic (as sodium arsenate) and 0.5 mg/kg arsenic on separate occasions. The arsenic solution was administered by gastric tube, and urine was collected over the next four days. The total recovery of arsenic, expressed as a percent of administered dose, is shown in Table 1 below.

Table 1. Arsenic Recovery After Oral Administration of Sodium Arsenate

	Percent Dose Recovered in Urine	
Animal ID	Arsenic, 1 mg/kg	Arsenic 0.5 mg/kg
7490	56.9	32.7
7630	37.7	77.0
7773	54.7	17.7
7597	19.3	6.1
7516	23.7	7.8
7499	58.0	54.7
7515	41.7	73.9

Recovery of arsenic in urine in these experiments was found to be quite variable, and in some cases much lower than expected. The ability to recover excreted arsenic from the metabolism cages was evaluated. Arsenic-spiked monkey urine was placed in empty metabolism cages, allowed to remain in the cage as it would during an actual experiment, and recovered using standard procedures. The procedures emphasized using only a small volume of rinsate to avoid dilution of the urine. The percent recoveries from three cages were 69, 70, and 79%. To increase recovery, the volume of rinsate was increased to 500 ml. Spiked urine was added as before to each of three cages, with urine recovered under simulated experimental conditions. This experiment was conducted twice. The resulting recoveries are summarized in Table 2.

Table 2. Efficiency of Recovery of Arsenic in Urine from the Metabolism Cage

	Percent Arsenic in Urine Recovered		
	Cage 1	Cage 2	Cage 3
Trial 1	93	99	95
Trial 2	96	95	95

Using the modified urine recovery protocol with larger rinsate volumes, monkeys were again dosed with 1 mg/kg or 0.5 mg/kg arsenic (as sodium arsentate) by

gastric tube. Urine was collected over the next four days. To assist in following mass recovery of the dose, fecal samples were also collected and analyzed. The results are summarized in the Table 3.

Table 3. Urinary and Fecal Recovery of Arsenic after an Oral Dose of Sodium Arsenate

Animal ID	Urine	Percent Dose Recovered Feces	Total
		Arsenic, 1.0 mg/kg	
7490	69.9	6.9	76.8
7630	34.6	67.3	101.9
7773	44.8	14.8	59.6
7597	38.8	46.5	85.3
7516	37.7	60.1	97.8
7499	69.5	21.9	91.4
7515	36.6	47.1	83.7
		Arsenic, 0.5 m/kg	
7490	49.9	3.0	53.0
7630	43.5	34.8	78.3
7773	37.0	19.5	56.5
7597	46.6	47.0	93.6
7516	32.5	55.2	87.6
7499	49.5	32.1	81.6
7515	47.0	14.4	61.4

Urinary recovery was more consistent than observed previously, although it was still rather variable among animals. Fecal recovery data indicated that much of the arsenic dose not in the urine was excreted in the feces.

An additional experiment has been conducted, although the analytical data (arsenic in urine and feces) have not yet been fully obtained. In this experiment, the protocol was modified to eliminate hydration of the animal with i.v. fluids to determine whether this might reduce inter-animal variability in urinary arsenic excretion. Fecal recovery data suggest less variability with this protocol (33 ± 10 percent, mean ± SD) compared with the observations shown in Table 3 (for 1.0 mg/kg: 38 ± 23 percent; for 0.5 mg/kg: 29 ± 18 percent). This represents a coefficient of variation that is less than half that in the previous experiments. Urinary data are not yet available.

Relative Bioavailability of Cadmium in Soil in Juvenile Swine

November 2004

Prepared by:

Stan W. Casteel, DVM, PhD, DABVT
Principal Investigator
Tim J. Evans, DVM, MS, PhD, DABVT, DACT
Co-Investigator
Veterinary Medical Diagnostic Laboratory
College of Veterinary Medicine
University of Missouri, Columbia
Columbia, Missouri

Michael V. Ruby
Yvette W. Lowney
Exponent
Boulder, Colorado

Rosalind A. Schoof
Integral Consulting Inc.
Mercer Island, Washington

Doc. no. 8601191.002 0302 1104 R271

Contents

	Page
Acknowledgments	**1**
Executive Summary	**2**
1 Introduction	**4**
1.1 Background	4
1.1.1 In Vivo Studies	4
1.1.2 Absolute and Relative Bioavailability	5
1.1.3 Selection of the Animal Model	6
1.1.4 Metallothionein Induction and Potential Effects on the Animal Model	6
2 Materials and Methods	**7**
2.1 Test Materials	7
2.2 Experimental Design and General Procedures	7
2.3 Data Analysis	9
3 Results	**10**
3.1 Test Soil Characterization	10
3.2 Blood Cadmium vs. Time	10
3.3 Dose-Response Patterns	11
3.3.1 Blood Cadmium	11
3.3.2 Tissue Cadmium	11
3.3.3 Relative Cadmium Bioavailability from Test Soils	11
4 References	**13**

Acknowledgments

The work described in this report is the product of a team effort that involved a number of people. In particular, the authors acknowledge the efforts and support of the following:

- Christie Loiacono, DVM, University of Missouri–Columbia, provided expert evaluation of the animals' health at necropsy.
- Margaret Dunsmore, BS, University of Missouri–Columbia, coordinated sample preparation, doughball preparation, and sample tracking activities, as well as sample analysis, data reduction, and report preparation.
- Chastidy Adams, BS, University of Missouri–Columbia, coordinated all in-life activities of the pigs in the study.
- Ashley Akeman, University of Missouri–Columbia, assisted in daily laboratory activities.
- Bill Brattin, Syracuse Research Corporation, assisted with data analysis methods.
- Melanie Edwards, Exponent, performed the statistical analyses on the data.

This study was funded by the Strategic Environmental Research & Development Program (SERDP).

Executive Summary

Young swine were used as test animals to measure the gastrointestinal absorption of cadmium from four contaminated soils: Pt. Mugu soil, Colorado smelter soil, Oklahoma smelter soil, and Dugway soil. Young swine were selected for use in the study, because the gastrointestinal physiology and overall size of young swine are similar to those of children, a population of concern for exposure to metals in soil. Groups of five swine were given oral doses of cadmium-contaminated soil sufficient to deliver doses ranging from 20 to 960 μg of Cd/kg-day for 15 days. Other groups of swine were given soluble cadmium (cadmium chloride) orally for comparison at doses of 0, 10, 60, or 320 μg Cd/kg-day. The U.S. Environmental Protection Agency (EPA) toxicity value for cadmium is based on human ingestion of soluble cadmium in water, and assumes that 5% of this cadmium will be absorbed (IRIS 2004). Therefore, soluble cadmium, as cadmium chloride, was used as the reference material in this relative cadmium bioavailability study.

The amount of cadmium absorbed by each animal was evaluated by measuring the amount of cadmium in the blood (only for the control animals, and those receiving cadmium doses of 60 μg Cd/kg/day or higher) and the amount of cadmium in liver and kidney (for all dose groups). The amount of cadmium present in blood or tissues of animals exposed to test soils was compared to that of animals exposed to cadmium chloride, to calculate the relative bioavailability of cadmium in soil. Separate relative bioavailability calculations were based on the area under the blood-cadmium-concentration-vs.-time curve (for Pt. Mugu soil only), terminal liver cadmium concentration, and terminal kidney cortex cadmium concentration. The relative bioavailability adjustment (RBA) results for the four samples from this investigation are summarized in Table ES-1, below.

The estimates of RBA based on kidney and liver are very similar for three of the soils (Colorado smelter soil was 0.89 for kidney and 0.66 for liver, Oklahoma smelter soil was 0.79 for kidney and 0.76 for liver, and Dugway soil was 0.18 for kidney and 0.09 for liver). Results for the Pt. Mugu soil are more variable, with the RBA for liver (0.96) being greater than the RBAs for kidney (0.60) and blood (0.56). Because the kidney is the primary target organ of toxicity for cadmium, RBA results for that tissue are considered most relevant for risk assessment. Greater reliance on the kidney RBAs is supported by the finding that either liver RBAs (for three soils) or blood RBA (for one soil) were in close agreement with the kidney RBA. The lower relative cadmium bioavailability for Dugway soil compared to the other soils may be related to the predominance of cadmium sulfate in that soil combined with the low cadmium concentrations and the predominance of very fine (clay) particles in the soil.

Table ES-1. Relative bioavailability estimates

	Pt. Mugu Soil	Colorado Smelter Soil	Oklahoma Smelter Soil	Dugway Soil
Kidney				
RBA	0.60	0.89	0.79	0.18
Lower bound	0.52	0.61	0.53	0.07
Upper bound	0.69	1.19	1.07	0.30
Standard Error	0.05	0.17	0.16	0.07
Liver				
RBA	0.96	0.66	0.76	0.09
Lower bound	0.80	0.33	0.40	–0.02
Upper bound	1.19	1.03	1.16	0.21
Standard Error	0.11	0.21	0.22	0.07
Blood AUC (bleed1)				
RBA[a]	0.56	NA	NA	NA
Lower bound	0.40	--	--	--
Upper bound	0.89	--	--	--
Standard Error	0.12	--	--	--

[a] RBA (relative bioavailability adjustment) for the blood AUC (area under the curve) was fit excluding the control (0 dose) data, because the responses at this dose were all non-detect.

NA – not analyzed

1 Introduction

This study was performed in accordance with the protocol titled, *Systemic Bioavailability of Cadmium in Soil in Juvenile Swine*, which was reviewed by the Navy Bureau of Medicine and Surgery (assigned Navy Research Database [NRD] No. 307), and approved on November 19, 2003.

1.1 Background

Cadmium is a ubiquitous environmental contaminant associated with renal, skeletal, and reproductive diseases in animals and humans. Cadmium is also classified as a probable human carcinogen. The average adult in the United States ingests approximately 30 µg of cadmium daily, and the biological half-life of cadmium is 10 to 30 years in humans (ATSDR 1999). It is estimated that environmental exposure to cadmium results in renal disease in 1% to 7% of the world's population (Klaassen et al. 1999; Satarug et al. 2000).

The likelihood of adverse health effects from chronic exposure to cadmium, and the potential for ingestion of contaminated soil by children, prompted this evaluation of the potential for human exposure to cadmium in soil matrices. Because the chemical form of a metal and its associated matrix will influence its intestinal absorption, bioavailability is an important variable in assessing exposure to metals in soil.

1.1.1 *In Vivo* Studies

In practice, oral relative bioavailability adjustments (RBAs) for metals in soil are based on the differences in specific metal concentrations in biological samples (blood, urine, and/or tissues) between animals ingesting metal-contaminated soil and animals orally exposed to a readily soluble salt of the metal (i.e., a reference compound). For the soil and soluble forms of the metal, doses that produce equivalent biological responses are used to determine the RBAs.

Distribution generally refers to the transport of a substance from its point of entry into the body to its deposition within the tissues. The distribution of an ingested metal is taken into consideration in determining which biological responses are measured in relative bioavailability studies. While the linear and nonlinear dose-dependent aspects of the distribution of lead within the blood, kidneys, liver, and femur of immature pigs have been described (Casteel et al. 1997a), there is a relative lack of information on the distribution of cadmium following repeated oral exposure of juvenile swine. These data gaps are especially evident with respect to the effects of dose, time since administration, and duration of exposure on blood levels of cadmium following intestinal absorption.

Approximately 2.5% and 5% of oral doses of cadmium are absorbed by humans from diet and drinking water, respectively (IRIS 2004), and somewhat lower absorption is reported in rats and mice. A substantial portion of an oral cadmium dose is initially retained in the gastrointestinal mucosa, and then is slowly excreted in the feces over a period of weeks. For this reason, whole-

body retention of cadmium shortly after dosing is not a reliable indicator of cadmium absorption. Cadmium is widely distributed in the body, with the major portion of the body burden located in the liver and kidney of humans and laboratory animals (ATSDR 1999). Most absorbed cadmium is excreted very slowly, with biological half-lives ranging from 20% to 50% of an animal's life span. Blood cadmium levels reflect mainly recent exposure to cadmium, rather than body burden. Thus, cadmium concentrations in blood, kidney, and liver are expected to provide indications of the degree of absorption of recent oral doses.

A literature search revealed a number of studies on cadmium bioavailability, but only a few on cadmium bioavailability from soil. A good review of the literature is available in the Agency for Toxic Substances and Disease Registry (ATSDR) toxicological profile for cadmium (ATSDR 1999). The two studies of cadmium bioavailability from soil include one in rats (Schoof and Freeman 1995) and one in the juvenile swine model that was used in this research (Schroder et al. 2003).

Metals, such as cadmium, occur in soil as a complex mixture of solid-phase compounds of varying particle size and morphology. The occurrence and relative distribution of an element among these various solid phases, and the physical relation between the phases and the soil, control an element's solubility and, hence, its bioavailability. To date, there have been a limited number of studies of cadmium bioavailability from soil, and therefore, relatively little is known about the factors that control this endpoint. The soil chemistry and cadmium forms in the soils used in this study were characterized, and this information is used to assess the factors that may be controlling oral cadmium bioavailability.

1.1.2 Absolute and Relative Bioavailability

Bioavailability is a concept that relates to the absorption of chemicals, and how absorption depends on the physical-chemical properties of the chemical and its medium (e.g., dust, soil, food, water, etc.) and the physiology of the exposed receptor. Bioavailability is normally described as the fraction (or percentage) of a chemical that enters into the blood following an exposure of some specified amount, duration, and route. In some cases, bioavailability may be measured using chemical levels in peripheral tissues such as liver, kidney, and bone, rather than blood. The fraction or percentage absorbed can be expressed either in absolute terms (i.e., absolute bioavailability) or in relative terms (i.e., relative bioavailability). Absolute bioavailability is measured by comparing the amount of chemical entering the blood (or other tissue) following oral exposure to the test material with the amount entering the blood (or other tissue) following intravenous exposure to an equal amount of some dissolved form of the chemical. In contrast, relative bioavailability is measured by comparing oral absorption of a test material to oral absorption of an appropriate reference material (generally a soluble form of the chemical). For example, if 100 μg of dissolved cadmium were administered in drinking water, and a total of 5 μg entered the blood, the absolute bioavailability would be 0.05 (5%). Likewise, if 100 μg of cadmium in soil were administered, and 3 μg entered the blood, the absolute bioavailability from soil would be 0.03 (3%). If the cadmium dissolved in water were used as the reference material for calculating the relative bioavailability of cadmium absorbed from soil, the relative bioavailability would be 0.03/0.05 = 0.60 (60%).

1.1.3 Selection of the Animal Model

Juvenile swine were selected for this study because of the similarity in gastrointestinal parameters between swine and humans. For example, feeding behavior, gastrointestinal anatomy, acid secretion, and the development of small-intestinal absorption mechanisms are all quite similar between swine and humans (Weis and LaVelle 1991). For these reasons, swine have been used as a surrogate for humans in the fields of pharmaceutical research and nutrition (Dodds 1982; Miller and Ullrey 1987). Juvenile animals were selected, because metals absorption is frequently greater in younger animals, and this model is designed to predict uptake in the most sensitive population of concern—children. The young swine model has been used to assess the oral bioavailability of both lead and arsenic in soil (Casteel et al. 1997a,b), and the results from these studies have been used to develop relative bioavailability adjustments for human health risk assessment by the U.S. Environmental Protection Agency (EPA).

1.1.4 Metallothionein Induction and Potential Effects on the Animal Model

Metallothioneins (MTs) are thought to play a role in a wide variety of physiological processes, including absorption and tissue distribution of dietary metals, essential metal homeostasis, heavy metal detoxification, free radical scavenging, and regulation of the cell cycle. MTs are low-molecular-weight (6 to 7 kD), heat-stable, metal-binding proteins that have been divided into four major isoforms (MT-1, MT-2, MT-3, and MT-4) in mammalian species. MT-1 and MT-2 are the most common MT isoforms and, depending on the species, can be further subdivided into subtypes (e.g., MT-1a and 1b, MT-1e to MT-1h, MT-1x and MT-2a in humans; MT-1a to -1g and MT-2a and -2b in swine; MT-1 and MT-2 in rodents).

Exposure to cadmium induces the expression of metallothioneins in a variety of tissues in humans and various animal models, including juvenile swine (ATSDR 1999). In addition to the similarities in the complexities of MT expression, humans and swine have 50-fold higher levels of hepatic MTs, less biliary excretion of cadmium, and enhanced resistance to cadmium toxicosis, compared to rodents. In addition to cadmium, MT-1 and MT-2 are induced by exposure to other metals such as bismuth, copper, mercury, silver, and zinc, as well as by hormones, cytokines, other xenobiotics, oxidative damage, inflammation, and stress (Davis and Cousins 2000; Ghoshal and Jacob 2001; Miles et al. 2000; Samson and Gedamu 1998).

Recently, MT induction in the juvenile swine model was shown to be associated with greater retention of cadmium within the renal cortices and, therefore, to be a potential determinant of the bioavailability of cadmium in soil matrices that contain other inducers of MT expression, when renal concentrations of cadmium are used to estimate exposure (Evans et al. 2004). Although MT induction was not measured in this study, this issue should be kept in mind when interpreting the study data for estimating the relative bioavailability of cadmium.

2 Materials and Methods

2.1 Test Materials

The samples tested in this study were provided by Exponent, Inc. and were surficial soils (0–3 in.) that had been collected from cadmium-contaminated sites. The test soils were: 1) a soil from Pt. Mugu, California (sample PTMG), a composite residential soil from near a smelter in Colorado (sample CO-SCS), a soil from near a zinc smelter in Oklahoma (sample OK-SS), and a composite soil from Dugway Proving Ground in Utah (sample DPGC). Anhydrous cadmium chloride ($CdCl_2$) was used as the soluble cadmium reference material in this study, and was obtained from Sigma.

Test soils were characterized for the following parameters, all of which were collected on the <250-μm size fraction (i.e., the size fraction dosed to the swine), with the exception of particle size distribution (sand, silt, clay), which was measured on the <2-mm size fraction: pH (EPA Method 9045C), total organic carbon (TOC; ASTM D4129-82), total carbon (ASTM D4129-82; from which total inorganic carbon [TIC] was calculated by difference between total carbon and TOC), cation exchange capacity (CEC; EPA Method 9081), cadmium concentration (in triplicate; EPA Method 7131), and metals concentrations (arsenic, chromium, copper, iron, lead, manganese, mercury, nickel, phosphorus, and zinc; EPA Method 6010B, with the exception of arsenic [EPA Method 7060A], lead [EPA Method 7421], and mercury [EPA Method 7471A]). Analysis for cadmium concentration was preceded by thorough mixing of the soils exactly as they were mixed prior to dose preparation. The bottle containing the soil was placed on a low-speed roller apparatus for 30 minutes; it was then removed, inverted five times, and allowed to stand a few minutes to settle, and the samples were then taken for analysis.

In addition, an aliquot of each soil sample was evaluated for cadmium mineralogy by electron microprobe, using the method described in Davis et al. (1993). This method involves establishing the chemistry of individual cadmium-bearing grains in the sample, until a representative number have been analyzed (generally 100–200), and the distribution of cadmium among the different cadmium forms in the soil can be established.

2.2 Experimental Design and General Procedures

Intact male pigs weighing 10–12 kg were provided by Chinn Farms (Clarence, Missouri) and were housed in individual stainless steel cages. The animals were weaned onto standard pig chow purchased from MFA Inc. (Columbia, Missouri). To minimize cadmium exposure from the diet, the animals were then transitioned gradually from the MFA feed to a special low-metal feed (purchased from Zeigler Brothers, Inc., Gardner, Pennsylvania) over the time interval from day –7 to day –3; they were maintained on this feed for the duration of the study. The feed was nutritionally complete and met all requirements of the National Institutes of Health – National Research Council. The typical nutritional components and chemical analysis of the feed are

presented in Table 2-1. Typically, the feed contained approximately 5.7% moisture and 1.7% fiber, and provided about 3.4 kcal of metabolizable energy per gram.

Each animal was given an amount of feed equal to 4% of the mean body weight of all animals in the dose group. Feed was administered in two equal portions at 1100 and 1700 hours daily. Drinking water was provided *ad libitum* via self-activated water nozzles in each cage. Analysis of samples from randomly selected drinking-water nozzles during previous studies has indicated that mean cadmium concentration (setting non-detects at one-half the detection limit) is less than 0.05 μg/L.

All doses were delivered daily for 15 consecutive days (Days 0–14 in Table 2) in a low-metal moistened feed. The cadmium chloride (in solution) was pipetted, and the cadmium in soil was weighed and placed in the center of a 10- to 20-gram moistened dough ball (prepared by mixing the powdered low-metal feed with water). Animals were given a divided dose at 0900 and 1500 hours, and were fed 2 hours after dosing. Animal weights were recorded, and dose and feed amounts were adjusted on days –1, 2, and every third day thereafter until study termination, to achieve constant doses and feed (as a percent of body weight) during the study.

For three of the soils (Colorado smelter soil, Oklahoma smelter soil, and Dugway soil), groups of four to five swine were given oral doses of 20 and 60 μg Cd/kg-day for 15 days. One soil with the highest cadmium concentrations (Pt. Mugu soil) was administered at doses of 240, 480, and 960 μg Cd/kg-day. Other groups of animals were given a soluble cadmium reference dose (as cadmium chloride) orally at doses of 0, 10, 60, and 320 μg Cd/kg-day for the same period of time. The doses were given according to the design outlined in Table 3.

Blood samples (6–8 mL) were drawn from the control animals into a plastic syringe by venipuncture of the anterior vena cava, and from those animals that received cadmium doses of 60 μg Cd/kg/day or higher (i.e., groups 1, 3–7, 9, 11, and 13) on days 0, 6, 8, 10, 12, and 14. The blood was then transferred immediately into Vacutainer® tubes containing EDTA. On each of the blood sampling days, blood samples were drawn just prior to the 0900 hour dosing (Bleed I) and 2 hours after the 0900 hour dosing (Bleed II).

On the morning of study day 15, following the last blood collection, all animals were humanely euthanized, and representative samples (approximately 30 grams of the medial lobe) of the liver and the right kidney cortex were collected and stored in cadmium-free plastic bags at –40 °C until being prepared for cadmium analysis. All animals were subjected to detailed examination at necropsy by a certified veterinary pathologist to assess overall animal health.

Concentrations of cadmium in the blood, liver, and kidney cortex samples were determined after acid digestion by graphite furnace atomic absorption spectroscopy (GFAAS), as well as flame AAS for liver and kidney samples to confirm the accuracy of dilutions on samples with high cadmium concentrations. The liver values from furnace AAS were used for data analysis, while for the kidney data, the cadmium concentrations were so high that the flame AAS values were deemed more accurate and were used for data analysis. Sample preparation and analytical methods are described in Appendix A, and the cadmium concentration data for blood (measured as described above) and for liver and kidney (measured at sacrifice on day 15) are provided in Appendix B.

2.3 Data Analysis

Regression methods were used to estimate the relative oral bioavailability of cadmium from four test soils relative to cadmium chloride. Because the response in the juvenile swine model was measured as cadmium concentrations in liver and kidney tissue, and the area-under-the-curve for blood cadmium concentration vs. time, relative bioavailability values were determined for each of these different types of responses.

A single simultaneous regression model was used to estimate the slope for each test material while restricting the intercept to be equal to the response from the control animals for all the test materials. This is appropriate because at zero dose all of the test materials should yield the same response. As is typical with animal data of this type, the variability in the response increases with increasing dose—a property known as heteroscedasticity. Because heteroscedasticity of the data is contrary to the underlying assumption of equal variance for a linear regression to be applicable, each dose-group was weighted by the inverse of the predicted variance for that dose group (average dose was assumed for each member of a dose group). The predicted variance is an estimate of expected variance as a function of the magnitude of the response data, and is considered a more robust measure of variance than the dose-group specific measured variance, because it is less affected by individual measurements. Weighting by the inverse of the predicted variance gives less weight to the more variable data points and achieves homogeneous variability across all dose-groups. A simultaneous linear regression model was then fit to each endpoint for the weighted data. The relative bioavailability adjustment (RBA) values for each response (liver, kidney, and blood) for each soil were then estimated as the ratio of the slope for the soil versus that for cadmium chloride. Fieller's formula was then used to estimate the uncertainty in these RBA estimates, as represented by the upper and lower 95th percentiles and standard error on the RBA estimates.

3 Results

3.1 Test Soil Characterization

Soil chemistry and metals concentrations in each test soil are provided in Table 4, and the cadmium mineralogy results are provided in Table 5. Reported cadmium concentrations are the average of triplicate analyses (Table 4), and were used for preparing soil doses to achieve the target dose levels specified in Table 3. Concentrations of other metals, some of which may affect enteric absorption of cadmium, are also presented for each of the test soils in Table 4. Cadmium concentrations were far higher in the Pt. Mugu soil compared to the other soils (4,109 mg/kg vs. 47–452 mg/kg for the others), and this soil was also high in chromium, nickel and phosphorus. The Oklahoma smelter soil had exceptionally high zinc concentrations.

Values of pH in three of the four test soils were near neutral (7.43 to 7.55), while the Dugway soil exhibited a more basic pH (9.06). TOC values ranged from 1.90% to 4.98%, while TIC ranged from less than 0.05% to 1.51%. CEC values did not range widely among the soils (52.2 to 70.1 meq/100 g). The Pt. Mugu soil contained the greatest proportion of sand (coarse, medium, and fine grained), while the Colorado smelter soil and the Oklahoma smelter soil contained greater proportions of silt-size particles. The Dugway soil was the only one of the four test soils that contained an appreciable quantity of clay-sized particles.

Cadmium mineralogy (Table 5) indicated that only a few forms dominated the cadmium-bearing mineral assemblage in these test soils. These mineralogic forms are cadmium-calcium-metal[1] oxide (Pt. Mugu and Colorado smelter), cadmium-metal oxide (Colorado smelter), cadmium-metal sulfate (Dugway), and cadmium-iron oxide (Oklahoma smelter). All other cadmium-bearing phases were found to account for less than 8% of cadmium mineral mass. The average particle size (based on long-axis dimension) of each cadmium-bearing phase in each sample is provided in parentheses in Table 5. These results suggest that oral cadmium bioavailability from soil in this study will be controlled by the solubility of only a few cadmium forms in the gastrointestinal tracts of the juvenile swine.

3.2 Blood Cadmium vs. Time

Figures 1 and 2 show the group mean blood cadmium concentrations for Bleed I (0800 hours) and Bleed II (1100 hours) at different times during the study. For Bleed I, blood cadmium concentrations were initially at or below the method detection limit (0.1 μg/L) in all groups, and remained at or below detection limits in the negative control animals. In animals given repeated oral doses of cadmium chloride (Groups 2–4) and Pt. Mugu soil (Groups 5–7) at doses of 60 μg/kg/day or greater, blood levels began to rise within 1–2 days, and continued to rise until the end of the study (day 15). For the other three soils (Colorado smelter, Oklahoma smelter

[1] "Metal" stands for other metals present at low concentrations, and generally consisted of combinations of aluminum, iron, lead, antimony, and zinc.

and and Dugway), only animals in the 60-μg/kg/day dose groups were sampled, and even at this dose, blood levels of cadmium were predominately at or below the detection limit (0.1 μg/L).

Although the same trends in blood cadmium concentrations were evident in Bleed II (taken at 2 hours post-dosing), as in Bleed I, the results were more variable (Figure 2). This is consistent with the rapidly changing blood cadmium concentrations associated with the absorption of cadmium after the dose was given (Bleed II was included in this study to try to capture data on peak blood cadmium concentrations, and the Bleed II values are indeed greater than the Bleed I values). As would be expected, the steep slope of the concentration-vs.-time curve during this interval leads to greater variability in the blood cadmium concentrations. Because of this increased variability, RBA calculations were based on data from Bleed I.

3.3 Dose-Response Patterns

3.3.1 Blood Cadmium

The measurement endpoint used to quantify the blood cadmium response was the area under the curve (AUC) for blood cadmium concentration vs. time (days 0–14). The AUC was selected because it is the standard pharmacokinetic index of chemical uptake into the blood compartment, and is relatively insensitive to small variations in daily blood cadmium levels. The AUC was calculated using the trapezoidal rule to estimate the AUC between each time point that a blood cadmium value was measured (days 0, 6, 8, 10, 12, and 14), and summing the areas across all time intervals in the study. This mean AUC for each pig was then plotted against the body weight–adjusted dose for that pig by dosing material.

As indicated in Figure 3, the dose-response patterns appear to be linear for the soluble reference material (cadmium chloride) and for the Pt. Mugu soil for Bleed I. It was not possible to prepare dose-response curves for the Colorado smelter, Oklahoma smelter, and Dugway soils, because blood cadmium results were at or below detection limits.

3.3.2 Tissue Cadmium

Tissue results for swine dosed with each test soil, and with the cadmium chloride reference material, were subjected to the weighted simultaneous linear regression data analysis method described in the Data Analysis section. Results from this analysis are shown graphically in Figures 4 through 12.

3.3.3 Relative Cadmium Bioavailability from Test Soils

Based on the results from the weighted simultaneous regressions described above, the RBA values for each response (liver, kidney, and blood) for each soil were calculated as the ratio of the slope for the soil versus that for cadmium chloride. The upper- and lower-bound values in Table 6 represent the upper and lower 95th percentile values on the RBA estimates (based on application of Fieller's formula).

The estimates of RBA based on kidney and liver are very similar for three of the soils (Colorado smelter soil was 0.89 for kidney and 0.66 for liver, Oklahoma smelter soil was 0.79 for kidney and 0.76 for liver, and Dugway soil was 0.18 for kidney and 0.09 for liver). Results for the Pt. Mugu soil are more variable, with the RBA for liver (0.96) being greater than the RBAs for kidney (0.60) and blood (0.56). Because the kidney is the primary target organ of toxicity for cadmium, RBA results for that tissue are considered most relevant for risk assessment. Greater reliance on the kidney RBAs is supported by the finding that either liver RBAs (for three soils) or blood RBA (for one soil) were in close agreement with the kidney RBA.

Assuming that the kidney results should be given the greatest weight, the three soils with the greatest cadmium concentrations (Pt. Mugu, Colorado smelter, and Oklahoma smelter) all yield similar RBA values (range of 0.60 to 0.89). In contrast, the Dugway soil yielded a considerably lower cadmium RBA of 0.18. An examination of soil characteristics and cadmium mineralogy suggests that this outcome may be due to the more basic soil pH of the Dugway soil, the high clay content of this soil, or the presence of most of the cadmium in the Dugway soil as cadmium-metal sulfate (a cadmium phase not found in the other soils).

4 References

ATSDR. 1999. Toxicological profile for cadmium. Agency for Toxic Substance and Disease Registry, Atlanta, GA. www.atsdr.cdc.gov/toxprofiles.

Casteel, S.W., R.P. Cowart, C.P. Weis, et al. 1997a. Bioavailability of lead to juvenile swine dosed with soil from the Smuggler Mountain NPL site of Aspen, Colorado. Fund. Appl. Toxicol. 36:177–187.

Casteel, S.W., L.D. Brown, M.E. Dunsmore, et al. 1997b. Relative bioavailability of arsenic in mining wastes. Prepared for U.S. Environmental Protection Agency, Region VIII, Denver, CO. Doc. Control No. 4500-88-AORH.

Davis, S.R., and R.J.Cousins. 2000. Metallothionein expression in animals: A physiological perspective on function. J. Nutr. 130:1085-1088.

Davis, A., J.W. Drexler, M.V. Ruby, and A. Nicholson. 1993. Micromineralogy of mine wastes in relation to lead bioavailability, Butte, Montana. Environ. Sci. Technol. 27(7):1415–1425.

Dodds, J.W. 1982. The pig model for biomedical research. Fed. Proc. 41:247–256.

Evans, T.J., M.S. Carlson, J.T. Turk, and S.W. Casteel. 2004. The effects of zinc pretreatment on the absorption and retention of ingested cadmium in acutely exposed juvenile swine. Immunohistochemical assessment of metallothionein induction by cadmium in orally exposed juvenile swine. Manuscript in preparation.

Ghoshal, K., and S.T. Jacob. 2001. Regulation of metallothionein gene expression. Prog. Nucleic Acid Res. Mol. Biol. 66:357-385.

IRIS. 2004. Integrated Risk Information System. U.S. Environmental Protection Agency, Washington, D.C. http://www.epa.gov/iris/subst/0141.htm.

Klaassen, C.D., J. Liu, and S. Choudhuri. 1999. Metallothionein: An intracellular protein to protect against cadmium toxicity. Annual Rev. Pharmacol. Toxicol. 39:267–294.

Miles, A.T., G.M. Hawksworth, J.H. Beattie, and V. Rodilla. 2000. Induction, regulation, degradation, and biological significance of mammalian metallothioneins. Crit. Rev. Biochem. Mol. Biol. 35:35–70.

Miller, E.R., and D.E. Ullrey. 1987. The pig as a model for human nutrition. Ann. Rev. Nutr. 7:381–382.

Satarug, S., M.R. Haswell-Elkins, and M.R. Moore. 2000. Safe levels of cadmium intake to prevent renal toxicity in human subjects. Brit. J. Nutr. 84:791–802.

Samson S. L-A., and L. Gedamu. 1998. Molecular analyses of metallothionein gene regulation. Prog. Nucleic Acid Res. Mol. Biol. 59:257-288.

Schroder, J.L., N.T. Basta, J. Si, et al. 2003. In vitro gastrointestinal method to estimate relative bioavailable cadmium in contaminated soil. Environ. Sci. Technol. 37(7):1365–1370.

Schoof, R.A., and G.B. Freeman. 1995. Oral bioavailability of lead and cadmium in soil from a smelter site. Seventh International Congress of Toxicology, Seattle, WA. July 3–6, 1995.

Weis, C.P., and J.M. LaVelle. 1991. Characteristics to consider when choosing an animal model for the study of lead bioavailability. Chem. Spec. Bioavail. 3(3/4):113–120.

Table 1. Typical feed composition[a]

Nutrient Name	Amount	Nutrient Name	Amount
Protein	20.1021%	Chlorine	0.1911%
Arginine	1.2070%	Magnesium	0.0533%
Lysine	1.4690%	Sulfur	0.0339%
Methionine	0.8370%	Manganese	20.4719 mg/kg
Met+Cys	0.5876%	Zinc	118.0608 mg/kg
Tryptophan	0.2770%	Iron	135.3710 mg/kg
Histidine	0.5580%	Copper	8.1062 mg/kg
Leucine	1.8160%	Cobalt	0.0110 mg/kg
Isoleucine	1.1310%	Iodine	0.2075 mg/kg
Phenylalanine	1.1050%	Selenium	0.3196 mg/kg
Phe+Tyr	2.0500%	Nitrogen Free Extract	60.2340%
Threonine	0.8200%	Vitamin A	5.1892 kIU/kg
Valine	1.1910%	Vitamin D3	0.6486 kIU/kg
Fat	4.4440%	Vitamin E	87.2080 IU/kg
Saturated Fat	0.5590%	Vitamin K	0.9089 mg/kg
Unsaturated Fat	3.7410%	Thiamine	9.1681 mg/kg
Linoleic 18:2:6	1.9350%	Riboflavin	10.2290 mg/kg
Linoleic 18:3:3	0.0430%	Niacin	30.1147 mg/kg
Crude Fiber	3.8035%	Pantothenic Acid	19.1250 mg/kg
Ash	4.3347%	Choline	1019.8600 mg/kg
Calcium	0.8675%	Pyridoxine	8.2302 mg/kg
Phos Total	0.7736%	Folacin	2.0476 mg/kg
Available Phosphorus	0.7005%	Biotin	0.2038 mg/kg
Sodium	0.2448%	Vitamin B12	23.4416 mg/kg
Potassium	0.3733%		

[a] Nutritional values provided by Zeigler Bros., Inc.

Table 2. Daily study schedule for dosing, weighing, and collecting blood

Study Day	Weigh	Dose Prep	Dose Administration	Bleed, Pre-Dose Groups 1,3-7, 9,11,13	Bleed, 2hrs Post-Dose Groups 1, 3-7, 9, 11, 13
–4	X				
–3					
–2					
–1	X	X			
0			X	X	X
1			X		
2	X	X	X		
3			X		
4			X		
5	X	X	X		
6			X	X	X
7			X		
8	X	X	X	X	X
9			X		
10			X	X	X
11	X	X	X		
12			X	X	X
13			X		
14	X		X	X	X
15	study termination				

Table 3. Dose administration for the relative cadmium bioavailability study

Dose Group	N	Treatment	Target Cadmium Intake (μg/kg/day)
1	4	Negative Control	0
2	4	$CdCl_2$ (anhydrous)	10
3	4	$CdCl_2$ (anhydrous)	60
4	4	$CdCl_2$ (anhydrous)	320
5	5	PTMG (4109 mg/kg Cd)	240
6	5	PTMG (4109 mg/kg Cd)	480
7	5	PTMG (4109 mg/kg Cd)	960
8	4	CO-SCS (452 mg/kg Cd)	20
9	4	CO-SCS (452 mg/kg Cd)	60
10	4	OK-SS (102 mg/kg Cd)	20
11	4	OK-SS (102 mg/kg Cd)	60
12	4	DPGC (46.8 mg/kg Cd)	20
13	4	DPGC (46.8 mg/kg Cd)	60

Table 4. Test soil characterization

Chemical	Units	Pt. Mugu Soil (PTMG)		CO Smelter Soil (CO-SCS)		OK Smelter Soil (OK-SS)		Dugway Soil (DPGC)	
Conventionals									
PH	s.u.	7.43		7.52		7.55		9.06	
Total organic carbon	%	1.90		2.21		4.98		2.87	
Total inorganic carbon	%	0.99		0.05	*U*	0.74		1.51	
Cation exchange capacity	meq/100g	65.9		54.1		70.1		52.2	
Particle Size Distribution[a]									
Very coarse sand (850–2,000 µm)	%	11.8		9.08		12.9		10.6	
Coarse sand (425–850 µm)	%	30.5		10.7		15.7		8.58	
Medium sand (250–425 µm)	%	30.3		12.8		15.1		8.18	
Fine sand (106–250 µm)	%	20.4		25.7		16.5		29.8	
Very fine sand (75–106 µm)	%	1.91		9.97		4.45		9.87	
Percent silt (4–75 µm)	%	2.22		27.5		31.8		3.31	
Percent clay (<4 µm)	%	3.34		3.18		1.73		29.7	
Cadmium Concentration[b]	mg/kg	4,109	±375	452	±7	102	±0.7	46.8	±0.4
Other Metal Concentrations									
Arsenic	mg/kg	165		416		77.2		8.50	
Chromium	mg/kg	16,300		26.0		19.4		41.8	
Copper	mg/kg	1,950		89.3		1,300		45.2	
Iron	mg/kg	15,600		20,500		22,500		14,100	
Lead	mg/kg	1,140		642		1,000		71.3	
Manganese	mg/kg	138		510		804		266	
Mercury	mg/kg	1.85		8.04		0.900		5.95	
Nickel	mg/kg	3,850		16.7		45.1		24.1	
Phosphorus	mg/kg	3,310		804		790		1,150	
Silver	mg/kg	171		2.0	*U*	24		2.5	
Zinc	mg/kg	1,370		1,310		28,500		394	

Soils sieved to <250 µm

U – undetected; value represents reporting limit

[a] Measured on <2-mm size fraction

[b] Reported cadmium concentrations are based on triplicate analyses.

Table 5. Cadmium mineralogy results

Cadmium Form	Pt. Mugu Soil (PTMG) Percent Mass Distribution	Pt. Mugu Soil (PTMG) Average Particle Size[a] (µm)	CO Smelter Soil (CO-SCS) Percent Mass Distribution	CO Smelter Soil (CO-SCS) Average Particle Size[a] (µm)	OK Smelter Soil (OK-SS) Percent Mass Distribution	OK Smelter Soil (OK-SS) Average Particle Size[a] (µm)	Dugway Soil (DPGC) Percent Mass Distribution	Dugway Soil (DPGC) Average Particle Size[a] (µm)
CdCa(M) oxide	47.6%	16	31.5%	21	--	--	--	--
$CdCl_2$	5.8%	12	--	--	--	--	--	--
Cd(M) oxide	--	--	44.2%	6.4	--	--	--	--
Cd(M) silicate	--	--	6.7%	14	--	--	--	--
Cd(M) sulfate	1.5% [b]	18 [b]	0.4%	2	--	--	99.4%	2.1
Cd oxide	42.6%	4.2	7.7%	3.0	--	--	--	--
Cd sulfide	1.3%	9	--	--	--	--	--	--
CdFe oxide	0.0%	23	1.7%	23	92.5%	34	0.6%	26
CdFe sulfate	0.1%	13	--	--	7.5%	24	--	--
CdPb(M) oxide	--	4	7.0%	4.6	--	--	--	--
No. particles counted	--	176	--	114	--	110	--	108

Note: -- – Not present
Forms contributing less than 1% of cadmium mass in any sample are not shown.
(M) stands for "metals" and generally consisted of a combination of Al, Fe, Pb, Sb, and/or Zn.

[a] Based on long-axis dimensions.
[b] Sum of CdMSO4 and CdSO4 values from CU data.

Table 6. Cadmium RBA estimates for test soils

	Pt. Mugu	CO-SCS	OK-SS	Dugway
Kidney				
RBA	0.60	0.89	0.79	0.18
Lower bound	0.52	0.61	0.53	0.07
Upper bound	0.69	1.19	1.07	0.30
Standard Error	0.05	0.17	0.16	0.07
Liver				
RBA	0.96	0.66	0.76	0.09
Lower bound	0.80	0.33	0.40	–0.02
Upper bound	1.19	1.03	1.16	0.21
Standard Error	0.11	0.21	0.22	0.07
Blood AUC (bleed1)				
RBA[a]	0.56	NA	NA	NA
Lower bound	0.40	--	--	--
Upper bound	0.89	--	--	--
Standard Error	0.12	--	--	--

[a] RBA based on blood AUC was fit excluding the control (0 dose) data, because the response at 0 dose was non-detect.

NA – not analyzed

Figure 1. Bleed I: Group Mean Blood Cadmium by Day

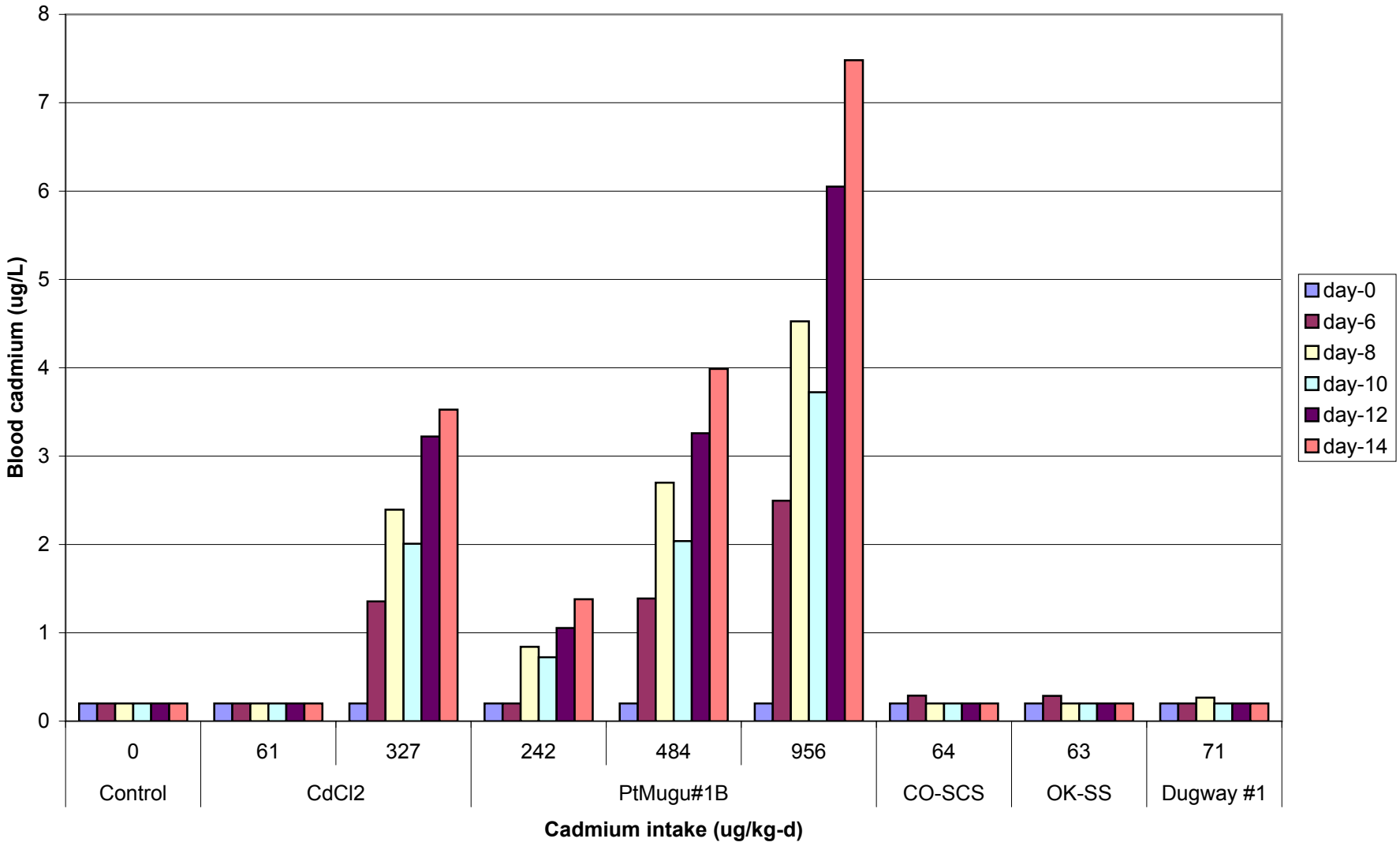


Figure 2. Bleed II: Group Mean Blood Cadmium Level

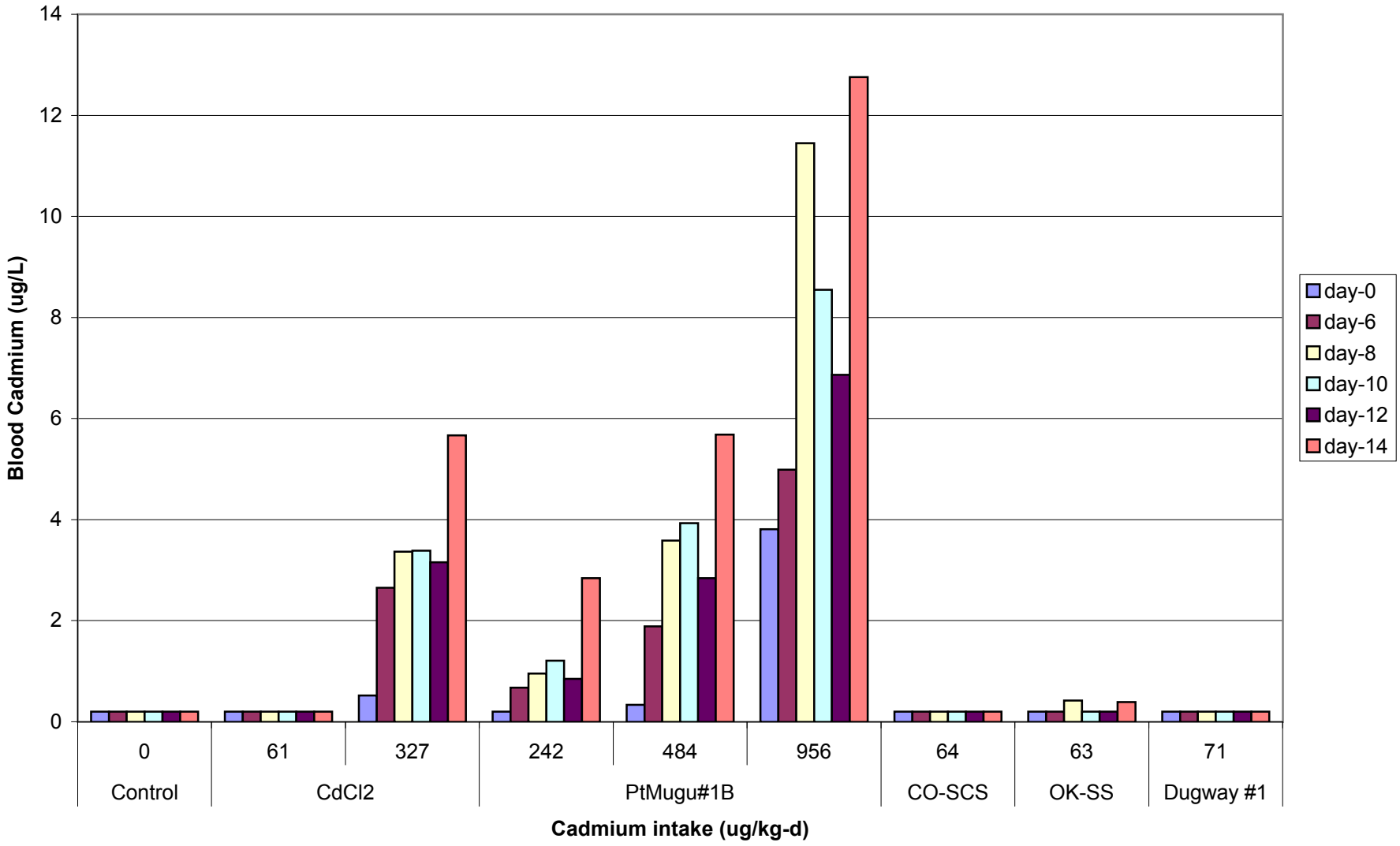


Figure 3. Blood AUC Dose-Response Relationships for Pt. Mugu Soil and Cadmium Chloride

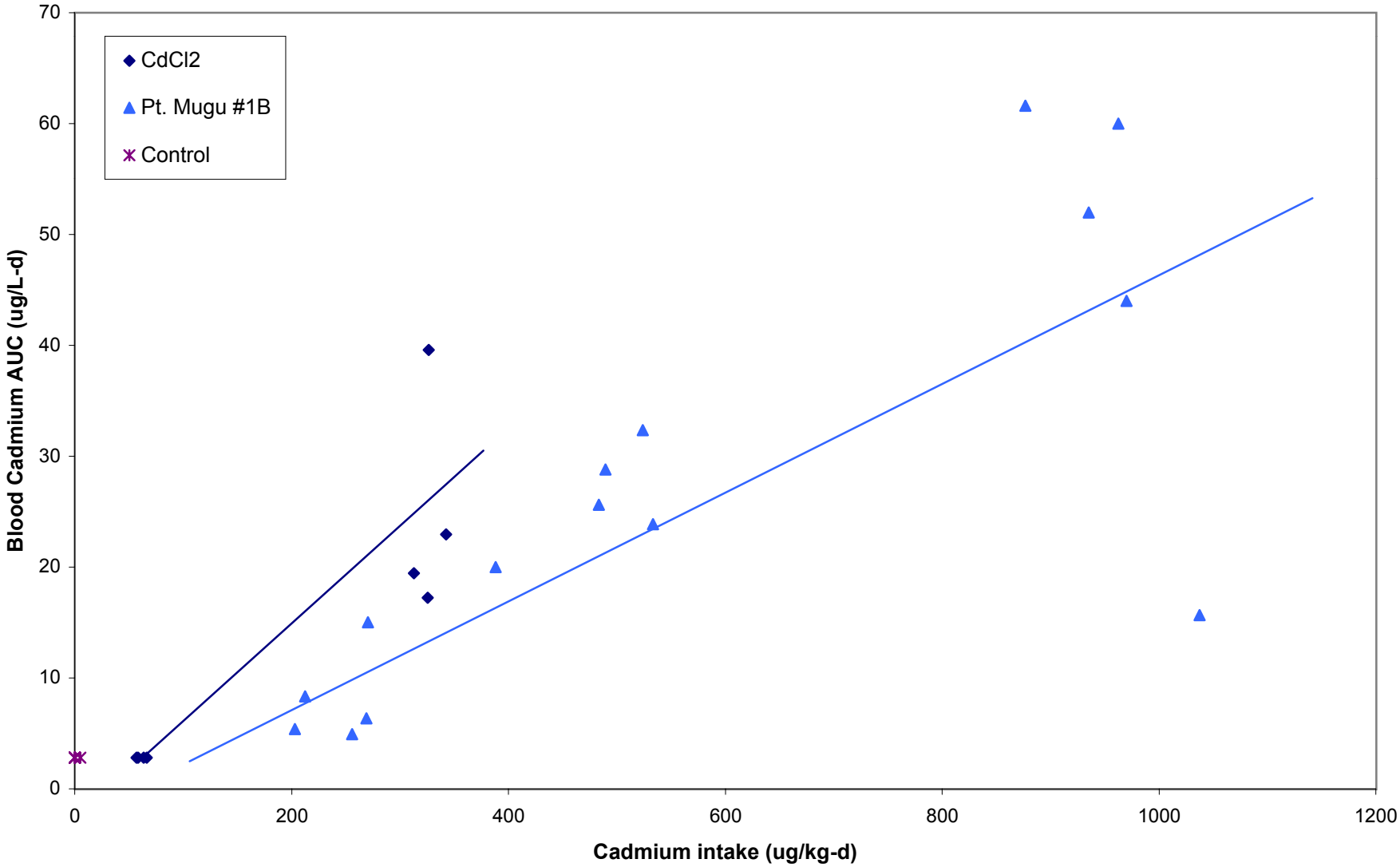


Figure 4. Liver Dose-Response Relationships for Pt. Mugu Soil and Cadmium Chloride

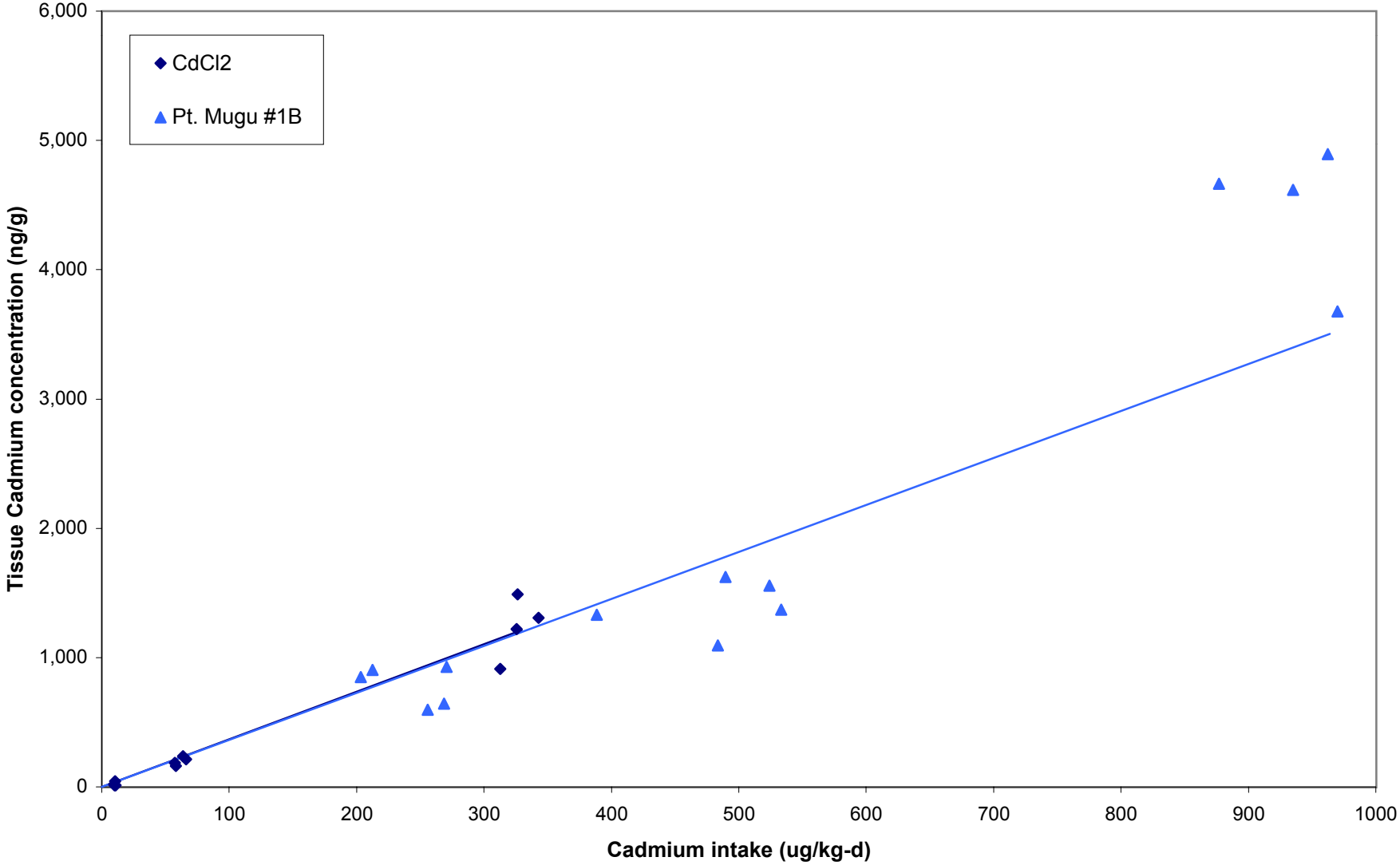


Figure 5. Kidney Dose-Response Relationships for Pt. Mugu Soil and Cadmium Chloride

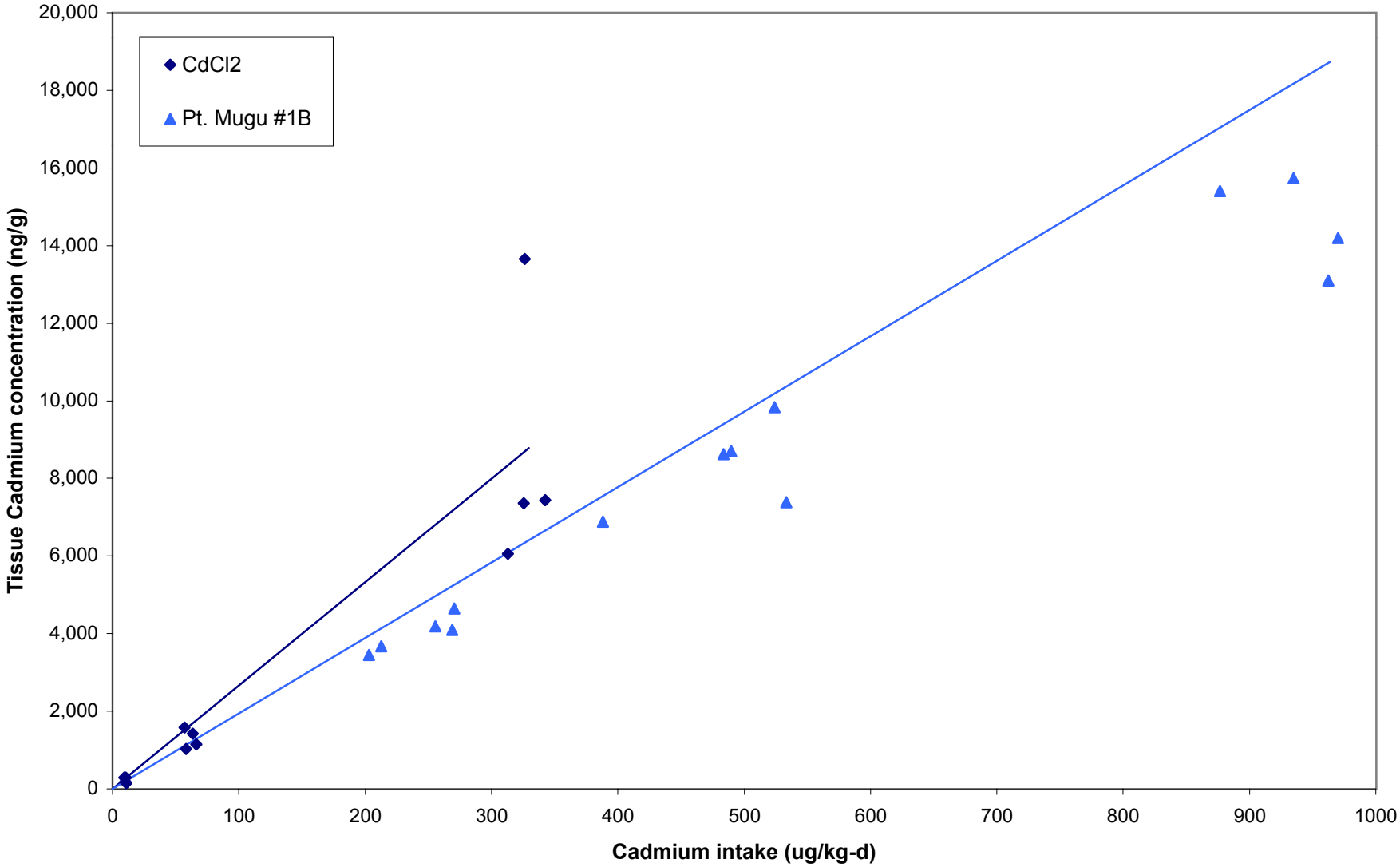


Figure 6. Liver Dose-Response Relationships for CO-SCS Soil and Cadmium Chloride

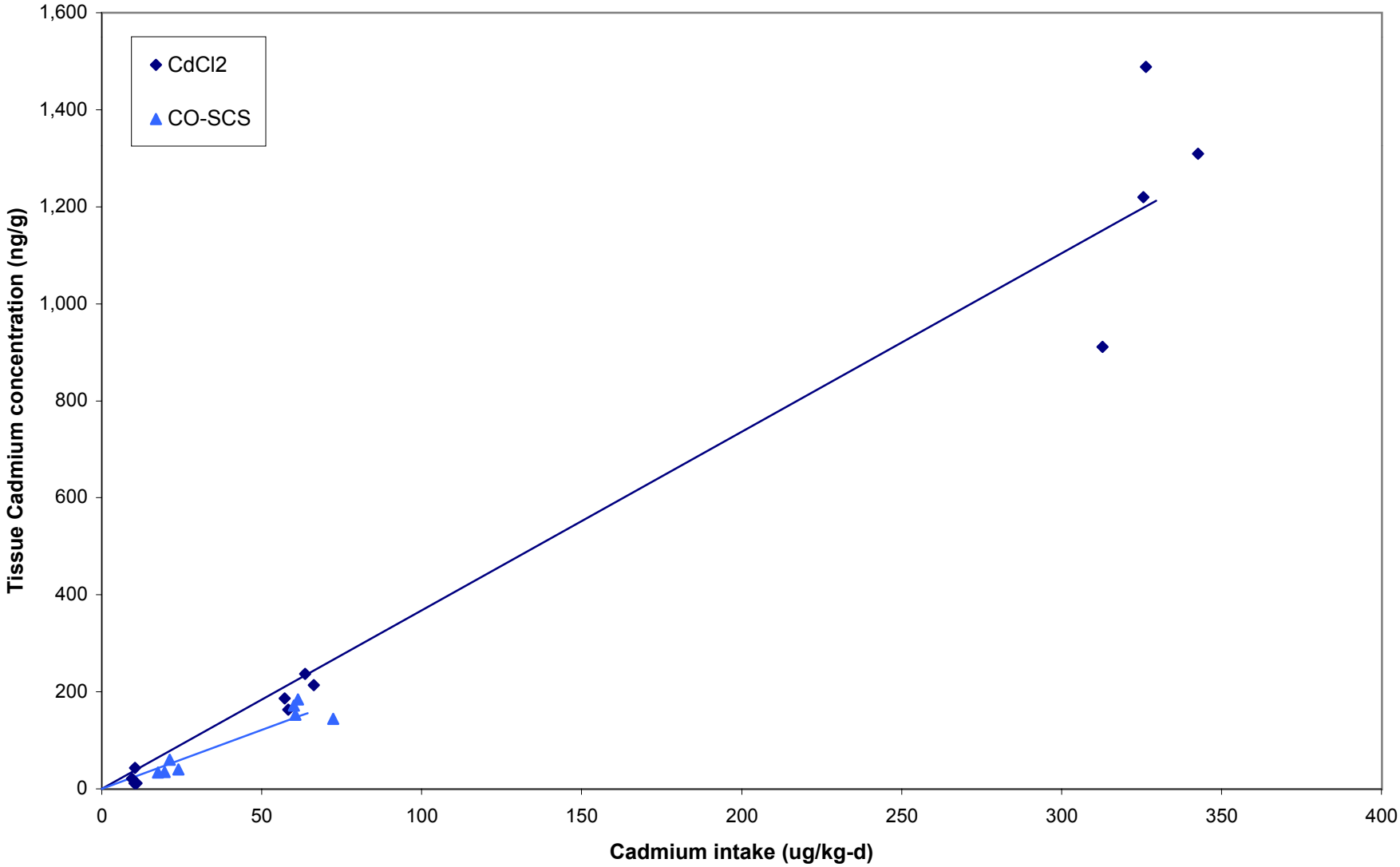


Figure 7. Kidney Dose-Response Relationships for CO-SCS Soil and Cadmium Chloride

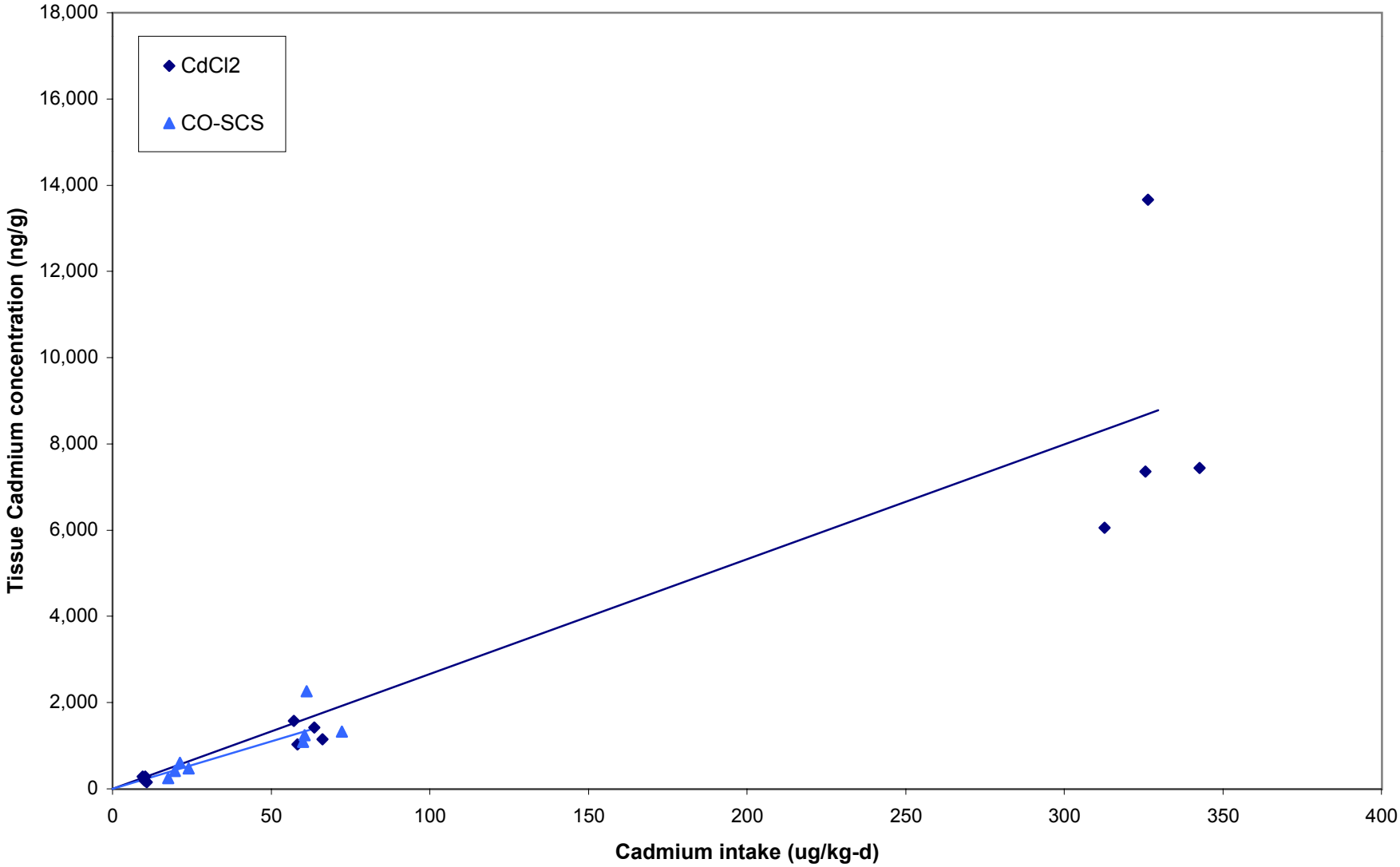


Figure 8. Liver Dose-Response Relationships for OK-SS Soil and Cadmium Chloride

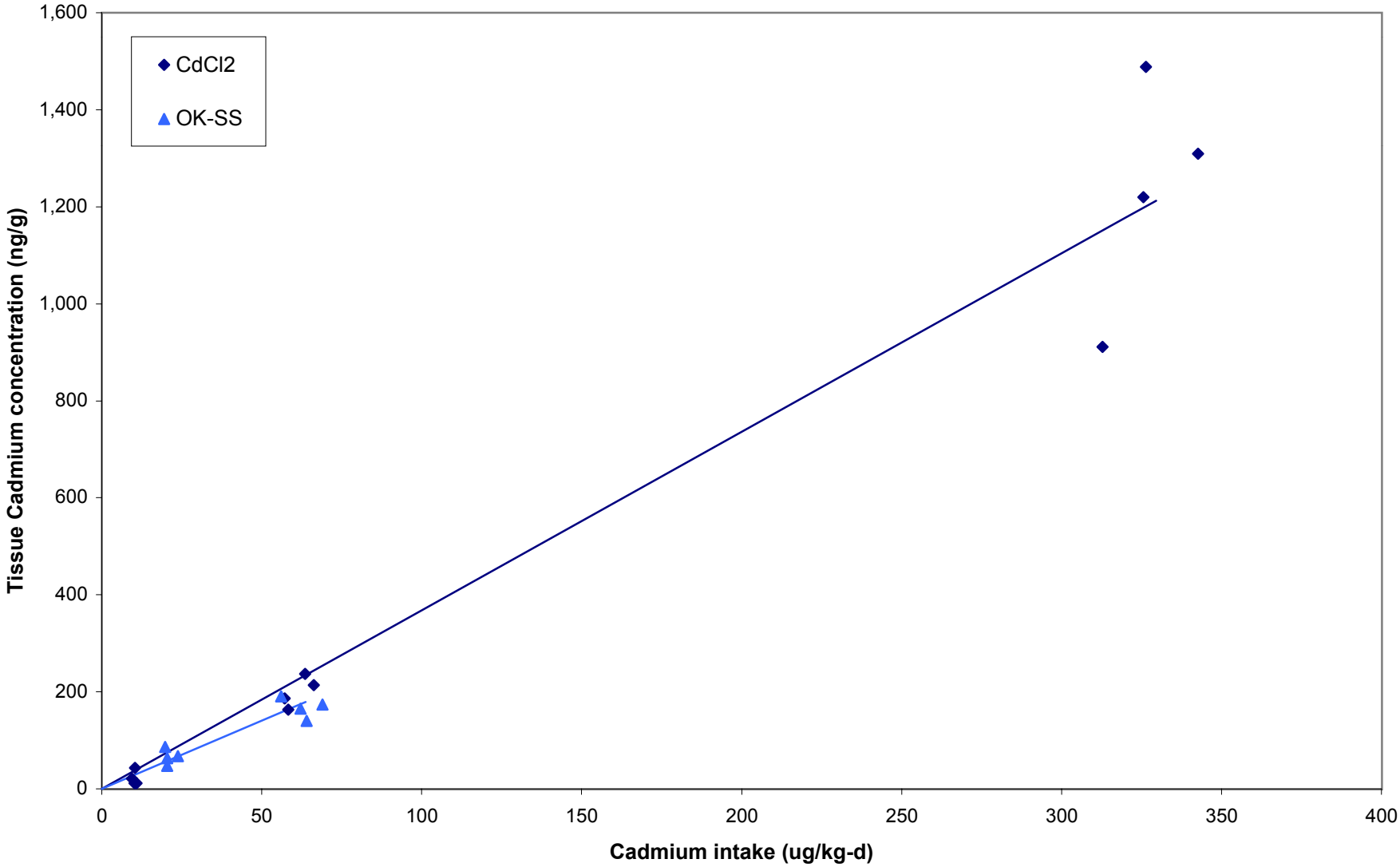


Figure 9. Kidney Dose-Response Relationships for OK-SS Soil and Cadmium Chloride

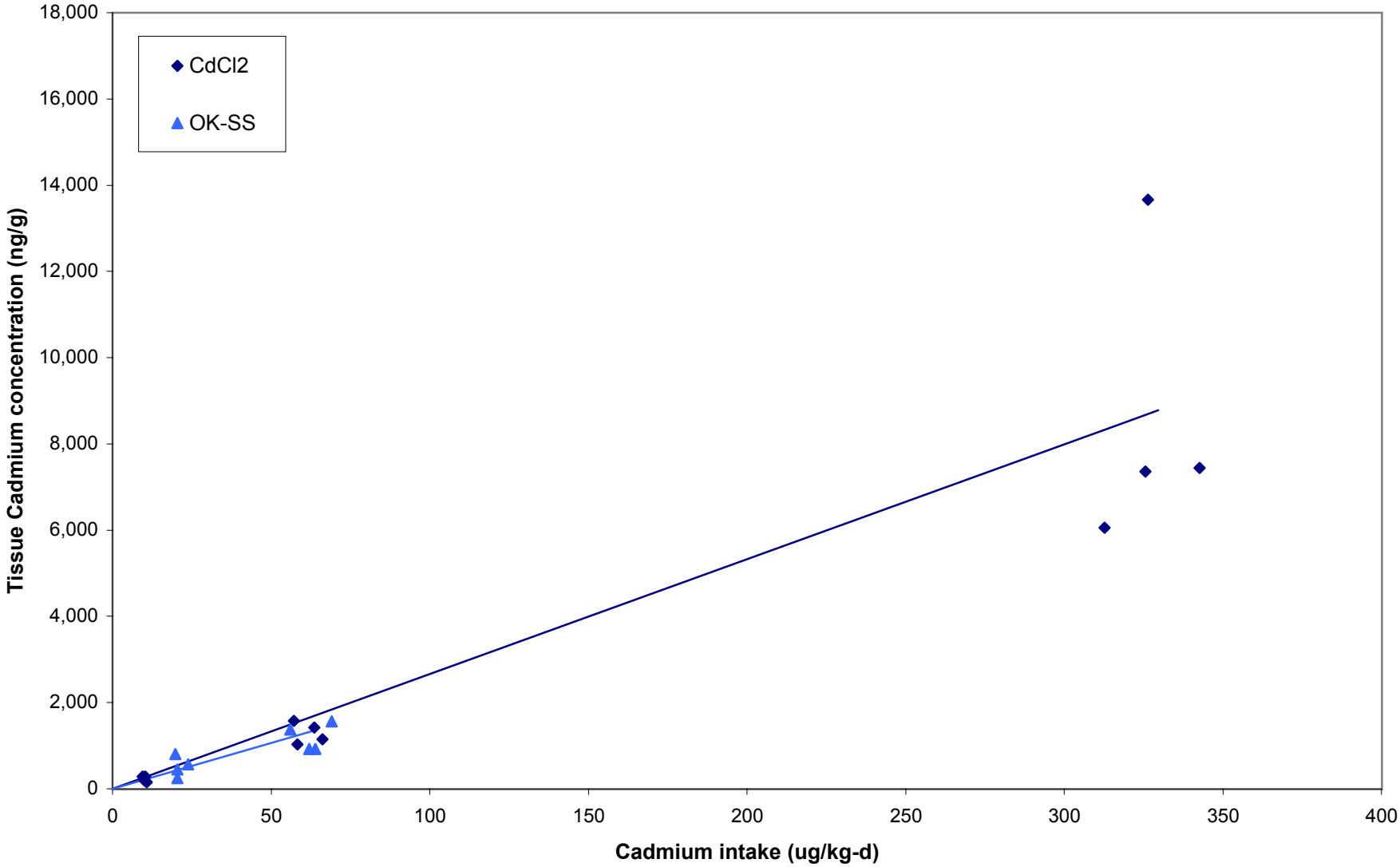


Figure 10. Liver Dose-Response Relationships for Dugway Soil and Cadmium Chloride

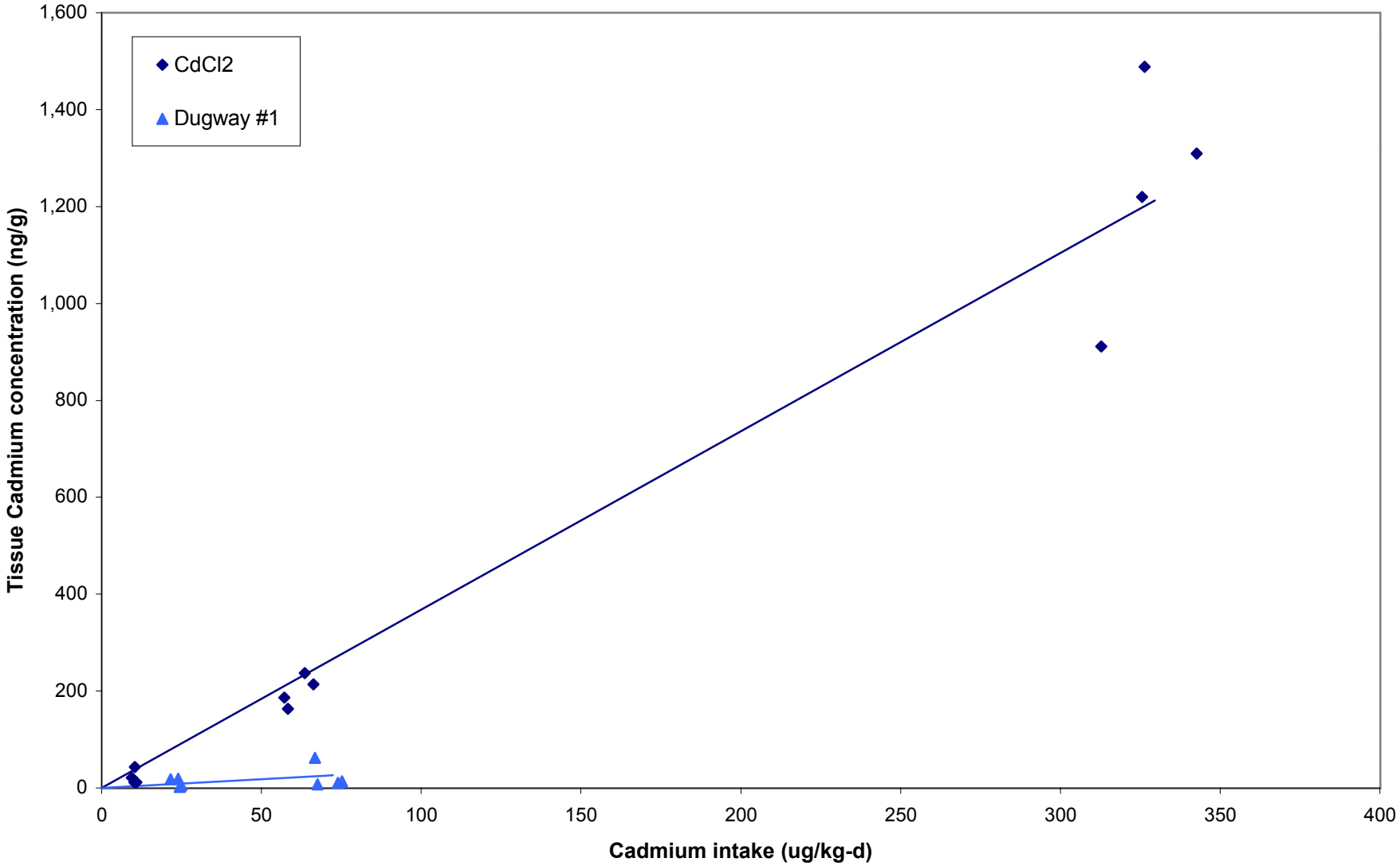


Figure 11. Kidney Dose-Response Relationships for Dugway Soil and Cadmium Chloride

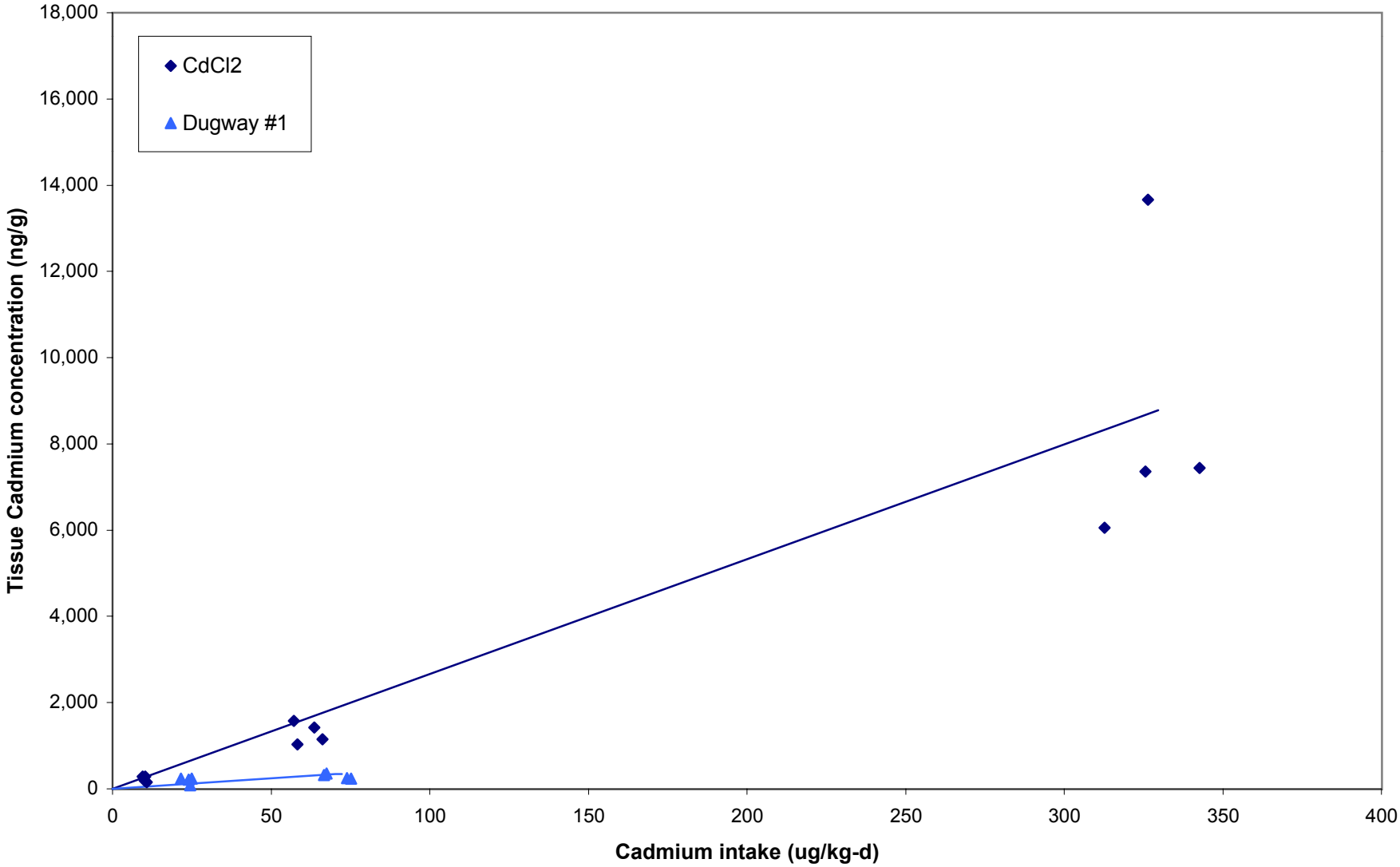
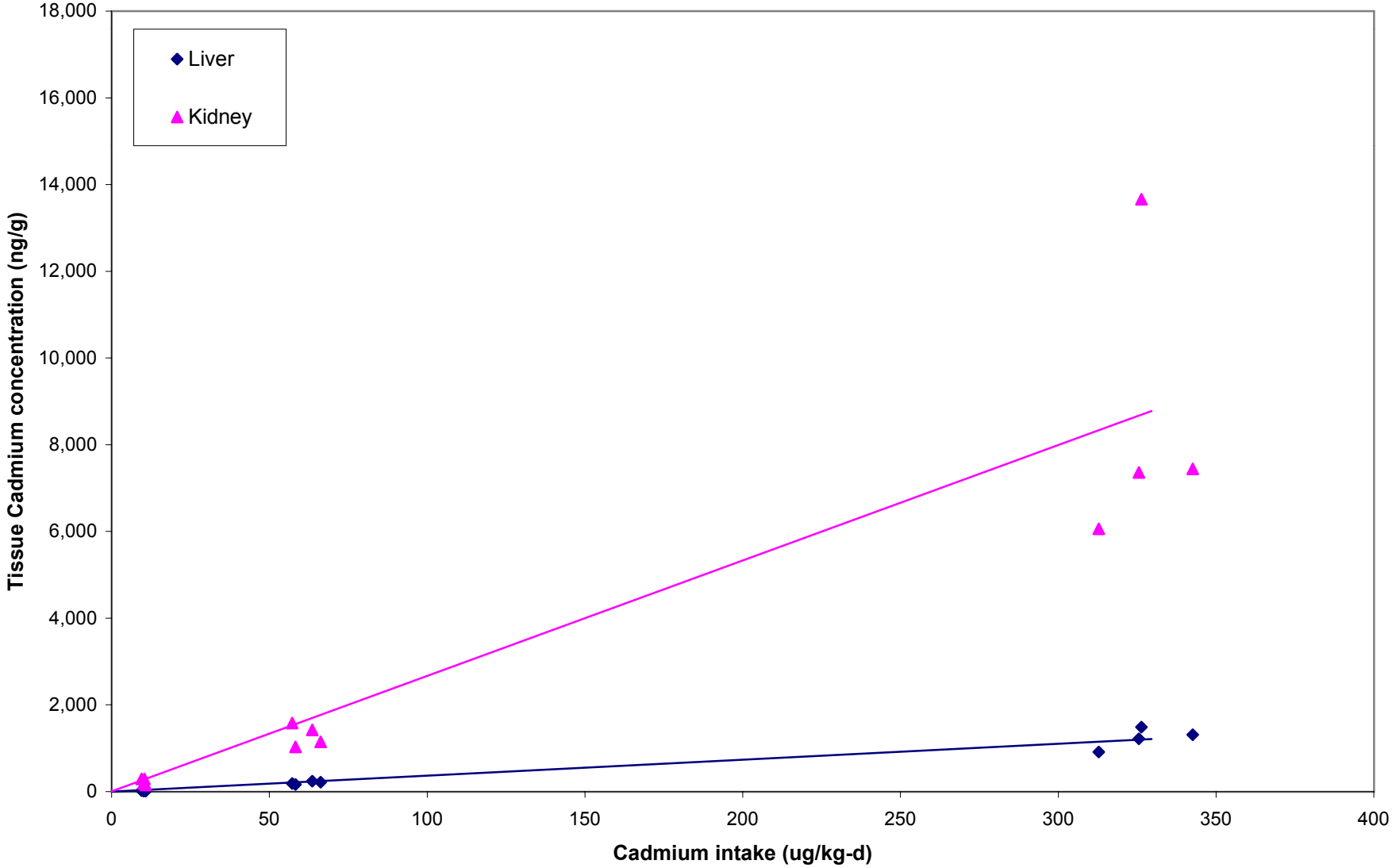


Figure 12. Liver and Kidney Cadmium Chloride Dose-Response Relationships



Cadmium Analysis in Tissue, Blood, and Soil Samples

Sample Preparation

Tissue

One gram of tissue (±5% by weight) was placed in a 5-mL Teflon screw-cap container. Two milliliters of trace-metal nitric acid (concentrated) was added and the cap was screwed tight. Each container was heated overnight at 90 °C in a laboratory oven. After the overnight heating, samples were allowed to cool and diluted to 10 mL with deionized distilled water. Analysis was performed by graphite furnace atomic absorption spectroscopy (GFAAS), as described below.

Blood

Blood was collected in 7-mL purple-top (EDTA) Vacutainers, and stored at 4 °C until analysis. One milliliter of gently mixed blood was pipetted into 3 mL of 1 M nitric acid (Trace Metal Grade) in a 15-mL Falcon® tube, and mixed by inversion or vortexing. Samples were then centrifuged at 2000 rpm for 10 minutes to settle the precipitate. Samples were stored at 4 °C until analysis.

Alternative analysis preparation was 1 mL of gently mixed blood pipetted into 3 mL of Matrix Modifier (recipe follows) in a 15-mL Falcon tube and mixed by inversion. Samples were stored at 4 °C until analysis.

The matrix modifier used for the blood analysis was developed by the Centers for Disease Control and Prevention (CDC; Miller et al. 1987) and consists of:

1. Deionized distilled water containing:
   - 0.2% v/v nitric acid
   - 0.5% Triton X-100
   - 0.2% ammonium phosphate, dibasic.

A single batch of matrix modifier was prepared, which was sufficient for the entire study, and was used to prepare all standards and blanks during blood analysis (standards and quality control [QC] samples were matrix matched). Blood samples were analyzed by GFAAS, as described below.

Soil

Digestion was performed by EPA Method 3050 (U.S. EPA 1997), and analysis by flame ionization spectroscopy, as described below.

GFAAS Analysis

Analysis was performed by graphite furnace atomic absorption with Zeeman background correction using a THGA tube and an appropriate modifier (ammonium nitrate or palladium nitrate), if necessary. Standard conditions from the Perkin-Elmer Analyst 800 manual were used as a starting point; however, these instrument parameters were optimized for this project.

The analytical sequence followed the standard U.S. EPA contract laboratory (CLP) procedures, with internal (ICV) and continuing (CCV) calibration verification analyses every 10 samples, recalibration every 15 (R value on calibration curve of 0.995 or better) and an instrument spike every 20 samples. If the ICV/CCV was outside the ±10% range, all samples since the last good ICV were reanalyzed, with ICV in range. Acceptable spike recovery range was ±15%. All tissue samples were analyzed in duplicate, and a preparation (or digestion) duplicate was performed every 15 samples. Each blood sample was analyzed singly, with a duplicate performed every 20 samples.

Flame Analysis

Samples with cadmium concentrations in the parts per million range (soils and kidney samples primarily) were analyzed by flame ionization spectroscopy. Duplicates, spikes, and quality control were as specified by EPA CLP procedures.

References

Miller, D.T., D.C. Paschal, E.W. Gunter, P.E. Stroud, and J. D'Angelo. 1978. Determination of lead in blood using electrothermal atomization atomic-absorption spectrometry with a L'vov platform and matrix modifier. Analyst 112:1701–1704.

U.S. EPA. 1997. Test Methods for Evaluating Solid Waste. SW-846. Version 2.0. U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response, Washington, DC.

Detailed Data and Summary

Overview

Performance of this study involved collection and reduction of a large amount of data. All of these data and the data reduction steps are contained in two Microsoft Excel® spreadsheets named "EXPCD-1Liver Kidney Regression Data.xls" and "EXPCD-1Blood Data and Analysis Regression.xls." Two additional spreadsheets, "EXPCD1MichiganFinalResults.xls" and "EXPCD1SwineBloodAUC.xls," contain the regression results and graphs supplied by Exponent. These files are intended to allow detailed review and evaluation of all aspects of this study. The following sections of this appendix present printouts of selected tables and graphs from these Excel files. These tables and graphs provide more detailed documentation of the individual animal data and the data reduction steps performed in this study than was presented in the main text. Any additional details of interest to a reader can be found in the spreadsheets.

Raw Data and Data Reduction Steps

Body Weights and Dose Calculations

Animals were weighed on day –1 (one day before exposure) and every three days thereafter during the course of the study (data in Table B-1). Doses of cadmium for the three days following each weighing were based on the group mean body weight, adjusted by addition of 1 kg to account for the expected weight gain over the interval before the next weighing. After completion of the experiment, body weights were estimated by interpolation for those days when measurements were not collected, and the actual administered doses (μg Cd/kg) were calculated for each day and then averaged across all days (Table B-2). If an animal missed a dose or was given an incorrect dose, the calculation of average dose was corrected for these factors. Throughout the duration of this study, only one animal was noted to have consumed only a partial dose (on one day). This was adjusted for when calculating the body-weight-adjusted doses for this experiment.

Blood Cadmium vs. Time

Blood cadmium values were measured in each animal on days 0, 6, 8, 10,12, and 14 in the negative control animals and in those receiving 60 μg Cd/kg/day or higher. There were two bleedings: Bleed I, one hour prior to the 0900 hour dose and Bleed II, 2 hours after the 0900 hour dose. The raw data, (reported as μg/L of diluted blood) are provided in Table B-3. These data were adjusted as follows: a) non-detects were evaluated by assuming a value equal to one-half the method detection limit, and b) the concentrations in diluted blood were converted to units of μg/L in whole blood by multiplying by a factor of 4, because 1 mL of blood was diluted to 4 mL with matrix modifier for analysis. The final concentration data are shown in the right-

hand column of Table B-3. Tables B-4 and B-5 show tabulated data for Bleeds I and II, respectively. Figures B-1 through B-3 plot the blood cadmium results for individual animals organized by group and by day for controls, $CdCl_2$, and Pt. Mugu.

Blood Cadmium AUC

The area under the blood-cadmium-vs.-time curve for each animal was calculated by finding the area under the curve for each time step using the trapezoidal rule:

$$AUC(di\ to\ dj) = 0.5*(ri+rj)*(dj-di)$$

where:

d = day number
r = response (blood cadmium value) on day i (r_i) or day j (r_j).

The areas were then summed for each of the time intervals to yield the final AUC for each animal. These calculations are shown in Tables B-6 (Bleed I) and B-7 (Bleed II). If a blood cadmium value was missing (either because of problems with sample preparation/analysis or bleeding), the blood cadmium value for that day was estimated by linear interpolation.

Liver and Kidney Cadmium Data

At sacrifice (day 15), samples of liver and kidney were removed and analyzed for cadmium concentrations. The raw data (expressed as μg Cd/L and/or mg Cd/L of prepared sample) are summarized in Tables B-8 and B-9, respectively. These data were adjusted as follows: a) non-detects were evaluated by assuming a value equal to one-half the method detection limit, and b) the concentrations in the prepared sample were converted to units of concentration in the original biological sample by dividing by the following factors:

- Liver: 0.1 kg wet weight/L prepared sample
- Kidney: 0.1 kg wet weight/L prepared sample.

The resulting values are shown in the right-hand columns of Tables B-8 and B-9.

Quality Assurance Data

A number of quality assurance samples were evaluated during this study to ensure the quality of the results, including 5% duplicates, 5% standards, and a program of inter-laboratory comparison. These steps are detailed below.

Duplicates

Duplicate samples were prepared and analyzed for about 5% of all samples generated during the study. Table B-10 lists the duplicate values for blood, liver, and kidney and the relative percent difference (RPD) for each pair of analyses.

Standards

Quality control standards from Environmental Resource Associates were used to verify instrument accuracy in the flame and furnace analysis of cadmium. ERA 697, Potable Water Metals was used for furnace analysis, and ERA 508, Flame AA Trace Metals was used for flame analysis. Quality assurance (QA) samples were analyzed every 10 samples, and QA results had to be within plus or minus 10% of the certified value for sample results to be acceptable. Cadmium spikes were run on the instrument, and spikes needed to be within ±15% to be considered acceptable.

A certified reference material from the National Research Council Canada, LUTS-1 (non-defatted lobster hepato-pancreas reference material), was used as a preparation QA material for the digestion and analysis of liver and kidney tissue.

Figure B-1
Bleed I: Blood Cadmium by Day
Control and CdCl2

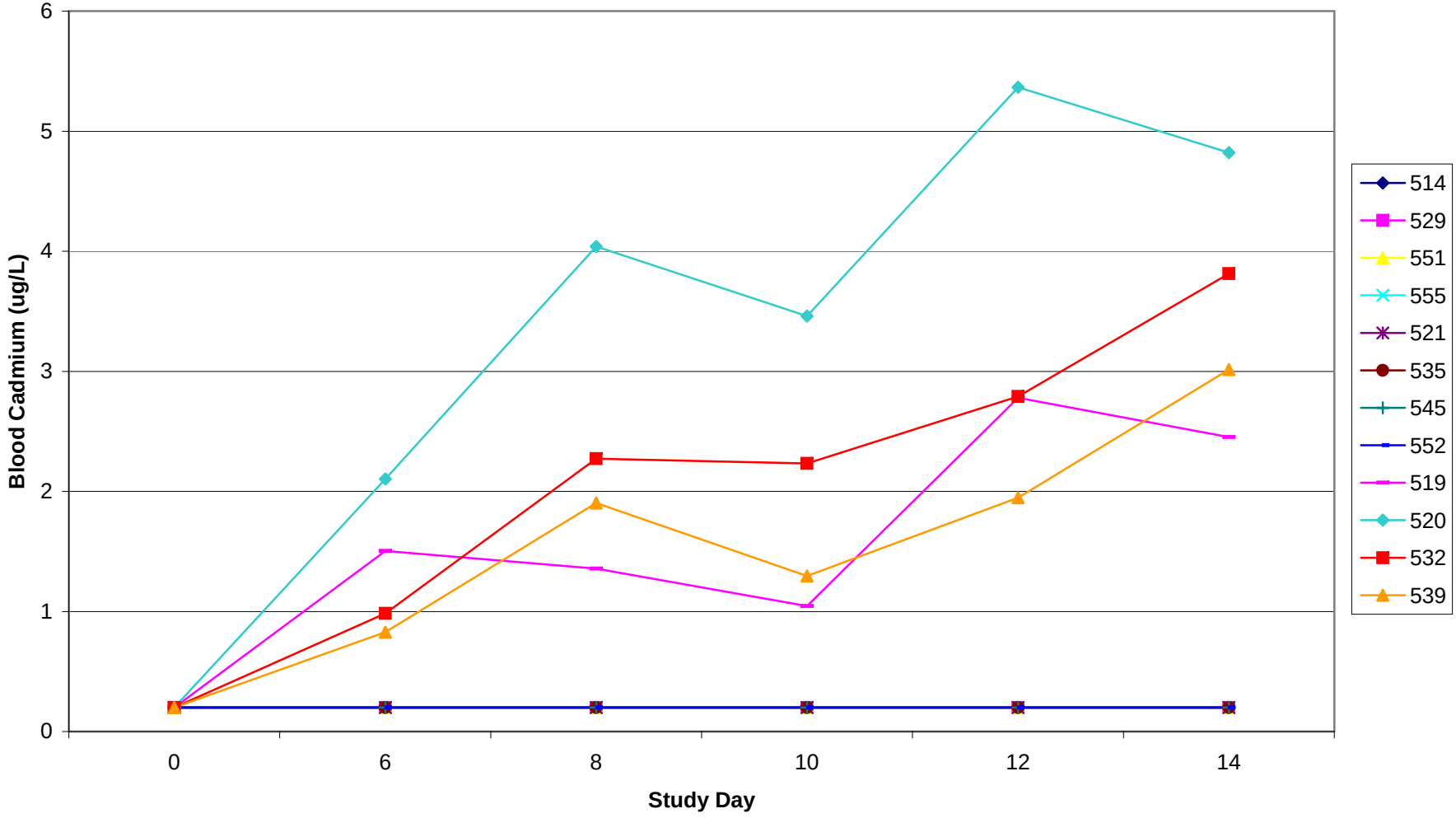


Figure B-2
Bleed I : Blood Cadmium by Day
PtMugu #1B

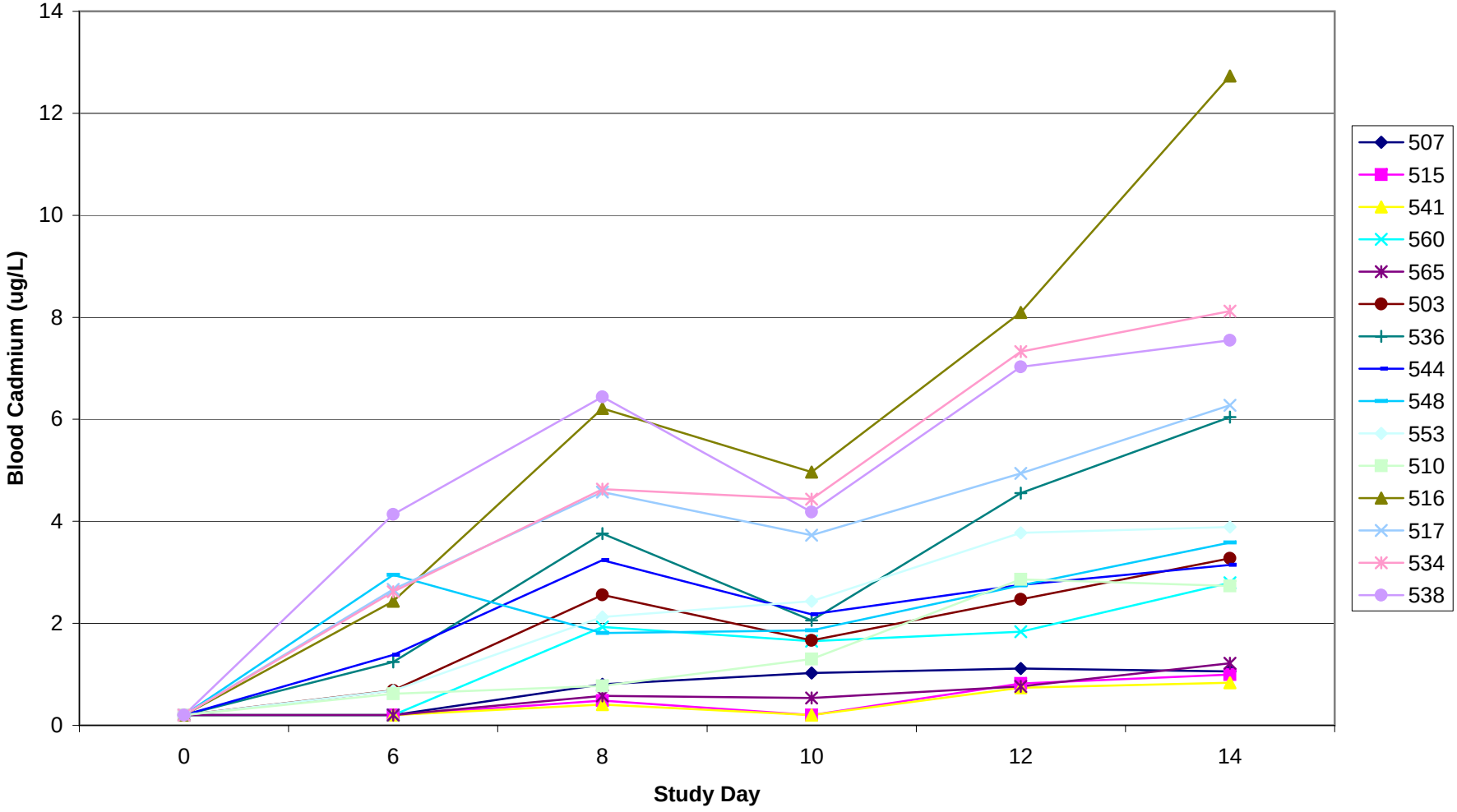


Figure B-3
Bleed II: Blood Cadmium by Day
Control and CdCl2

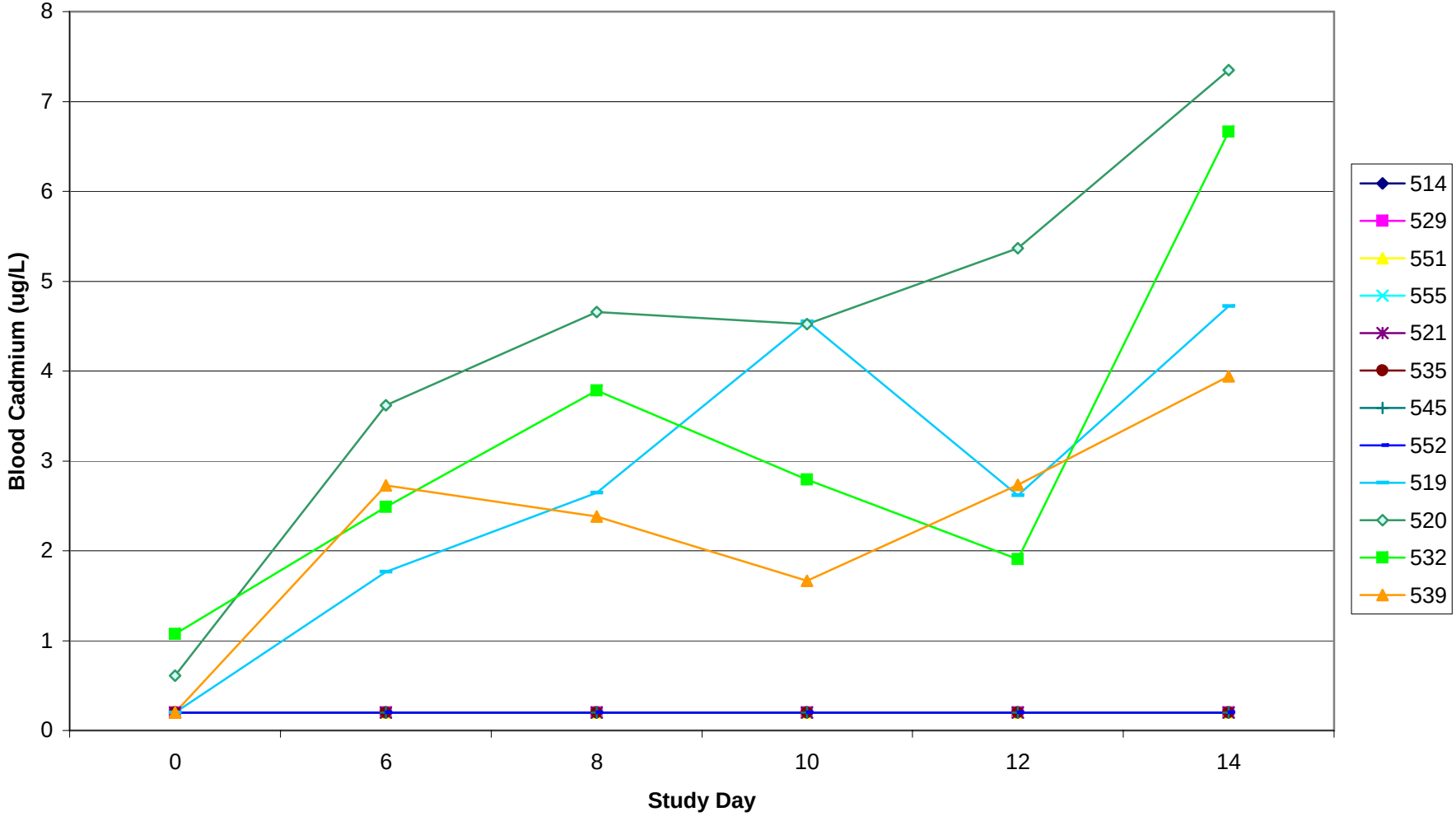


Figure B-4
Bleed II: Blood Cadmium by Day
PtMugu #1B

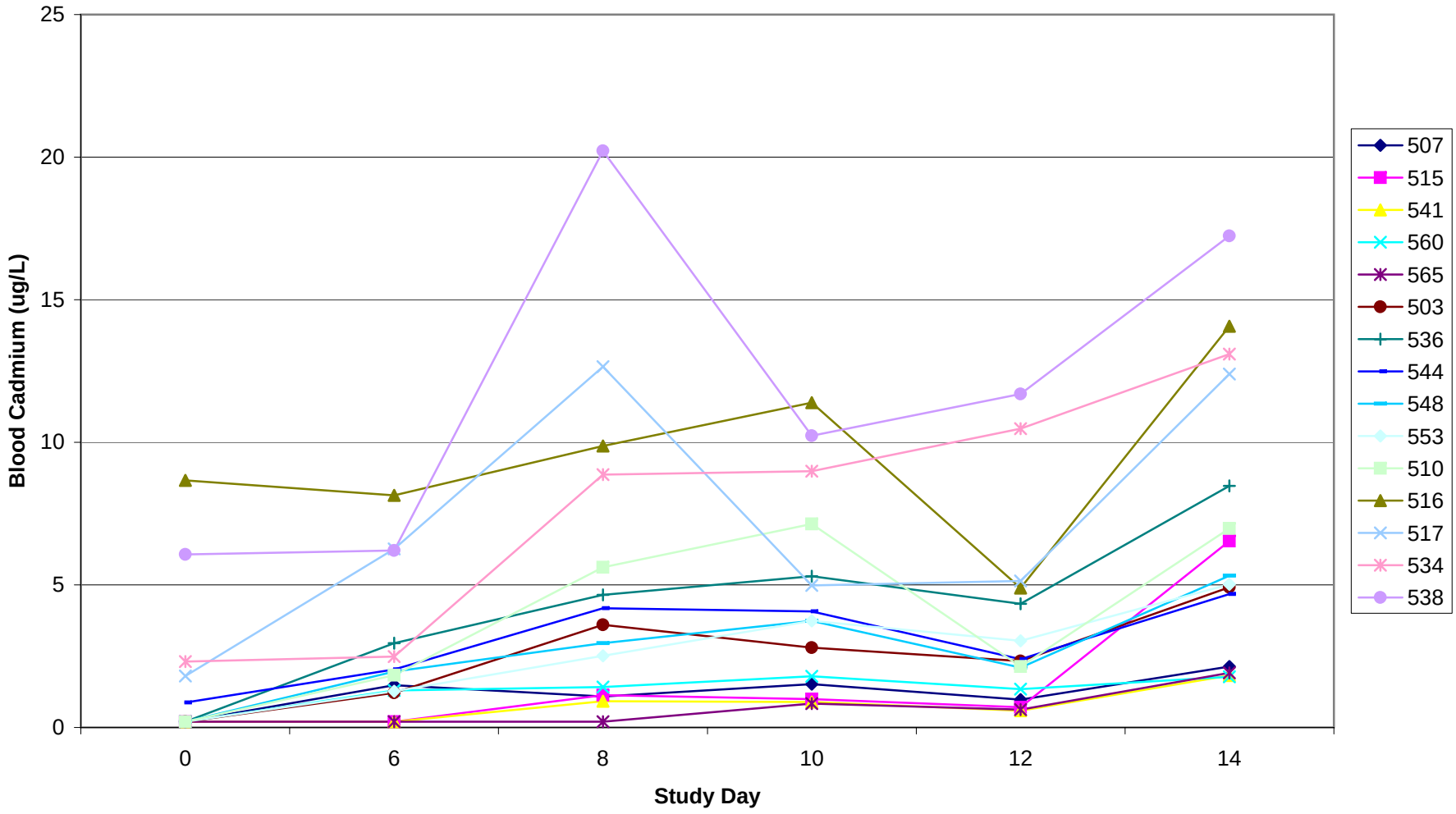


TABLE B-1 BODY WEIGHTS AND ADMINISTERED DOSES, BY DAY

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

Group	ID #	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		Day 15		
		BW (kg)	ug Pb per day	BW (kg)	ug Pb per day	BW (kg)	ug Pb per day	BW (kg)	ug Pb per day	BW (kg)	ug Pb per day	BW (kg)	ug Pb per day	BW (kg)	ug Pb per day	BW (kg)	ug Pb per day	BW (kg)	ug Pb per day	BW (kg)	ug Pb per day	BW (kg)	ug Pb per day	BW (kg)	ug Pb per day	BW (kg)	ug Pb per day	BW (kg)	ug Pb per day	BW (kg)	ug Pb per day	BW (kg)	ug Pb per day	BW (kg)	ug Pb per day	
1	514	9.90	0	10.2	0	10.4	0	10.70	0	11.1	0	11.6	0	12	0	12.4	0	12.8	0	13.15	0	13.5	0	13.9	0	14.2	0	14.9	0	15.6	0	16.3	0	16.9	0	**0.4**
1	529	8.45	0	8.7	0	9.0	0	9.30	0	9.6	0	9.9	0	10.2	0	10.5	0	10.9	0	11.2	0	11.7	0	12.1	0	12.6	0	13.3	0	14.0	0	14.7	0	15.3	0	**0.4**
1	551	8.50	0	8.8	0	9.1	0	9.35	0	9.7	0	10.1	0	10.5	0	10.8	0	11.1	0	11.45	0	11.9	0	12.4	0	12.9	0	13.6	0	14.2	0	14.9	0	15.5	0	**0.4**
1	555	9.00	0	9.1	0	9.3	0	9.40	0	9.8	0	10.2	0	10.65	0	11.0	0	11.4	0	11.8	0	12.3	0	12.8	0	13.3	0	14.0	0	14.8	0	15.5	0	16.2	0	**0.4**
2	508	9.65	0	9.9	108	10.1	108	10.35	108	10.7	115	11.1	115	11.45	115	11.9	126	12.4	126	12.9	126	13.4	138	13.9	138	14.35	138	15.0	151	15.6	151	16.2	151	16.8	0	**0.4**
2	528	9.90	0	10.1	108	10.2	108	10.35	108	10.8	115	11.2	115	11.55	115	11.9	126	12.3	126	12.6	126	12.9	138	13.1	138	13.35	138	14.1	151	14.9	151	15.7	151	16.4	0	**0.4**
2	540	10.90	0	11.1	108	11.3	108	11.50	108	11.8	115	12.1	115	12.45	115	12.9	126	13.3	126	13.7	126	14.2	138	14.6	138	15.1	138	15.6	151	16.0	151	16.5	151	17.0	0	**0.3**
2	550	8.60	0	9.0	108	9.3	108	9.70	108	10.1	115	10.5	115	10.85	115	11.3	126	11.7	126	12.05	126	12.5	138	13.0	138	13.45	138	14.2	151	15.0	151	15.7	151	16.5	0	**0.4**
3	521	10.00	0	10.2	594	10.3	594	10.45	594	10.8	640	11.2	640	11.5	640	11.9	706	12.2	706	12.6	706	13.0	775	13.4	775	13.85	775	14.6	849	15.3	849	16.1	849	16.8	0	**0.4**
3	535	8.15	0	8.5	594	8.8	594	9.10	594	9.5	640	9.9	640	10.25	640	10.7	706	11.2	706	11.6	706	12.0	775	12.5	775	12.9	775	13.6	849	14.2	849	14.9	849	15.5	0	**0.4**
3	545	7.75	0	8.1	594	8.4	594	8.70	594	9.1	640	9.5	640	9.95	640	10.3	706	10.7	706	11.1	706	11.6	775	12.1	775	12.55	775	13.1	849	13.6	849	14.1	849	14.6	0	**0.4**
3	552	9.70	0	9.9	594	10.2	594	10.40	594	10.7	640	11.0	640	11.35	640	11.7	706	12.0	706	12.35	706	12.7	775	13.0	775	13.3	775	14.2	849	15.1	849	16.1	849	17.0	0	**0.4**
4	519	10.10	0	10.3	3392	10.5	3392	10.65	3392	11.0	3604	11.3	3604	11.55	3604	12.0	3872	12.5	3872	13	3872	13.4	4288	13.8	4288	14.15	4288	15.0	4696	15.8	4696	16.6	4696	17.4	0	**0.4**
4	520	9.50	0	9.7	3392	9.9	3392	10.15	3392	10.5	3604	10.8	3604	11.15	3604	11.6	3872	12.0	3872	12.45	3872	12.9	4288	13.4	4288	13.85	4288	14.4	4696	15.0	4696	15.6	4696	16.1	0	**0.4**
4	532	9.15	0	9.4	3392	9.6	3392	9.80	3392	10.1	3604	10.4	3604	10.7	3604	11.1	3872	11.4	3872	11.8	3872	12.2	4288	12.5	4288	12.9	4288	13.6	4696	14.2	4696	14.9	4696	15.5	0	**0.4**
4	539	9.65	0	9.9	3392	10.2	3392	10.45	3392	10.6	3604	10.8	3604	11	3604	11.5	3872	11.9	3872	12.35	3872	12.8	4288	13.3	4288	13.8	4288	14.4	4696	15.0	4696	15.6	4696	16.2	0	**0.4**
5	507	10.55	0	10.8	2363	11.1	2363	11.40	2363	11.7	2560	12.0	2560	12.3	2560	12.7	2778	13.1	2778	13.55	2778	14.0	3071	14.4	3071	14.8	3071	15.4	3394	16.0	3394	16.7	3394	17.3	0	**0.4**
5	515	10.85	0	11.2	2363	11.6	2363	11.90	2363	12.3	2560	12.6	2560	12.95	2560	13.4	2778	13.8	2778	14.15	2778	14.6	3071	15.1	3071	15.6	3071	16.2	3394	16.8	3394	17.4	3394	18.0	0	**0.4**
5	541	8.45	0	8.7	2363	9.0	2363	9.20	2363	9.5	2560	9.7	2560	9.95	2560	10.4	2778	10.8	2778	11.25	2778	11.7	3071	12.2	3071	12.7	3071	13.4	3394	14.1	3394	14.8	3394	15.4	0	**0.4**
5	560	7.50	0	7.8	2363	8.1	2363	8.35	2363	8.7	2560	9.1	2560	9.5	2560	10.0	2778	10.5	2778	10.95	2778	11.4	3071	11.8	3071	12.15	3071	12.8	3394	13.5	3394	14.2	3394	14.9	0	**0.4**
5	565	8.05	0	8.3	2363	8.5	2363	8.75	2363	9.0	2560	9.3	2560	9.55	2560	9.9	2778	10.3	2778	10.6	2778	11.1	3071	11.6	3071	12.15	3071	12.7	3394	13.3	3394	13.9	3394	14.5	0	**0.4**
6	503	10.80	0	11.1	4440	11.5	4440	11.80	4440	12.2	4782	12.6	4782	13	4782	13.3	5354	13.7	5354	14	5354	14.5	5888	14.9	5888	15.4	5888	16.1	6526	16.8	6526	17.6	6526	18.3	0	**0.4**
6	536	7.65	0	7.8	4440	8.0	4440	8.20	4440	8.6	4782	9.0	4782	9.35	4782	9.8	5354	10.2	5354	10.6	5354	11.0	5888	11.5	5888	11.9	5888	12.6	6526	13.3	6526	14.0	6526	14.7	0	**0.4**
6	544	8.10	0	8.4	4440	8.7	4440	8.95	4440	9.4	4782	9.8	4782	10.25	4782	10.7	5354	11.2	5354	11.6	5354	12.1	5888	12.5	5888	12.95	5888	13.5	6526	14.1	6526	14.6	6526	15.2	0	**0.4**
6	548	8.30	0	8.5	4440	8.8	4440	9.00	4440	9.4	4782	9.8	4782	10.15	4782	10.5	5354	10.9	5354	11.25	5354	11.7	5888	12.2	5888	12.6	5888	13.2	6526	13.8	6526	14.4	6526	14.9	0	**0.4**
6	553	7.50	0	7.7	4440	7.9	4440	8.05	4440	8.5	4782	8.9	4782	9.35	4782	9.7	5354	10.0	5354	10.35	5354	10.8	5888	11.3	5888	11.75	5888	12.3	6526	12.9	6526	13.5	6526	14.0	0	**0.4**
7	510	8.60	0	8.7	9526	8.9	9526	9.00	9526	9.3	10257	9.7	10257	10	10257	10.4	11232	10.9	11232	11.3	11232	11.8	12470	12.2	12470	12.7	12470	13.3	13736	13.8	13736	14.4	13736	14.9	0	**0.4**
7	516	9.80	0	10.2	9526	10.6	9526	10.95	9526	11.4	10257	11.8	10257	12.2	10257	12.6	11232	13.0	11232	13.45	11232	13.8	12470	14.2	12470	14.6	12470	15.2	13736	15.8	13736	16.4	13736	17.0	0	**0.4**
7	517	9.30	0	9.4	9526	9.6	9526	9.70	9526	10.0	10257	10.3	10257	10.55	10257	11.0	11232	11.5	11232	12	11232	12.5	12470	13.0	12470	13.55	12470	14.3	13736	15.0	13736	15.7	13736	16.4	0	**0.4**
7	534	9.40	0	9.7	9526	10.0	9526	10.35	9526	10.7	10257	11.0	10257	11.3	10257	11.7	11232	12.0	11232	12.4	11232	12.8	12470	13.2	12470	13.6	12470	14.4	13736	15.1	13736	15.9	13736	16.6	0	**0.4**
7	538	8.70	0	9.0	9526	9.4	9526	9.70	9526	10.1	10257	10.5	10257	10.85	10257	11.4	11232	11.9	11232	12.35	11232	12.8	12470	13.3	12470	13.8	12470	14.3	13736	14.8	13736	15.4	13736	15.9	0	**0.4**
8	513	9.15	0	9.5	189	9.8	189	10.10	189	10.4	207	10.7	207	11.05	207	11.4	233	11.8	233	12.15	233	12.6	255	13.0	255	13.35	255	13.8	285	14.3	285	14.8	285	15.3	0	**0.3**
8	537	8.10	0	8.4	189	8.7	189	9.00	189	9.4	207	9.7	207	10.1	207	10.5	233	10.9	233	11.25	233	11.7	255	12.2	255	12.65	255	13.2	285	13.8	285	14.4	285	14.9	0	**0.4**
8	559	6.70	0	7.0	189	7.2	189	7.45	189	7.9	207	8.4	207	8.9	207	9.3	233	9.7	233	10.1	233	10.7	255	11.2	255	11.75	255	12.3	285	12.8	285	13.3	285	13.8	0	**0.4**
8	562	8.50	0	8.8	189	9.1	189	9.35	189	9.8	207	10.3	207	10.75	207	11.0	233	11.3	233	11.6	233	12.1	255	12.6	-255	13.1	255	14.0	285	14.8	285	15.7	285	16.5	0	**0.5**
9	501	9.00	0	9.3	609	9.5	609	9.80	609	10.2	660	10.6	660	11	660	11.5	728	12.0	728	12.45	728	13.0	807	13.5	807	13.95	807	14.5	892	15.1	892	15.7	892	16.3	0	**0.4**
9	511	9.25	0	9.5	609	9.8	609	10.05	609	10.5	660	11.0	660	11.4	660	11.8	728	12.2	728	12.55	728	13.0	807	13.5	807	13.95	807	14.7	892	15.5	892	16.2	892	17.0	0	**0.4**
9	522	9.65	0	9.9	609	10.2	609	10.40	609	10.7	660	11.0	660	11.25	660	11.7	728	12.1	728	12.5	728	12.9	807	13.2	807	13.55	807	14.1	892	14.7	892	15.3	892	15.9	0	**0.4**
9	543	7.20	0	7.5	609	7.8	609	8.10	609	8.4	660	8.7	660	9.05	660	9.5	728	9.9	728	10.3	728	10.8	807	11.3	807	11.8	807	13.1	892	14.3	892	15.6	892	16.9	0	**0.6**
10	505	8.20	0	8.5	218	8.7	218	9.00	218	9.3	235	9.6	235	9.95	235	10.4	260	10.8	260	11.2	260	11.7	282	12.1	282	12.55	282	13.3	311	14.1	311	14.8	311	15.6	0	**0.4**
10	518	10.50	0	10.7	218	10.9	218	11.10	218	11.6	235	12.0	235	12.5	235	12.7	260	13.0	260	13.2	260	13.7	282	14.2	282	14.65	282	15.3	311	15.9	311	16.6	311	17.2	0	**0.4**
10	533	9.60	0	10.0	218	10.3	218	10.65	218	11.1	235	11.6	235	12	235	12.4	260	12.7	260	13.1	260	13.6	282	14.0	282	14.45	282	15.1	311	15.7	311	16.3	311	16.9	0	**0.4**
10	546	9.75	0	10.0	218	10.3	218	10.60	218	11.0	235	11.4	235	11.75	235	12.2	260	12.6	260	13.05	260	13.5	282	14.0	282	14.45	282	15.2	311	16.0	311	16.8	311	17.6	0	**0.4**
11	502	10.65	0	11.0	646	11.3	646	11.60	646	12.1	694	12.5	694	12.95	694	13.4	783	13.8	783	14.25	783	14.7	864	15.2	864	15.7	864	16.7	951	17.7	951	18.7	951	19.7	0	**0.5**
11	509	8.40	0	8.6	646	8.7	646	8.90	646	9.4	694	9.8	694	10.3	694	10.8	783	11.3	783	11.8	783	12.4	864	12.9	864	13.45	864	14.2	951	14.9	951	15.6	951	16.3	0	**0.5**
11	542	9.35	0	9.5	646	9.7	646	9.85	646	10.4	694	11.0	694	11.55	694	11.9	783	12.3	783	12.65	783	13.0	864	13.4	864	13.8	864	14.6	951	15.5	951	16.3	951	17.1	0	**0.4**
11	556	9.20	0	9.6	646	10.0	646	10.35	646	10.8	694	11.2	694	11.6	694	12.0	783	12.5	783	12.9	783	13.4	864	13.8	864	14.25	864	15.4	951	16.6	951	17.7	951	18.9	0	**0.5**
12	526	9.20	0	9.6	220	9.9	220	10.25	220	10	240	11.1	240	11.5	240	12.1	271	12.6	271	13.15	271	13.7	302	14.3	302	14.8	302	15.4	338	16.0	338	16.6	338	17.2	0	**0.5**
12	527	8.30	0	8.6	220	8.9	220	9.20	220	9.6	240	10.1	240	10.5	240	10.9	271	11.3	271	11.7	271	12.2	302	12.7	302	13.2	302	13.7	338	14.2	338	14.7	338	15.2	1	**0.4**
12	549	8.00	0	8.3	220	8.6	220	8.85	220	9.3	240	9.7	240	10.15	240	10.6	271	11.0	271	11.45	271	12.0	302	12.5	302	13.05	302	13.8	338	14.6	338	15.3	338	16.1	2	**0.5**
12	564	8.05	0	8.3	220	8.5	220	8.70	220	9.2	240	9.6	240	10.1	240	10.5	271	10.9	271	11.25	271	11.7	302	12.2	302	12.7	302	13.3	338	13.8	338	14.4	338	15.0	3	**0.4**
13	506	9.55	0	9.7	711	9.8	711	9.95	711	10.6	747	11.2	747	11.75	747	12.4	850	13.0	850	13.6	850	14.1	970	14.5	970	14.95	970	15.8	1085	16.7	1085	17.5	1085	18.4	4	**0.5**
13	524	10.30	0	10.3	711	10.3	711	10.35	711	10.7	747	11.1	747	11.5	747	12.2	850	12.9	850	13.55	850	14.1	970	14.7	970	15.25	970	16.0	1085	16.7	1085	17.4	1085	18.1	5	**0.5**
13	531	8.05	0	8.4	711	8.8	711	9.15	711	9.7	747	10.3	747	10.8	747	11.3	850	11.8	850	12.25	850	12.8	970	13.4	970	13.95	970	14.7	1085	15.4	1085	16.1	1085	16.8	6	**0.5**
13	558	8.60	0	8.8	711	8.9	711	9.10	711	9.5	747	10.0	747	10.4	747	10.9	850	11.4	850	11.85	850	12.5	970	13.1	970	13.7	970	14.3	1085	15.0	1085	15.6	1085	16.2	7	**0.4**

Table B-2. Body Weight Adjusted Doses
(Dose for Day/BW for Day)

Group	ID #	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	Avg Dose	Target Dose	% Target	Avg %	Group Mean Avg dose
1	514	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0			
1	529	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0			
1	551	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0			
1	555	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0			0
2	508	10.89	10.64	10.40	10.71	10.35	10.02	10.54	10.13	9.75	10.32	9.96	9.63	10.08	9.69	9.33	10.16	10	102		
2	528	10.71	10.55	10.40	10.67	10.29	9.94	10.57	10.27	9.98	10.75	10.54	10.35	10.67	10.12	9.62	10.36	10	104		
2	540	9.70	9.52	9.36	9.71	9.46	9.22	9.77	9.47	9.18	9.75	9.44	9.15	9.68	9.39	9.13	9.46	10	95		
2	550	12.00	11.53	11.10	11.38	10.96	10.58	11.18	10.79	10.44	11.04	10.64	10.27	10.61	10.08	9.59	10.81	10	108	102	10
3	521	58.52	57.67	56.84	59.24	57.38	55.63	59.47	57.69	56.01	59.52	57.67	55.94	58.22	55.43	52.90	57.21	60	95		
3	535	70.16	67.63	65.27	67.46	64.84	62.41	65.96	63.30	60.84	64.38	62.15	60.06	62.66	59.79	57.17	63.60	60	106		
3	545	73.64	70.85	68.28	70.17	67.11	64.30	68.30	65.86	63.58	66.88	64.21	61.73	65.06	62.66	60.43	66.20	60	110		
3	552	59.80	58.43	57.12	59.70	57.98	56.37	60.41	58.73	57.15	61.16	59.67	58.25	59.72	56.10	52.90	58.23	60	97	102	61
4	519	329.85	324.08	318.50	329.13	320.36	312.03	321.77	309.35	297.85	320.40	311.48	303.04	313.76	297.53	282.89	312.80	320	98		
4	520	349.09	341.48	334.19	343.78	333.19	323.23	334.27	322.22	311.00	331.97	320.40	309.60	325.73	313.41	301.99	326.37	320	102		
4	532	362.14	353.95	346.12	356.83	346.54	336.82	349.88	338.66	328.14	352.44	342.13	332.40	346.57	330.70	316.23	342.64	320	107		
4	539	342.05	333.09	324.59	338.93	333.19	327.64	338.17	325.38	313.52	334.13	322.00	310.72	326.11	313.07	301.03	325.57	320	102	102	327
5	507	218.10	212.54	207.26	218.77	213.30	208.10	218.42	211.49	204.99	219.85	213.48	207.47	220.16	211.69	203.85	212.63	100	213		
5	515	210.96	204.57	198.55	208.95	203.15	197.65	208.06	202.01	196.30	209.84	203.13	196.83	209.51	202.03	195.06	203.11	100	203		
5	541	271.58	263.99	256.82	270.86	263.88	257.25	267.51	256.79	246.90	261.70	251.35	241.78	253.61	241.29	230.11	255.69	100	256		
5	560	303.56	292.90	282.96	293.09	280.76	269.43	278.23	265.38	253.66	270.54	261.33	252.73	264.47	251.10	239.02	270.61	100	271		
5	565	285.24	277.42	270.03	283.88	275.72	268.02	280.57	270.99	262.04	276.22	263.95	252.73	266.55	254.88	244.18	268.83	100	269	242	242
6	503	398.76	387.17	376.23	391.94	379.50	367.82	401.52	391.73	382.40	407.01	394.29	382.34	404.90	387.66	371.83	388.34	200	194		
6	536	566.74	553.78	541.40	557.09	533.28	511.41	548.16	525.73	505.06	533.66	513.50	494.80	517.91	490.65	466.12	523.95	200	262		
6	544	529.56	512.25	496.03	509.60	487.10	466.51	500.34	480.15	461.52	488.64	471.05	454.68	483.38	464.46	446.96	483.48	200	242		
6	548	520.25	506.41	493.28	509.60	489.60	471.11	509.06	491.91	475.88	503.25	484.62	467.31	494.99	474.02	454.75	489.74	200	245		
6	553	577.81	564.34	551.49	563.66	536.27	511.41	552.87	534.47	517.26	544.35	521.84	501.11	529.82	506.52	485.18	533.23	200	267	242	484
7	510	1090.76	1074.35	1058.44	1098.99	1061.10	1025.73	1076.58	1033.65	994.01	1059.77	1019.34	981.89	1036.66	995.34	957.19	1037.59	400	259		
7	516	935.44	901.51	869.95	902.40	870.49	840.76	890.28	861.82	835.12	901.44	877.14	854.11	903.67	869.35	837.54	876.73	400	219		
7	517	1009.82	995.74	982.06	1027.44	999.08	972.25	1018.04	975.31	936.03	996.27	956.78	920.29	963.91	918.78	877.68	969.97	400	242		
7	534	980.37	949.43	920.38	961.62	933.89	907.72	962.77	933.44	905.84	974.22	944.70	916.91	957.19	909.65	866.61	934.98	400	234		
7	538	1054.53	1017.00	982.06	1017.25	979.99	945.37	989.64	947.88	909.50	971.69	936.42	903.62	959.42	926.00	894.84	962.35	400	241	239	956
8	513	20.00	19.36	18.75	19.90	19.31	18.76	20.39	19.75	19.16	20.33	19.70	19.11	20.60	19.90	19.25	19.62	20	98		
8	537	22.54	21.77	21.04	22.13	21.30	20.52	22.20	21.42	20.69	21.77	20.94	20.17	21.56	20.67	19.86	21.24	20	106		
8	559	27.25	26.30	25.42	26.13	24.63	23.29	25.03	24.00	23.04	23.95	22.78	21.71	23.23	22.29	21.43	24.03	20	120		
8	562	21.56	20.89	20.25	21.12	20.16	19.28	21.10	20.57	20.07	21.08	-20.25	19.47	20.43	19.25	18.21	17.55	20	88	103	21
9	501	65.76	63.93	62.19	64.71	62.27	60.01	63.39	60.83	58.46	62.34	60.03	57.88	61.40	59.03	56.84	61.27	60	102		
9	511	64.04	62.29	60.64	62.86	60.28	57.90	61.77	59.83	58.00	62.03	59.88	57.88	60.70	57.75	55.08	60.06	60	100		
9	522	61.56	60.04	58.60	61.79	60.19	58.67	62.39	60.24	58.23	62.83	61.16	59.58	63.14	60.63	58.32	60.49	60	101		
9	543	81.26	78.13	75.24	78.42	75.58	72.94	76.89	73.65	70.67	74.76	71.45	68.42	68.29	62.25	57.20	72.34	60	121	106	64
10	505	25.72	24.93	24.20	25.21	24.38	23.60	25.08	24.11	23.21	24.25	23.35	22.51	23.40	22.15	21.03	23.81	20	119		
10	518	20.35	19.98	19.62	20.30	19.52	18.79	20.42	20.05	19.69	20.64	19.94	19.28	20.36	19.55	18.81	19.82	20	99		
10	533	21.89	21.14	20.45	21.16	20.33	19.57	21.02	20.42	19.84	20.85	20.18	19.55	20.66	19.84	19.09	20.40	20	102		
10	546	21.70	21.11	20.54	21.38	20.66	19.99	21.34	20.60	19.92	20.90	20.20	19.55	20.43	19.43	18.53	20.42	20	102	106	21
11	502	58.93	57.28	55.71	57.63	55.56	53.63	58.51	56.67	54.95	58.63	56.77	55.02	56.93	53.72	50.84	56.05	60	93		
11	509	75.44	74.00	72.62	74.14	70.62	67.42	72.50	69.29	66.36	69.94	66.96	64.22	67.11	63.88	60.95	69.03	60	115		
11	542	67.91	66.74	65.61	66.67	63.23	60.13	65.71	63.74	61.90	66.28	64.38	62.59	64.97	61.47	58.33	63.98	60	107		
11	556	67.44	64.84	62.44	64.50	62.10	59.87	65.07	62.81	60.70	64.70	62.59	60.62	61.74	57.45	53.72	62.04	60	103	105	63
12	526	23.00	22.19	21.43	23.40	21.64	20.86	22.45	21.47	20.58	22.01	21.16	20.38	21.94	21.11	20.35	21.60	20	108		
12	527	25.54	24.68	23.88	24.90	23.83	22.84	24.82	23.94	23.13	24.72	23.75	22.85	24.66	23.79	22.98	24.02	20	120		
12	549	26.52	25.64	24.82	25.84	24.68	23.63	25.56	24.56	23.63	25.17	24.09	23.11	24.48	23.22	22.08	24.47	20	122		
12	564	26.57	25.89	25.25	26.17	24.90	23.75	25.81	24.90	24.05	25.70	24.68	23.75	25.47	24.42	23.46	24.98	20	125	119	24
13	506	73.40	72.40	71.43	70.78	66.97	63.55	68.76	65.49	62.52	69.01	66.87	64.86	68.70	65.19	62.03	67.47	60	112		
13	524	68.90	68.78	68.67	69.57	67.17	64.94	69.79	66.09	62.75	68.69	66.04	63.58	68.05	65.19	62.56	66.72	60	111		
13	531	84.45	80.92	77.68	76.98	72.85	69.14	75.36	72.26	69.41	75.65	72.45	69.51	74.01	70.56	67.42	73.91	60	123		
13	558	81.08	79.56	78.11	78.33	74.93	71.80	78.13	74.81	71.76	77.78	74.11	70.78	75.73	72.53	69.58	75.27	60	125	118	71

Table B-3 RAW AND ADJUSTED BLOOD CADMIUM DATA
EXPCD-1, MAY 2003
BLEEDS I AND II

Detection Limit (DL) 0.1ug/L 1ml blood, 3ml 1MHNO3
possible outlier

matrix	index	group	Material Administered	Dosage	pig number	tag number	Qualifier	Result (ug/L)	day	Dilution Adjusted Value ug/L)
blood	1	1	Control	0.0	514	EXPCD-1-0149	<	0.1	0-I	0.2
blood	2	1	Control	0.0	529	EXPCD-1-0150	<	0.1	0-I	0.2
blood	3	1	Control	0.0	551	EXPCD-1-0126	<	0.1	0-I	0.2
blood	4	1	Control	0.0	555	EXPCD-1-0135	<	0.1	0-I	0.2
blood	5	3	CdCl2	61.3	521	EXPCD-1-0156	<	0.1	0-I	0.2
blood	6	3	CdCl2	61.3	535	EXPCD-1-0132	<	0.1	0-I	0.2
blood	7	3	CdCl2	61.3	545	EXPCD-1-0152	<	0.1	0-I	0.2
blood	8	3	CdCl2	61.3	552	EXPCD-1-0153	<	0.1	0-I	0.2
blood	9	4	CdCl2	326.8	519	EXPCD-1-0148	<	0.1	0-I	0.2
blood	10	4	CdCl2	326.8	520	EXPCD-1-0160	<	0.1	0-I	0.2
blood	11	4	CdCl2	326.8	532	EXPCD-1-0136	<	0.1	0-I	0.2
blood	12	4	CdCl2	326.8	539	EXPCD-1-0139	<	0.1	0-I	0.2
blood	13	5	PtMugu#1B	242.2	507	EXPCD-1-0120	<	0.1	0-I	0.2
blood	14	5	PtMugu#1B	242.2	515	EXPCD-1-0157	<	0.1	0-I	0.2
blood	15	5	PtMugu#1B	242.2	541	EXPCD-1-0143	<	0.1	0-I	0.2
blood	16	5	PtMugu#1B	242.2	560	EXPCD-1-0141	<	0.1	0-I	0.2
blood	17	5	PtMugu#1B	242.2	565	EXPCD-1-0125	<	0.1	0-I	0.2
blood	18	6	PtMugu#1B	483.7	503	EXPCD-1-0129	<	0.1	0-I	0.2
blood	19	6	PtMugu#1B	483.7	536	EXPCD-1-0155	<	0.1	0-I	0.2
blood	20	6	PtMugu#1B	483.7	544	EXPCD-1-0140	<	0.1	0-I	0.2
blood	21	6	PtMugu#1B	483.7	548	EXPCD-1-0159	<	0.1	0-I	0.2
blood	22	6	PtMugu#1B	483.7	553	EXPCD-1-0147	<	0.1	0-I	0.2
blood	23	7	PtMugu#1B	956.3	510	EXPCD-1-0158	<	0.1	0-I	0.2
blood	24	7	PtMugu#1B	956.3	516	EXPCD-1-0131	<	0.1	0-I	0.2
blood	25	7	PtMugu#1B	956.3	517	EXPCD-1-0137	<	0.1	0-I	0.2
blood	26	7	PtMugu#1B	956.3	534	EXPCD-1-0130	<	0.1	0-I	0.2
blood	27	7	PtMugu#1B	956.3	538	EXPCD-1-0123	<	0.1	0-I	0.2
blood	28	9	CO-SCS	63.5	501	EXPCD-1-0142	<	0.1	0-I	0.2
blood	29	9	CO-SCS	63.5	511	EXPCD-1-0151	<	0.1	0-I	0.2
blood	30	9	CO-SCS	63.5	522	EXPCD-1-0146	<	0.1	0-I	0.2
blood	31	9	CO-SCS	63.5	543	EXPCD-1-0138	<	0.1	0-I	0.2
blood	32	11	OK-SS	62.8	502	EXPCD-1-0128	<	0.1	0-I	0.2
blood	33	11	OK-SS	62.8	509	EXPCD-1-0124	<	0.1	0-I	0.2
blood	34	11	OK-SS	62.8	542	EXPCD-1-0133	<	0.1	0-I	0.2
blood	35	11	OK-SS	62.8	556	EXPCD-1-0154	<	0.1	0-I	0.2
blood	36	13	Dugway #1	70.8	506	EXPCD-1-0122	<	0.1	0-I	0.2
blood	37	13	Dugway #1	70.8	524	EXPCD-1-0134	<	0.1	0-I	0.2
blood	38	13	Dugway #1	70.8	531	EXPCD-1-0121	<	0.1	0-I	0.2
blood	39	13	Dugway #1	70.8	558	EXPCD-1-0145	<	0.1	0-I	0.2
blood	49	11			2556	EXPCD-1-0161	<	0.1	0-I	0.2
blood	50	7			2517	EXPCD-1-0144	<	0.1	0-I	0.2
blood	51	4			2519	EXPCD-1-0127	<	0.1	0-I	0.2
blood	54	1	Control	0.0	514	EXPCD-1-0178	<	0.1	0-II	0.2
blood	55	1	Control	0.0	529	EXPCD-1-0167	<	0.1	0-II	0.2
blood	56	1	Control	0.0	551	EXPCD-1-0180	<	0.1	0-II	0.2
blood	57	1	Control	0.0	555	EXPCD-1-0187	<	0.1	0-II	0.2
blood	58	3	CdCl2	61.3	521	EXPCD-1-0195	<	0.1	0-II	0.2
blood	59	3	CdCl2	61.3	535	EXPCD-1-0194	<	0.1	0-II	0.2
blood	60	3	CdCl2	61.3	545	EXPCD-1-0189	<	0.1	0-II	0.2
blood	61	3	CdCl2	61.3	552	EXPCD-1-0170		0.1	0-II	0.2
blood	62	4	CdCl2	326.8	519	EXPCD-1-0201	<	0.1	0-II	0.2
blood	63	4	CdCl2	326.8	520	EXPCD-1-0200		0.152	0-II	0.609
blood	64	4	CdCl2	326.8	532	EXPCD-1-0173		0.268	0-II	1.074
blood	65	4	CdCl2	326.8	539	EXPCD-1-0164		0.1	0-II	0.2
blood	66	5	PtMugu#1B	242.2	507	EXPCD-1-0183		0.1	0-II	0.2
blood	67	5	PtMugu#1B	242.2	515	EXPCD-1-0162	<	0.1	0-II	0.2
blood	68	5	PtMugu#1B	242.2	541	EXPCD-1-0191	<	0.1	0-II	0.2
blood	69	5	PtMugu#1B	242.2	560	EXPCD-1-0177		0.1	0-II	0.2
blood	70	5	PtMugu#1B	242.2	565	EXPCD-1-0174	<	0.1	0-II	0.2
blood	71	6	PtMugu#1B	483.7	503	EXPCD-1-0192	<	0.1	0-II	0.2
blood	72	6	PtMugu#1B	483.7	536	EXPCD-1-0199	<	0.1	0-II	0.2
blood	73	6	PtMugu#1B	483.7	544	EXPCD-1-0163		0.218	0-II	0.872
blood	74	6	PtMugu#1B	483.7	548	EXPCD-1-0175	<	0.1	0-II	0.2
blood	75	6	PtMugu#1B	483.7	553	EXPCD-1-0179	<	0.1	0-II	0.2
blood	76	7	PtMugu#1B	956.3	510	EXPCD-1-0166		0.1	0-II	0.2
blood	77	7	PtMugu#1B	956.3	516	EXPCD-1-0184		2.166	0-II	8.663
blood	78	7	PtMugu#1B	956.3	517	EXPCD-1-0186		0.45	0-II	1.801
blood	79	7	PtMugu#1B	956.3	534	EXPCD-1-0202		0.578	0-II	2.312

Table B-3 RAW AND ADJUSTED BLOOD CADMIUM DATA
EXPCD-1, MAY 2003
BLEEDS I AND II

Detection Limit (DL) 0.1ug/L 1ml blood, 3ml 1MHNO3
possible outlier

matrix	index	group	Material Administered	Dosage	pig number	tag number	Qualifier	Result (ug/L)	day	Dilution Adjusted Value ug/L)
blood	80	7	PtMugu#1B	956.3	538	EXPCD-1-0169		1.502	0-II	6.07
blood	81	9	CO-SCS	63.5	501	EXPCD-1-0185	<	0.1	0-II	0.2
blood	82	9	CO-SCS	63.5	511	EXPCD-1-0193	<	0.1	0-II	0.2
blood	83	9	CO-SCS	63.5	522	EXPCD-1-0176	<	0.1	0-II	0.2
blood	84	9	CO-SCS	63.5	543	EXPCD-1-0171	<	0.1	0-II	0.2
blood	85	11	OK-SS	62.8	502	EXPCD-1-0172	<	0.1	0-II	0.2
blood	86	11	OK-SS	62.8	509	EXPCD-1-0203	<	0.1	0-II	0.2
blood	87	11	OK-SS	62.8	542	EXPCD-1-0196	<	0.1	0-II	0.2
blood	88	11	OK-SS	62.8	556	EXPCD-1-0197	<	0.1	0-II	0.2
blood	89	13	Dugway #1	70.8	506	EXPCD-1-0182	<	0.1	0-II	0.2
blood	90	13	Dugway #1	70.8	524	EXPCD-1-0190	<	0.1	0-II	0.2
blood	91	13	Dugway #1	70.8	531	EXPCD-1-0168	<	0.1	0-II	0.2
blood	92	13	Dugway #1	70.8	558	EXPCD-1-0198	<	0.1	0-II	0.2
blood	93	4			2532	EXPCD-1-0188		0.239	0-II	0.956
blood	94	7			2516	EXPCD-1-0181		1.915	0-II	7.662
blood	95	1			2555	EXPCD-1-0165	<	0.1	0-II	0.2
blood	96	1	Control	0.0	514	EXPCD-1-0236	<	0.1	6-I	0.2
blood	97	1	Control	0.0	529	EXPCD-1-0216	<	0.1	6-I	0.2
blood	98	1	Control	0.0	551	EXPCD-1-0240	<	0.1	6-I	0.2
blood	99	1	Control	0.0	555	EXPCD-1-0238	<	0.1	6-I	0.2
blood	100	3	CdCl2	61.3	521	EXPCD-1-0245	<	0.1	6-I	0.2
blood	101	3	CdCl2	61.3	535	EXPCD-1-0212	<	0.1	6-I	0.2
blood	102	3	CdCl2	61.3	545	EXPCD-1-0207	<	0.1	6-I	0.2
blood	103	3	CdCl2	61.3	552	EXPCD-1-0243	<	0.1	6-I	0.2
blood	104	4	CdCl2	326.8	519	EXPCD-1-0209		0.376	6-I	1.504
blood	105	4	CdCl2	326.8	520	EXPCD-1-0232		0.526	6-I	2.104
blood	106	4	CdCl2	326.8	532	EXPCD-1-0227		0.246	6-I	0.984
blood	107	4	CdCl2	326.8	539	EXPCD-1-0234		0.207	6-I	0.828
blood	108	5	PtMugu#1B	242.2	507	EXPCD-1-0225	<	0.1	6-I	0.2
blood	109	5	PtMugu#1B	242.2	515	EXPCD-1-0235	<	0.1	6-I	0.2
blood	110	5	PtMugu#1B	242.2	541	EXPCD-1-0220	<	0.1	6-I	0.2
blood	111	5	PtMugu#1B	242.2	560	EXPCD-1-0228		contaminate	6-I	0.2
blood	112	5	PtMugu#1B	242.2	565	EXPCD-1-0222	<	0.1	6-I	0.2
blood	113	6	PtMugu#1B	483.7	503	EXPCD-1-0211		0.172	6-I	0.688
blood	114	6	PtMugu#1B	483.7	536	EXPCD-1-0241		0.311	6-I	1.244
blood	115	6	PtMugu#1B	483.7	544	EXPCD-1-0204		0.345	6-I	1.38
blood	116	6	PtMugu#1B	483.7	548	EXPCD-1-0217		0.737	6-I	2.948
blood	117	6	PtMugu#1B	483.7	553	EXPCD-1-0239		0.17	6-I	0.68
blood	118	7	PtMugu#1B	956.3	510	EXPCD-1-0242		0.155	6-I	0.62
blood	119	7	PtMugu#1B	956.3	516	EXPCD-1-0244		0.609	6-I	2.436
blood	120	7	PtMugu#1B	956.3	517	EXPCD-1-0205		0.666	6-I	2.664
blood	121	7	PtMugu#1B	956.3	534	EXPCD-1-0208		0.655	6-I	2.62
blood	122	7	PtMugu#1B	956.3	538	EXPCD-1-0214		1.034	6-I	4.136
blood	123	9	CO-SCS	63.5	501	EXPCD-1-0224	<	0.1	6-I	0.2
blood	124	9	CO-SCS	63.5	511	EXPCD-1-0237		0.039	6-I	0.2
blood	125	9	CO-SCS	63.5	522	EXPCD-1-0206		0.138	6-I	0.552
blood	126	9	CO-SCS	63.5	543	EXPCD-1-0221	<	0.1	6-I	0.2
blood	127	11	OK-SS	62.8	502	EXPCD-1-0213	<	0.1	6-I	0.2
blood	128	11	OK-SS	62.8	509	EXPCD-1-0219	<	0.1	6-I	0.2
blood	129	11	OK-SS	62.8	542	EXPCD-1-0215	<	0.1	6-I	0.2
blood	130	11	OK-SS	62.8	556	EXPCD-1-0229	<	0.135	6-I	0.54
blood	131	13	Dugway #1	70.8	506	EXPCD-1-0223	<	0.1	6-I	0.2
blood	132	13	Dugway #1	70.8	524	EXPCD-1-0231		0.1	6-I	0.2
blood	133	13	Dugway #1	70.8	531	EXPCD-1-0233	<	0.1	6-I	0.2
blood	134	13	Dugway #1	70.8	558	EXPCD-1-0230	<	0.1	6-I	0.2
blood	135	13			2558	EXPCD-1-0210	<	0.1	6-I	0.2
blood	136	9			2543	EXPCD-1-0218	<	0.1	6-I	0.2
blood	137	5			2565	EXPCD-1-0226	<	0.1	6-I	0.2
blood	138	1	Control	0.0	514	EXPCD-1-0269	<	0.1	6-II	0.2
blood	139	1	Control	0.0	529	EXPCD-1-0271	<	0.1	6-II	0.2
blood	140	1	Control	0.0	551	EXPCD-1-0249	<	0.1	6-II	0.2
blood	141	1	Control	0.0	555	EXPCD-1-0282	<	0.1	6-II	0.2
blood	142	3	CdCl2	61.3	521	EXPCD-1-0285	<	0.1	6-II	0.2
blood	143	3	CdCl2	61.3	535	EXPCD-1-0278	<	0.1	6-II	0.2
blood	144	3	CdCl2	61.3	545	EXPCD-1-0260	<	0.1	6-II	0.2
blood	145	3	CdCl2	61.3	552	EXPCD-1-0273	<	0.1	6-II	0.2
blood	146	4	CdCl2	326.8	519	EXPCD-1-0254		0.442	6-II	1.768
blood	147	4	CdCl2	326.8	520	EXPCD-1-0259		0.905	6-II	3.62

Table B-3 RAW AND ADJUSTED BLOOD CADMIUM DATA
EXPCD-1, MAY 2003
BLEEDS I AND II

Detection Limit (DL) 0.1ug/L 1ml blood, 3ml 1MHNO3
possible outlier

matrix	index	group	Material Administered	Dosage	pig number	tag number	Qualifier	Result (ug/L)	day	Dilution Adjusted Value ug/L)
blood	148	4	CdCl2	326.8	532	EXPCD-1-0263		0.622	6-II	2.488
blood	149	4	CdCl2	326.8	539	EXPCD-1-0251		0.682	6-II	2.728
blood	150	5	PtMugu#1B	242.2	507	EXPCD-1-0279		0.371	6-II	1.484
blood	151	5	PtMugu#1B	242.2	515	EXPCD-1-0277	<	0.1	6-II	0.2
blood	152	5	PtMugu#1B	242.2	541	EXPCD-1-0252	<	0.1	6-II	0.2
blood	153	5	PtMugu#1B	242.2	560	EXPCD-1-0270		0.324	6-II	1.296
blood	154	5	PtMugu#1B	242.2	565	EXPCD-1-0287	<	0.1	6-II	0.2
blood	155	6	PtMugu#1B	483.7	503	EXPCD-1-0246		0.305	6-II	1.22
blood	156	6	PtMugu#1B	483.7	536	EXPCD-1-0284		0.738	6-II	2.952
blood	157	6	PtMugu#1B	483.7	544	EXPCD-1-0247		0.508	6-II	2.032
blood	158	6	PtMugu#1B	483.7	548	EXPCD-1-0265		0.491	6-II	1.964
blood	159	6	PtMugu#1B	483.7	553	EXPCD-1-0256		0.317	6-II	1.268
blood	160	7	PtMugu#1B	956.3	510	EXPCD-1-0280		0.458	6-II	1.832
blood	161	7	PtMugu#1B	956.3	516	EXPCD-1-0253		2.036	6-II	8.144
blood	162	7	PtMugu#1B	956.3	517	EXPCD-1-0258		1.566	6-II	6.264
blood	163	7	PtMugu#1B	956.3	534	EXPCD-1-0267		0.621	6-II	2.484
blood	164	7	PtMugu#1B	956.3	538	EXPCD-1-0275		1.551	6-II	6.204
blood	165	9	CO-SCS	63.5	501	EXPCD-1-0261	<	0.1	6-II	0.2
blood	166	9	CO-SCS	63.5	511	EXPCD-1-0286	<	0.1	6-II	0.2
blood	167	9	CO-SCS	63.5	522	EXPCD-1-0262	<	0.1	6-II	0.2
blood	168	9	CO-SCS	63.5	543	EXPCD-1-0248	<	0.1	6-II	0.2
blood	169	11	OK-SS	62.8	502	EXPCD-1-0281	<	0.1	6-II	0.2
blood	170	11	OK-SS	62.8	509	EXPCD-1-0272	<	0.1	6-II	0.2
blood	171	11	OK-SS	62.8	542	EXPCD-1-0276	<	0.1	6-II	0.2
blood	172	11	OK-SS	62.8	556	EXPCD-1-0283	<	0.1	6-II	0.2
blood	173	13	Dugway #1	70.8	506	EXPCD-1-0255	<	0.1	6-II	0.2
blood	174	13	Dugway #1	70.8	524	EXPCD-1-0274	<	0.1	6-II	0.2
blood	175	13	Dugway #1	70.8	531	EXPCD-1-0264	<	0.1	6-II	0.2
blood	176	13	Dugway #1	70.8	558	EXPCD-1-0250	<	0.1	6-II	0.2
blood	177	9			2511	EXPCD-1-0268	<	0.1	6-II	0.2
blood	178	1			2551	EXPCD-1-0266	<	0.1	6-II	0.2
blood	179	13			2531	EXPCD-1-0257	<	0.1	6-II	0.2
blood	180	1	Control	0.0	514	EXPCD-1-0329	<	0.1	8-I	0.2
blood	181	1	Control	0.0	529	EXPCD-1-0314	<	0.1	8-I	0.2
blood	182	1	Control	0.0	551	EXPCD-1-0289	<	0.1	8-I	0.2
blood	183	1	Control	0.0	555	EXPCD-1-0327	<	0.1	8-I	0.2
blood	184	3	CdCl2	61.3	521	EXPCD-1-0302	<	0.1	8-I	0.2
blood	185	3	CdCl2	61.3	535	EXPCD-1-0318	<	0.1	8-I	0.2
blood	186	3	CdCl2	61.3	545	EXPCD-1-0324	<	0.1	8-I	0.2
blood	187	3	CdCl2	61.3	552	EXPCD-1-0308	<	0.1	8-I	0.2
blood	188	4	CdCl2	326.8	519	EXPCD-1-0304		0.339	8-I	1.356
blood	189	4	CdCl2	326.8	520	EXPCD-1-0321		1.01	8-I	4.04
blood	190	4	CdCl2	326.8	532	EXPCD-1-0322		0.568	8-I	2.272
blood	191	4	CdCl2	326.8	539	EXPCD-1-0315		0.476	8-I	1.904
blood	192	5	PtMugu#1B	242.2	507	EXPCD-1-0323		0.201	8-I	0.804
blood	193	5	PtMugu#1B	242.2	515	EXPCD-1-0290		0.121	8-I	0.484
blood	194	5	PtMugu#1B	242.2	541	EXPCD-1-0301		0.102	8-I	0.408
blood	195	5	PtMugu#1B	242.2	560	EXPCD-1-0320		0.482	8-I	1.928
blood	196	5	PtMugu#1B	242.2	565	EXPCD-1-0316		0.145	8-I	0.58
blood	197	6	PtMugu#1B	483.7	503	EXPCD-1-0328		0.639	8-I	2.556
blood	198	6	PtMugu#1B	483.7	536	EXPCD-1-0326		0.94	8-I	3.76
blood	199	6	PtMugu#1B	483.7	544	EXPCD-1-0319		0.81	8-I	3.24
blood	200	6	PtMugu#1B	483.7	548	EXPCD-1-0292		0.453	8-I	1.812
blood	201	6	PtMugu#1B	483.7	553	EXPCD-1-0294		0.531	8-I	2.124
blood	202	7	PtMugu#1B	956.3	510	EXPCD-1-0311		0.193	8-I	0.772
blood	203	7	PtMugu#1B	956.3	516	EXPCD-1-0296		1.554	8-I	6.216
blood	204	7	PtMugu#1B	956.3	517	EXPCD-1-0303		1.143	8-I	4.572
blood	205	7	PtMugu#1B	956.3	534	EXPCD-1-0313		1.157	8-I	4.628
blood	206	7	PtMugu#1B	956.3	538	EXPCD-1-0299		1.61	8-I	6.44
blood	207	9	CO-SCS	63.5	501	EXPCD-1-0305	<	0.1	8-I	0.2
blood	208	9	CO-SCS	63.5	511	EXPCD-1-0307	<	0.1	8-I	0.2
blood	209	9	CO-SCS	63.5	522	EXPCD-1-0310	<	0.1	8-I	0.2
blood	210	9	CO-SCS	63.5	543	EXPCD-1-0300	<	0.1	8-I	0.2
blood	211	11	OK-SS	62.8	502	EXPCD-1-0295	<	0.1	8-I	0.2
blood	212	11	OK-SS	62.8	509	EXPCD-1-0288	<	0.1	8-I	0.2
blood	213	11	OK-SS	62.8	542	EXPCD-1-0317	<	0.1	8-I	0.2
blood	214	11	OK-SS	62.8	556	EXPCD-1-0298	<	0.1	8-I	0.2
blood	215	13	Dugway #1	70.8	506	EXPCD-1-0325		0.116	8-I	0.464

Table B-3 RAW AND ADJUSTED BLOOD CADMIUM DATA
EXPCD-1, MAY 2003
BLEEDS I AND II

Detection Limit (DL) 0.1ug/L 1ml blood, 3ml 1MHNO3
possible outlier

matrix	index	group	Material Administered	Dosage	pig number	tag number	Qualifier	Result (ug/L)	day	Dilution Adjusted Value ug/L)
blood	216	13	Dugway #1	70.8	524	EXPCD-1-0297	<	0.1	8-I	0.2
blood	217	13	Dugway #1	70.8	531	EXPCD-1-0306	<	0.1	8-I	0.2
blood	218	13	Dugway #1	70.8	558	EXPCD-1-0291	<	0.1	8-I	0.2
blood	219	6			2536	EXPCD-1-0293		0.83	8-I	3.32
blood	220	9			2501	EXPCD-1-0312	<	0.1	8-I	0.2
blood	221	5			2515	EXPCD-1-0309	<	0.1	8-I	0.2
blood	222	1	Control	0.0	514	EXPCD-1-0358	<	0.1	8-II	0.2
blood	223	1	Control	0.0	529	EXPCD-1-0366	<	0.1	8-II	0.2
blood	224	1	Control	0.0	551	EXPCD-1-0360	<	0.1	8-II	0.2
blood	225	1	Control	0.0	555	EXPCD-1-0337	<	0.1	8-II	0.2
blood	226	3	CdCl2	61.3	521	EXPCD-1-0330	<	0.1	8-II	0.2
blood	227	3	CdCl2	61.3	535	EXPCD-1-0363	<	0.1	8-II	0.2
blood	228	3	CdCl2	61.3	545	EXPCD-1-0352	<	0.1	8-II	0.2
blood	229	3	CdCl2	61.3	552	EXPCD-1-0341	<	0.1	8-II	0.2
blood	230	4	CdCl2	326.8	519	EXPCD-1-0365		0.661	8-II	2.644
blood	231	4	CdCl2	326.8	520	EXPCD-1-0354		1.164	8-II	4.656
blood	232	4	CdCl2	326.8	532	EXPCD-1-0353		0.946	8-II	3.784
blood	233	4	CdCl2	326.8	539	EXPCD-1-0346		0.595	8-II	2.38
blood	234	5	PtMugu#1B	242.2	507	EXPCD-1-0348		0.272	8-II	1.088
blood	235	5	PtMugu#1B	242.2	515	EXPCD-1-0362		0.284	8-II	1.136
blood	236	5	PtMugu#1B	242.2	541	EXPCD-1-0355		0.231	8-II	0.924
blood	237	5	PtMugu#1B	242.2	560	EXPCD-1-0350		0.355	8-II	1.42
blood	238	5	PtMugu#1B	242.2	565	EXPCD-1-0359	<	0.1	8-II	0.2
blood	239	6	PtMugu#1B	483.7	503	EXPCD-1-0347		0.901	8-II	3.604
blood	240	6	PtMugu#1B	483.7	536	EXPCD-1-0339		1.163	8-II	4.652
blood	241	6	PtMugu#1B	483.7	544	EXPCD-1-0344		1.046	8-II	4.184
blood	242	6	PtMugu#1B	483.7	548	EXPCD-1-0351		0.74	8-II	2.96
blood	243	6	PtMugu#1B	483.7	553	EXPCD-1-0345		0.628	8-II	2.512
blood	244	7	PtMugu#1B	956.3	510	EXPCD-1-0338		1.405	8-II	5.62
blood	245	7	PtMugu#1B	956.3	516	EXPCD-1-0335		2.467	8-II	9.868
blood	246	7	PtMugu#1B	956.3	517	EXPCD-1-0340		3.164	8-II	12.656
blood	247	7	PtMugu#1B	956.3	534	EXPCD-1-0357		2.218	8-II	8.872
blood	248	7	PtMugu#1B	956.3	538	EXPCD-1-0371		5.055	8-II	20.22
blood	249	9	CO-SCS	63.5	501	EXPCD-1-0361	<	0.1	8-II	0.2
blood	250	9	CO-SCS	63.5	511	EXPCD-1-0349	<	0.1	8-II	0.2
blood	251	9	CO-SCS	63.5	522	EXPCD-1-0332	<	0.1	8-II	0.2
blood	252	9	CO-SCS	63.5	543	EXPCD-1-0368	<	0.1	8-II	0.2
blood	253	11	OK-SS	62.8	502	EXPCD-1-0356	<	0.1	8-II	0.2
blood	254	11	OK-SS	62.8	509	EXPCD-1-0364		0.271	8-II	1.084
blood	255	11	OK-SS	62.8	542	EXPCD-1-0331	<	0.1	8-II	0.2
blood	256	11	OK-SS	62.8	556	EXPCD-1-0333	<	0.1	8-II	0.2
blood	257	13	Dugway #1	70.8	506	EXPCD-1-0367	<	0.1	8-II	0.2
blood	258	13	Dugway #1	70.8	524	EXPCD-1-0334	<	0.1	8-II	0.2
blood	259	13	Dugway #1	70.8	531	EXPCD-1-0369	<	0.1	8-II	0.2
blood	260	13	Dugway #1	70.8	558	EXPCD-1-0336		0.1	8-II	0.2
blood	261	3			2521	EXPCD-1-0342	<	0.1	8-II	0.2
blood	262	1			2529	EXPCD-1-0343	<	0.1	8-II	0.2
blood	263	6			2544	EXPCD-1-0370		1.088	8-II	4.352
blood	264	1	Control	0.0	514	EXPCD-1-0393	<	0.1	10-I	0.2
blood	265	1	Control	0.0	529	EXPCD-1-0398	<	0.1	10-I	0.2
blood	266	1	Control	0.0	551	EXPCD-1-0404	<	0.1	10-I	0.2
blood	267	1	Control	0.0	555	EXPCD-1-0401	<	0.1	10-I	0.2
blood	268	3	CdCl2	61.3	521	EXPCD-1-0409	<	0.1	10-I	0.2
blood	269	3	CdCl2	61.3	535	EXPCD-1-0408	<	0.1	10-I	0.2
blood	270	3	CdCl2	61.3	545	EXPCD-1-0395	<	0.1	10-I	0.2
blood	271	3	CdCl2	61.3	552	EXPCD-1-0376	<	0.1	10-I	0.2
blood	272	4	CdCl2	326.8	519	EXPCD-1-0402		0.261	10-I	1.044
blood	273	4	CdCl2	326.8	520	EXPCD-1-0372		0.865	10-I	3.46
blood	274	4	CdCl2	326.8	532	EXPCD-1-0387		0.558	10-I	2.232
blood	275	4	CdCl2	326.8	539	EXPCD-1-0378		0.324	10-I	1.296
blood	276	5	PtMugu#1B	242.2	507	EXPCD-1-0389		0.257	10-I	1.028
blood	277	5	PtMugu#1B	242.2	515	EXPCD-1-0412	<	0.1	10-I	0.2
blood	278	5	PtMugu#1B	242.2	541	EXPCD-1-0400	<	0.1	10-I	0.2
blood	279	5	PtMugu#1B	242.2	560	EXPCD-1-0388		0.413	10-I	1.652
blood	280	5	PtMugu#1B	242.2	565	EXPCD-1-0375		0.134	10-I	0.536
blood	281	6	PtMugu#1B	483.7	503	EXPCD-1-0394		0.417	10-I	1.668
blood	282	6	PtMugu#1B	483.7	536	EXPCD-1-0407		0.514	10-I	2.056
blood	283	6	PtMugu#1B	483.7	544	EXPCD-1-0377		0.544	10-I	2.176

Table B-3 RAW AND ADJUSTED BLOOD CADMIUM DATA
EXPCD-1, MAY 2003
BLEEDS I AND II

Detection Limit (DL) 0.1ug/L 1ml blood, 3ml 1MHNO3
possible outlier

matrix	index	group	Material Administered	Dosage	pig number	tag number	Qualifier	Result (ug/L)	day	Dilution Adjusted Value ug/L)
blood	284	6	PtMugu#1B	483.7	548	EXPCD-1-0373		0.465	10-I	1.86
blood	285	6	PtMugu#1B	483.7	553	EXPCD-1-0390		0.608	10-I	2.432
blood	286	7	PtMugu#1B	956.3	510	EXPCD-1-0405		0.325	10-I	1.3
blood	287	7	PtMugu#1B	956.3	516	EXPCD-1-0399		1.241	10-I	4.964
blood	288	7	PtMugu#1B	956.3	517	EXPCD-1-0374		0.932	10-I	3.728
blood	289	7	PtMugu#1B	956.3	534	EXPCD-1-0381		1.109	10-I	4.436
blood	290	7	PtMugu#1B	956.3	538	EXPCD-1-0410		1.046	10-I	4.184
blood	291	9	CO-SCS	63.5	501	EXPCD-1-0379	<	0.1	10-I	0.2
blood	292	9	CO-SCS	63.5	511	EXPCD-1-0392	<	0.1	10-I	0.2
blood	293	9	CO-SCS	63.5	522	EXPCD-1-0380	<	0.1	10-I	0.2
blood	294	9	CO-SCS	63.5	543	EXPCD-1-0385	<	0.1	10-I	0.2
blood	295	11	OK-SS	62.8	502	EXPCD-1-0406	<	0.1	10-I	0.2
blood	296	11	OK-SS	62.8	509	EXPCD-1-0411	<	0.1	10-I	0.2
blood	297	11	OK-SS	62.8	542	EXPCD-1-0413	<	0.1	10-I	0.2
blood	298	11	OK-SS	62.8	556	EXPCD-1-0384	<	0.1	10-I	0.2
blood	299	13	Dugway #1	70.8	506	EXPCD-1-0386	<	0.1	10-I	0.2
blood	300	13	Dugway #1	70.8	524	EXPCD-1-0403	<	0.1	10-I	0.2
blood	301	13	Dugway #1	70.8	531	EXPCD-1-0383	<	0.1	10-I	0.2
blood	302	13	Dugway #1	70.8	558	EXPCD-1-0391	<	0.1	10-I	0.2
blood	303	13			2506	EXPCD-1-0396		2.103	10-I	8.412
blood	304	13			2524	EXPCD-1-0397	<	0.1	10-I	0.2
blood	305	11			2502	EXPCD-1-0382	<	0.1	10-I	0.2
blood	306	1	Control	0.0	514	EXPCD-1-0450	<	0.1	10-II	0.2
blood	307	1	Control	0.0	529	EXPCD-1-0435	<	0.1	10-II	0.2
blood	308	1	Control	0.0	551	EXPCD-1-0451	<	0.1	10-II	0.2
blood	309	1	Control	0.0	555	EXPCD-1-0427	<	0.1	10-II	0.2
blood	310	3	CdCl2	61.3	521	EXPCD-1-0434	<	0.1	10-II	0.2
blood	311	3	CdCl2	61.3	535	EXPCD-1-0445	<	0.1	10-II	0.2
blood	312	3	CdCl2	61.3	545	EXPCD-1-0447	<	0.1	10-II	0.2
blood	313	3	CdCl2	61.3	552	EXPCD-1-0423	<	0.1	10-II	0.2
blood	314	4	CdCl2	326.8	519	EXPCD-1-0453		1.138	10-II	4.552
blood	315	4	CdCl2	326.8	520	EXPCD-1-0441		1.131	10-II	4.524
blood	316	4	CdCl2	326.8	532	EXPCD-1-0431		0.698	10-II	2.792
blood	317	4	CdCl2	326.8	539	EXPCD-1-0433		0.417	10-II	1.668
blood	318	5	PtMugu#1B	242.2	507	EXPCD-1-0421		0.38	10-II	1.52
blood	319	5	PtMugu#1B	242.2	515	EXPCD-1-0446		0.248	10-II	0.992
blood	320	5	PtMugu#1B	242.2	541	EXPCD-1-0437		0.223	10-II	0.892
blood	321	5	PtMugu#1B	242.2	560	EXPCD-1-0440		0.45	10-II	1.8
blood	322	5	PtMugu#1B	242.2	565	EXPCD-1-0444		0.21	10-II	0.84
blood	323	6	PtMugu#1B	483.7	503	EXPCD-1-0438		0.699	10-II	2.796
blood	324	6	PtMugu#1B	483.7	536	EXPCD-1-0428		1.326	10-II	5.304
blood	325	6	PtMugu#1B	483.7	544	EXPCD-1-0454		1.017	10-II	4.068
blood	326	6	PtMugu#1B	483.7	548	EXPCD-1-0439		0.935	10-II	3.74
blood	327	6	PtMugu#1B	483.7	553	EXPCD-1-0430		0.935	10-II	3.74
blood	328	7	PtMugu#1B	956.3	510	EXPCD-1-0449		1.785	10-II	7.14
blood	329	7	PtMugu#1B	956.3	516	EXPCD-1-0436		2.847	10-II	11.388
blood	330	7	PtMugu#1B	956.3	517	EXPCD-1-0417		1.246	10-II	4.984
blood	331	7	PtMugu#1B	956.3	534	EXPCD-1-0424		2.247	10-II	8.988
blood	332	7	PtMugu#1B	956.3	538	EXPCD-1-0415		2.557	10-II	10.228
blood	333	9	CO-SCS	63.5	501	EXPCD-1-0414	<	0.1	10-II	0.2
blood	334	9	CO-SCS	63.5	511	EXPCD-1-0418	<	0.1	10-II	0.2
blood	335	9	CO-SCS	63.5	522	EXPCD-1-0448	<	0.1	10-II	0.2
blood	336	9	CO-SCS	63.5	543	EXPCD-1-0420	<	0.1	10-II	0.2
blood	337	11	OK-SS	62.8	502	EXPCD-1-0442	<	0.1	10-II	0.2
blood	338	11	OK-SS	62.8	509	EXPCD-1-0443	<	0.1	10-II	0.2
blood	339	11	OK-SS	62.8	542	EXPCD-1-0422	<	0.1	10-II	0.2
blood	340	11	OK-SS	62.8	556	EXPCD-1-0429	<	0.1	10-II	0.2
blood	341	13	Dugway #1	70.8	506	EXPCD-1-0425	<	0.1	10-II	0.2
blood	342	13	Dugway #1	70.8	524	EXPCD-1-0426	<	0.1	10-II	0.2
blood	343	13	Dugway #1	70.8	531	EXPCD-1-0419	<	0.1	10-II	0.2
blood	344	13	Dugway #1	70.8	558	EXPCD-1-0432	<	0.1	10-II	0.2
blood	345	7			2510	EXPCD-1-0416		1.585	10-II	6.34
blood	346	1			2514	EXPCD-1-0455	<	0.1	10-II	0.2
blood	347	3			2545	EXPCD-1-0452	<	0.1	10-II	0.2
blood	348	1	Control	0.0	514	EXPCD-1-0457		0.1	12-I	0.2
blood	349	1	Control	0.0	529	EXPCD-1-0461		0.1	12-I	0.2
blood	350	1	Control	0.0	551	EXPCD-1-0487		0.1	12-I	0.2
blood	351	1	Control	0.0	555	EXPCD-1-0465		0.1	12-I	0.2

Table B-3 RAW AND ADJUSTED BLOOD CADMIUM DATA
EXPCD-1, MAY 2003
BLEEDS I AND II

Detection Limit (DL) 0.1ug/L 1ml blood, 3ml 1MHNO3
possible outlier

matrix	index	group	Material Administered	Dosage	pig number	tag number	Qualifier	Result (ug/L)	day	Dilution Adjusted Value ug/L)
blood	352	3	CdCl2	61.3	521	EXPCD-1-0492		0.1	12-I	0.2
blood	353	3	CdCl2	61.3	535	EXPCD-1-0480		0.1	12-I	0.2
blood	354	3	CdCl2	61.3	545	EXPCD-1-0464		0.1	12-I	0.2
blood	355	3	CdCl2	61.3	552	EXPCD-1-0497		0.1	12-I	0.2
blood	356	4	CdCl2	326.8	519	EXPCD-1-0495		0.695	12-I	2.78
blood	357	4	CdCl2	326.8	520	EXPCD-1-0473		1.341	12-I	5.366
blood	358	4	CdCl2	326.8	532	EXPCD-1-0475		0.698	12-I	2.791
blood	359	4	CdCl2	326.8	539	EXPCD-1-0482		0.487	12-I	1.948
blood	360	5	PtMugu#1B	242.2	507	EXPCD-1-0474		0.279	12-I	1.116
blood	361	5	PtMugu#1B	242.2	515	EXPCD-1-0493		0.205	12-I	0.82
blood	362	5	PtMugu#1B	242.2	541	EXPCD-1-0470		0.184	12-I	0.737
blood	363	5	PtMugu#1B	242.2	560	EXPCD-1-0460		0.459	12-I	1.835
blood	364	5	PtMugu#1B	242.2	565	EXPCD-1-0463		0.19	12-I	0.762
blood	365	6	PtMugu#1B	483.7	503	EXPCD-1-0483		0.618	12-I	2.472
blood	366	6	PtMugu#1B	483.7	536	EXPCD-1-0469		1.138	12-I	4.551
blood	367	6	PtMugu#1B	483.7	544	EXPCD-1-0466		0.689	12-I	2.755
blood	368	6	PtMugu#1B	483.7	548	EXPCD-1-0467		0.685	12-I	2.742
blood	369	6	PtMugu#1B	483.7	553	EXPCD-1-0481		0.944	12-I	3.776
blood	370	7	PtMugu#1B	956.3	510	EXPCD-1-0477		0.715	12-I	2.86
blood	371	7	PtMugu#1B	956.3	516	EXPCD-1-0485		2.024	12-I	8.097
blood	372	7	PtMugu#1B	956.3	517	EXPCD-1-0459		1.235	12-I	4.94
blood	373	7	PtMugu#1B	956.3	534	EXPCD-1-0491		1.832	12-I	7.329
blood	374	7	PtMugu#1B	956.3	538	EXPCD-1-0489		1.757	12-I	7.03
blood	375	9	CO-SCS	63.5	501	EXPCD-1-0472		0.1	12-I	0.2
blood	376	9	CO-SCS	63.5	511	EXPCD-1-0471		0.1	12-I	0.2
blood	377	9	CO-SCS	63.5	522	EXPCD-1-0496		0.1	12-I	0.2
blood	378	9	CO-SCS	63.5	543	EXPCD-1-0486		0.1	12-I	0.2
blood	379	11	OK-SS	62.8	502	EXPCD-1-0484		0.1	12-I	0.2
blood	380	11	OK-SS	62.8	509	EXPCD-1-0476		0.1	12-I	0.2
blood	381	11	OK-SS	62.8	542	EXPCD-1-0488		0.1	12-I	0.2
blood	382	11	OK-SS	62.8	556	EXPCD-1-0458		0.1	12-I	0.2
blood	383	13	Dugway #1	70.8	506	EXPCD-1-0494		0.1	12-I	0.2
blood	384	13	Dugway #1	70.8	524	EXPCD-1-0479		0.1	12-I	0.2
blood	385	13	Dugway #1	70.8	531	EXPCD-1-0468		0.1	12-I	0.2
blood	386	13	Dugway #1	70.8	558	EXPCD-1-0478		0.1	12-I	0.2
blood	387	3			2535	EXPCD-1-0462		0.1	12-I	0.2
blood	388	9			2522	EXPCD-1-0456		0.1	12-I	0.2
blood	389	11			2542	EXPCD-1-0490		0.1	12-I	0.2
blood	390	1	Control	0.0	514	EXPCD-1-0522		0.1	12-II	0.2
blood	391	1	Control	0.0	529	EXPCD-1-0530		0.1	12-II	0.2
blood	392	1	Control	0.0	551	EXPCD-1-0537		0.1	12-II	0.2
blood	393	1	Control	0.0	555	EXPCD-1-0518		0.1	12-II	0.2
blood	394	3	CdCl2	61.3	521	EXPCD-1-0505		0.1	12-II	0.2
blood	395	3	CdCl2	61.3	535	EXPCD-1-0521		0.1	12-II	0.2
blood	396	3	CdCl2	61.3	545	EXPCD-1-0525		0.1	12-II	0.2
blood	397	3	CdCl2	61.3	552	EXPCD-1-0539		0.1	12-II	0.2
blood	398	4	CdCl2	326.8	519	EXPCD-1-0514		0.654	12-II	2.614
blood	399	4	CdCl2	326.8	520	EXPCD-1-0504		1.342	12-II	5.367
blood	400	4	CdCl2	326.8	532	EXPCD-1-0531		0.476	12-II	1.905
blood	401	4	CdCl2	326.8	539	EXPCD-1-0515		0.683	12-II	2.732
blood	402	5	PtMugu#1B	242.2	507	EXPCD-1-0532		0.243	12-II	0.972
blood	403	5	PtMugu#1B	242.2	515	EXPCD-1-0508		0.177	12-II	0.707
blood	404	5	PtMugu#1B	242.2	541	EXPCD-1-0538		0.147	12-II	0.59
blood	405	5	PtMugu#1B	242.2	560	EXPCD-1-0516		0.337	12-II	1.347
blood	406	5	PtMugu#1B	242.2	565	EXPCD-1-0519		0.157	12-II	0.627
blood	407	6	PtMugu#1B	483.7	503	EXPCD-1-0511		0.582	12-II	2.329
blood	408	6	PtMugu#1B	483.7	536	EXPCD-1-0528		1.083	12-II	4.333
blood	409	6	PtMugu#1B	483.7	544	EXPCD-1-0509		0.599	12-II	2.394
blood	410	6	PtMugu#1B	483.7	548	EXPCD-1-0535		0.525	12-II	2.102
blood	411	6	PtMugu#1B	483.7	553	EXPCD-1-0513		0.759	12-II	3.036

Table B-3 RAW AND ADJUSTED BLOOD CADMIUM DATA
EXPCD-1, MAY 2003
BLEEDS I AND II

Detection Limit (DL) 0.1ug/L 1ml blood, 3ml 1MHNO3
possible outlier

matrix	index	group	Material Administered	Dosage	pig number	tag number	Qualifier	Result (ug/L)	day	Dilution Adjusted Value ug/L)
blood	412	7	PtMugu#1B	956.3	510	EXPCD-1-0527		0.534	12-II	2.136
blood	413	7	PtMugu#1B	956.3	516	EXPCD-1-0520		1.223	12-II	4.89
blood	414	7	PtMugu#1B	956.3	517	EXPCD-1-0529		1.285	12-II	5.138
blood	415	7	PtMugu#1B	956.3	534	EXPCD-1-0499		2.62	12-II	10.48
blood	416	7	PtMugu#1B	956.3	538	EXPCD-1-0500		2.922	12-II	11.69
blood	417	9	CO-SCS	63.5	501	EXPCD-1-0524		0.1	12-II	0.2
blood	418	9	CO-SCS	63.5	511	EXPCD-1-0533		0.1	12-II	0.2
blood	419	9	CO-SCS	63.5	522	EXPCD-1-0526		0.1	12-II	0.2
blood	420	9	CO-SCS	63.5	543	EXPCD-1-0498		0.1	12-II	0.2
blood	421	11	OK-SS	62.8	502	EXPCD-1-0510		0.1	12-II	0.2
blood	422	11	OK-SS	62.8	509	EXPCD-1-0512		0.1	12-II	0.2
blood	423	11	OK-SS	62.8	542	EXPCD-1-0523		0.1	12-II	0.2
blood	424	11	OK-SS	62.8	556	EXPCD-1-0534		0.1	12-II	0.2
blood	425	13	Dugway #1	70.8	506	EXPCD-1-0506		0.1	12-II	0.2
blood	426	13	Dugway #1	70.8	524	EXPCD-1-0536		0.1	12-II	0.2
blood	427	13	Dugway #1	70.8	531	EXPCD-1-0502		0.1	12-II	0.2
blood	428	13	Dugway #1	70.8	558	EXPCD-1-0501		0.1	12-II	0.2
blood	429	6			2503	EXPCD-1-0503		0.659	12-II	2.635
blood	430	7			2538	EXPCD-1-0517		1.7	12-II	6.801
blood	431	7			2534	EXPCD-1-0507		2.074	12-II	8.295
blood	432	1	Control	0.0	514	EXPCD-1-0548		0.1	14-I	0.2
blood	433	1	Control	0.0	529	EXPCD-1-0576		0.1	14-I	0.2
blood	434	1	Control	0.0	551	EXPCD-1-0575		0.1	14-I	0.2
blood	435	1	Control	0.0	555	EXPCD-1-0551		0.1	14-I	0.2
blood	436	3	CdCl2	61.3	521	EXPCD-1-0561		0.1	14-I	0.2
blood	437	3	CdCl2	61.3	535	EXPCD-1-0581		0.1	14-I	0.2
blood	438	3	CdCl2	61.3	545	EXPCD-1-0569		0.1	14-I	0.2
blood	439	3	CdCl2	61.3	552	EXPCD-1-0550		0.1	14-I	0.2
blood	440	4	CdCl2	326.8	519	EXPCD-1-0557		0.613	14-I	2.453
blood	441	4	CdCl2	326.8	520	EXPCD-1-0563		1.205	14-I	4.82
blood	442	4	CdCl2	326.8	532	EXPCD-1-0572		0.953	14-I	3.813
blood	443	4	CdCl2	326.8	539	EXPCD-1-0578		0.754	14-I	3.015
blood	444	5	PtMugu#1B	242.2	507	EXPCD-1-0555		0.264	14-I	1.055
blood	445	5	PtMugu#1B	242.2	515	EXPCD-1-0570		0.249	14-I	0.994
blood	446	5	PtMugu#1B	242.2	541	EXPCD-1-0552		0.209	14-I	0.835
blood	447	5	PtMugu#1B	242.2	560	EXPCD-1-0559		0.699	14-I	2.797
blood	448	5	PtMugu#1B	242.2	565	EXPCD-1-0543		0.305	14-I	1.218
blood	449	6	PtMugu#1B	483.7	503	EXPCD-1-0545		0.819	14-I	3.275
blood	450	6	PtMugu#1B	483.7	536	EXPCD-1-0579		1.511	14-I	6.043
blood	451	6	PtMugu#1B	483.7	544	EXPCD-1-0562		0.787	14-I	3.148
blood	452	6	PtMugu#1B	483.7	548	EXPCD-1-0540		0.896	14-I	3.583
blood	453	6	PtMugu#1B	483.7	553	EXPCD-1-0565		0.972	14-I	3.888
blood	454	7	PtMugu#1B	956.3	510	EXPCD-1-0553		0.683	14-I	2.731
blood	455	7	PtMugu#1B	956.3	516	EXPCD-1-0571		3.183	14-I	12.73
blood	456	7	PtMugu#1B	956.3	517	EXPCD-1-0556		1.569	14-I	6.275
blood	457	7	PtMugu#1B	956.3	534	EXPCD-1-0558		2.031	14-I	8.124
blood	458	7	PtMugu#1B	956.3	538	EXPCD-1-0547		1.887	14-I	7.548
blood	459	9	CO-SCS	63.5	501	EXPCD-1-0541		0.1	14-I	0.2
blood	460	9	CO-SCS	63.5	511	EXPCD-1-0574		0.1	14-I	0.2
blood	461	9	CO-SCS	63.5	522	EXPCD-1-0577		0.1	14-I	0.2
blood	462	9	CO-SCS	63.5	543	EXPCD-1-0573		0.1	14-I	0.2
blood	463	11	OK-SS	62.8	502	EXPCD-1-0568		0.1	14-I	0.2
blood	464	11	OK-SS	62.8	509	EXPCD-1-0567		0.1	14-I	0.2
blood	465	11	OK-SS	62.8	542	EXPCD-1-0554		0.1	14-I	0.2
blood	466	11	OK-SS	62.8	556	EXPCD-1-0546		0.1	14-I	0.2
blood	467	13	Dugway #1	70.8	506	EXPCD-1-0564		0.1	14-I	0.2
blood	468	13	Dugway #1	70.8	524	EXPCD-1-0566		0.1	14-I	0.2
blood	469	13	Dugway #1	70.8	531	EXPCD-1-0580		0.1	14-I	0.2
blood	470	13	Dugway #1	70.8	558	EXPCD-1-0542		0.1	14-I	0.2
blood	471	4			2520	EXPCD-1-0549		1.642	14-I	6.497
blood	472	3			2552	EXPCD-1-0560		0.1	14-I	0.2
blood	473	6			2553	EXPCD-1-0544		0.986	14-I	3.945
blood	474	1	Control	0.0	514	EXPCD-1-0597		<dl	14-II	0.2
blood	475	1	Control	0.0	529	EXPCD-1-0585		<dl	14-II	0.2
blood	476	1	Control	0.0	551	EXPCD-1-0584		<dl	14-II	0.2
blood	477	1	Control	0.0	555	EXPCD-1-0617		<dl	14-II	0.2
blood	478	3	CdCl2	61.3	521	EXPCD-1-0618		<dl	14-II	0.2
blood	479	3	CdCl2	61.3	535	EXPCD-1-0620		<dl	14-II	0.2

Table B-3 RAW AND ADJUSTED BLOOD CADMIUM DATA
EXPCD-1, MAY 2003
BLEEDS I AND II

Detection Limit (DL) 0.1ug/L 1ml blood, 3ml 1MHNO3
possible outlier

matrix	index	group	Material Administered	Dosage	pig number	tag number	Qualifier	Result (ug/L)	day	Dilution Adjusted Value ug/L)
blood	480	3	CdCl2	61.3	545	EXPCD-1-0595		<dl	14-II	0.2
blood	481	3	CdCl2	61.3	552	EXPCD-1-0602		<dl	14-II	0.2
blood	482	4	CdCl2	326.8	519	EXPCD-1-0590		1.181	14-II	4.724
blood	483	4	CdCl2	326.8	520	EXPCD-1-0589		1.837	14-II	7.348
blood	484	4	CdCl2	326.8	532	EXPCD-1-0605		1.665	14-II	6.66
blood	485	4	CdCl2	326.8	539	EXPCD-1-0586		0.986	14-II	3.944
blood	486	5	PtMugu#1B	242.2	507	EXPCD-1-0609		0.535	14-II	2.14
blood	487	5	PtMugu#1B	242.2	515	EXPCD-1-0610		1.634	14-II	6.536
blood	488	5	PtMugu#1B	242.2	541	EXPCD-1-0588		0.456	14-II	1.824
blood	489	5	PtMugu#1B	242.2	560	EXPCD-1-0604		0.445	14-II	1.78
blood	490	5	PtMugu#1B	242.2	565	EXPCD-1-0583		0.479	14-II	1.916
blood	491	6	PtMugu#1B	483.7	503	EXPCD-1-0587		1.23	14-II	4.92
blood	492	6	PtMugu#1B	483.7	536	EXPCD-1-0615		2.117	14-II	8.468
blood	493	6	PtMugu#1B	483.7	544	EXPCD-1-0582		1.17	14-II	4.68
blood	494	6	PtMugu#1B	483.7	548	EXPCD-1-0591		1.33	14-II	5.32
blood	495	6	PtMugu#1B	483.7	553	EXPCD-1-0606		1.255	14-II	5.02
blood	496	7	PtMugu#1B	956.3	510	EXPCD-1-0614		1.745	14-II	6.98
blood	497	7	PtMugu#1B	956.3	516	EXPCD-1-0603		3.518	14-II	14.072
blood	498	7	PtMugu#1B	956.3	517	EXPCD-1-0621		3.099	14-II	12.396
blood	499	7	PtMugu#1B	956.3	534	EXPCD-1-0608		3.273	14-II	13.092
blood	500	7	PtMugu#1B	956.3	538	EXPCD-1-0622		4.31	14-II	17.24
blood	501	9	CO-SCS	63.5	501	EXPCD-1-0623		0.047	14-II	0.2
blood	502	9	CO-SCS	63.5	511	EXPCD-1-0616		<dl	14-II	0.2
blood	503	9	CO-SCS	63.5	522	EXPCD-1-0601		<dl	14-II	0.2
blood	504	9	CO-SCS	63.5	543	EXPCD-1-0611		<dl	14-II	0.2
blood	505	11	OK-SS	62.8	502	EXPCD-1-0593		<dl	14-II	0.2
blood	506	11	OK-SS	62.8	509	EXPCD-1-0600		0.114	14-II	0.456
blood	507	11	OK-SS	62.8	542	EXPCD-1-0613		<dl	14-II	0.2
blood	508	11	OK-SS	62.8	556	EXPCD-1-0607		0.174	14-II	0.696
blood	509	13	Dugway #1	70.8	506	EXPCD-1-0619		<dl	14-II	0.2
blood	510	13	Dugway #1	70.8	524	EXPCD-1-0594		<dl	14-II	0.2
blood	511	13	Dugway #1	70.8	531	EXPCD-1-0592		<dl	14-II	0.2
blood	512	13	Dugway #1	70.8	558	EXPCD-1-0596		<dl	14-II	0.2
blood	513	5			2560	EXPCD-1-0612		<dl	14-II	0.2
blood	514	11			2509	EXPCD-1-0598		<dl	14-II	0.2
blood	515	6			2548	EXPCD-1-0599		<dl	14-II	0.2

TABLE B-4 **BLOOD CADMIUM VALUES**

Bleed I

ug/L

Raw Data Bleeding I

group	Material Administered	Dosage (BW Adjusted)	pig number	0	6	8	10	12	14
1	Control	0.00	514	0.2	0.2	0.2	0.2	0.2	0.2
1	Control	0.00	529	0.2	0.2	0.2	0.2	0.2	0.2
1	Control	0.00	551	0.2	0.2	0.2	0.2	0.2	0.2
1	Control	0.00	555	0.2	0.2	0.2	0.2	0.2	0.2
3	CdCl2	57.21	521	0.2	0.2	0.2	0.2	0.2	0.2
3	CdCl2	63.60	535	0.2	0.2	0.2	0.2	0.2	0.2
3	CdCl2	66.20	545	0.2	0.2	0.2	0.2	0.2	0.2
3	CdCl2	58.23	552	0.2	0.2	0.2	0.2	0.2	0.2
4	CdCl2	312.80	519	0.2	1.504	1.356	1.044	2.78	2.453
4	CdCl2	326.37	520	0.2	2.104	4.04	3.46	5.366	4.82
4	CdCl2	342.64	532	0.2	0.984	2.272	2.232	2.791	3.813
4	CdCl2	325.57	539	0.2	0.828	1.904	1.296	1.948	3.015
5	PtMugu#1B	212.63	507	0.2	0.2	0.804	1.028	1.116	1.055
5	PtMugu#1B	203.11	515	0.2	0.2	0.484	0.2	0.82	0.994
5	PtMugu#1B	255.69	541	0.2	0.2	0.408	0.2	0.737	0.835
5	PtMugu#1B	270.61	560	0.2	0.2	1.928	1.652	1.835	2.797
5	PtMugu#1B	268.83	565	0.2	0.2	0.58	0.536	0.762	1.218
6	PtMugu#1B	388.34	503	0.2	0.688	2.556	1.668	2.472	3.275
6	PtMugu#1B	523.95	536	0.2	1.244	3.76	2.056	4.551	6.043
6	PtMugu#1B	483.48	544	0.2	1.38	3.24	2.176	2.755	3.148
6	PtMugu#1B	489.74	548	0.2	2.948	1.812	1.86	2.742	3.583
6	PtMugu#1B	533.23	553	0.2	0.68	2.124	2.432	3.776	3.888
7	PtMugu#1B	1037.59	510	0.2	0.62	0.772	1.3	2.86	2.731
7	PtMugu#1B	876.73	516	0.2	2.436	6.216	4.964	8.097	12.73
7	PtMugu#1B	969.97	517	0.2	2.664	4.572	3.728	4.94	6.275
7	PtMugu#1B	934.98	534	0.2	2.62	4.628	4.436	7.329	8.124
7	PtMugu#1B	962.35	538	0.2	4.136	6.44	4.184	7.03	7.548
9	CO-SCS	61.27	501	0.2	0.2	0.2	0.2	0.2	0.2
9	CO-SCS	60.06	511	0.2	0.2	0.2	0.2	0.2	0.2
9	CO-SCS	60.49	522	0.2	0.552	0.2	0.2	0.2	0.2
9	CO-SCS	72.34	543	0.2	0.2	0.2	0.2	0.2	0.2
11	OK-SS	56.05	502	0.2	0.2	0.2	0.2	0.2	0.2
11	OK-SS	69.03	509	0.2	0.2	0.2	0.2	0.2	0.2
11	OK-SS	63.98	542	0.2	0.2	0.2	0.2	0.2	0.2
11	OK-SS	62.04	556	0.2	0.54	0.2	0.2	0.2	0.2
13	Dugway #1	67.47	506	0.2	0.2	0.464	0.2	0.2	0.2
13	Dugway #1	66.72	524	0.2	0.2	0.2	0.2	0.2	0.2
13	Dugway #1	73.91	531	0.2	0.2	0.2	0.2	0.2	0.2
13	Dugway #1	75.27	558	0.2	0.2	0.2	0.2	0.2	0.2

TABLE B-5 **BLOOD CADMIUM OUTLIERS**

Bleed II

ug/L

Raw Data Bleeding II

group	Material Administered	Dosage (BW Adjusted)	pig number	0	6	8	10	12	14
1	Control	0.00	514	0.2	0.2	0.2	0.2	0.2	0.2
1	Control	0.00	529	0.2	0.2	0.2	0.2	0.2	0.2
1	Control	0.00	551	0.2	0.2	0.2	0.2	0.2	0.2
1	Control	0.00	555	0.2	0.2	0.2	0.2	0.2	0.2
3	CdCl2	57.21	521	0.2	0.2	0.2	0.2	0.2	0.2
3	CdCl2	63.60	535	0.2	0.2	0.2	0.2	0.2	0.2
3	CdCl2	66.20	545	0.2	0.2	0.2	0.2	0.2	0.2
3	CdCl2	58.23	552	0.2	0.2	0.2	0.2	0.2	0.2
4	CdCl2	312.80	519	0.2	1.768	2.644	4.552	2.614	4.724
4	CdCl2	326.37	520	0.609	3.62	4.656	4.524	5.367	7.348
4	CdCl2	342.64	532	1.074	2.488	3.784	2.792	1.905	6.66
4	CdCl2	325.57	539	0.2	2.728	2.38	1.668	2.732	3.944
5	PtMugu#1B	212.63	507	0.2	1.484	1.088	1.52	0.972	2.14
5	PtMugu#1B	203.11	515	0.2	0.2	1.136	0.992	0.707	6.536
5	PtMugu#1B	255.69	541	0.2	0.2	0.924	0.892	0.59	1.824
5	PtMugu#1B	270.61	560	0.2	1.296	1.42	1.8	1.347	1.78
5	PtMugu#1B	268.83	565	0.2	0.2	0.2	0.84	0.627	1.916
6	PtMugu#1B	388.34	503	0.2	1.22	3.604	2.796	2.329	4.92
6	PtMugu#1B	523.95	536	0.2	2.952	4.652	5.304	4.333	8.468
6	PtMugu#1B	483.48	544	0.872	2.032	4.184	4.068	2.394	4.68
6	PtMugu#1B	489.74	548	0.2	1.964	2.96	3.74	2.102	5.32
6	PtMugu#1B	533.23	553	0.2	1.268	2.512	3.74	3.036	5.02
7	PtMugu#1B	1037.59	510	0.2	1.832	5.62	7.14	2.136	6.98
7	PtMugu#1B	876.73	516	8.663	8.144	9.868	11.388	4.89	14.072
7	PtMugu#1B	969.97	517	1.801	6.264	12.656	4.984	5.138	12.396
7	PtMugu#1B	934.98	534	2.312	2.484	8.872	8.988	10.48	13.092
7	PtMugu#1B	962.35	538	6.07	6.204	20.22	10.228	11.69	17.24
9	CO-SCS	61.27	501	0.2	0.2	0.2	0.2	0.2	0.2
9	CO-SCS	60.06	511	0.2	0.2	0.2	0.2	0.2	0.2
9	CO-SCS	60.49	522	0.2	0.2	0.2	0.2	0.2	0.2
9	CO-SCS	72.34	543	0.2	0.2	0.2	0.2	0.2	0.2
11	OK-SS	56.05	502	0.2	0.2	0.2	0.2	0.2	0.2
11	OK-SS	69.03	509	0.2	0.2	1.084	0.2	0.2	0.456
11	OK-SS	63.98	542	0.2	0.2	0.2	0.2	0.2	0.2
11	OK-SS	62.04	556	0.2	0.2	0.2	0.2	0.2	0.696
13	Dugway #1	67.47	506	0.2	0.2	0.2	0.2	0.2	0.2
13	Dugway #1	66.72	524	0.2	0.2	0.2	0.2	0.2	0.2
13	Dugway #1	73.91	531	0.2	0.2	0.2	0.2	0.2	0.2
13	Dugway #1	75.27	558	0.2	0.2	0.2	0.2	0.2	0.2

TABLE B-6 Area Under Curve Determinations

Bleed I

Calculated using interpolated values for excluded data.

Material Administered	Bleeding I group	pig#	AUC (ug/dL-days) For Time Span Shown 0-6	6-8	8-10	10-12	12-14	AUC Total (ug/dL-days)
Control	1	514	1.2	0.4	0.4	0.4	0.4	2.8
Control	1	529	1.2	0.4	0.4	0.4	0.4	2.8
Control	1	551	1.2	0.4	0.4	0.4	0.4	2.8
Control	1	555	1.2	0.4	0.4	0.4	0.4	2.8
CdCl2	3	521	1.2	0.4	0.4	0.4	0.4	2.8
CdCl2	3	535	1.2	0.4	0.4	0.4	0.4	2.8
CdCl2	3	545	1.2	0.4	0.4	0.4	0.4	2.8
CdCl2	3	552	1.2	0.4	0.4	0.4	0.4	2.8
CdCl2	4	519	5.112	2.86	2.4	3.824	5.233	19.429
CdCl2	4	520	6.912	6.144	7.5	8.826	10.186	39.568
CdCl2	4	532	3.552	3.256	4.504	5.023	6.604	22.939
CdCl2	4	539	3.084	2.732	3.2	3.244	4.963	17.223
PtMugu#1B	5	507	1.2	1.004	1.832	2.144	2.171	8.351
PtMugu#1B	5	515	1.2	0.684	0.684	1.02	1.814	5.402
PtMugu#1B	5	541	1.2	0.608	0.608	0.937	1.572	4.925
PtMugu#1B	5	560	1.2	2.128	3.58	3.487	4.632	15.027
PtMugu#1B	5	565	1.2	0.78	1.116	1.298	1.98	6.374
PtMugu#1B	6	503	2.664	3.244	4.224	4.14	5.747	20.019
PtMugu#1B	6	536	4.332	5.004	5.816	6.607	10.594	32.353
PtMugu#1B	6	544	4.74	4.62	5.416	4.931	5.903	25.61
PtMugu#1B	6	548	9.444	4.76	3.672	4.602	6.325	28.803
PtMugu#1B	6	553	2.64	2.804	4.556	6.208	7.664	23.872
PtMugu#1B	7	510	2.46	1.392	2.072	4.16	5.591	15.675
PtMugu#1B	7	516	7.908	8.652	11.18	13.061	20.827	61.628
PtMugu#1B	7	517	8.592	7.236	8.3	8.668	11.215	44.011
PtMugu#1B	7	534	8.46	7.248	9.064	11.765	15.453	51.99
PtMugu#1B	7	538	13.008	10.576	10.624	11.214	14.578	60
CO-SCS	9	501	1.2	0.4	0.4	0.4	0.4	2.8
CO-SCS	9	511	1.2	0.4	0.4	0.4	0.4	2.8
CO-SCS	9	522	2.256	0.752	0.4	0.4	0.4	4.208
CO-SCS	9	543	1.2	0.4	0.4	0.4	0.4	2.8
OK-SS	11	502	1.2	0.4	0.4	0.4	0.4	2.8
OK-SS	11	509	1.2	0.4	0.4	0.4	0.4	2.8
OK-SS	11	542	1.2	0.4	0.4	0.4	0.4	2.8
OK-SS	11	556	2.22	0.74	0.4	0.4	0.4	4.16
Dugway #1	13	506	1.2	0.664	0.664	0.4	0.4	3.328
Dugway #1	13	524	1.2	0.4	0.4	0.4	0.4	2.8
Dugway #1	13	531	1.2	0.4	0.4	0.4	0.4	2.8
Dugway #1	13	558	1.2	0.4	0.4	0.4	0.4	2.8

TABLE B-7 Area Under Curve Determinations

BLEED II

Calculated using interpolated values for excluded data.

Material Administered	Bleeding II group	pig#	AUC (ug/dL-days) For Time Span Shown 0-6	6-8	8-10	10-12	12-14	AUC Total (ug/dL-days)
Control	1	514	1.2	0.4	0.4	0.4	0.4	2.8
Control	1	529	1.2	0.4	0.4	0.4	0.4	2.8
Control	1	551	1.2	0.4	0.4	0.4	0.4	2.8
Control	1	555	1.2	0.4	0.4	0.4	0.4	2.8
CdCl2	3	521	1.2	0.4	0.4	0.4	0.4	2.8
CdCl2	3	535	1.2	0.4	0.4	0.4	0.4	2.8
CdCl2	3	545	1.2	0.4	0.4	0.4	0.4	2.8
CdCl2	3	552	1.2	0.4	0.4	0.4	0.4	2.8
CdCl2	4	519	5.904	4.412	7.196	7.166	7.338	32.016
CdCl2	4	520	12.687	8.276	9.18	9.891	12.715	52.749
CdCl2	4	532	10.686	6.272	6.576	4.697	8.565	36.796
CdCl2	4	539	8.784	5.108	4.048	4.4	6.676	29.016
PtMugu#1B	5	507	5.052	2.572	2.608	2.492	3.112	15.836
PtMugu#1B	5	515	1.2	1.336	2.128	1.699	7.243	13.606
PtMugu#1B	5	541	1.2	1.124	1.816	1.482	2.414	8.036
PtMugu#1B	5	560	4.488	2.716	3.22	3.147	3.127	16.698
PtMugu#1B	5	565	1.2	0.4	1.04	1.467	2.543	6.65
PtMugu#1B	6	503	4.26	4.824	6.4	5.125	7.249	27.858
PtMugu#1B	6	536	9.456	7.604	9.956	9.637	12.801	49.454
PtMugu#1B	6	544	8.712	6.216	8.252	6.462	7.074	36.716
PtMugu#1B	6	548	6.492	4.924	6.7	5.842	7.422	31.38
PtMugu#1B	6	553	4.404	3.78	6.252	6.776	8.056	29.268
PtMugu#1B	7	510	6.096	7.452	12.76	9.276	9.116	44.7
PtMugu#1B	7	516	50.421	18.012	21.256	16.278	18.962	124.929
PtMugu#1B	7	517	24.195	18.92	17.64	10.122	17.534	88.411
PtMugu#1B	7	534	14.388	11.356	17.86	19.468	23.572	86.644
PtMugu#1B	7	538	36.822	26.424	30.448	21.918	28.93	144.542
CO-SCS	9	501	1.2	0.4	0.4	0.4	0.4	2.8
CO-SCS	9	511	1.2	0.4	0.4	0.4	0.4	2.8
CO-SCS	9	522	1.2	0.4	0.4	0.4	0.4	2.8
CO-SCS	9	543	1.2	0.4	0.4	0.4	0.4	2.8
OK-SS	11	502	1.2	0.4	0.4	0.4	0.4	2.8
OK-SS	11	509	1.2	1.284	1.284	0.4	0.656	4.824
OK-SS	11	542	1.2	0.4	0.4	0.4	0.4	2.8
OK-SS	11	556	1.2	0.4	0.4	0.4	0.896	3.296
Dugway #1	13	506	1.2	0.4	0.4	0.4	0.4	2.8
Dugway #1	13	524	1.2	0.4	0.4	0.4	0.4	2.8
Dugway #1	13	531	1.2	0.4	0.4	0.4	0.4	2.8
Dugway #1	13	558	1.2	0.4	0.4	0.4	0.4	2.8

Table B-8
EXPCD-1, MAY 2003

Liver Furnace and Flame Data

Matrix	Index	Group	Pig Number	Treatment	Cadmium Intake ug/kg/day	Sample Number	Tag Number	Furnace DL=.04ug/L Furnace Cd, ug/L(corrected for 1/10dilution)	Furnace Cd, ng/g	Flame DL=.02mg/L Flame Cd, mg/L	Flame,Cd, ng/g
liver	580	1	514	Control	0	EXPCD-1-514-(15)-L	EXPCD-1-0749	<dl	2	<dl	<dl
liver	581	1	529	Control	0	EXPCD-1-529-(15)-L	EXPCD-1-0733	<dl	2	<dl	<dl
liver	582	1	551	Control	0	EXPCD-1-551-(15)-L	EXPCD-1-0761	<dl	2	<dl	<dl
liver	583	1	555	Control	0	EXPCD-1-555-(15)-L	EXPCD-1-0714	<dl	2	<dl	<dl
liver	584	2	508	CdCl2	10	EXPCD-1-508-(15)-L	EXPCD-1-0760	1.185	11.85	<dl	<dl
liver	585	2	528	CdCl2	10	EXPCD-1-528-(15)-L	EXPCD-1-0711	4.342	43.42	0.012	120
liver	586	2	540	CdCl2	10	EXPCD-1-540-(15)-L	EXPCD-1-0743	2.112	21.12	<dl	<dl
liver	587	2	550	CdCl2	10	EXPCD-1-550-(15)-L	EXPCD-1-0751	1.167	11.67	<dl	<dl
liver	588	3	521	CdCl2	60	EXPCD-1-521-(15)-L	EXPCD-1-0738	18.67	186.7	0.026	260
liver	589	3	535	CdCl2	60	EXPCD-1-535-(15)-L	EXPCD-1-0737	23.72	237.2	0.032	320
liver	590	3	545	CdCl2	60	EXPCD-1-545-(15)-L	EXPCD-1-0709	21.38	213.8	0.026	260
liver	591	3	552	CdCl2	60	EXPCD-1-552-(15)-L	EXPCD-1-0736	16.35	163.5	0.017	170
liver	592	4	519	CdCl2	320	EXPCD-1-519-(15)-L	EXPCD-1-0728	91.07	910.7	0.093	930
liver	593	4	520	CdCl2	320	EXPCD-1-520-(15)-L	EXPCD-1-0744	148.8	1488	0.156	1560
liver	594	4	532	CdCl2	320	EXPCD-1-532-(15)-L	EXPCD-1-0731	130.9	1309	0.134	1340
liver	595	4	539	CdCl2	320	EXPCD-1-539-(15)-L	EXPCD-1-0725	122	1220	0.128	1280
liver	596	5	507	Pt. Mugu #1B	234	EXPCD-1-507-(15)-L	EXPCD-1-0720	90.33	903.3	0.091	910
liver	597	5	515	Pt. Mugu #1B	234	EXPCD-1-515-(15)-L	EXPCD-1-0724	84.96	849.6	0.097	970
liver	598	5	541	Pt. Mugu #1B	234	EXPCD-1-541-(15)-L	EXPCD-1-0730	59.5	595	0.062	620
liver	599	5	560	Pt. Mugu #1B	234	EXPCD-1-560-(15)-L	EXPCD-1-0755	92.88	928.8	0.094	940
liver	600	5	565	Pt. Mugu #1B	234	EXPCD-1-565-(15)-L	EXPCD-1-0715	64.52	645.2	0.071	710
liver	601	6	503	Pt. Mugu #1B	468	EXPCD-1-503-(15)-L	EXPCD-1-0753	133	1330	0.128	1280
liver	602	6	536	Pt. Mugu #1B	468	EXPCD-1-536-(15)-L	EXPCD-1-0732	155.5	1555	0.161	1610
liver	603	6	544	Pt. Mugu #1B	468	EXPCD-1-544-(15)-L	EXPCD-1-0719	109.5	1095	0.124	1240
liver	604	6	548	Pt. Mugu #1B	468	EXPCD-1-548-(15)-L	EXPCD-1-0708	162.5	1625	0.163	1630
liver	605	6	553	Pt. Mugu #1B	468	EXPCD-1-553-(15)-L	EXPCD-1-0722	137.2	1372	0.145	1450
liver	606	7	510	Pt. Mugu #1B	936	EXPCD-1-510-(15)-L	EXPCD-1-0739	207	2070	0.23	2300
liver	607	7	516	Pt. Mugu #1B	936	EXPCD-1-516-(15)-L	EXPCD-1-0707	466.6	4666	0.399	3990
liver	608	7	517	Pt. Mugu #1B	936	EXPCD-1-517-(15)-L	EXPCD-1-0763	367.9	3679	0.297	2970
liver	609	7	534	Pt. Mugu #1B	936	EXPCD-1-534-(15)-L	EXPCD-1-0710	461.9	4619	0.39	3900
liver	610	7	538	Pt. Mugu #1B	936	EXPCD-1-538-(15)-L	EXPCD-1-0756	489.4	4894	0.369	3690
liver	611	8	513	CO-SCS	20.8	EXPCD-1-513-(15)-L	EXPCD-1-0752	3.488	34.88	<dl	<dl
liver	612	8	537	CO-SCS	20.8	EXPCD-1-537-(15)-L	EXPCD-1-0762	6.038	60.38	0.004	40
liver	613	8	559	CO-SCS	20.8	EXPCD-1-559-(15)-L	EXPCD-1-0706	3.956	39.56	0.011	110
liver	614	8	562	CO-SCS	20.8	EXPCD-1-562-(15)-L	EXPCD-1-0757	3.383	33.83	<dl	<dl
liver	615	9	501	CO-SCS	62.4	EXPCD-1-501-(15)-L	EXPCD-1-0750	18.41	184.1	0.021	210
liver	616	9	511	CO-SCS	62.4	EXPCD-1-511-(15)-L	EXPCD-1-0758	17.14	171.4	0.012	120
liver	617	9	522	CO-SCS	62.4	EXPCD-1-522-(15)-L	EXPCD-1-0721	15.27	152.7	0.015	150
liver	618	9	543	CO-SCS	62.4	EXPCD-1-543-(15)-L	EXPCD-1-0717	14.4	144	0.018	180
liver	619	10	505	OK-SS	20.6	EXPCD-1-505-(15)-L	EXPCD-1-0713	6.785	67.85	0.013	130
liver	620	10	518	OK-SS	20.6	EXPCD-1-518-(15)-L	EXPCD-1-0746	8.635	86.35	0.011	110
liver	621	10	533	OK-SS	20.6	EXPCD-1-533-(15)-L	EXPCD-1-0723	4.778	47.78	0.011	110
liver	622	10	546	OK-SS	20.6	EXPCD-1-546-(15)-L	EXPCD-1-0754	6.286	62.86	0.005	50
liver	623	11	502	OK-SS	61.8	EXPCD-1-502-(15)-L	EXPCD-1-0726	19.07	190.7	0.028	280
liver	624	11	509	OK-SS	61.8	EXPCD-1-509-(15)-L	EXPCD-1-0741	17.41	174.1	0.026	260
liver	625	11	542	OK-SS	61.8	EXPCD-1-542-(15)-L	EXPCD-1-0748	13.98	139.8	0.017	170
liver	626	11	556	OK-SS	61.8	EXPCD-1-556-(15)-L	EXPCD-1-0745	16.56	165.6	0.023	230
liver	627	12	526	Dugway #1	23.4	EXPCD-1-526-(15)-L	EXPCD-1-0735	1.799	17.99	0.006	60
liver	628	12	527	Dugway #1	23.4	EXPCD-1-527-(15)-L	EXPCD-1-0734	1.905	19.05	0.004	40
liver	629	12	549	Dugway #1	23.4	EXPCD-1-549-(15)-L	EXPCD-1-0716	<dl	2	<dl	<dl
liver	630	12	564	Dugway #1	23.4	EXPCD-1-564-(15)-L	EXPCD-1-0740	0.508	5.08	0.007	70
liver	631	13	506	Dugway #1	70.2	EXPCD-1-506-(15)-L	EXPCD-1-0742	0.701	7.01	<dl	<dl
liver	632	13	524	Dugway #1	70.2	EXPCD-1-524-(15)-L	EXPCD-1-0727	6.264	62.64	0.01	100
liver	633	13	531	Dugway #1	70.2	EXPCD-1-531-(15)-L	EXPCD-1-0729	1.08	10.8	0.01	100
liver	634	13	558	Dugway #1	70.2	EXPCD-1-558-(15)-L	EXPCD-1-0747	1.363	13.63	0.023	230
Duplicates											
liver	635	4	2539			EXPCD-1-2539-(15)-L	EXPCD-1-0718	129.1	1291	0.132	1320
liver	636	5	2541			EXPCD-1-2541-(15)-L	EXPCD-1-0712	58.59	585.9	0.064	640
liver	637	5	2507			EXPCD-1-2507-(15)-L	EXPCD-1-0759	91.92	919.2	0.091	910

Flame dl is .02mg/L=200ng/g

Table B-9
EXPCD-1, MAY 2003

Kidney Raw Furnace and Flame Data

Matrix	Index	Group	Pig Number	Treatment	Cadmium Intake ug/kg/day	Sample Number	Tag Number	Furnace DL=.04ug/L Furnace Cd, ug/L (corrected for 1/10dilution)	1g in 10ml Furnace Cd, ng/g	Flame Cd, mg/L	Flame DL=.02mg/L Flame & Furnace combined Cd, ng/g
kidney	529	1	514	Control	0	EXPCD-1-514-(15)-K	EXPCD-1-0652	<dl	2	<dl	2
kidney	530	1	529	Control	0	EXPCD-1-529-(15)-K	EXPCD-1-0654	<dl	2	<dl	2
kidney	531	1	551	Control	0	EXPCD-1-551-(15)-K	EXPCD-1-0700	<dl	2	<dl	2
kidney	532	1	555	Control	0	EXPCD-1-555-(15)-K	EXPCD-1-0690	<dl	2	<dl	2
kidney	533	2	508	CdCl2	10	EXPCD-1-508-(15)-K	EXPCD-1-0659	18.73	187.3	0.023	187.3
kidney	534	2	528	CdCl2	10	EXPCD-1-528-(15)-K	EXPCD-1-0680	32.26	322.6	0.029	290
kidney	535	2	540	CdCl2	10	EXPCD-1-540-(15)-K	EXPCD-1-0672	27.04	270.4	0.029	290
kidney	536	2	550	CdCl2	10	EXPCD-1-550-(15)-K	EXPCD-1-0694	15.1	151	<dl	151
kidney	537	3	521	CdCl2	60	EXPCD-1-521-(15)-K	EXPCD-1-0650	151.5	1515	0.158	1580
kidney	538	3	535	CdCl2	60	EXPCD-1-535-(15)-K	EXPCD-1-0682	122.9	1229	0.142	1420
kidney	539	3	545	CdCl2	60	EXPCD-1-545-(15)-K	EXPCD-1-0669	105.9	1059	0.115	1150
kidney	540	3	552	CdCl2	60	EXPCD-1-552-(15)-K	EXPCD-1-0695	101.8	1018	0.103	1030
kidney	541	4	519	CdCl2	320	EXPCD-1-519-(15)-K	EXPCD-1-0681	140.3	1403	0.606	6060
kidney	542	4	520	CdCl2	320	EXPCD-1-520-(15)-K	EXPCD-1-0667	1576	15760	1.366	13660
kidney	543	4	532	CdCl2	320	EXPCD-1-532-(15)-K	EXPCD-1-0678	892.8	8928	0.744	7440
kidney	544	4	539	CdCl2	320	EXPCD-1-539-(15)-K	EXPCD-1-0691	1214	12140	0.736	7360
kidney	545	5	507	Pt. Mugu #1B	234	EXPCD-1-507-(15)-K	EXPCD-1-0648	395.9	3959	0.367	3670
kidney	546	5	515	Pt. Mugu #1B	234	EXPCD-1-515-(15)-K	EXPCD-1-0660	450.9	4509	0.345	3450
kidney	547	5	541	Pt. Mugu #1B	234	EXPCD-1-541-(15)-K	EXPCD-1-0674	487.1	4871	0.419	4190
kidney	548	5	560	Pt. Mugu #1B	234	EXPCD-1-560-(15)-K	EXPCD-1-0663	691.1	6911	0.465	4650
kidney	549	5	565	Pt. Mugu #1B	234	EXPCD-1-565-(15)-K	EXPCD-1-0702	475.3	4753	0.41	4100
kidney	550	6	503	Pt. Mugu #1B	468	EXPCD-1-503-(15)-K	EXPCD-1-0675	863.7	8637	0.688	6880
kidney	551	6	536	Pt. Mugu #1B	468	EXPCD-1-536-(15)-K	EXPCD-1-0661	1387	13870	0.984	9840
kidney	552	6	544	Pt. Mugu #1B	468	EXPCD-1-544-(15)-K	EXPCD-1-0703	983.9	9839	0.863	8630
kidney	553	6	548	Pt. Mugu #1B	468	EXPCD-1-548-(15)-K	EXPCD-1-0662	1231	12310	0.87	8700
kidney	554	6	553	Pt. Mugu #1B	468	EXPCD-1-553-(15)-K	EXPCD-1-0649	931.4	9314	0.738	7380
kidney	555	7	510	Pt. Mugu #1B	936	EXPCD-1-510-(15)-K	EXPCD-1-0688	1123	11230	0.952	9520
kidney	556	7	516	Pt. Mugu #1B	936	EXPCD-1-516-(15)-K	EXPCD-1-0656	1730	17300	1.541	15410
kidney	557	7	517	Pt. Mugu #1B	936	EXPCD-1-517-(15)-K	EXPCD-1-0686	1599	15990	1.419	14190
kidney	558	7	534	Pt. Mugu #1B	936	EXPCD-1-534-(15)-K	EXPCD-1-0698	2088	20880	1.573	15730
kidney	559	7	538	Pt. Mugu #1B	936	EXPCD-1-538-(15)-K	EXPCD-1-0697	1700	17000	1.31	13100
kidney	560	8	513	CO-SCS	20.8	EXPCD-1-513-(15)-K	EXPCD-1-0657	39.48	394.8	0.042	420
kidney	561	8	537	CO-SCS	20.8	EXPCD-1-537-(15)-K	EXPCD-1-0664	59.92	599.2	0.061	610
kidney	562	8	559	CO-SCS	20.8	EXPCD-1-559-(15)-K	EXPCD-1-0665	47.24	472.4	0.047	470
kidney	563	8	562	CO-SCS	20.8	EXPCD-1-562-(15)-K	EXPCD-1-0676	25.88	258.8	0.025	250
kidney	564	9	501	CO-SCS	62.4	EXPCD-1-501-(15)-K	EXPCD-1-0666	282.8	2828	0.226	2260
kidney	565	9	511	CO-SCS	62.4	EXPCD-1-511-(15)-K	EXPCD-1-0670	102.5	1025	0.109	1090
kidney	566	9	522	CO-SCS	62.4	EXPCD-1-522-(15)-K	EXPCD-1-0683	37.15	371.5	0.124	1240
kidney	567	9	543	CO-SCS	62.4	EXPCD-1-543-(15)-K	EXPCD-1-0696	140.6	1406	0.133	1330
kidney	568	10	505	OK-SS	20.6	EXPCD-1-505-(15)-K	EXPCD-1-0699	61.28	612.8	0.057	570
kidney	569	10	518	OK-SS	20.6	EXPCD-1-518-(15)-K	EXPCD-1-0658	86.15	861.5	0.081	810
kidney	570	10	533	OK-SS	20.6	EXPCD-1-533-(15)-K	EXPCD-1-0705	31.38	313.8	0.025	250
kidney	571	10	546	OK-SS	20.6	EXPCD-1-546-(15)-K	EXPCD-1-0692	49.07	490.7	0.045	450
kidney	572	11	502	OK-SS	61.8	EXPCD-1-502-(15)-K	EXPCD-1-0653	135	1350	0.138	1380
kidney	573	11	509	OK-SS	61.8	EXPCD-1-509-(15)-K	EXPCD-1-0685	169.5	1695	0.156	1560
kidney	574	11	542	OK-SS	61.8	EXPCD-1-542-(15)-K	EXPCD-1-0677	94.94	949.4	0.093	930
kidney	575	11	556	OK-SS	61.8	EXPCD-1-556-(15)-K	EXPCD-1-0655	83.83	838.3	0.093	930
kidney	576	12	526	Dugway #1	23.4	EXPCD-1-526-(15)-K	EXPCD-1-0668	23.61	236.1	0.024	240
kidney	577	12	527	Dugway #1	23.4	EXPCD-1-527-(15)-K	EXPCD-1-0679	20.68	206.8	0.021	210
kidney	578	12	549	Dugway #1	23.4	EXPCD-1-549-(15)-K	EXPCD-1-0693	8.111	81.11	<dl	81.11
kidney	579	12	564	Dugway #1	23.4	EXPCD-1-564-(15)-K	EXPCD-1-0651	17.74	177.4	0.024	240
kidney	580	13	506	Dugway #1	70.2	EXPCD-1-506-(15)-K	EXPCD-1-0671	31.93	319.3	0.036	360
kidney	581	13	524	Dugway #1	70.2	EXPCD-1-524-(15)-K	EXPCD-1-0684	37.18	371.8	0.032	320
kidney	582	13	531	Dugway #1	70.2	EXPCD-1-531-(15)-K	EXPCD-1-0687	25.56	255.6	0.025	250
kidney	583	13	558	Dugway #1	70.2	EXPCD-1-558-(15)-K	EXPCD-1-0689	23.13	231.3	0.016	231.3
Duplicates											
kidney	584	10	2505			EXPCD-1-2505-(15)-K	EXPCD-1-0673	62.41	624.1	0.063	630
kidney	585	2	2528			EXPCD-1-2528-(15)-K	EXPCD-1-0701	31.48	314.8	0.027	270
kidney	586	8	2562			EXPCD-1-2562-(15)-K	EXPCD-1-0704	30.09	300.9	0.021	210

Range 0.5-10ug/L

From Furnace,1/10dilution, many samples out of range

Table B-10 Intralaboratory Duplicates
EXPCD-1, MAY 2003 RPD=Relative % difference=100*(Orig-Dup)/((Orig+Dup)/2)
BLEEDS I AND II

Matrix	Pig Number	Day	Original Dilution Adjusted Value (ug/L)	Duplicate Dilution Adjusted Value (ug/L)	RPD	Avg RPD for tissue
blood	519	0-I	0.2	0.2	0%	
blood	517	0-I	0.2	0.2	0%	
blood	556	0-I	0.2	0.2	0%	
blood	555	0-II	0.2	0.2	0%	
blood	532	0-II	1.074	0.956	12%	
blood	516	0-II	8.663	7.662	12%	
blood	565	6-I	0.2	0.2	0%	
blood	543	6-I	0.2	0.2	0%	
blood	558	6-I	0.2	0.2	0%	
blood	551	6-II	0.2	0.2	0%	
blood	511	6-II	0.2	0.2	0%	
blood	531	6-II	0.2	0.2	0%	
blood	515	8-I	0.484	0.2	83%	
blood	536	8-I	3.76	3.32	12%	
blood	501	8-I	0.2	0.2	0%	
blood	529	8-II	0.2	0.2	0%	
blood	521	8-II	0.2	0.2	0%	
blood	544	8-II	4.184	4.352	-4%	
blood	502	10-I	0.2	0.2	0%	
blood	506	10-I	0.2	8.412	-191%	
blood	524	10-I	0.2	0.2	0%	
blood	514	10-II	0.2	0.2	0%	
blood	545	10-II	0.2	0.2	0%	
blood	510	10-II	7.14	6.34	12%	
blood	535	12-I	0.2	0.2	0%	
blood	522	12-I	0.2	0.2	0%	
blood	542	12-I	0.2	0.2	0%	
blood	503	12-II	2.329	2.635	-12%	
blood	534	12-II	10.48	8.295	23%	
blood	538	12-II	11.69	6.801	53%	
blood	552	14-I	0.2	0.2	0%	
blood	520	14-I	4.82	6.497	-30%	
blood	553	14-I	3.888	3.945	-1%	
blood	560	14-II	1.78	0.2	160%	
blood	548	14-II	5.32	0.2	186%	
blood	509	14-II	0.456	0.2	78%	11%
liver	539	15	1220	1291	-6%	
liver	507	15	903.3	919.2	-2%	
liver	541	15	595	585.9	2%	-2%
kidney	528	CdCl2	290	270	7%	
kidney	505	OK-SS	570	630	-10%	
kidney	559	CO-SCS	470	210	76%	25%

Soil Cadmium Bioavailability as a Function of Mineralogy and Soil Characteristics

Rosalind A. Schoof[1], Michael V. Ruby[3], Stan W. Casteel [2], Tim J. Evans[2], Yvette W. Lowney[3], Alma M. Cardenas[1]*

Integral Consulting, Inc., 7900 SE 28th St., Suite 300, Mercer Island, WA 98040, rschoof@integral-corp.com

RECEIVED DATE

1. Integral Consulting, Inc., 7900 SE 28th St., Suite 300, Mercer Island, WA 98040, rschoof@integral-corp.com, telephone: (206) 230-9600, fax: (206) 230-9601; 2. University of Missouri, Veterinary Medical Diagnostic Laboratory, College of Veterinary Medicine, Columbia, MO 65211, casteels@missouri.edu; evanst@missouri.edu; 3. Exponent, Inc., Boulder, CO 80301, rubym@exponent.com ; lowneyy@exponent.com

ABSTRACT

A juvenile swine model was used to assess the relative oral bioavailability of cadmium in soil from four sites with varying soil characteristics. Groups of swine were given oral doses of soil containing 20 to 960 µg of Cd/kg-day for 15 days. The reference material was soluble cadmium chloride, administered at doses of 10, 60, or 320 µg Cd/kg day. Concentrations of cadmium in blood, liver, and kidney were evaluated to determine the bioavailability of cadmium from soils relative to the reference material. Results indicate that soil-specific factors control the relative bioavailability of cadmium: three of the soils studied exhibited modest reductions in bioavailability relative to cadmium chloride, while a fourth soil yielded considerably lower relative bioavailability. In order to understand the factors controlling the bioavailability of cadmium, each test sample was evaluated for soil chemistry, including cadmium mineralogy. Results indicate that the reduced bioavailability may be due to the occurrence of cadmium as less soluble cadmium-metal sulfates, with a higher soil pH possibly contributing to the formation of these less soluble phases. Particle size evaluation suggests that the solubility of the predominant cadmium phases may be a more significant factor in controlling relative bioavailability than is particle size.

KEYWORDS cadmium, oral bioavailability, juvenile swine

Introduction

Cadmium is a ubiquitous soil constituent, and may be present at higher concentrations in lead, zinc, and copper ores. Although ingestion of cadmium in food is the primary exposure route for humans, residents and workers in the vicinity of smelter sites and other industrial operations using cadmium containing materials may have increased potential for exposure to cadmium in soil. The study reported herein examines the influence of cadmium mineralogy and soil characteristics on the bioavailability of ingested soil cadmium relative to the bioavailability of a water soluble form of cadmium, i.e., the relative bioavailability.

The oral bioavailability of cadmium is low, with approximately 3-5 % of an oral dose of cadmium absorbed from diet or drinking water by humans, and somewhat lower absorption reported in rats and mice (*1*). A substantial portion of an oral cadmium dose is initially retained in the gastrointestinal mucosa, and then slowly excreted over a period of weeks. For this reason whole body retention of cadmium shortly after dosing is not a reliable indicator of cadmium absorption. Absorbed cadmium is accumulated in kidney cortex and liver, and is slowly excreted, with a biological half-life of 20 to 50 percent of an animal's lifetime (*1*).

It has been estimated that environmental exposure to cadmium results in renal disease in 1% to 7% of the world's population (*2, 3*), principally due to consumption of rice and other foods that accumulate cadmium, with smokers being at higher risk due to having higher body burdens. Renal disease is thought to be triggered after renal cortex concentrations exceed some threshold concentration, often late in life (*4*).

The potential for chronic exposure to cadmium from soil to significantly increase body burden and lead to increased incidence or earlier onset of disease will be partly dependent on the bioavailability of cadmium in this matrix. The relative bioavailability of metals from soil is dependent on multiple factors, including metal phases present and soil characteristics. In soil, cadmium can occur as a complex mixture of solid-phase compounds of varying particle size and morphology including discrete

mineral phases, coprecipitated and sorbed species associated with soil minerals or organic matter, and dissolved species that may be complexed by a variety of organic and inorganic ligands. These characteristics affect the solubility, and hence, relative bioavailability of metals from soil. For example, cadmium carbonate in soil is highly soluble while cadmium sulfate and cadmium sulfide complexes are less soluble (*5*). pH is another important factor, with cadmium solubility in soil decreasing as soil pH increases due to cadmium adsorption to soil particles and formation of irreversibly insoluble complexes.

Studies that have demonstrated reduced relative bioavailability of cadmium from soil have been conducted in rats (*5*) and in juvenile swine (*6*). An in vitro bioaccessibility model has also been used (*6*). Swine are useful in assessing bioavailability because of the similarity in gastrointestinal parameters between swine and humans. Feeding behavior, gastrointestinal anatomy, acid secretion, and the development of small-intestinal absorption mechanisms are quite similar between swine and humans (*7*). For these reasons, swine have been used as a surrogate for humans in the fields of pharmaceutical research and nutrition (*8, 9*). Juvenile animals are preferred because metal absorption is frequently greater in younger animals and thus predicts uptake in children, who may have greater exposure than adults. The juvenile swine model has been used to assess the oral bioavailability of both lead and arsenic in soil, and the results from these studies have been used to develop relative bioavailability adjustments (RBA) for human health risk assessment by the U.S. Environmental Protection Agency (*5, 10*); however, the model has not previously been modified to reflect the toxicokinetic behavior of cadmium. As note above, cadmium accumulates in the kidney and liver. Thus, cadmium concentrations in blood, kidney and liver may be used to estimate relative bioavailability of test substrates. While the linear and nonlinear dose-dependent aspects of the distribution of lead within the blood, kidneys, liver, and femur of immature pigs have been described (*11*), there is a relative lack of information on the distribution of cadmium following repeated oral exposure of juvenile swine. In addition to assessing relative bioavailability of cadmium from soils, this study examines the effects of dose and time-since-administration on blood levels of cadmium following ingestion.

Materials and Methods

Test Materials. The samples tested in this study were surficial soils (0–3 in.) from sites with elevated levels of cadmium in the soil, including Pt. Mugu, California (sample PTMG), smelter sites in Colorado (sample CO-SCS) and Oklahoma (sample OK-SS), and Dugway Proving Ground in Utah (sample DPGC). Anhydrous cadmium chloride ($CdCl_2$, Sigma) was used as the soluble cadmium reference material. The samples were sieved to <2 mm, and the particle size distribution was measured. The samples were then sieved to <250 µm (the size fraction used in oral bioavailability studies because it is more likely to adhere to hands and be ingested) and tested for the following parameters: pH (EPA Method 9045C); total organic carbon (TOC; ASTM D4129-82); total carbon (ASTM D4129-82), from which total inorganic carbon (TIC) was calculated by difference between total carbon and TOC; cation exchange capacity (CEC; EPA Method 9081); cadmium concentration (in triplicate; EPA Method 7131); and metals concentrations (arsenic, chromium, copper, iron, lead, manganese, mercury, nickel, phosphorus, and zinc; EPA Method 6010B, with the exception of arsenic [EPA Method 7060A], lead [EPA Method 7421], and mercury [EPA Method 7471A]).

Analysis for cadmium concentration was preceded by thorough mixing of the soils by placing a bottle containing the soil on a low-speed roller apparatus for 30 minutes inverting it five times, and allowing it to stand a few minutes to settle prior to sampling. The same mixing procedure was followed prior to dose preparation. An aliquot of each soil sample was also evaluated for cadmium mineralogy by electron microprobe, using the method described in Davis et al. (*12*). This method involves establishing the chemistry of individual cadmium-bearing grains in the sample, until a representative number have been analyzed (generally 100–200), and the distribution of cadmium among the different cadmium forms in the soil can be established.

Experimental Design and General Procedures. Intact male pigs weighing 10–12 kg were provided by Chinn Farms (Clarence, Missouri) and were housed in individual stainless steel cages. The animals were weaned onto standard pig chow purchased from MFA Inc. (Columbia, Missouri). To minimize

cadmium exposure from the diet, the animals were transitioned gradually from the MFA feed to a special low-metal feed (purchased from Zeigler Brothers, Inc., Gardner, Pennsylvania) over the time interval from day –7 to day –3. They were maintained on this feed for the duration of the study. The feed was nutritionally complete and met all requirements of the National Institutes of Health – National Research Council. Each animal was given an amount of feed equal to 4% of the mean body weight of all animals in the dose group. Drinking water was provided ad libitum via self-activated water nozzles in each cage. Analysis of samples from randomly selected drinking-water nozzles during previous studies has indicated that mean cadmium concentration (setting non-detects at one-half the detection limit) is less than 0.05 µg/L.

All doses were delivered daily for 15 consecutive days. The cadmium chloride (in solution) was pipetted, and the cadmium in soil was weighed and placed in the center of a 10- to 20-gram moistened dough ball (prepared by mixing the powdered low-metal feed with water). Animals were given a divided dose at 0900 and 1500 hours, and were fed 2 hours after dosing. Animal weights were recorded, and dose and feed amounts were adjusted on days –1, 2, and every third day thereafter until study termination, to achieve constant doses and feed (as a percent of body weight) during the study.

For three of the soils (CO-SCS soil, OK-SS soil, and DPGC soil), groups of four swine were given oral doses of 20 and 60 µg Cd/kg-day for 15 days. One soil with the highest cadmium concentrations (PTMG soil) was administered to groups of five swine at doses of 240, 480 and 960 µg Cd/kg-day. Cadmium chloride was administered orally to groups of four swine at doses of 10, 60, and 320 µg Cd/kg-day for the same period of time.

Blood samples (6–8 mL) were drawn from the control animals into a plastic syringe by venipuncture of the anterior vena cava, and from those animals that received cadmium doses of 60 µg Cd/kg/day or higher on days 0, 6, 8, 10, 12, and 14. The blood was then transferred immediately into Vacutainer® tubes containing EDTA. On each of the blood sampling days, blood samples were drawn just prior to the 0900 hour dosing (bleed I) and 2 hours after the 0900 hour dosing (bleed II).

On the morning of study day 15, following the last blood collection, all animals were humanely euthanized, and representative samples of the liver (approximately 30 grams of the medial lobe), and the right kidney cortex were collected and stored in cadmium-free plastic bags at –40 °C until being prepared for cadmium analysis. All animals were subjected to detailed examination at necropsy by a certified veterinary pathologist to assess overall animal health.

Concentrations of cadmium in the blood, liver, and kidney cortex samples were determined after acid digestion by graphite furnace atomic absorption spectroscopy (GFAAS), as well as flame AAS for liver and kidney samples to confirm the accuracy of dilutions on samples with high cadmium concentrations. The liver values from furnace AAS were used for data analysis, while for the kidney tissue data the cadmium concentrations were so high that the flame AAS values were deemed more accurate.

Data Analysis. Regression methods were used to estimate the oral bioavailability of cadmium from four test soils relative to cadmium chloride based on cadmium concentrations in liver and kidney tissue, and the area-under-the-curve for blood cadmium concentration vs. time. A single simultaneous regression model was used to estimate the slope for each test material while restricting the intercept to be equal to the response from the control animals for all the test materials. This model is appropriate because at zero dose all of the test materials should yield the same response. As is typical with animal data of this type, the variability in the response increases with increasing dose, a property known as heteroscedasticity. Because heteroscedasticity of the data is contrary to the underlying assumption of equal variance for a linear regression to be applicable, each dose-group was weighted by the inverse of the predicted variance for that dose group (average dose was assumed for each member of a dose group) and analyzed according to procedures from USEPA (*13*). The predicted variance is an estimate of expected variance as a function of the magnitude of the response data, and is considered a more robust measure of variance than the dose-group specific measured variance because it is less affected by individual measurements. Weighting by the inverse of the predicted variance gives less weight to the more variable data points and achieves homogeneous variability across all dose-groups. A simultaneous linear regression model was then fit to each endpoint for the weighted data. The RBA values for each

response (liver, kidney, and blood) for each soil were then estimated as the ratio of the slope for the soil vs. that for cadmium chloride. Fieller's formula was then used to estimate the uncertainty in these RBA estimates, as represented by the upper and lower 95th percentiles and standard error on the RBA estimates.

Results

Soil chemistry and metals concentrations in each test soil are provided in Table 1. Reported cadmium concentrations are the average of triplicate analyses and were used for preparing soil doses to achieve the target dose levels. Concentrations of other metals, some of which may affect enteric absorption of cadmium, are also presented for each of the test soils in Table 1. Cadmium concentrations were far higher in the PTMG soil (4,109 mg/kg) compared to the other soils (47 to 452 mg/kg) and the PTMG soil was also high in chromium, nickel and phosphorus. The OK-SS soil had exceptionally high zinc concentrations. Values of pH in three of the four test soils were near neutral (7.43 to 7.55), while the DPGC soil exhibited a more basic pH (9.06). TOC values ranged from 1.90% to 4.98%, while TIC ranged from less than 0.05% to 1.51%. CEC values did not range widely among the soils, 52.2 to 70.1 meq/100 g. The PTMG soil contained the greatest proportion of sand (coarse, medium, and fine grained), while the CO-SCS and OK-SS soils contained greater proportions of silt-size particles. The DPGC soil was the only one of the four test soils that contained an appreciable quantity of clay-sized particles.

Each test soil exhibited distinct cadmium mineralogy (Table 2) with only a few forms dominating the cadmium-bearing mineral assemblage. These mineralogic forms are cadmium oxide (PTMG), cadmium-calcium-metal oxide (PTMG and CO-SCS), cadmium-metal oxide (CO-SCS), cadmium-metal sulfate (DPGC), and cadmium-iron oxide (OK-SS). All other cadmium-bearing phases were found to account for less than 8% of cadmium mineral mass. The average particle sizes based on long-axis dimension of each predominant cadmium-bearing phase ranged from 2.1 to 34 microns (Table 2).

Blood cadmium concentrations were initially at or below the method detection limit of 0.1 µg/L in all groups and remained at or below detection limits in the control group animals. In animals given repeated oral doses of cadmium chloride and PTMG soil at doses of 60 µg/kg/day or greater, blood levels began to rise within 1 to 2 days, and continued to rise until the end of the study, day 15 (bleed I data for all days shown in Figure 1). For the other three soils (CO-SCS, OK-SS, and DPGC), only animals in the 60 µg/kg/day dose groups were sampled, and even at this dose, blood levels of cadmium were predominately at or below the detection limit of 0.1 µg/L for the duration of the study.

Although the same trends in blood cadmium concentrations were evident in bleed I (collected prior to the first daily dose) and bleed II (collected 2 hours after the second daily dose), the results for bleed II were more variable (data not shown). This result is consistent with the rapidly changing blood cadmium concentrations associated with the absorption of cadmium after the dose was given. Bleed II was included in this study to try to capture data on peak blood cadmium concentrations, and the bleed II values were indeed greater than the bleed I values for the dose groups exhibiting a response in bleed I. As would be expected, the steep slope of the concentration vs. time curve during this interval leads to greater variability in the blood cadmium concentrations. Because of this increased variability in bleed II data, RBA calculations were based on data from bleed I.

The measurement endpoint used to quantify the blood cadmium response was the area-under-the-curve (AUC) for blood cadmium concentration vs. time for days 0 to 14. The AUC was selected because it is the standard pharmacokinetic index of chemical uptake into the blood compartment, and is relatively insensitive to small variations in daily blood cadmium levels. The AUC was calculated using the trapezoidal rule to estimate the AUC between each time point that a blood cadmium value was measured (days 0, 6, 8, 10, 12, and 14), and summing the areas across all time intervals in the study. The mean AUC for each pig was then plotted against the body weight–adjusted dose for that pig by dosing material. The dose-response patterns appear to be linear for the soluble reference material (cadmium chloride) and for the PTMG soil for bleed I (Figure 2). It was not possible to prepare dose-

response curves based on blood data for the CO-SCS, OK-SS, and DPGC soils, because blood cadmium results were at or below detection limits.

Liver and kidney data were subjected to the weighted simultaneous linear regression data analysis described above. Results from this analysis for kidney are shown graphically in Figure 3. The RBA values for each response endpoint (liver, kidney, and blood) for each soil are presented in Table 3. The RBAs for three of the soils (CO-SCS, OK-SS, PTMG) were greater than 0.5, while the RBAs for the DPGC soil were substantially lower. For the CO-SCS, OK-SS and DPGC soils, kidney and liver RBA estimates were in good agreement. For the PTMG soil, kidney and blood AUC RBA estimates were in good agreement, while liver estimates were much higher. The liver results for this soil appear to be an anomaly.

Discussion

In this study, the bioavailability of cadmium from four contaminated site soils was determined relative to soluble cadmium chloride in the blood, kidney, and liver of juvenile swine. All inorganic cadmium forms commonly present in soils induce toxicity by the same mechanism, so these forms may be considered together when assessing bioavailability. The oral toxicity reference values for cadmium are based on a number of chronic studies of renal disease in humans that formed the basis for a toxicokinetic model that was used to estimate the no observed adverse effect level (NOAEL) from cumulative lifetime exposures (*14*). Because the kidney is the primary target organ of toxicity for cadmium, RBA results for that tissue are considered most relevant for risk assessment.

Assuming that the kidney results should be given the greatest weight, the three soils with the greatest cadmium concentrations (PTMG, CO-SCS, and OK-SS) demonstrate modest reductions in bioavailability relative to cadmium chloride (RBA values of 0.60, 0.89, and 0.79, for each soil, respectively). In contrast, the DPGC soil yielded a considerably lower cadmium RBA of 0.18 based on kidney data. An examination of soil characteristics and cadmium mineralogy suggests that this outcome may be due to the more basic soil pH, high clay content of this soil, and the occurrence of a cadmium

form not found in the other soils. Cadmium was present in the PTMG, CO-SCS, and OK-SS soils in a variety of cadmium oxide phases (cadmium-calcium-metal oxide, cadmium-metal oxide, cadmium oxide, and cadmium-iron oxide) and for the DPGC soil as cadmium-metal sulfate. The basic pH in DPGC soil coupled with the high clay content decreases metal solubility and may have contributed to the lower RBA values. Schroder et al. (*6*) reported greater variation in RBAs for juvenile swine exposed to cadmium in ten soils, with RBA values ranging from 10 percent to greater than 100 percent. The soil origin, characteristics and mineralogy were not reported so that study does not yield insights to the factors controlling relative bioavailability of cadmium from soil.

The cadmium containing soils tested in the present study also had elevated concentrations of other metals that might induce the expression of metallothioneins (MTs), proteins that have been reported to affect cadmium toxicity or toxicokinetics (*2, 15, 16*). Recently, renal and hepatic MT induction was shown to be increased in juvenile swine dosed with soils containing elevated zinc concentrations, while a similar induction was not observed in animals receiving cadmium chloride and no excess zinc (*17*). Based on the present study's finding that the soil that had much higher zinc (OK-SS) has RBAs similar to the other two soils dominated by cadmium oxides, it does not appear that MT induction is a critical factor affecting cadmium bioavailability from soil in this model. The ten soils tested by Schroder et al. (*6*) also included a mixture of high and low zinc soils, and reported RBA values did not correlate with zinc concentration.

The relative bioavailability of soil cadmium has also been assessed in rats, resulting in RBA estimates somewhat lower than those from the juvenile swine model. Schilderman et al. (*18*) tested an artificial soil that had been spiked with cadmium chloride and mixed on a mechanical rotator for a two-week period (final concentration of 4,400 mg/kg). The soil mixture was administered with 5 percent gum acacia to 8-week-old male rats in a single gavage dose. Bioavailability for the soil mixture was estimated to be 43 percent relative to cadmium in saline based on the area under the curve of blood concentration vs. time. Cadmium concentrations in the liver and kidneys of the soil cadmium-treated rats were significantly lower than in those of the saline cadmium dosed group. Another study evaluated

the absorption of cadmium from a residential soil sample with 174 mg/kg cadmium collected from residential yards near a historic zinc smelter (*19*). Four-week-old weanling rats were fed diets containing four dose levels of either soil cadmium or soluble cadmium chloride for a period of 30 days. Based on a comparison of liver and kidney data, cadmium in soil was estimated to be 33 percent bioavailable relative to soluble cadmium. Soil characteristics for this zinc smelter soil were generally similar to the zinc smelter soil tested in the present study (OK-SS), and cadmium was present primarily as cadmium metal oxides and sphalerite (ZnS) (unpublished data).

Anatomic and physiologic differences between rats and swine may account for differences in reported relative bioavailability for similar soils. As discussed in Weis and LaVelle (*7*), swine consume discrete, periodic meals, followed by a period of gastric emptying whereas rodents are continuous feeders. Additionally, swine do not exhibit coprophagic behavior that contributes uncertainty associated with dose of the test substance and intake of essential nutrients. The anatomy of the swine gastrointestinal system more closely resembles that of humans than do rodents, which have a forestomach that does not secrete digestive acids and stores gastric flora. High biliary excretion of lead by rats results in uncertainty associated with body burden estimates and may also be a factor for cadmium absorption by rats. Swine, like humans, do not exhibit the levels of biliary excretion seen in rats. Nevertheless, both animal models produce relative bioavailability estimates that are not markedly different. Because the same soil has not be dosed across the different animal models, it is impossible to determine whether the differences in reported RBAs from the different models result from responses of the animals, or from differences in bioavailability from the specific soils tested.

This study provides further evidence of the value of the juvenile swine model in assessing the relative bioavailability of soil cadmium, as well as arsenic, lead, and perhaps other metals, and reinforces the importance of including soil characterization and mineralogic analyses in these studies. The three soils with similar chemical and physical characteristics yielded similar kidney RBA values, ranging from 0.60 to 0.89. Little difference was observed in RBAs for all the oxides of cadmium in these neutral pH soils regardless of mean particle size for the phase. In contrast, the alkaline soil with a cadmium sulfate

phase had a much lower RBA despite having the smallest mean particle size. This finding suggests that the solubility of the predominant cadmium phases may be a more significant factor in controlling relative bioavailability than is particle size.

ACKNOWLEDGMENT

Funding for this study was provided by the Strategic Environmental Research and Development Program (SERDP, Project CU-1165). This study was performed in accordance with the protocol, Systemic Bioavailability of Cadmium in Soil in Juvenile Swine, which was reviewed by the Navy Bureau of Medicine and Surgery. The mineralogy analyses were performed by Dr. John Drexler at the University of Colorado, Boulder. Statistical analyses were performed by Ms. Melanie Edwards.

REFERENCES

(1) ATSDR. 1999. Toxicological Profile for Cadmium. Prepared by Research Triangle Institute, for U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, Atlanta, GA. PB/99/166621. www.atsdr.cdc.gov/toxprofiles.

(2) Klaassen, C.D., Liu, J., and Choudhuri, S. *Annu. Rev. Pharmacol. Toxicol*. **1999**. 39, 267–294.

(3) Satarug, S., Haswell-Elkins, M.R., and Moore, M.R. *Br. J. Nutr*. **2000**. 84, 791–802.

(4) Goyer, R.A., and T.W. Clarkson. 2001. Toxic effects of metals. In: Klassen, C.D. (Ed.). Casarett & Doull's Toxicology: The Basic Science of Poisons, 6th ed. McGraw-Hill, New York, pp. 811–867.

(5) Kelley, M.E., S.E. Brauning, R.A. Schoof, and M.V. Ruby. 2002. Assessing oral bioavailability of metals in soil. Battelle Press, Columbus, OH.

(6) Schroder, J.L., Basta, N.T., Si, J., Casteel, S.W., Evans, T., and Payton, M. *Environ. Sci. Technol*. **2003**. 37, 1365–1370.

(7) Weis, C.P. and LaVelle, J.M. *Chem. Spec. Bioavail*. **1991**. 3, 113–120.

(8) Dodds, J.W. *Fed. Proc*. **1982**. 41, 247–256.

(9) Miller, E.R. and Ullrey, D.E. *Annu. Rev. Nutr*. **1987** 7, 381–382.

(10) NRC. 2003. Bioavailability of Contaminants in Soils and Sediments: Processes, Tools, and Applications. National Research Council. National Academy Press. Washington, DC.

(11) Casteel, S.W., Cowart, R.P., Weis, C.P., Henningsen, G.M., Hoffman, E., Brattin, W.J., Guzman, R.E., Starost, M.F., Payne, J.T., Stockham, S.L., Becker, S.V., Drexler, J.W., and Turk, J.R. *Fundam. Appl. Toxicol*. **1997**. 36, 177–187.

(12) Davis, A., Drexler, J.W., Ruby, M.V., and Nicholson, A. *Environ. Sci. Technol*. **1993**. 27, 1415–1425.

(13) USEPA. 2003. Estimation of relative bioavailability of lead in soil and soil-like materials using *in vivo* and *in vitro* methods. External Review Draft. U.S. Environmental Protection Agency, Washington, DC.

(14) USEPA. 2004. Cadmium. Integrated Risk Information System (IRIS). U.S. Environmental Protection Agency. Available at http://www.epa.gov/iris/.

(15) Miles, A.T., Hawksworth, G.M., Beattie, J.H., and Rodilla, V. *Crit. Rev. Biochem. Mol. Biol*. **2000**. 35, 35–70.

(16) Pinot, F., S.E. Kreps, M. Bachelet, et al. *Rev. Environ. Health* **2000**.15, 229–323.

(17) Turk, J.T., Evans, T.J., Casteel, S.W., and Basta, N.T. *Toxicol. Sci*. **2001**. 60(Suppl.), 414 (Abstract).

(18) Schilderman, P.A.E.L., Moonen, E.J.C., Kempkers, P., and Kleinjans, J.C.S. *Environ. Health Perspect*. **1997**.105, 234-238.

(19) Schoof, R.A. and Freeman, G.B. 1995. Oral bioavailability of lead and cadmium in soil from a smelter site. Seventh International Congress of Toxicology, Seattle, WA. July 3–6, 1995.

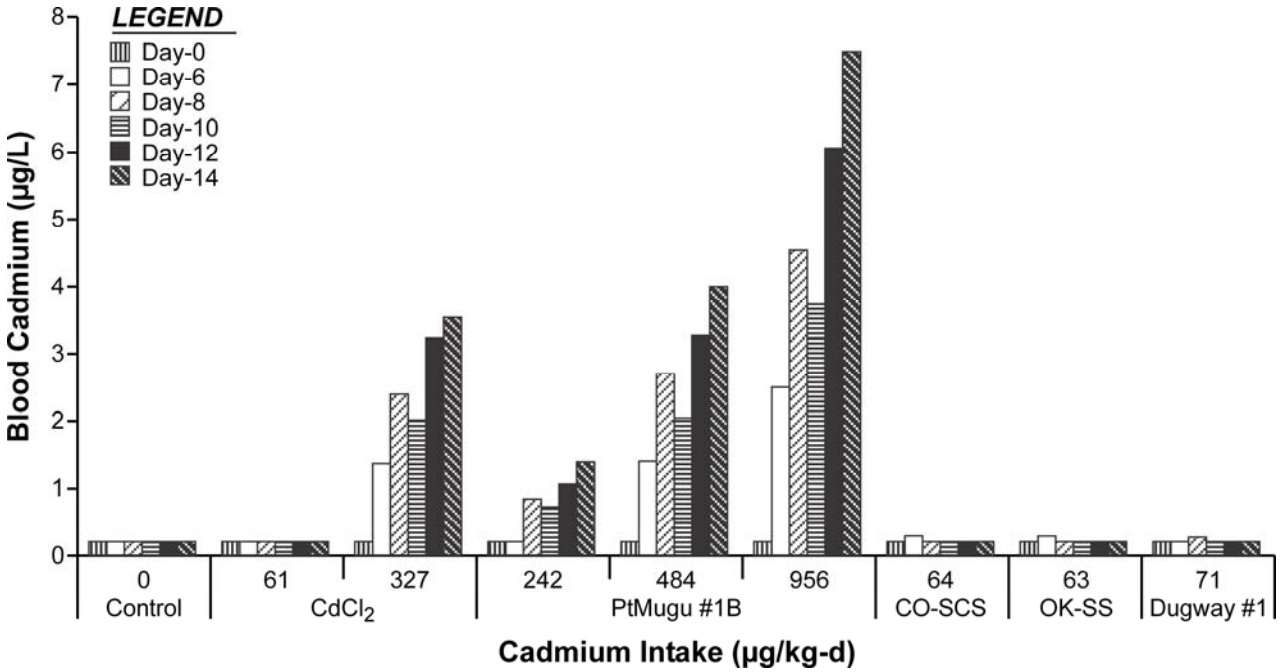


Figure 1. Group mean blood cadmium by day for bleed I. The time course of changes in blood cadmium concentrations are shown for the cadmium chloride reference groups and the Pt. Mugu soil group. Group means are shown.

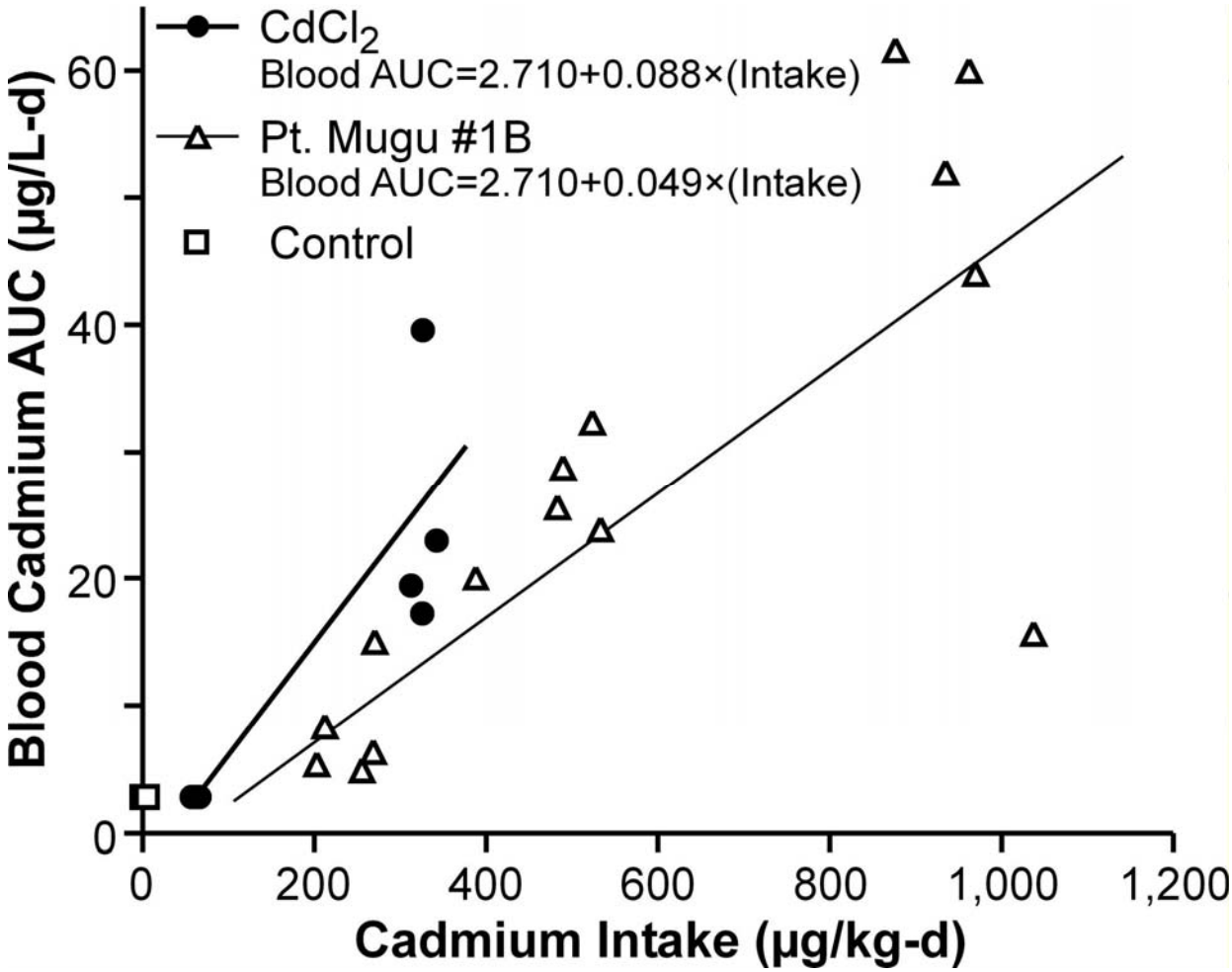


Figure 2. Blood cadmium AUC dose-response for Pt. Mugu soil and cadmium chloride. AUC was calculated using the trapezoidal rule to estimate the AUC at each time point (i.e., days 0, 6, 8, 10, 12, and 14), and summing the areas across all time intervals in the study. Individual animal mean AUC is shown plotted against the body weight-adjusted dose for that animal, along with regression lines from weighted simultaneous linear regression, with each dose group weighted by the inverse of the predicted variance.

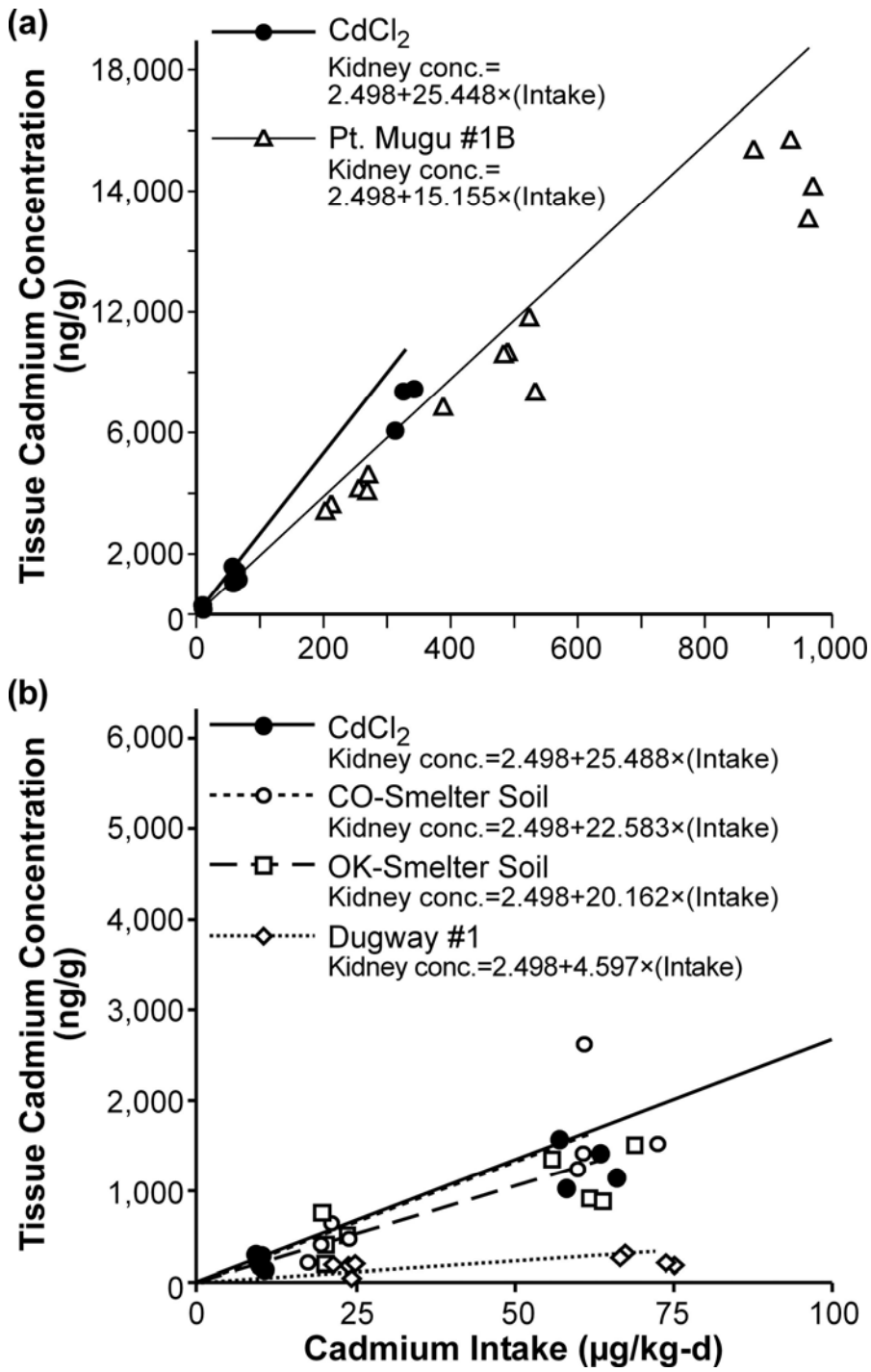


Figure 3. Kidney cadmium dose-response.

Panel A shows kidney cadmium concentration vs. cadmium intakes for the cadmium chloride reference group vs. Pt. Mugu soil, and panel B shows kidney cadmium concentration vs. cadmium intakes for the other three soils as compared to the cadmium chloride reference group. Individual

animal data points are shown along with regression lines from weighted simultaneous linear regression, with each dose group weighted by the inverse of the predicted variance and the soil intercept restricted to be equal to the response from the control animals.

Table 1. Soil Characterization

Chemical	Units	Pt. Mugu Soil (PTMG)		CO Smelter Soil (CO-SCS)		OK Smelter Soil (OK-SS)		Dugway Soil (DPGC)	
Conventionals									
PH	s.u.	8		8		8		9	
Total organic carbon	%	2		2		5		3	
Total inorganic carbon	%	1		0	*U*	1		2	
Cation exchange capacity	meq/100 g	66		54		70		52	
Particle Size Distribution[a]									
Very coarse sand (850–2,000 µm)	%	12		9		13		11	
Coarse sand (425–850 µm)	%	31		11		16		9	
Medium sand (250–425 µm)	%	30		13		15		8	
Fine sand (106–250 µm)	%	20		26		17		30	
Very fine sand (75–106 µm)	%	2		10		4		10	
Percent silt (4–75 µm)	%	2		28		32		3	
Percent clay (<4 µm)	%	3		3		2		30	
Cadmium Concentration[b]	mg/kg	4,109	±375	452	±7	102	±0.7	47	±0.4
Other Metal Concentrations									
Arsenic	mg/kg	165		416		77		9	
Chromium	mg/kg	16,300		26		19		42	
Copper	mg/kg	1,950		89		1,300		45	
Iron	mg/kg	15,600		20,500		22,500		14,100	
Lead	mg/kg	1,140		642		1,000		71	
Manganese	mg/kg	138		510		804		266	
Mercury	mg/kg	2		8		0.900		6	
Nickel	mg/kg	3,850		17		45		24	
Phosphorus	mg/kg	3,310		804		790		1,150	
Silver	mg/kg	171		2	*U*	24		3	
Zinc	mg/kg	1,370		1,310		28,500		394	

Soils sieved to <250 µm
U – undetected; value represents reporting limit
[a] Measured on <2-mm size fraction
[b] Reported cadmium concentrations are based on triplicate analyses.

Table 2. Cadmium Mineralogy

Cadmium Form	Pt. Mugu Soil (PTMG) Percent Mass Distribution	Pt. Mugu Soil (PTMG) Average Particle Size[a] (µm)	CO Smelter Soil (CO-SCS) Percent Mass Distribution	CO Smelter Soil (CO-SCS) Average Particle Size[a] (µm)	OK Smelter Soil (OK-SS) Percent Mass Distribution	OK Smelter Soil (OK-SS) Average Particle Size[a] (µm)	Dugway Soil (DPGC) Percent Mass Distribution	Dugway Soil (DPGC) Average Particle Size[a] (µm)
CdCa(M) oxide	47.6%	16	31.5%	21	--	--	--	--
$CdCl_2$	5.8%	12	--	--	--	--	--	--
Cd(M) oxide	--	--	44.2%	6.4	--	--	--	--
Cd(M) silicate	--	--	6.7%	14	--	--	--	--
Cd(M) sulfate	1.5% [b]	18 [b]	0.4%	2	--	--	99.4%	2.1
Cd oxide	42.6%	4.2	7.7%	3.0	--	--	--	--
Cd sulfide	1.3%	9	--	--	--	--	--	--
CdFe oxide	0.0%	23	1.7%	23	92.5%	34	0.6%	26
CdFe sulfate	0.1%	13	--	--	7.5%	24	--	--
CdPb(M) oxide	--	4	7.0%	4.6	--	--	--	--
No. particles counted	--	176	--	114	--	110	--	108

Note: -- – Not present

Forms contributing less than 1% of cadmium mass in any sample are not shown.

(M) stands for "metals" and generally consisted of a combination of Al, Fe, Pb, Sb, and/or Zn.

[a] Based on long-axis dimensions.

[b] Sum of $CdMSO_4$ and $CdSO_4$ data.

Table 3. Cadmium Relative Bioavailability Estimates

	Pt. Mugu Soil (PTMG)	CO Smelter Soil (CO-SCS)	OK Smelter Soil (OK-SS)	Dugway Soil (DPGC)
Kidney				
RBA[a]	0.60	0.89	0.79	0.18
Lower bound[b]	0.52	0.61	0.53	0.07
Upper bound	0.69	1.19	1.07	0.30
Standard error	0.05	0.17	0.16	0.07
Liver				
RBA	0.96	0.66	0.76	0.09
Lower bound	0.80	0.33	0.40	–0.02
Upper bound	1.19	1.03	1.16	0.21
Standard error	0.11	0.21	0.22	0.07
Blood AUC (bleed1)				
RBA[c]	0.56	NA	NA	NA
Lower bound	0.40	--	--	--
Upper bound	0.89	--	--	--
Standard error	0.12	--	--	--

[a] RBA – Relative bioavailability adjustment

[b] The upper- and lower-bound values represent the upper and lower 95th percentile values on the RBA estimates (based on application of Fieller's formula).

[c] RBA based on blood area–under-the-curve (AUC) was fit excluding the control (0 dose) data, because the response at 0 dose was non-detect.

NA – not analyzed

Supplemental Materials for Section 5

Study Design: Dermal Absorption of Cadmium from Soil in Human Cadaver Skin

Introduction

The physical form of cadmium occurring in soils (i.e., its speciation) is believed to determine the extent to which this element may be absorbed into a human receptor. To date, there have been no studies of dermal cadmium absorption from environmentally contaminated soil, and little is therefore known about the mineralogical and soil factors that control this endpoint. In the Wester et al. (1992) study, dermal cadmium uptake was measured using cadmium chloride freshly mixed with soil. In this study, cadmium chloride was dissolved in water and mixed with the test soil (Yolo County loam; 180–300 μm size fraction) in the laboratory, and then applied to human cadaver skin *in vitro* (loadings of 4 and 40 mg/cm^2) for 16 hours. Dermal absorption of cadmium was estimated to be 0.08 to 0.20%, based on the percent of the applied dose present in the skin and receptor fluid combined.

The *in vitro* studies conducted for this project will use a similar approach to the Wester et al. (1992) study, but will use environmentally contaminated soils to provide estimates of dermal cadmium absorption from soils as they exist in the environment. Exposure conditions in this study have been chosen to be representative of human exposures in the environment. These include a 24-hour exposure period, use of the fine soil fraction (i.e., <150 μm), and a soil loading rate of 4 mg/cm^2. The resultant data will be used to evaluate whether cadmium weathered into soil exhibits significantly lower dermal absorption than cadmium freshly mixed with soil.

Study Design

Dermal absorption of cadmium in soil will be studied in human cadaver skin at the Dermatology Department, University of California, San Francisco. The goal of this study is to replicate the work of Wester et al. (1992), while using cadmium-bearing soils from two environmental sites (provided by Exponent). Experimental procedures will generally be those provided in Wester et al. (1992), with the exceptions specified below.

The test soils for this research will each be sieved to <150 μm (100 mesh) prior to use, and total cadmium concentration will be determined on triplicate aliquots of each sieved soil. The two environmentally contaminated soils to be used in this study will contain cadmium concentrations in the range of 200–1,500 mg cadmium/kg soil. The reference material, consisting of cadmium chloride freshly mixed with Yolo County loam (<150 μm size fraction) (per the method of Wester et al. 1993) will have a concentration in the

range of the environmental soils. Immediately prior to topical application, each test soil will be moistened with 15% (w/w) of de-ionized water.

This study will evaluate two environmentally contaminated soils, and a reference material consisting of cadmium chloride freshly mixed with Yolo County loam (per the method described in Wester et al. 1993). A negative control, consisting of the Yolo County loam with no cadmium applied will also be evaluated. Each of the four substrates will be tested in duplicate using human cadaver skin obtained from four different sources (Table 1). This will result in 32 tests.

Table 1. Study matrix for dermal cadmium absorption study

Human Skin	Formulations							
Source:	A	A	B	B	C	C	D	D
1	X	X	X	X	X	X	X	X
2	X	X	X	X	X	X	X	X
3	X	X	X	X	X	X	X	X
4	X	X	X	X	X	X	X	X

A and B are cadmium-bearing soils (supplied by Exponent), C is the Yolo County soil (used in Wester et al. 1992) mixed with cadmium chloride, and D is the blank control.

Human cadaver skin from four different sources will be dermatomed to 500 μm and stored refrigerated. Flow-through cells with a 5-cm^2 skin surface area will be used, with Eagles MEM (flow rate of 3.0 mL/hr) as the receptor fluid. The test soil (20 mg, <150 μm) will be evenly distributed on the skin surface with a glass rod, to achieve a loading rate of 4 mg/cm^2. Receptor fluid will be collected for a 24-hour exposure period (72 mL sample). At the end of the 24-hour period, the experiment will be stopped, the post-exposure soil will be collected, and the skin surface will be washed once with 1 mL of liquid soap and twice with 1 mL of distilled water. The wash waters will be pooled for analysis. The skin will be completely solubilized in Soluene 350 and 1M HCl, in preparation for analysis. This will result in four analytical samples (1 receptor fluid, 1 post-exposure soil, 1 wash water, and 1 skin sample) per test.

To ensure that cadmium will be detectable in the receptor fluid using the above design, a pilot study will be performed using two different aliquots of the reference material (cadmium chloride mixed with Yolo County soil; <150 μm size fraction) prepared to contain 400 and 2,000 mg cadmium/kg soil (dry weight). Each of these reference materials will be evaluated for percutaneous absorption of cadmium in one cadaver skin type, with each test conducted in duplicate. Duplicate blank control samples (Yolo County soil alone) will also be evaluated during this pilot study. The receptor fluid and skin from these six tests, along with the two samples of reference material, will be analyzed for total cadmium concentration.

All samples generated during this study will be shipped to Battelle Pacific NW Labs in Sequim, Washington, for analysis of total cadmium concentration by inductively coupled plasma/mass spectrometry (ICP/MS; EPA Method 1638). This method will achieve a detection limit of 0.02 ug/g (dry weight) for solid samples (soil and skin), and 0.05 ug/L for the skin wash solution. Receptor fluid samples will be subjected to a preconcentration step (using iron/palladium coprecipitation) to reduce the detection limit in these samples to approximately 0.006 ug/L.

Detection Limit Calculation

Assuming exposure to 24 mg of soil (soil loading of 4 mg/cm^2 on 6 cm^2) with 200 mg Cd/kg soil, and 0.02% absorption into the receptor fluid, we'd have 9.6E-7 mg Cd in the 72 mL of fluid, or about 0.013 ug/L. This is about 2-fold higher than Eric's detection limit for Cd in receptor fluid (even with the preconcentration step). We should be in good shape with the skin samples. I calculate that with 0.1% absorption into the skin sample, we'd have about 0.50 ug Cd/g skin (dry weight, assuming skin density is 0.8 g/cc and 75% water content), or about 25-fold over Eric's detection limit.

FUNDAMENTAL AND APPLIED TOXICOLOGY 20, 336–340 (1993)

In Vivo and *in Vitro* Percutaneous Absorption and Skin Decontamination of Arsenic from Water and Soil

RONALD C. WESTER,* HOWARD I. MAIBACH,* LENA SEDIK,* JOSEPH MELENDRES,* AND MICHAEL WADE†

*Department of Dermatology, University of California School of Medicine, San Francisco, California 94143; and †Department of Toxic Substances Control, State of California, Sacramento, California 95812

Received August 12, 1992; accepted November 18, 1992

In Vivo and *in Vitro* Percutaneous Absorption and Skin Decontamination of Arsenic from Water and Soil. WESTER, R. C., MAIBACH, H. I., SEDIK, L., MELENDRES, J., AND WADE, M. (1993). *Fundam. Appl. Toxicol.* 20, 336–340.

The objective was to determine the percutaneous absorption of arsenic-73 as $H_3A_sO_4$ from water and soil. Soil (Yolo County 65-California-57-8) was passed through 10-, 20-, and 48-mesh sieves. Soil retained by 80 mesh was mixed with radioactive arsenic-73 at a low (trace) level of 0.0004 $\mu g/cm^2$ (micrograms arsenic per square centimeter skin surface area) and a higher dose of 0.6 $\mu g/cm^2$. Water solutions of arsenic-73 at a low (trace) level of 0.000024 $\mu g/cm^2$ and a higher dose of 2.1 $\mu g/cm^2$ were prepared for comparative analysis. *In vivo* in Rhesus monkey a total of 80.1 ± 6.7% (SD) intravenous arsenic-73 dose was recovered in urine over 7 days; the majority of the dose was excreted in the first day. With topical administration for 24 hr, absorption of the low dose from water was 6.4 ± 3.9% and 2.0 ± 1.2% from the high dose. *In vitro* percutaneous absorption of the low dose from water with human skin resulted in 24-hr receptor fluid (phosphate-buffered saline) accumulation of 0.93 ± 1.1% dose and skin concentration (after washing) of 0.98 ± 0.96%. Combining receptor fluid accumulation and skin concentration gave a combined amount of 1.9%, a value less than that *in vivo* (6.4%) in the Rhesus monkey. From soil, receptor fluid accumulation was 0.43 ± 0.54% and skin concentration was 0.33 ± 0.25%. Combining receptor fluid plus skin concentrations gave an absorption value of 0.8%, an amount less than that with *in vivo* absorption (4.5%) in the Rhesus. These absorption values did not match current EPA default assumptions. Washing with soap and water readily removed residual skin surface arsenic, both *in vitro* and *in vivo*. The partition coefficient of arsenic in water to powdered human stratum corneum was 1.1×10^4 and from water to soil it was 2.5×10^4. This relative similarity in arsenic binding to powdered human stratum corneum and soil may indicate why arsenic absorption was similar from water and soil. This powdered human stratum corneum partition coefficient model may provide a facile method for such predictions. © 1993 Society of Toxicology.

Arsenic was the first metal identified as a carcinogen. Strong epidemiological evidence supports the role of arsenic in the induction of skin and lung cancer (Magos, 1991). Skin carcinomas are assumed to be the result of prolonged ingestion of inorganic arsenic in medicine or drinking water. Arsenic exists in soil (naturally and in toxic dump sites) as well as in water. Human skin has continual direct exposure to soil and water during daily activities, work, or play. Chemicals, including metals (Skog and Wallberg, 1964; Wester *et al.*, 1992c), touching skin have the potential to be absorbed into the body. Chemicals can be absorbed into skin from both water and soil matrices (Wester *et al.*, 1987, 1990, 1992b,c). Our objective was to determine percutaneous absorption of arsenic as $H_3A_sO_4$ from water and soil into and through human skin utilizing *in vitro* and *in vivo* technology. Such data aid risk assessment for human health from arsenic-contaminated soil and water. Little data regarding the skin absorption of metals from soil or water are currently available. Recently we have shown that cadmium is absorbed through human skin with both short-term (30 min) and longer-term (24 hr) exposure (Wester *et al.*, 1992c). Risk assessments presently ignore or rely on broad assumptions when estimating exposure via the skin route. Such exclusions or assumptions can lead to significant over- or underexposure estimates (Wester *et al.*, 1992c).

MATERIALS AND METHODS

Formulations. The soil used in these studies has been designated as Yolo County soil sample 65-California-57-8 (26% sand, 26% clay, 48% silt, 0.9% organic). Soil was passed through 10-, 20-, and 48-mesh sieves. Soil retained by 80 mesh was used for studies by first lightly moistening and then adding arsenic-73 as $H_3A_sO_4$ in water. Soil was then mixed well by hand, open to air. The soil load on the skin was 0.04 g soil/cm^2 skin area. The concentration of arsenic in soil was prepared at two dose levels. The low dose was the minimum arsenic that could be used given the specific activity of the compound. This represents general background arsenic. The high dose is representative of what would be encountered in more contaminated areas. This higher dose is also equal in mass to other compounds experimentally dosed on skin. This can be used for comparative purposes. The low (trace) dose gave an arsenic skin concentration of 0.00004 $\mu g/cm^2$. The high dose gave an arsenic skin concentration of 0.6 $\mu g/cm^2$. Arsenic-73 was obtained from Los Alamos Laboratories as $H_3A_sO_4$. Arsenic-73 has a half-life of 80.3 days, so the material was used immediately upon arrival.

0272-0590/93 $5.00
Copyright © 1993 by the Society of Toxicology.
All rights of reproduction in any form reserved.

Corresponding water formulations of arsenic were prepared for comparison. The water load on skin was 5 $\mu l/cm^2$ skin area. This amount of water is a thin layer of water which covers the skin but does not run off the skin. It is similar to a thin layer of other dermatological doses (cream, ointment). The low (trace) dose was 0.000024 $\mu g/cm^2$. The high dose gave an arsenic skin concentration of 2.1 $\mu g/cm^2$.

The intravenous dose given to Rhesus monkeys was 3.0 μCi ^{73}As in 0.5 ml normal saline injected into the saphenous vein.

In vivo. Female Rhesus monkeys (n = 3 or 4) were placed in metabolic chairs and each topical dose was applied to a premeasured 12-cm^2 area of abdominal skin. The skin site area was dosed with soil or water formulation and then it was covered with a nonocclusive cover to stop soil from falling off the skin. The cover was two standard human aluminum eye patches with large holes permeating the surface. Sandwiched between the two eye patches was a sheet of water vapor-permeable membrane (Goretex; 0.2 μm pore size) that allowed free passage of moisture but retained the soil at the skin application site. The monkeys had free access to food and water, but were restricted from touching their abdominal areas by barrier plates. Urine was collected for 24 hr in the pan under the metabolic chair. The cover on the skin site of applications was then washed with soap and water and the monkeys were transferred subsequently to metabolic cages for continued urine collection during the next 6 days. The *in vivo* wash procedure was soap (50/50, v/v, water), water, soap, water, and water. *In vivo* percutaneous absorption is determined by the ratio of urinary excretion following topical application to that following intravenous administration. Therefore, 3 μCi ^{73}As in water was administered intravenously to four monkeys, and urine was collected from the animals in metabolic cages (Wester and Maibach, 1975).

In vitro. Three separate donor skin sources with three replicates per each experiment were used. Small cells were of the flow-through design with a 1-cm^2 surface area. Phosphate-buffered saline at a flow rate of 3.0 ml/hr (reservoir volume) served as receptor fluid. Human cadaver skin was dermatomed to 500 μm and stored refrigerated at 4°C in Eagle's minimum essential medium to preserve skin viability. The skin was used within 5 days. This preservation/use regimen follows that used by the human skin transplant bank (Hurst *et al.*, 1984) and in the work of Bronaugh *et al.* (1989).

^{73}As in soil (0.04 g) formulation as the low dose was applied to the skin surface mounted in three diffusion cells. The soil was distributed evenly on the surface with a glass rod. Glass rods were then rinsed with water, and the radioactivity was determined by scintillation counting. ^{73}As in water formulation as the low dose was applied with a micropipette to the surface of the skin of the next three cells. Standards for each formulation were made by dissolving 0.04 g of soil formulation and 5 μl of water formulation in 10 ml of scintillation cocktail. At the end of a 24-hr period, the system was stopped. The residual soil or water remaining in the cells was collected and analyzed. The skin surface was washed once with 1 ml liquid soap (50/50 water, v/v; Ivory Liquid, Proctor & Gamble, Cincinnati, OH) and twice with 1 ml of distilled water, and the wash solutions were analyzed by scintillation counting. Cells were disassembled. Cell tops were rinsed three times with 1 ml of water. The inner surface of the skin was swabbed with cotton balls and counted. The skin itself was completely solubilized in Soluene 3540 (Packard Instruments, Downders Grove, IL), and 1 M HCl was added to neutralize the homogenate. The receptor fluid samples from the permeation cells' residual fluid, the skin surface washes, the cotton balls, the glass apparatus, and the skin itself were assayed for ^{73}As content by liquid scintillation counting.

Binding to powdered human stratum corneum. The binding behavior of arsenic in water to powdered stratum corneum and to soil was determined (Wester *et al.*, 1987). Powdered stratum corneum was prepared as follows: Callus (obtained from the California College of Podiatic Medicine) was cut into fine pieces with scissors and pulverized in a mortar and pestle containing dry ice. Particles of stratum corneum that would pass through a 48-mesh sieve but were retained by an 80-mesh sieve were used (180 to 300 μm). In a plastic microcentrifuge tube, 1.0 mg of powdered stratum corneum or 1 mg of soil was mixed with 1.0 ml of arsenic chloride solution by vortexing. After a given contact time, the mixture was separated by centrifugation and the supernate removed. Four tubes were prepared for each test.

Scintillation counting. The scintillation cocktail was Universol-ES (ICN, Costa Rica, CA). Background control samples and test samples were counted in a Packard Instruments Model 4640 or Model 1500 counter. Control and test sample counts were transferred to a computer program (Appleworks/Apple IIE computer, Apple Computer Co., Mountain View, CA) which subtracted background control samples and generated a spreadsheet with the data reported under Results. The counting process and computer program have been verified to be accurate by a quality assurance officer.

RESULTS

Figure 1 shows the urinary arsenic excretion in Rhesus monkeys following intravenous administration. A total of 80.1 ± 6.7% (SD) was recovered over 7 days and excretion was completed during that time period. The majority of the dose (65.0 ± 5.1%) was excreted in the first day. This value of 80.1% was used to correct *in vivo* data in Table 1 for excretion by routes other than urine and for body retention.

Table 1 gives the *in vivo* percutaneous absorption of arsenic from water and soil. Absorption of the low dose from water was 6.4 ± 3.9%. A lesser although not significantly ($p > 0.05$; Student's t test) different 2.0 ± 1.2% was absorbed from the high dose. Percutaneous absorption of arsenic from soil was 4.5 ± 3.2% from the low dose and 3.2 ± 1.9% from the high dose (nonsignificant difference).

Figure 2 shows the arsenic urinary excretion following topical application for 24 hr from water and soil formula-

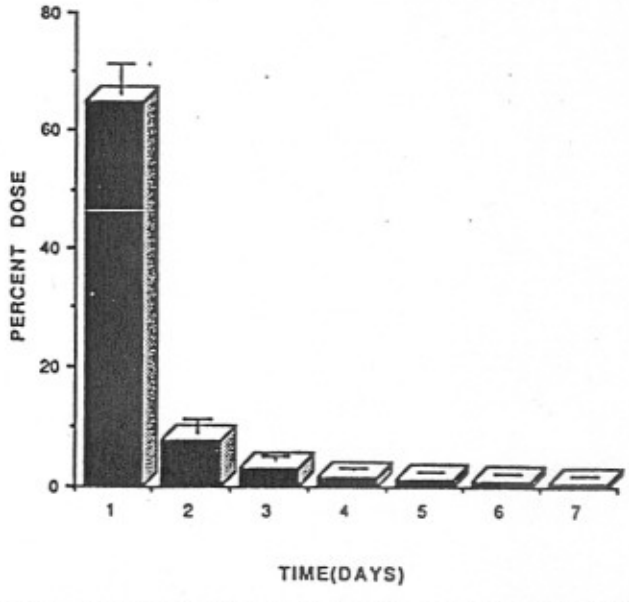


FIG. 1. Arsenic-73 urinary excretion following intravenous administration to Rhesus monkeys (mean ± SD).

TABLE 1
In Vivo Percutaneous Absorption of Arsenic from Water and Soil on Rhesus Monkey

Percent applied dose			
Water		Soil	
Low dose	High dose	Low dose	High dose
6.4 ± 3.9[a]	2.0 ± 1.2[a]	4.5 ± 3.2[b]	3.2 ± 1.9[b]

[a] $n = 3$ (mean + standard deviation).
[b] $n = 4$ (mean + standard deviation).

tions for the low and high doses. Excretion was complete by Day 7 for both soil doses and the water high dose. The water low dose showed some continued arsenic excretion at the end of the urine collection period.

Figure 3 shows the arsenic skin decontamination of the topical low dose after 24 hr skin residence time. A total of 76.3 ± 37.8% was recovered from the water dose and 68.8 ± 11.2% was recovered from the soil dose. The majority of the surface arsenic was recovered with the first soap and water washes. Figure 4 shows the decontamination results for the topical high dose after 24 hr. Surface accountability for the water dose was 77.7 ± 13.6 and 76.6 ± 10.32% for the soil dose. The first soap and water washes were able to remove most of the surface residual arsenic. Combined first soap and water washes removed 62% of the water dose and 59.8% of the soil dose.

Table 2 gives the *in vitro* percutaneous absorption of arsenic from water and soil on human skin. After 24 hr with soil formulation, 0.43 ± 0.54% was recovered in phosphate-buffered saline receptor fluid. After soap and water wash, there was a residual 0.33 ± 0.25% dose in skin. The soap and water wash removed 99.2 ± 1.1%. With water formulation, 0.93 ± 1.1% accumulated in the receptor fluid and 0.98 ± 0.96% was in skin after surface wash. The soap and water wash accounted for 69.8 ± 16.4%. If *in vitro* percutaneous absorption is calculated as receptor fluid accumulation plus residual skin concentration (after soap and water wash) then the absorption in human skin is 0.76% from soil and 1.9% from water.

Table 3 gives the partition coefficient of arsenic from water to powdered human stratum corneum and from water to soil. Arsenic chloride preferred water to both stra-

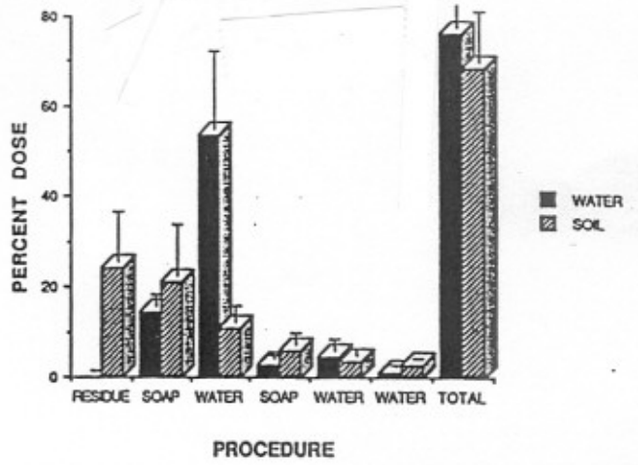


FIG. 3. *In vivo* skin decontamination following 24 hr arsenic-73 topical application of low dose (mean ± SD).

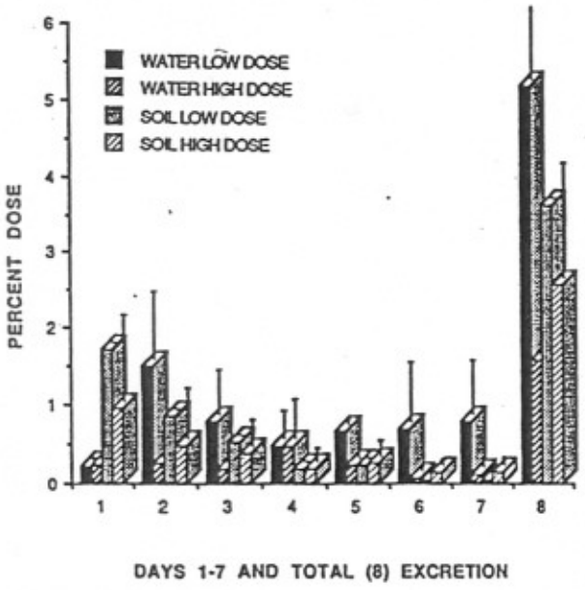


FIG. 2. Daily and total urinary arsenic-73 excretion following topical administration to Rhesus monkeys (mean ± SD).

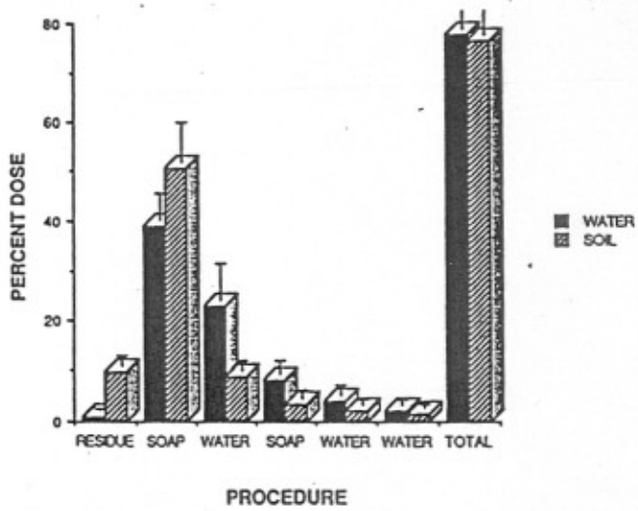


FIG. 4. *In vivo* skin decontamination following 24 hr arsenic-73 topical application of high dose (mean ± SD).

tum corneum and soil. In contrast, arsenic chloride preferred soil over stratum corneum approximately 2.5:1.

DISCUSSION

The *in vivo* percutaneous absorption in the Rhesus monkey of arsenic from soil was 3.2–4.5%, and a not so comparable 0.8% was determined *in vitro* with combined receptor fluid plus human skin. From water the *in vivo* percutaneous absorption was 2.0–6.4% and a somewhat comparable 1.9% was determined *in vitro* with human skin. There are no literature values with which to compare these results. A recent EPA publication (EPA, 1992) suggests a default assumption for inorganics of $K_p^w = 10^{-3}$ cm/hr permeability constant to determine percutaneous absorption. For the high dose from water used in this present study (2.1 μg/5 μl/cm^2 = 420 μg/ml/cm^2) a K_p^w of 1×10^{-3} cm/hr gives an estimated absorption rate of 0.42 μg/cm^2/hr. For 24 hr an absorption of 10.1 μg/cm^2 would be predicted. In actuality only 0.04 μg/cm^2 (2% of 2.1 μg/cm^2) was absorbed, a 250-fold difference between assumption value and measured value. Part of this difference is due to assumption versus actual data. Another cause is the volume used to calculate available dose. The permeability constant uses 1 cm^3 (ml) as the available volume. The present study uses a volume of 5 μl/cm^2, which is a thin film of water over the surface of the skin. This study thus uses a finite dose and the EPA assumes an infinite exposure dose that produces a steady-state rate of absorption. Further laboratory validation of assumed default absorption is obviously needed.

The *in vitro* percutaneous absorption of cadmium from water was 9–12% dose (Wester *et al.*, 1992c) (when receptor fluid and skin concentrations are added), an amount greater than that of arsenic. This may be due to the physical and chemical characteristics of the metals. Arsenic was topically dosed as the soluble $H_3A_sO_4$, and cadmium was dosed as the soluble $CdCl_2$. Arsenic is able to undergo metabolic interconversions (Magos, 1991). Whether the various transformations of arsenic can occur in skin or affect percutaneous absorption has not been determined in the present studies. Technology for such determination does exist (Ademola *et al.*, 1992).

TABLE 2
In Vitro Percutaneous Absorption of Arsenic from Water and Soil on Human Skin

	Percent dose absorbed[a]			
Vehicle	Skin	Surface wash	Receptor fluid	Total
Soil	0.33 ± 0.25	99.2 ± 27.3	0.43 ± 0.54	99.9 ± 27.5
Water	0.98 ± 0.96	69.8 ± 16.4	0.93 ± 1.1	71.7 ± 15.4

[a] Mean ± standard deviation; $n = 9$ (3 skin sources × 3 replicates each).

TABLE 3
Partition Coefficient of Arsenic in Human Powdered Stratum Corneum/Water and Soil/Water

Test material	Partition coefficient[a]
Stratum corneum	10,928
Soil	24,882

[a] Partition coefficient = $\frac{\text{Concentration of arsenic-73 in 1000 mg HPSC (soil)}}{\text{Concentration of arsenic-73 in 1000 ml water}}$.

The percutaneous absorption of arsenic was about 1% from soil *in vitro* and 3–5% from soil *in vivo*. There was no difference between the absorption of arsenic from soil and the absorption from water. Using the same soil source and same techniques, absorption differs between the metals arsenic and cadmium, just as it differs between organic compounds (DDT, BaP, chlordane, and pentachlorophenol) studied in the same systems (Wester *et al.*, 1990, 1992b,c). The compounds, whether inorganic or organic, seem to retain individuality when partitioning from soil or water into and through skin.

Washing with soap and water readily removed most of the surface-resident arsenic for both soil and water vehicles. This seems positive for long-term potential health hazard risk when daily exposure can be limited by simple bathing. Skin decontamination seems related to physical rubbing and solubility in water and soap. The water-soluble glyphosate is readily removed from skin with water-only wash or soap and water (Wester *et al.*, 1991). Lipid-soluble alachlor differs in its skin decontamination for water-only wash and soap and water (Wester *et al.*, 1992b) and is dependent upon the amount of soap (Bucks *et al.*, 1989) (more comes off skin with increased soap concentration).

The partition coefficient of arsenic chloride from water to powdered human stratum corneum was 1.1×10^4 and from water to soil it was 2.5×10^4. This gives an approximate 1:2.5 ratio for stratum corneum and soil. The percutaneous absorption from water and soil to skin was also about equal. In contrast the partition coefficient for cadmium chloride to powdered human stratum corneum was 3.6×10^1 and from water to soil it was 1.0×10^5 (Wester *et al.*, 1992a). Therefore, soil had a greater affinity for cadmium, and not so coincidentally, the *in vitro* absorption of cadmium from soil was much less than that from water. Certainly chemical–soil contamination cannot be disregarded in human health risk assessment.

ACKNOWLEDGMENTS

We thank Dr. James Forsell for the human skin, Dr. James Nickolopoulos for the human callus, and Dr. Steve DiZio for his support to the soil project.

REFERENCES

Ademola, J. I., Wester, R. C., and Maibach, H. I. (1992). Cutaneous metabolism of theophilline by the human skin. *J. Invest. Dermatol.* **98**, 310–314.

Bronaugh, R. L., Stewart, R. F., and Storm, J. E. (1989). Extent of cutaneous metabolism during percutaneous absorption of xenobiotics. *Toxicol. Appl. Pharmacol.* **99**, 534–543.

Bucks, D. A. W., Wester, R. C., Mobayen, M. M., Yang, D., Maibach, H. I., and Coleman, D. L. (1989). *In vitro* percutaneous absorption and stratum corneum binding of alachlor: Effect of formulation dilution with water. *Toxicol. Appl. Pharmacol.* **100**, 417–423.

EPA. (1992). *Dermal Exposure Assessment: Principles and Applications*, EPA/600/8-91/011B, pp. 5-48–5-49. Office of Research and Development, Washington, DC.

Hurst, L. N., Brown, D. H., and Murray, K. A. (1984). Prolonged life and improved quality for stored skin grafts. *Plast. Reconstr. Surg.* **73**, 105–109.

Magos, L. (1991). Epidemiological and experimental aspects of metal carcinogenesis: Physicochemical properties, kinetics, and the active species. *Environ. Health Perspect.* **95**, 157–189.

Skog, E., and Wahlberg, J. E. (1964). A comparative investigation of the percutaneous absorption of metal compounds in the guinea pig by means of the radioactive isotopes: ^{51}Cr, ^{58}Co, ^{65}Zn, ^{110m}Ag, ^{115m}Cd, ^{203}Hg. *J. Invest. Dermatol.* **43**, 187–192.

Wester, R. C., and Maibach, H. I. (1975). Percutaneous absorption in the Rhesus monkey compared to man. *Toxicol. Appl. Pharmacol.* **32**, 394–398.

Wester, R. C., Mobayen, M., and Maibach, H. I. (1987). *In vivo* and *in vitro* absorption and binding to powdered stratum corneum as methods to evaluate skin absorption of environmental chemical contaminant from ground and surface water. *J. Toxicol. Environ. Health* **21**, 367–374.

Wester, R. C., Maibach, H. I., Bucks, D. A. W., Sedik, L., Melendres, J., Liao, C., and DiZio, S. (1990). Percutaneous absorption of [^{14}C]DDT and [^{14}C]benzo(*a*)pyrene from soil. *Fundam. Appl. Toxicol.* **15**, 510–516.

Wester, R. C., Melendres, J., Sarason, R., McMaster, J., and Maibach, H. I. (1991). Glyphosate skin binding, absorption, residual tissue distribution, and skin decontamination. *Fundam. Appl. Toxicol.* **16**, 725–735.

Wester, R. C., Melendres, J., and Maibach, H. I. (1992a). *In vivo* percutaneous absorption and skin decontamination of alachlor in Rhesus monkey. *J. Toxicol. Environ. Health* **36**, 1–12.

Wester, R. C., Maibach, H. I., Sedik, L., Melendres, J., Liao, C., and DiZio, S. (1992b). Percutaneous absorption of [^{14}C]chlordane from soil. *J. Toxicol. Environ. Health* **35**, 269–277.

Wester, R. C., Maibach, H. I., Sedik, L., Melendres, J., DiZio, S., and Wade, M. (1992c). *In vitro* percutaneous absorption of cadmium from water and soil into human skin. *Fundam. Appl. Toxicol.* **19**, 1–5.

TOXICOLOGICAL SCIENCES **79,** 287–295 (2004)
DOI: 10.1093/toxsci/kfh114
Advance Access publication March 31, 2004

In Vivo Percutaneous Absorption of Arsenic from Water and CCA-Treated Wood Residue

Ronald C. Wester,*,[1] Xiaoying Hui,† Sherry Barbadillo,† Howard I. Maibach,† Yvette W. Lowney,‡ Rosalind A. Schoof,§ Stewart E. Holm,¶ and Michael V. Ruby‡

*University of California, San Francisco, Dermatology Department, PO Box 0989, San Francisco, California 94143-0989; †University of California, San Francisco, California 94143-0989; ‡Exponent, Boulder, Colorado 80301; §Integral Consulting, Inc., Mercer Island, Washington; and ¶Georgia-Pacific Corporation, Atlanta, Georgia 30303-1847

Received September 30, 2003; accepted February 5, 2004

This study was conducted to evaluate the dermal absorption of arsenic from residues present on the surface of wood preserved with chromated copper arsenate (CCA). The research reported herein used methods parallel to those of earlier research on the dermal absorption of radiolabeled arsenic (R. C. Wester *et al.,* 1993, *Fund. Appl. Toxicol.* 20, 336–340), with modifications to allow use of environmental matrices that are not radiolabeled. These modifications include the surface area of application and dietary intake of arsenic, thus maximizing the potential for detection of dermally absorbed arsenic in exposed animals above diet-associated background levels of exposure. Two forms of arsenic were administered in this work. The first, arsenic in solution, was applied to the skin of monkeys to calibrate the model against prior absorption research and to serve as the basis of comparison for absorption of arsenic from CCA-treated wood residues. The second substrate was residue that resides on the surface of CCA-treated wood. Results from this research indicate that this study methodology can be used to evaluate dermally absorbed arsenic without the use of a radiolabel. Urinary excretion of arsenic above background levels can be measured following application of soluble arsenic, and absorption rates (0.6–4.4% absorption) are consistent with prior research using the more sensitive, radiolabeled technique. Additionally, the results show that arsenic is poorly absorbed from CCA-treated wood residues (i.e., does not result in urinary arsenic excretion above background levels).

Key Words: **dermal arsenic absorption; CCA; arsenic exposure; environmental arsenic.**

Prior research on the dermal absorption of soluble arsenic administered in water, and soluble arsenic mixed with soil, in Rhesus monkeys (Wester *et al.,* 1993) produced mean dermal absorption rates for soluble arsenic in the range of 2.0–6.4% of the applied dose. Percent absorption did not vary across five orders of magnitude in the applied dose. Also, in Wester *et al.* (1993), the absorption rates for arsenic from the test soil fell within the range of the rates for percutaneous absorption of the arsenic administered in water. The research method was based on dermal application and subsequent urinary excretion of radiolabeled arsenic (As^{73}), thereby permitting detection of very small amounts of absorbed arsenic in the urine. Subsequent to this research, questions arose as to whether the data on dermal absorption of soluble arsenic mixed with soil immediately prior to dermal application are representative of arsenic absorption from environmental media (U.S. EPA, 2001a). Specifically, this issue affects the ongoing discussion of dermal absorption of arsenic from wood treated with chromated copper arsenate (CCA). Currently, the U.S. EPA is evaluating whether children who repeatedly contact playground equipment or decks made from CCA-treated wood may face increased risks from the associated arsenic exposures (U.S. EPA 2001a, 2003). The U.S. EPA assessment currently relies on dermal arsenic absorption data generated for soluble arsenic and soluble arsenic mixed with soil, and may not be representative of exposures associated with contact with CCA-treated wood. This paper used a methodology similar to that used by Wester *et al.* (1993) to assess dermal arsenic absorption from the residues that would adhere to an individual's skin after contacting the surface of CCA-treated wood.

Among several challenges associated with studying exposure to arsenic from environmental media is the large degree of exposure to background levels of arsenic from the diet (Schoof, 1999a,b; Yost *et al.*, 2004). Typical daily urinary arsenic excretion for Rhesus monkeys consuming the standard diet of Purina monkey chow is 5–15 μg As/day. In the Wester *et al.* (1993) research, the use of a radiolabeled arsenic source circumvented the confounding effects of concomitant dietary exposures and associated difficulties in data interpretation. For study of environmental samples (e.g., contaminated soils or treated wood), it is not practicable to use a radiolabeled source. Therefore, a new research protocol was designed, incorporating a low-arsenic diet. Urine samples were analyzed using inductively coupled plasma/mass spectrometry, which pro-

[1] To whom correspondence should be addressed. Fax: (415) 753-5304. E-mail: rcwgx@itsa.ucsf.edu.

Toxicological Sciences vol. 79 no. 2 © Society of Toxicology 2004; all rights reserved.

vided an adequately low detection limit for total arsenic in urine. This alteration in the study design allows for a sensitive evaluation of dermal arsenic absorption from natural environmental media.

The research reported herein describes the use of the Rhesus monkey model to measure the dermal absorption of arsenic from water and from residues collected from the surface of CCA-treated wood. The Rhesus monkey is a relevant animal model for *in vivo* human percutaneous absorption (Wester and Maibach, 1975, 1989).

MATERIALS AND METHODS

Formulations and dosing rates. An open crossover design was used, in which each animal is dosed in each of the trials (soluble arsenic in solution applied to the skin, CCA residue applied to the skin, and iv injection), with a washout period of at least 14 days between each dose. This design allows for each animal to serve as its own internal control.

The iv dose (1060 μg arsenic/monkey) was administered as a solution of sodium arsenate heptahydrate in deionized (DI) water (2120 mg/l arsenic). For the iv dose, each monkey received 0.5 ml of the dosing solution injected into the saphenous vein. The iv dose was given while the monkeys were in their metabolic cages, so the monkeys did not spend any time in the metabolic restraint chairs, as they did with the topical doses.

For the soluble arsenic dose, arsenic was administered in water onto the monkey's skin at an application rate of 5 μl/cm^2 evenly applied across 100 cm^2 of skin, to achieve a total dermal dose of 1430 μg arsenic (Table 1). The solution was prepared from sodium arsenate heptahydrate in DI water, which was acidified with 1% nitric acid (trace-metal grade). The soluble arsenic dose was designed to match the arsenic dose applied in the CCA-treated wood residue.

The CCA residue used in this study is the easily dislodgeable material present on the surface of CCA-treated wood, and was collected from the surface of CCA-treated wood that had been weathered in the environment. This represents the material that a human might contact during play or use of a CCA-treated wooden structure. Consideration was given to using an actual piece of CCA-treated wood in this research, but we elected to use the "collected residue" for the following reasons:

- If actual wood were used, it would be impossible to accurately characterize the dose of arsenic applied to the skin.
- There was concern that the environment of the skin (e.g., transdermal water loss, irritation) may be modified if a solid structure such as wood was applied directly.
- We could not ensure adequate wood-to-skin contact for a solid wood material. If the wood was not held in good contact with the skin, then the results would be biased low.
- Prior to using the "collected residue," we evaluated the chemical structure of arsenic in the residue and on the surface of CCA-treated wood (new and aged). These results indicate that the nature of the arsenic in the residue is identical to the arsenic on the surface of the wood (Nico *et al.,* 2003).
- Because the form of arsenic in the residue was the same as in the treated wood samples, and because use of the residue circumvented the issues associated with items 1–3 above, we determined that use of the residue provided the best study matrix.

The residue, in the form of a fine particulate, was supplied by the American Chemistry Council (ACC, 2003), and represents the material present on the surface of CCA-treated wood, which an individual might contact during use of, or play on, structures made of treated wood. In collecting the "residue" from the surface of the wood, efforts were made to collect the material on the surface of the wood that might be dislodged during direct human contact with the wood. Specifically, CCA-treated boards consisting of either Southern Yellow Pine or Ponderosa Pine were removed from in-service residential decks in Michigan and Georgia. Deck structures ranged from one to four years of age and had no coatings applied. Aged structures were selected, because they were believed to best represent the material that an individual might contact over time. As described below, recent chemical characterization work indicates that the chemical structure of the arsenic in the residue collected from the surface of decks is indistinguishable from the form of arsenic in newly treated or aged CCA-treated wood structures. A total of 1456 board sections (each 2 ft. long) were collected and shipped to Michigan State University, where the residue was collected as a single composite from multiple boards. The residue was collected by wiping the boards with a soft-bristle test-tube brush while rinsing with DI water. The rinsate and residue collected in this manner were filtered through glass wool, concentrated by rotary evaporation under vacuum at 46°C, and then air dried in a fume hood at 22°C and 65% humidity. The dried residue was irradiated using Cobalt-60 irradiation for 3 h, to eliminate possible microbial contamination of the sample.

Duplicate aliquots of the residue material used in the dermal dosing studies were analyzed for arsenic, chromium, copper, iron, and manganese concentrations, which involved digestion in refluxing nitric acid and analysis by inductively coupled plasma mass spectroscopy (ICP-MS; EPA Method 6010B; U.S. EPA, 1997). This analytical method was used to ensure adequate sensitivity for all metals of interest. As a means of comparing the composition of the

TABLE 1
Arsenic Doses Given during This Study and Earlier Dermal Absorption Studies

Study	Concentration[a]	Volume[b]	Arsenic mass: Dosed (μg)	Arsenic mass: Per unit area (μg/cm^2)
Soluble dose	2860 mg/l[c]	0.5 ml	1430	14.3
CCA residue	3555 mg/kg[c]	400 mg	1422	14.2
Intravenous dose	2120 mg/l	0.5 ml	1060	—
Soluble dose (Wester *et al.,* 1993)				
High dose	—	0.06 ml	76	2.1
Low dose	—	0.06 ml	0.00086	0.000024

Note. —, not available or not applicable.
[a]Arsenic concentration in dosing material.
[b]Volume of dosing material administered.
[c]Average of duplicate analyses.

CCA residue with the composition of treated wood, samples (a 1-cm^2 wood chip from the top 0.2 cm of wood surface) of newly treated wood and a sample of weathered wood from a five-year-old CCA-treated residential deck were subjected to identical digestion and analyses.

For very fine soil (i.e., silty clay), a loading of 5.4 mg/cm^2 of skin results in a monolayer (U.S. EPA, 2001b). Because the residue appears similar in particle size distribution to silty clay, and a loading rate of 4 mg/cm^2 of the residue provides complete coverage on a flat surface, a loading rate of 4 mg/cm^2 was selected for this study. Application of 4 mg/cm^2 on 100 cm^2 of skin area resulted in a total dose of 1422 μg arsenic (Table 1). The residue was applied as a dry powder, and spread in an even layer across the exposure area.

In Vivo *Model.* Female Rhesus monkeys were selected for this research because of their ability to duplicate the biodynamics of percutaneous absorption in humans, and because previous studies of percutaneous arsenic absorption have used this same model. Prior research indicates that percutaneous absorption in the Rhesus monkey is similar to absorption in humans across a variety of chemicals and range of dermal penetration characteristics (Wester and Maibach, 1975). This research indicates that measurements from the monkey are just slightly higher than their counterparts in the human. Results from other species (pig, rat, rabbit) are not nearly as close to the values measured in humans, and indicate that, of the species tested, absorption in the monkey is closest to that in the human.

The monkeys were approximately 20 years old, which is the same approximate age as the monkeys used in the previous dermal arsenic absorption research (Wester *et al.,* 1993). The animals reside within the monkey colony maintained by the University of California, San Francisco, and have not been used for active research for 18 months. Prior to the beginning of the current series of studies, no topical doses had been applied to the skin of these animals for more than four years.

Each topical dose was applied to a pre-measured 100-cm area of abdominal skin of three monkeys. The dosing area was demarked by "masking" the boundaries with a single layer of Tegaderm (a water-vapor-permeable adhesive membrane available from 3M Health Care, St. Paul, MN) and then was dosed by spreading the fluid (5 $\mu l/cm^2$) or residue (4 mg/cm^2) evenly across the 100-cm^2 dosing area. The dosing area was then covered with a layer of Tegaderm to ensure that the material remained in contact with the skin. The Tegaderm patch over the dosing area extended well beyond the boundaries of the exposure area. In addition to the Tegaderm patch, the abdomen of each monkey was wrapped with Spandage Instant Stretch Bandage (MEDI-TECH International Corp., Brooklyn, NY) to ensure that the applied dose was kept in direct contact with the skin throughout the dosing period. This bandage is of a web construction; most of the Tegaderm was exposed to the open air for moisture and air exchange. Following application of the topical doses, the monkeys were placed in metabolic restraint chairs for the duration of the eight-h dosing period. The eight-h dosing period was selected to represent an upper bound of time that an individual might remain in contact with residues, and is also the upper limit of time that the monkey can remain in the metabolic restraint chair. During this time, the monkeys had free access to water, but were restricted from touching their abdominal area. Researchers remained in the room and interacted with the monkeys, and the monkeys were hand fed bananas and liquid diet during this stage.

Urine was collected during the 8-h dosing period in a pan under the metabolic chair. After 8 h, the monkeys were removed from the chairs, the Spandage bandage and Tegaderm patch were removed, and the applied doses were removed using a soap and water wash (50/50 v/v, soap and water, followed by water, soap, and two final water washes). The monkeys were then transferred to metabolic cages for continued urine collection over the following seven days.

As with humans, significant exposure to arsenic occurs from the normal diet (Schoof, 1999a,b; Yost, 2004). Urinary excretion of total arsenic for Rhesus monkeys on the standard diet of Purina Monkey Chow falls in the range of 5 to 15 μg/day—levels that would obscure accurate detection of the arsenic that might be absorbed following topical application of arsenic. Therefore, the monkeys were provided a low-arsenic diet (Primate Liquidiet from BioServe, Inc.) for seven days prior to each dose. The powdered Liquidiet formulation also was prepared into meal bars, which were provided *ad libitum* to the monkeys during the research period (seven days prior to dosing through seven days after dosing). The diet was supplemented with pieces of banana and apple, which are both known to be low in total arsenic (Schoof *et al.,* 1999a). DI water was provided *ad libitum.* The liquid diet was provided as both liquid and solid forms. Preference was for the solid form. The monkeys maintained their body weight during the study.

The monkey urine samples were preserved with nitric acid (2%) at the time of collection, and shipped to Battelle Pacific Northwest Laboratories in Sequim, Washington, for analysis. At Battelle, the urine samples were acidified with an additional 2% (by volume) of concentrated nitric acid and analyzed for total arsenic by ICP/MS (Method 1638, U.S. EPA, 2002). This method provides a method detection limit (MDL) of approximately 0.1 μg/l arsenic in monkey urine. Quality assurance and quality control (QA/QC) samples included a method blank, duplicates, matrix spikes, and a laboratory control sample at a 5% frequency of analysis.

RESULTS

Total metals concentrations of arsenic, chromium, and copper in the residue are presented in Table 2, along with corresponding data for a sample of newly treated wood (recently purchased from a local retailer), and a sample of weathered wood from a five-year-old residential deck. The relative concentrations of these three metals are similar for all three samples, indicating that the residue contains a proportion of the CCA metals that is similar to both freshly treated and aged wood. As expected, concentrations of all three metals are somewhat lower in the wood-chip samples than in residue. Although the residue is largely composed of decayed wood from the wood surface, larger wood fragments were removed from the sample during preparation of the residue, when the residue is filtered through glass wool. In contrast, the wood-chip samples contained a larger proportion of wood matter. More instructive is the ratio of the different metals from these analyses, which are similar across the samples.

Data for the mass of urinary arsenic excreted by the monkeys following dermal dosing are presented in Table 3 (soluble arsenic), Table 4 (CCA residue), and Table 5 (iv dose). Data on the background arsenic excretion for each monkey for the days prior to the dosing period are included. The value reported for the 0- to 24-h period is the combined arsenic mass from the urine collected during the 8-h dosing period, a wash of the

TABLE 2
Metal Concentrations in CCA Residue and Wood

Sample	Arsenic (mg/kg)	Chromium (mg/kg)	Copper (mg/kg)
CCA residue			
Sample 1	3600	4120	2260
Sample 2	3510	4070	2220
Weathered CCA-treated wood	1760	2700	942
Freshly-treated CCA wood	2730[a]	3080[a]	1545[a]

[a]Average of lab duplicates.

TABLE 3
Urinary Arsenic Data following Dermal Application of Arsenic in Soluble Dose

	24-h Mass excreted	
	(μg)	Corrected[a] (μg)
Animal 1		
Background		
24–48 h	5.07	0.00
0–24 h	1.56	0.00
0–24 h	41.58[b]	35.50
24–48 h	7.22	1.13
48–72 h	8.08	1.99
72–96 h	7.21	1.12
Total arsenic mass excreted (0–96 h)		39.74
Total arsenic mass excreted with urinary arsenic excretion fraction correction (0–96 h)		48.41[c]
Percent absorption (0–96 h)		3.4%[d]
Animal 2		
Background		
24–48 h	6.30	0.82
0–24 h	7.08	1.61
0–24 h	10.22[b]	4.75
24–48 h	6.96	1.48
48–72 h	5.32	0.00
72–96 h	6.53	1.05
Total arsenic mass excreted (0–96 h)		7.28
Total arsenic mass excreted with urinary arsenic excretion fraction correction (0–96 h)		8.87[c]
Percent absorption (0–96 h)		0.62%[d]
Animal 3		
Background		
24–48 h	5.20	1.79
0–24 h	3.07	0.00
0–24 h	30.35[b]	26.94
24–48 h	20.98	17.56
48–72 h	4.52	1.10
72–96 h	9.16	5.75
Total arsenic mass excreted (0–96 h)		51.35
Total arsenic mass excreted with urinary arsenic excretion fraction correction (0–96 h)		62.55[c]
Percent absorption (0–96 h)		4.4%[d]

[a]Corrected mass calculated by subtracting median of the eight background arsenic masses for each monkey. If corrected mass is calculated less than zero, corrected mass is set to zero.

[b]Sum of (0–8 h), pan wash, and (8–24 h). Pan wash concentration is calculated using pan wash concentration minus average of wash water concentrations.

[c]Calculated by correcting excreted mass for fractional excretion of arsenic from iv dose (i.e., 0.821 or 82.1%).

[d]Percent absorption calculated using soluble applied dose mass of 1430 μg.

urine collection pan, and the urine collected from 8 h to 24 h after the monkeys were returned to their cages. The right-most column in each of these tables presents the mass of arsenic excreted for each 24-h period, corrected for background levels

TABLE 4
Urinary Arsenic Data following Dermal Application of Arsenic in CCA Residue

	24-h Mass excreted	
	(μg)	Corrected[a] (μg)
Animal 1		
Background		
96–120 h	7.88	1.79
48–72 h	6.44	0.35
0–24 h	5.73	0.00
0–24 h	4.84[b]	0.00
24–48 h	4.90	0.00
48–72 h	4.86	0.00
72–96 h	5.89[c]	0.00
Total arsenic mass excreted (0–96 h)		0.00
Total arsenic mass excreted with urinary arsenic excretion fraction correction (0–96 hs)		0.00[d]
Percent absorption (0–96 h)		0.00%[e,f]
Animal 2		
Background		
96–120 h	5.79	0.32
48–72 h	1.92	0.00
0–24 h	4.59	0.00
0–24 h	4.17[b]	0.00
24–48 h	2.93	0.00
48–72 h	3.77	0.00
72–96 h	3.78[c]	0.00
Total arsenic mass excreted (0–96 h)		0.00
Total arsenic mass excreted with urinary arsenic excretion fraction correction (0–96 h)		0.00[d]
Percent absorption (0–96 h)		0.00%[e,f]
Animal 3		
Background		
96–120 h	4.40	0.99
48–72 h	4.88	1.47
0–24 h	3.44	0.03
0–24 h	4.24[b]	0.83
24–48 h	3.26	0.00
48–72 h	3.94	0.53
72–96 h	3.39[c]	0.00
Total arsenic mass excreted (0–96 h)		1.37
Total arsenic mass excreted with urinary arsenic excretion fraction correction (0–96 h)		1.66[d]
Percent absorption (0–96 h)		0.12%[e,f]

[a]Corrected mass calculated by subtracting median of the eight background arsenic masses for each monkey. If corrected mass is calculated less than zero, corrected mass is set to zero.

[b]Sum of (0–8 h), pan wash, and (8–24 h). Pan wash concentration is calculated using pan wash concentration minus average of wash water concentrations.

[c]24-h mass excreted is estimated as ¼ of 72–168 h sample mass.

[d]Calculated by correcting excreted mass for fractional excretion of arsenic from iv dose (i.e., 0.821 or 82.1%).

[e]Percent absorption calculated using CCA residue applied dose mass of 1422 μg.

[f]Not statistically different from background.

TABLE 5
Urinary Arsenic Data following Intravenous Arsenic Dose

	24-h Mass excreted	
	μg	Corrected[a] (μg)
Animal 1		
Background		
96–120 h	5.14	0.00
48–72 h	8.64	2.55
0–24 h	7.10	1.01
0–24 h	767.28[b]	761.19
24–48 h	65.88	59.79
48–72 h	19.54[c]	13.45
72–96 h	19.54[c]	13.45
Total arsenic mass excreted (0–96 h)		847.88
Percent absorption (0–96 h)		80.0%[d]
Animal 2		
Background		
96–120 h	5.16	0.00
48–72 h	7.26	1.79
0–24 h	4.54	0.00
0–24 h	761.84[b]	756.36
24–48 h	80.45	74.97
48–72 h	24.60[c]	19.13
72–96 h	24.60[c]	19.13
Total arsenic mass excreted (0–96 h)		869.59
Percent absorption (0–96 h)		82.0%[d]
Animal 3		
Background		
96–120 h	2.25	0.00
48–72 h	2.91	0.00
0–24 h	3.38	0.00
0–24 h	706.09[b]	702.68
24–48 h	123.50	120.09
48–72 h	38.68[c]	35.26
72–96 h	38.68[c]	35.26
Total arsenic mass excreted (0–96 h)		893.29
Percent absorption (0–96 h)		84.3%[d]

[a]Corrected mass calculated by subtracting median of the eight background arsenic masses for each monkey. If corrected mass is calculated less than zero, corrected mass is set to zero.

[b]Sum of (0–8 h), cage wash, and (8–24 h). Cage wash concentration is calculated using cage wash concentration minus average of wash water concentrations. [iv-dosed monkeys did not use the metabolic chair, and the cage wash was collected from below the cages after collection of the (0–8 h) sample.]

[c]24-h mass excreted is estimated as ½ of 48–96 hr sample mass.

[d]Percent absorption calculated using intravenous dose of 1060 μg.

of arsenic in urine by subtracting out the median of the eight background data points for each monkey, on a monkey-specific basis. (In other words, the eight background values for each monkey were compiled, and the median was calculated for each monkey. The median values of 6.09, 5.48, and 3.41 μg arsenic/24-h period for monkeys 1, 2, and 3, respectively, were subtracted out of the 24-h urine value to yield "background-corrected" values.) The median value was selected because it is the best representation of the central tendency of background urinary arsenic excretion over time, and is less sensitive to potential outlier effects (Fig. 1). This correction was applied to the data to reduce the influence of dietary arsenic on the excreted arsenic mass. The mass of arsenic excreted that is associated with the dermally applied dose is calculated by adding the mass excreted from the time of dosing through 96 h after dosing. After 96 h, the arsenic excretion has returned to background levels.

Prior research indicates that for female Rhesus monkeys, urinary excretion of an iv dose of arsenic was 80 ± 6.7% of the administered dose (Wester *et al.,* 1993). The iv dose given during this study resulted in 82.1 ± 2.2% of the administered arsenic dose excreted in urine (Table 5). The average urinary arsenic excretion value from this study (82.1%) was used to adjust the assumed total mass of arsenic excreted over the 96-h collection period, by dividing the calculated mass excreted by 0.821. This correction is intended to account for the fraction of arsenic that might be retained within the body or excreted by other routes (e.g., feces). This calculated mass excreted was then divided by the applied dose to calculate the percent of the applied dose that was absorbed for each animal and each dosing substrate. The percent absorption of arsenic was calculated in the following manner:

$$\text{Percent absorption} = \frac{(\text{Corrected mass excreted}_{0-96\text{ hours}} \div \text{Urinary Excretion Fraction})}{\text{Applied dose}} \times 100 \quad (1)$$

For the soluble dose, absorption rates were 3.4, 0.62, and 4.4% for the three monkeys in the study (Table 3). Dosing levels used in our earlier research on the dermal absorption of arsenic are compared to those used in this study in Table 1. Despite the nearly seven-fold difference in the dermal loading rate between the two studies, the average absorption rate for the group dosed with soluble arsenic (2.8%) is consistent with results from Wester *et al.* (1993) (Table 6). These results are consistent with the previous study, wherein absorption rates were relatively consistent (range of 2–6.4%) despite a five-orders-of-magnitude change in the dose levels (i.e., an applied dose range of 0.000024 to 2.1 μg/cm^2). These data strongly support the suggestion that the difference in the measured absorption rates in the Wester *et al.* (1993) research reflects experimental variability rather than dose-related differences in absorption (U.S. EPA, 2001b). This is consistent with our understanding of individual variability in percutaneous absorption in humans and animals (Wester and Maibach, 1991, 1997).

Converse to the results for soluble arsenic, data from dermal application of CCA residue indicate virtually no absorption. Absorption rates following dermal application of residue are presented in Table 4. These data show that urinary excretion of arsenic following dermal application of the CCA residue does

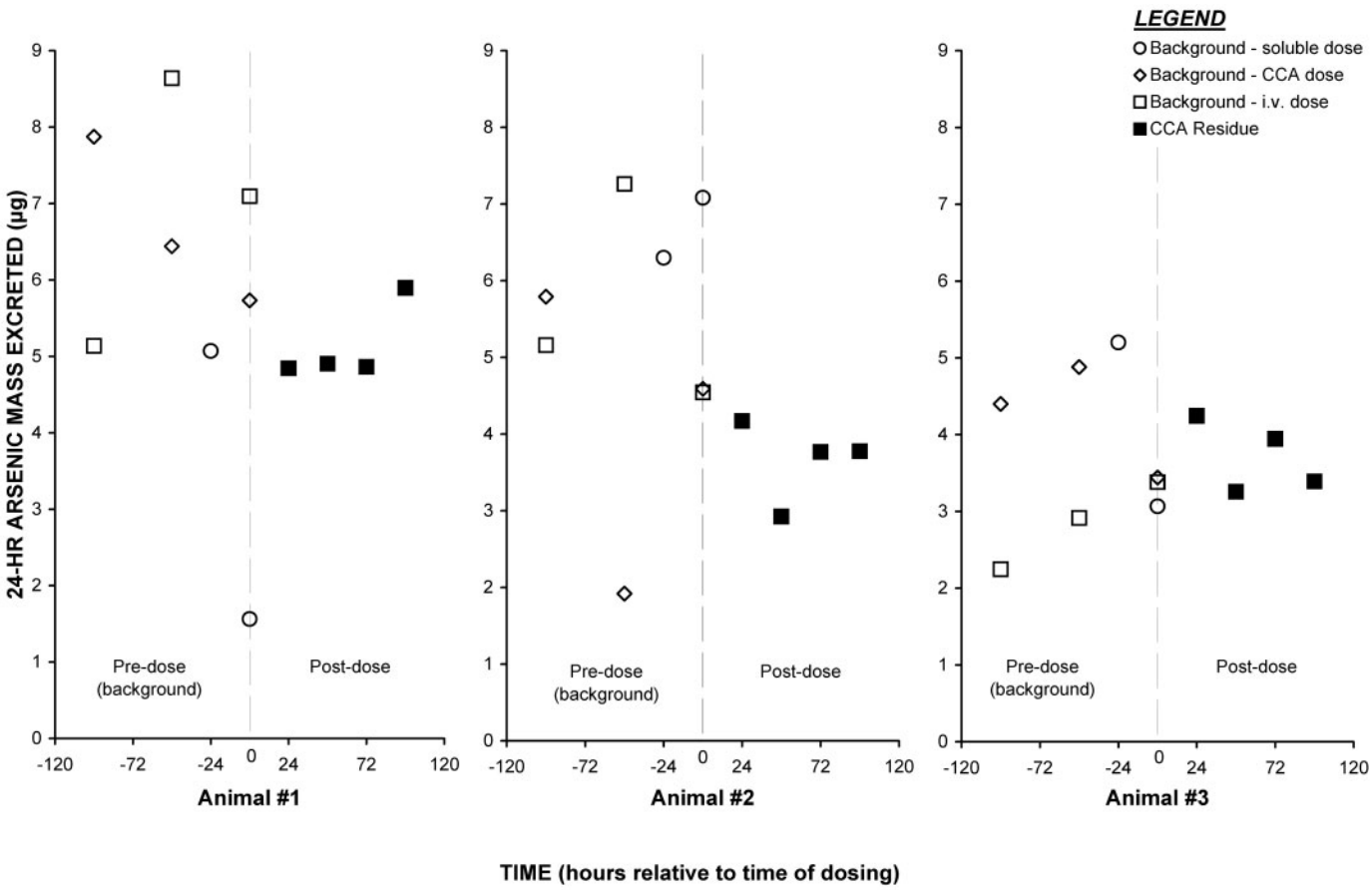


FIG. 1. Background urinary arsenic mass excretion in comparison to excretion following dosing with CCA residue.

not cause a detectable increase in urinary arsenic excretion, despite the fact that equivalent doses of arsenic were applied for both soluble arsenic and residue.

TABLE 6
Summary of Dermal Arsenic Absorption Values from Various Dosing Substrates

Substrate	Percent absorption	
	Average ± SD	(Range)
Soluble dose	2.8 ± 1.9	(0.62–4.4)
CCA residue	0.04 ± 0.07[a]	(0.00–0.12)
Wester *et al.* (1993)		
Soluble		
Low dose	6.4 ± 3.9	—
High dose	2.0 ± 1.2	—
Soluble mixed with soil		
Low dose	4.5 ± 3.2	—
High dose	3.2 ± 1.9	—

Note. —, not available or not applicable.
[a]Not statistically different from background for any monkey.

The time profiles for urinary arsenic excretion by each monkey are provided in Figure 2. These charts show a consistent time course for the three monkeys; peak excretion of arsenic occurs within 24 h of the dermal application of the soluble dose, with a rapid return to near-background levels of excretion within 48 to 72 h. Peak 24-h urinary arsenic excretion following the soluble dose ranged up to a maximum value of 41.6 μg. The time profile for arsenic excretion following dermal application of the CCA residue is also consistent across all three monkeys. Figure 2 depicts that, following application of the CCA residue, there is no increase in urinary arsenic excretion, followed out through time.

Because the number of animals that can be used in primate research is constrained, the crossover study design—wherein each individual animal is dosed in each dose group, and data from each individual monkey can be used as its own "comparison control"—was specifically selected for use in this research. This study design optimizes the potential to observe statistically significant results despite the small sample size. It does necessitate, however, use of specific statistical approaches that are consistent with the study design. To determine whether the difference in the

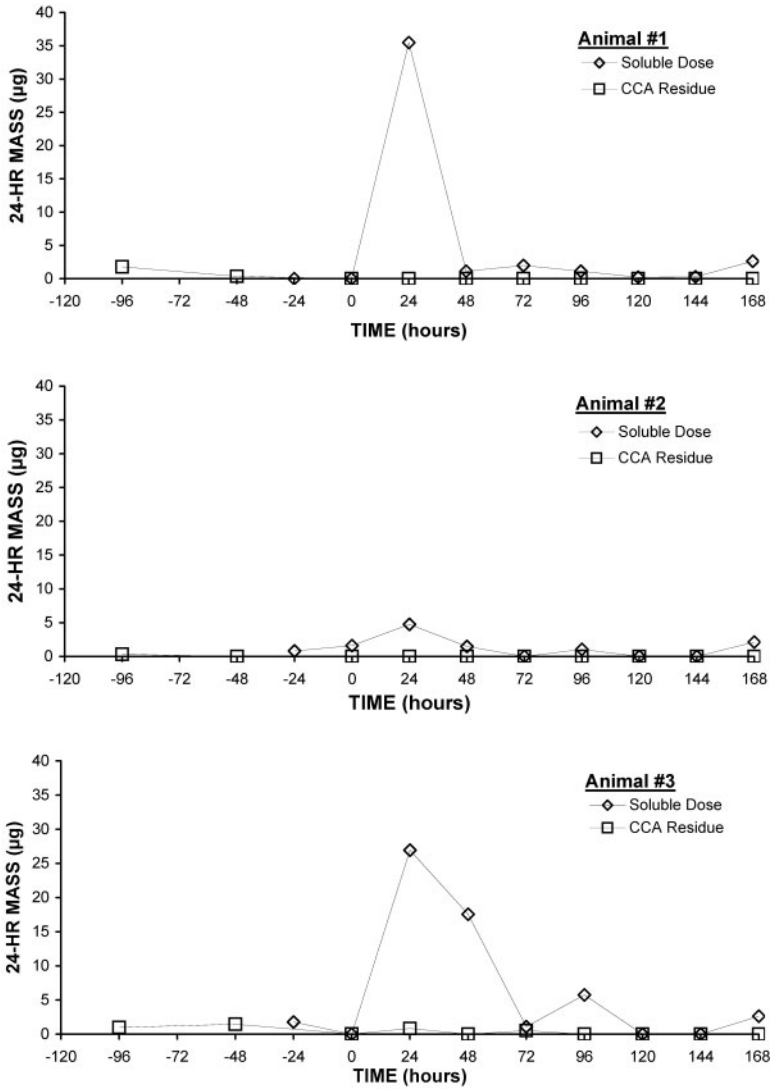


FIG. 2. Urinary arsenic mass excretion in 24-h increments.

results for the two dermal exposure groups was statistically different from background or from each other, an ANOVA analysis followed by a Tukey's multiple comparison test was conducted. In a study with a small number of animals, the variability between animals could be greater than the differences in absorption for different treatment groups; thus, statistical differences should be assessed after accounting for overall differences between monkeys. Because of the sequential nature of the data generated (i.e., at specified time points after dosing), analyses must also account for any time-dependent patterns present over the sampling period evaluated (e.g., comparing data within a given timepoint). The ANOVA model used to evaluate these data included factors for monkey, time, and treatment group. The factor for monkey controls for inter-monkey differences in mass excreted, allowing each monkey to serve as its own control. Monkey number was included as a random factor, because the monkeys tested were not specifically of interest but rather a random selection of monkeys. In order to incorporate the sampling order, time period was included in the ANOVA model as an ordered factor. After accounting for monkey and time period differences, the treatment factor (i.e., soluble or residue dose group) was assessed for significance and followed by Tukey's multiple comparison test to identify which treatments are different from one another, using an overall significance level of 0.05 or 95% confidence. Results indicate that the urinary arsenic excretion levels in the animals exposed to the CCA residue are not statistically greater than background. This is also depicted in Figure 1, which shows a scatter plot of the daily urinary excretion values for each monkey, including background urinary excretion for each animal (i.e., prior to dosing trials), in comparison to the daily urinary excretion following exposure to the CCA residue. This figure demonstrates that the range of daily urinary excretion following exposure to CCA residue falls well within the range of background

urinary arsenic excretion. Conversely, the urinary arsenic excretion in the animals exposed to soluble arsenic in solution is significantly greater than background, and significantly greater than the residue exposure group.

DISCUSSION

The results from this research indicate that the methodology described above can be used to evaluate dermally absorbed arsenic from environmental samples. The development of this method was challenging because of the high degree of background arsenic exposure from the diet, and the potential for that background exposure to obscure any signal from a dermally applied dose. Use of the low-arsenic diet resulted in an approximately four-fold decrease in urinary arsenic excretion relative to the standard primate diet, and allowed for detection above background of a dermally applied dose of arsenic.

Although the results indicate that the urinary arsenic levels following topical administration of arsenic in CCA residues are not distinguishable from background, the non-zero values for background urinary arsenic excretion, and the variability of the measured background values, impose some limits regarding the sensitivity of the model to detect an absorbed dose. A statistical evaluation using a comparison of means (*t*-test) for our data indicates that the absorbed dose would need to be in the range of 0.10 to 0.16% of the applied dose, at the dosing levels used in this study, for daily arsenic excretion levels to be detectable above background. Thus, while these data suggest that there may not be any dermal absorption of arsenic from CCA residue (no monkey demonstrated urinary arsenic excretion that was statistically different from background), the uncertainty associated with this research model tells us that dermal absorption of arsenic from CCA residues is at least an order of magnitude lower than absorption of soluble arsenic from solution.

Extensive chemical analyses indicate that the arsenic present in the CCA residue used in this study is structurally and chemically identical to the arsenic present on the surface of newly treated or aged CCA-treated wood (Nico *et al.,* 2003), thus making it an appropriate study substrate for understanding the potential dermal absorption of arsenic following contact with CCA-treated wood. The negligible absorption of arsenic from the CCA residues derives from the fact that this arsenic is chemically bound with other metals (particularly chromium) and ultimately to the wood structure (Bull, 2001; Nico *et al.,* 2003). The physico-chemical conditions on the surface of the skin do not result in the liberation of arsenic from the residue, thus precluding absorption. These results indicate that percutaneous absorption of arsenic from environmental media can be significantly different from soluble arsenic or even soluble arsenic mixed with environmental media (Table 6). Therefore, it is not appropriate to apply generic assumptions regarding dermal absorption to these unique matrices, and medium-specific analysis may be required to understand the dermal absorption from them (and potential associated risks). This appears to be true for arsenic, and may be true for other metals that form similarly stable complexes in the environment. The latter point should not be overgeneralized until additional metals have been thoroughly studied.

ACKNOWLEDGMENTS

The authors appreciate the help received from the American Chemistry Council, who provided the collected residue that was used in this research, as well as partial funding for the study. We also appreciate the support of Eric Crecelius and his staff at Battelle Marine Sciences Labs for their assistance and technical expertise in evaluating arsenic in urine. Finally, we acknowledge Georgia-Pacific Corporation for the funding they provided.

REFERENCES

American Chemistry Council (ACC) (2003). Dislodgeable material collection procedure. CCA-Treated Wood Work Group. May 12.

Bull, D. C. (2001). The chemistry of chromated copper arsenate. II. Preservative-wood interactions. *Wood Sci. Technol.* **34,** 459–466.

Nico, P. S., Fendorf, S. E., Lowney, Y. W., Holm, S. E., and Ruby, M. V. (2003). Chemical structure of arsenic and chromium in CCA-treated wood: Implications of environmental weathering. *Environ. Sci. Technol.* (in press)

Schoof, R. A., Yost, L. J., Eickhoff, J., Crecelius, E. A., Cragin, D. W., Meacher, D. M., and Menzel, D. B. (1999). A market basket survey of inorganic arsenic in food. *Food Chem. Toxicol.* **37,** 839–846.

Schoof, R. A., Eickhoff, J., Yost, L. J,, Crecelius, E. A., Cragin, D. W., Meacher, D. M., and Menzel, D. B. (1999). Dietary exposure to inorganic arsenic. In Proc. Third International Conference on Arsenic Exposure and Health Effects. W. R. Chappell, C. O. Abernathy, and R. L. Calderon (eds). pp. 81–88. Elsevier Science Ltd.

U.S. Environmental Protection Agency (EPA) (1997). Test methods for evaluating solid waste-physical/chemical methods, SW-846. Revised methods. Version 2. Integrated Manual/Update III. Washington, DC.

U.S. Environmental Protection Agency (EPA) (2001a). A set of scientific issues being considered by the Environmental Protection Agency regarding: Preliminary evaluation of the non-dietary hazards and exposure to children from contact with chromated copper arsenate (CCA)-treated wood playground structures and CCA-contaminated soil. FIFRA Scientific Advisory Panel (SAP) Meeting, October 23–25, 2001, Arlington, VA. SAP Report No. 2001-12, December 12.

U.S. Environmental Protection Agency (EPA) (2001b). Risk assessment guidance for Superfund. Volume I: Human health evaluation manual (Part E, Supplemental guidance for dermal risk assessment), Interim Draft, September. Office of Emergency and Remedial Response. Washington, DC.

U.S. Environmental Protection Agency (EPA) (2002). Analysis of multimedia, multi-concentration samples for metals by 200-Series metals methods and 1600-series trace metals methods. Office of Water, Washington, DC.

U.S. Environmental Protection Agency (EPA) (2003). A probabilistic risk assessment for children who contact CCA-treated playsets and decks. Draft final report. Office of Pesticide Programs, Antimicrobials Division. November 10.

Wester, R. C., and Maibach H. I. (1975). Percutaneous absorption in the rhesus monkey compared to man. *Tox. Appl. Pharmacol.* **32,** 394–398.

Wester, R. C., and Maibach, H. I. (1989). *In vivo* animal models for percutaneous absorption. In *Percutaneous Absorption,* 2nd ed. (R. Brouaugh and H. Maibach, Eds.), pp. 221–238. Marcel Dekker, New York.

Wester, R. C., and Maibach, H. I. (1991). Individual and regional variation with *in vitro* percutaneous absorption. In *In Vitro Percutaneous Absorption* (R. Brouaugh and H. Maibach, Eds.), pp. 25–30. CRC Press, Boca Raton, FL.

Wester, R. C., and Maibach, H. I. (1997). Toxicokinetics: Dermal exposure and absorption of toxicants. In *Comprehensive Toxicology, Vol. 1, General Principles* (J. Bond, Ed.), pp. 99–114. Elsevier Science, Oxford, U.K.

Wester, R. C., Maibach, H. I., Sedik, L., Melendres, J., and Wade, M. (1993). In vivo and in vitro percutaneous absorption and skin decontamination of arsenic from water and soil. *Fund. Appl. Toxicol*. **20,** 336–340.

Yost, L. J., Tao, S. H., Egan, S. K., Barraj, L. M., Smith, K. M., Tsuji, J. S., Lowney, Y. W., Schoof, R. A., and Rachman, N. J. (2004). Estimation of dietary intake of inorganic arsenic in U.S. children. *Human and Ecological Risk Assessment*. (in press)

Supplemental Materials for Section 6

STANDARDIZED DoD PROTOCOL FORMAT

PROTOCOL TITLE: Evaluating the Oral Bioavailability of Metals from Soil and Earthworms to American Robins

PRINCIPAL INVESTIGATOR:

Mr. Michael V. Ruby, Exponent

CO-INVESTIGATORS:

Mr. Richard Poche, Genesis Laboratories

SCIENTIFIC REVIEW:

Dr. Robert Pastorok, Exponent

ATTENDING/CONSULTING VETERINARIAN:

Dr. Tracey Jensen, Consulting Veterinarian to Genesis

STATISTICAL REVIEW:

Ms. Melanie Edwards, Exponent

Evaluating the Oral Bioavailability of Metals from Soil and Earthworms to American Robins (NRD-292)

PRINCIPAL INVESTIGATOR: Mr. Michael V. Ruby, Exponent

CO-INVESTIGATOR(S): Mr. Richard Poche, Genesis Laboratories

I. NON-TECHNICAL SYNOPSIS: The wildlife receptors for which ecological risk assessment models consistently indicate the greatest level of potential exposure to metals in soil are small mammals (short-tailed shrew and cottontail rabbit) and two avian species (American robin and woodcock). Both small mammal and avian receptors receive much of their metals exposure either from direct soil ingestion or from consumption of earthworms. In assessing risks to these receptors from soil contamination, the standard method is to assume that the efficiency of contaminant uptake (including metals) from ingested soils is equal to the assimilation efficiency in laboratory tests conducted to determine toxicity thresholds (i.e., a relative bioavailability of 100%). However, a growing body of research indicates that many chemicals—including metals—are less bioavailable from ingested soil than from soluble forms (i.e., the form typically used in laboratory toxicity tests), when dosed in a similar manner. Therefore, this study will evaluate the bioavailability of metals in birds exposed to soil via the oral pathway, relative to the bioavailability of the same metals when dosed in soluble forms. This will include bioavailability following direct soil ingestion, and ingestion of worms cultured in metal-containing soil. The target metals for this research are lead, zinc, chromium, and cadmium. The results of this study will serve as the basis for subsequent research to develop simple extraction tests (i.e., laboratory tests that do not use animals) to predict exposures to American robin (and other avian species) from metals in soil.

II. BACKGROUND:

A. Background: Very limited research has been conducted on the bioavailability of metals from soil to wildlife. Given this lack of information, ecological risk assessments generally assume that metals in soil are equally bioavailable as in the critical toxicity study, potentially resulting in overestimates of risk. The research described in this protocol is being conducted on behalf of the Strategic Environmental Research and Development Program (SERDP) to begin to address this data gap.

B. Literature Search: To prevent unnecessary duplication of previous research, two database searches have been performed.

1. **Literature Source(s) Searched:** Searches of the Biomedical Research Database (BRD), the Computer Retrieval of Information on Scientific Projects (CRISP) database, and Medline (National Library of Medicine) have been performed.

2. **Date and Number of Search:** The CRISP database search was performed on January 17, 2002. The search was completed for current and historical research awards granted between fiscal years 1972 and 2002. The BRD database search was also performed on January 17, 2002. The search was completed for all responsible and performing organizations during fiscal years 1998, 1999, and 2000. The PubMed Medline database search was performed on February 19, 2003, and the search encompassed literature published on veterinary medicine, life sciences, biology, environmental science, marine biology, plant and animal science, biophysics, chemistry, pre-clinical sciences, pharmacy, and other disciplines from 1966 to present.

3. **Key Words of Search:** Bioavailability (or bioavailable) and metal(s), bioavailability (or bioavailable) and soil, metal(s) and soil, robin and metals bioavailability, robin and cadmium, robin and zinc, robin and chromium, robin and lead.

4. **Results of Search:** The searches of both databases revealed no research that is related or similar to that proposed in this protocol.

III. OBJECTIVE/HYPOTHESIS:

The study described herein will yield data on the relative bioavailability of metals in soil and associated prey species to American robin, which will be useful for improving the accuracy of ecological risk estimates.

IV. MILITARY RELEVANCE:

The proposed research will yield estimates of the relative bioavailability of metals in soil to American robin, which can be used in ecological risk assessments at DoD sites. Given that EPA considers the American robin to be a sentinel ecological receptor for terrestrial ecological risk assessments, this will help to improve the accuracy of risk estimates and risk-based corrective actions currently being made at DoD sites. In addition, this study will form the basis for subsequent work to develop simple extraction tests that are predictive of metals bioavailability from soil to robin. These tools will then be available to DoD personnel for site-specific evaluation of metals bioavailability from soil at contaminated sites and will result in more accurate exposure and risk estimates that are still protective of ecological receptors.

V. MATERIALS AND METHODS:

A. Experimental Design and General Procedures:

In this research, the relative bioavailability of metals from soil to robins will be investigated for three soils from DoD facilities (following either direct soil consumption,

or consumption of earthworms grown in contaminated soil). The term "relative bioavailability" is used to describe the amount of a metal that is absorbed into systemic circulation relative to the amount absorbed following exposure to a soluble form of the metal.

Relative bioavailability from soil will be assessed by comparing the absorption of each target metal by female American robins (*Turdus migratorius*) after direct soil ingestion (represented by Test Group #2 in Figure 1), as compared to absorption of soluble forms of the metals (represented by Test Group #1 in Figure 1). Relative bioavailability will also be assessed by comparing the absorption of each target metal by American robins after consumption of earthworms exposed to target metals in soil (represented by Test Group #3), as compared to absorption of soluble forms of the metals (Test Group #1).

To conduct this research, three metal-contaminated soils will be collected from Department of Defense (DoD) sites. Each of the three DoD test soils will be tested in birds at three levels: 100% contaminated, 50% contaminated, and 25% contaminated (Figure 2).

Metal absorption in robins will be evaluated following direct soil ingestion (Test Group #2) and after robins consume earthworms that have been exposed to the contaminated soils (represented by Test Group #3 in Figure 1). This work will establish whether the bioavailability of metals is different following direct soil ingestion versus ingestion of earthworms living in that soil (includes metals in earthworm tissue and the soils contained within the earthworms). Depurated earthworms will not be used because the goals of this study are 1) to study exposures of metals to robins under field-like conditions (to the extent possible), and 2) to establish if the bioavailability of metals from direct soil ingestion is significantly different from the bioavailability of metals due to ingestion of earthworms (i.e., soil in worms and worm tissues that contain metals). Collection of both kinds of bioavailability data will allow for an understanding of what sources of exposure (worm or soil) contribute more to overall exposure.

For all three test groups (e.g., soluble metal spikes, test soils, or earthworms) the test material will be mixed into standard avian lab diet.

- Test Group #1 will serve as the positive control for this study (Figure 1). For this test group, de-ionized (DI) water adjusted to pH 2.0 with nitric acid will be mixed with target metals to create a metal spiking solution that is specific to each test soil. This solution will be spiked into the lab food to create doses matched to the individual test soils, and will be designed to deliver doses equivalent to those delivered in the test soil (Figure 2).
- Robins in Test Group #2 will consume metal-containing soil mixed with lab food at the three specified dose levels (Figure 1).
- Robins in Test Group #3 will consume earthworms that have been exposed to contaminated soils mixed with lab food (Figure 1).

- Robins in Test Group #4 will serve as the negative control for this study. Depending on the test group for which these animals serve as a control, they will receive either standard diet, standard diet mixed with reference soil, or standard diet mixed with clean earthworms (Figure 1).

Soil Concentrations Required for Detection

To ensure that the DoD site soils will contain adequate metals concentrations to be detectable in robin tissue, Exponent calculated the minimum concentration of each metal in soil required to detect that metal in robin tissue at the end of the study. Based on analytical detection limits for metals in tissue, the minimum concentration of each metal in soil needed to detect absorption in robins was calculated using the following equation:

$$\text{Concentration}_{SOIL} = \frac{\text{Concentration}_{TISSUE} \times BW}{\text{Intake}_{SOIL} \times RBA \times \%\ \text{Retained} \times ET}$$

where,

$\text{Concentration}_{TISSUE}$ = lowest concentration that can be detected using given analytical method (μg metal per g tissue)

BW = body weight (grams)

Intake_{SOIL} = daily soil intake for robins (grams/day)

RAF = relative absorption factor

% Retained = percentage of bioavailable metal that can be retained in the tissues

ET = exposure time (days)

For all calculations, body weight was assumed to be 81 grams (U.S. EPA 1993), RAF was estimated at 50%, and soil intake was equal to 0.1 grams/day. For all metals except lead, it was assumed that the metals accumulated for two days, and then reached steady-state. After that, the amount retained was assumed to be equal to the amount excreted. Therefore, the exposure time was set to two days, and the percent retained was set to 100%. For lead, it was assumed that 50% is retained over the entire 28-day experiment. Therefore, the exposure time for lead was set to 28 days, and the percent retained was set to 50%.

The calculations suggest that based on analysis using inductively-coupled plasma/mass spectrometry (ICP/MS), except for chromium, which is analyzed by ICP, each metal must be present in soils at the following minimum concentrations:

Metal	ICP/MS Detection Limit (ppm dry weight)	Minimum Soil Concentration Needed to Detect Metals (ppm dry weight)
Lead	0.02	0.11
Zinc	0.5	19.8
Cadmium	0.05	1.98
Chromium	0.5[a]	19.8

[a] Analysis of chromium by ICP.

In order to ensure detection, the test soils used in this research will have metal concentrations that are one to two orders of magnitude higher than these values.

Laboratories Involved in Research

Four different laboratories will work together to complete this research. The four labs include the Exponent Boulder Lab (Exponent), Genesis Labs in Wellington, Colorado (Genesis), Texas A&M University lab (Texas A&M), and Columbia Analytical Services (CAS). All spike solutions and soils for dosing robins will be prepared at the Exponent lab and sent to Genesis Labs in Wellington, Colorado. Soils will also be shipped from Exponent to Texas A&M, where the earthworms will be cultured in the contaminated soils. After the earthworms have been cultured, they will be freeze-dried and shipped to Genesis for dosing to robins. Genesis will conduct all of the animal work for this study, and the approval from their Institutional Animal Care and Use Committee (IACUC) to conduct this study, as well the results from their most recent USDA inspection are provided in Appendix A. Genesis will euthanize all the robins and skin each carcass when the dosing experiments are completed. Genesis will then send each robin carcass to Columbia Analytical Services (CAS) where each carcass will be ground in a laboratory meat grinder, freeze dried, homogenized and the required analytical work will be performed.

1. Soil Preparation:

The target metals of interest for this research are lead, zinc, chromium, and cadmium. Three DoD soils containing adequate concentrations of these target metals will be obtained by Exponent. A clean background soil will also be obtained for use in this research.

The three DoD soils will each be air-dried and sieved through a 2-mm sieve. Bulk soil material, and 500 g of the <2-mm size fraction of each sample will be archived. Each sample will then be sieved to <500 μm. This particle size represents a soil fraction that earthworms may ingest (Edwards and Bohlen 1992) and that robins are likely to ingest during feeding and grooming (due to adherence of soil to feathers). Each bulk soil

sample (<2 mm) will be characterized for metals of interest (lead, zinc, cadmium and chromium), pH, total organic carbon (TOC), and particle size distribution (sand, silt, clay). In addition, the fine soil fraction (<500-µm size) will be analyzed in triplicate for the metals of interest, other metals that may affect gastrointestinal absorption of the target metals (calcium, copper, iron, manganese, potassium, selenium, and sodium), methylmercury and hexavalent chromium concentration, pH, total organic carbon (TOC), total inorganic carbon (TIC), particle size distribution (sand, silt, clay), cation exchange capacity (CEC), and extractable concentrations of iron, aluminum, and manganese oxides/hydroxides.

As mentioned above, three different "dosing levels" will be produced for each DoD test soil: 100% contaminated soil, 50% contaminated soil, and 25% contaminated soil (Figure 2). The different "dosing levels" will be achieved by cutting the test soil with a clean reference soil. This will results in an equal mass of soil in each feeding experiment. Prior to adding test soil to the diet, each soil will be heated to 80°C for four hours prior to feeding, to minimize potential for infectious agents that could affect the research.

2. Care and Treatment of American Robins:

American robins will be captured using standard mist-netting techniques, and will be maintained at the animal facilities of Genesis Laboratories, in Wellington, Colorado. Only female adult robins will be used for the study. The robins will be kept in a test room supplied with full spectrum lighting at an approximate light intensity of not less than six foot candles measurable at the front of each cage. A photoperiod of 12 hours light : 12 hours dark will be maintained for the duration of the test. Test animals shall be housed individually in 51 x 25 x 25.5 cm galvanized steel pens with sloping floors. Beneath each row of pens a galvanized steel tray containing absorbent material shall be used to collect waste. The pens will be cleaned once/week by removing the woodchips underneath the cages and replacing them with fresh ones. Pens shall be arranged in such a way as to eliminate the chance of cross-contamination among groups.

Robins will be acclimatized to the laboratory for at least one week prior to beginning the study. The robins will be offered food and water with minimal metals concentrations to reduce tissue metal concentrations to background levels. The food will also be augmented with background soil to help the birds acclimate to soil in their food. Only some of the robins will be used for soil dosing studies, but the selection process won't occur until after the robins are acclimated to the laboratory and diet. When the actual dosing studies begin, only the birds in the Test Group #2 will continue to consume soil-augmented diets.

After the acclimatization period, the robins will be weighed and divided into test groups, so that a similar array of robin sizes are represented in each test group. During the

dosing studies, the robins will be fed twice daily, using a feeding and fasting regime that is designed to encourage them to consume all of the dosed lab food. Between 0800 and 1000 hours each day, no food will be offered to the robins, causing them to fast for two hours prior to receiving dosed lab food. A two-hour fasting period will be sufficient to cause hunger in the robins so that they will consume the dosed lab food, without causing them discomfort. At 1000 hours, each robin will receive 4 g of dosed lab food in the form of a "feed ball". After all the dosed lab food is consumed, the birds will be offered non-dosed lab food *ad libitum* until the next morning when the cycle starts over. In this manner, each robin should consume the same amount of dosed food. However, if any dosed lab food remains in the cage, the amount of remaining food will be recorded. Remaining food will be characterized as either ¼, ½, ¾ consumed. All remaining food will be collected and stored individually for each bird in a refrigerator and weighed at the end of the study. Deionized water for drinking will be provided *ad libitum*. On the last day of the study (Day 28), all groups of robins will be provided standard "clean" lab food at 1700 hours. At 0800 the following day the robins will be sacrificed.

The individual robin carcasses will be weighed, skinned, weighed again, and ground in a laboratory meat grinder. The ground carcasses will be freeze-dried and homogenized. Because the animal skins are difficult to clean and/or digest completely, the animals will be skinned to minimize variability in the body burden results. A representative subsample will be collected and analyzed for metal content by U.S. EPA Methods 200.8 and 6010. The robin skins and whole-body homogenates will be archived (frozen) for future analysis, if necessary.

3. Test Groups and Dosing Preparation:

a. Test Group #1 (Soluble Spikes; Positive Control):

Test Group #1 will serve as positive controls for the soil (Test Group #2) and earthworm dose groups (Test Group #3).[1] In Test Group #1, nine dose groups of robins, each consisting of six animals (54 robins total), will be fed lab food spiked with soluble metal salt solution (Table 1). Each dose group will receive a spike solution consisting of the four target metals (lead, zinc, chromium, or cadmium) designed to represent the amount of metal present in each of the three DoD test soils (Test Group #2) at one of three concentrations (equivalent to 100%, 50%, and 25% of the metal masses present in each contaminated soil; Figure 2). Each metal spike solution will consist of DI water adjusted to a pH of 2.0 with nitric acid. Metals will be added into the nitric acid solution at appropriate concentrations. The spike solution is intended to deliver the same mass of metals to robins as the birds in Test Group #2 will receive from the soil. Exponent

[1] A "dose group" is defined as each group of robins that receives a unique dose of metals via a particular dosing material (e.g., soluble metal spike, soil, or earthworms).

will prepare the metal spike solutions and will ship them in separate high-density polyethylene (HDPE) bottles to Genesis Labs in Wellington, Colorado, where the robins will be housed.

The following metal salts will be used to prepare the soluble metal spike solutions for each target metal:

- Lead: lead acetate trihydrate [$(CH_3CO_2)_2Pb^* 3H_2O$]
- Zinc: zinc chloride [$ZnCl_2$]
- Cadmium: cadmium chloride [$CdCl_2$]
- Chromium: chromium acetate hydroxide [$(CH_3CO_2)_7Cr_3(OH)_2$]

Each metal spike solution will be mixed into a separate batch of lab food, and the spiked food will be fed to the robins daily (4 g food/robin/day). All of the food that will be used for Test Group #1 will be prepared prior to the onset of the experiments, and the feed balls will be frozen in labeled Ziploc baggies. Feed balls will be thawed for at least 24 hours before they are used. To enhance the palatability of the food, feed balls will be rolled in peanut oil prior to being presented to the birds.

The food for each dose group of six birds that will be dosed with metal spike solution will be created using the following ingredients:

580 grams Mazuri soft-billed bird diet
4 mLs pre-mixed spike solution (prepared by Exponent)
416 grams deionized water

The Mazuri soft-billed bird diet will first be ground to a powder-like consistency using a UDY Mill (UDY Corp, Ft. Collins, Colorado). Five hundred eighty grams of the ground Mazuri diet will be placed in a table-top Kitchenaid mixer. The spike solution (4 mLs) will be diluted to 50 mL with 46 mL of de-ionized water, and this fluid will be slowly added to the Mazuri diet, with the mixer running at a speed setting of 2. Subsequently, another 50 mL of deinoized water will gradually be added to the mixer, with mixing at a speed setting of 2. The food, water, and spike solution will then be blended on a speed setting of 2 for another 10 minutes. Subsequently, the final 320 mLs of deionized water will be added and the mixture will be blended on speed setting of 3 until dough is formed. The dough will be kneaded for 5 minutes and then formed into 4.0 gram (wet weight) balls, bagged in ziploc freezer bags, and placed in a freezer until used in the dosing experiments.

Three of the 4.0 gram dough balls (i.e., three separate samples) from the 100%, 50% and 25% metal spiked feed will be submitted to Columbia Analytical Services (CAS) in Kelso, Washington for analysis of cadmium, chromium, lead, and zinc concentrations, to test for homogeneity of the diet/metal spike mixture. The initial spike solution will also be submitted to CAS for analysis of cadmium, chromium, lead, and zinc, to confirm the concentrations of these metals in the 100% metal spike.

The recipe above will create enough food for one dose group of six robins, and for the triplicate 4-gram samples of spiked food to be collected for metals analysis. For each batch of food that is made, a different spike solution will be added to the food, but the volume (4 mLs) of spike solution will remain the same. All appliances and substrates that come into contact with the food will be decontaminated between batches.

b. <u>Test Group #2 (Soil Exposure):</u>

Test Group #2 will consist of robins dosed with contaminated site soils. In Test Group #2, nine dose groups of robins, each consisting of six animals (54 animals total), will be fed lab food spiked with DoD test soil (Table 1). Each dose group will consume lab food augmented with either 100% contaminated soil, 50% contaminated soil, or 25% contaminated soil from each of the three DoD sites (Figure 2). Exponent will prepare and ship the nine test soils in separate high-density polyethylene (HDPE) bottles to Genesis Labs.

Each DoD test soil will be mixed into a separate batch of lab food, and the soil-augmented food will be fed to the robins daily (4 g food/robin/day). Each soil-augmented feed ball will contain 0.1 grams of soil. To prepare the lab food that will be used in Test Group #2, it will be necessary to prepare nine separate batches of food (one batch for each dose group). All of the food that will be used for Test Group #2 will be prepared prior at the onset of the experiments, and the feed balls will be frozen in labeled Ziploc baggies. Feed balls will be thawed for at least 24 hours before they are used. Feed balls will be rolled in peanut oil prior to being presented to the birds.

The food for each dose group of six birds that will be dosed with DoD test soils will be created using the following ingredients:

555 grams Mazuri soft-billed bird diet
25 grams DoD test soil (prepared by Exponent)
420 grams deionized water

The Mazuri soft-billed bird diet will first be ground to a powder-like consistency using a UDY Mill (UDY Corp, Ft. Collins, Colorado). In a table-top Kitchenaid mixer, 555 grams of the ground Mazuri diet will be blended with 25 grams of DoD test soil (mixer speed setting of 1 for 5 minutes). Deionized water (50 mL) will then be gradually added to the Mazuri diet/soil mixture in the mixer, with the mixer running at a speed setting of 1. The

food, soil, and water will then be blended at a speed setting of 2 for 10 minutes. Subsequently, another 50 mL of deinoized water will gradually be added to the mixture (speed setting of 2). After blending for 10 minutes, the final 320 mls of deionized water will be added and the mixture will be blended on a speed setting of 3 until dough is formed. The dough will be kneaded for 5 minutes and then formed into 4.0 gram (wet weight) balls, bagged in ziploc freezer bags, and placed in a freezer until used in the dosing experiments.

Three of the 4.0 gram dough balls (i.e., three separate samples) from the 100%, 50%, and 25% soil spiked feed will be submitted to CAS for analysis of cadmium, chromium, lead, and zinc concentrations, to test for homogeneity of the diet/soil mixture.

The recipe above will create enough food for one dose group of robins, and the triplicate 4-gram samples of soil-augmented food to be collected for metals analysis. For each batch of food that is made, a different DoD test soil/concentration will be added to the food, but the amount of soil used in the recipe will be the same. All appliances and substrates that come into contact with the food will be decontaminated between batches.

c. Test Group #3 (Earthworm Exposure):

Robins belonging to Test Group #3 will consume desiccated earthworms that have been exposed to DoD test soils for 28 days. To accomplish this component of the research, earthworms will be grown in uncontaminated medium, and then transferred to three separate DoD test soils (the same soils that were used for Test Group #2) for 28 days. The worms will then be desiccated and fed to robins. The dose group for each test soil will consist of six robins each. The procedures associated with Test Group #3 are expanded in the next three sections.

i. Earthworm Stock Cultures

Fully clitellate, adult earthworms (*Eisenia fetida*) of similar age and size will be laboratory-bred at Texas A&M University from juvenile earthworms obtained from a single source: either Carolina Biological Supply Co. (Burlington, North Carolina) or Flowerfield Enterprises (Kalamazoo, Michigan). This species was selected for these studies based on its short generation time, prodigious production, ease of culturing in the laboratory, and availability of data on metal toxicity (Hartenstein et al. 1979; Neuhauser et al. 1980; Hartenstein et al. 1981; Haque and Ebing 1983; Stafford et al. 1987; Goats and Edwards 1988). In this study, each robin in Test Group #3 will be fed 0.1 grams of dessicated earthworms per day. This will result in robins in Test Groups #2 and #3 consuming similar amounts of soil and worm tissue, respectively. Daily consumption is estimated at 0.1 grams of dessicated worms per day per robin.

Earthworms will be reared in a bedding of artificial soil consisting of sphagnum peat moss (10%, dry weight), kaolin clay (20%), and silica sand (70%). The mixture will be pH adjusted to 7.0 with pure calcium carbonate and hydrated with reverse osmosis

(RO)-purified water (Edwards 1984). Plastic trays measuring approximately 34×28×14 cm will be used to hold the cultures. The trays will be covered with plastic to prevent drying, and held under continuous lighting at 22 ±3 °C. The worms will be seeded at 0.03 g/cm^3 soil and will be fed with commercial rabbit food pellets, which will be placed on top of the soil once per week (Fitzpatrick et al. 1996). At feeding time, any food that remains from the previous feeding will be removed and discarded. The bedding will be inspected weekly for condition of the worms and the bedding. Dead worms will be removed if noticed. Infected trays will be discarded, because biocides have potential effects on earthworm health and testing sensitivity.

Stock cultures of earthworms will be kept in the artificial soil for a minimum of one month before introducing them into test-soil containers. Metal concentrations in the bedding material will be determined in triplicate at the beginning and end of the earthworm culture period. This will be accomplished by collecting three cores of soil from one sample container, and subjecting each soil core to metals analysis. Baseline total body metal contents of 10 desiccated earthworms (Table 2), homogenized to yield one analytical sample, will be determined prior to their introduction into the test soils (i.e., day 0). Worms will not be washed or depurated prior to desiccation.

ii. Earthworm Test Soil Cultures

Earthworms will be cultivated in contaminated test soils before feeding to the robins in Test Group #3. The air-dried test soil (prepared as described above) will be mixed thoroughly with RO-purified water, to yield a moisture content of 25% by mass. The wetted soil will be put into test containers consisting of plexiglass cylinders 15 cm in diameter and 30 cm in height, as described in ASTM (1998) – Standard guide for conducting laboratory soil toxicity or bioaccumulation tests with the lumbricid earthworm *Eisenia fetida*. The ends of the cylinders (test containers) will be closed with cheesecloth held in place by polypropylene bands. One end of each test container will be placed in a polystyrene dish filled with RO-purified water to a depth of 3–5 cm, to maintain a moisture level gradient in the soil (Marquenie and Simmers 1988). The stock earthworms will be added to these test containers.

The test containers will be maintained in the same manner as described for the stock cultures. Constant light will be used to encourage burrowing, as described in ASTM (1998). A minimum of 70 earthworms of average weight (2.5 g wet weight) can be cultured per test container, according to ASTM (1998), for 28 days. [The earthworms that will be utilized in this study weigh less than 2.5 grams, and therefore it is likely that more than 70 earthworms can be cultured per test container.] Ten desiccated earthworms (homogenized into a single analytical sample) per test soil will be analyzed for trace metal contents on days 14 and 28 of the exposure period (Table 2). Worms will not be depurated or washed prior to desiccation.

iii. Dosing Earthworms to Robins

Test Group #3 will consist of three dose groups of robins, with six animals/dose group (total of 18 animals; Table 1). Each dose group will consume desiccated earthworms that were exposed to the contaminated DoD test soils. The amount of dessicated earthworms (0.1 grams earthworm) offered to each bird will be equivalent to the amount of soil that was offered to each bird in Test Group #2, "100% soil" (i.e., 0.1 grams soil).

Earthworms will be desiccated in the following manner: They will be removed from the culture vessels. Worms will not be depurated or washed prior to desiccation. Sample bags (Ziploc® baggies) will be labeled and tared, and samples of 20–50 identically treated earthworms will be placed into each sample bag. Filled sample bags will be weighed and placed in a –20 °C freezer until frozen solid. Once the sample is frozen, it will be placed in a chilled freeze-dryer for desiccation. The samples will be dried to constant weight (i.e., until the sample weight changes by less than 0.2 g for an initial weight, and a second weight determined after an additional hour of drying), and moisture content will be determined. The dried sample will then be gently ground to obtain a material than can be mixed with robin feed, and stored at –20 °C.

Each batch of food will be prepared using the following ingredients in the following manner:

555 grams Mazuri soft-billed bird diet
25 grams dessicated earthworms (dry weight)
420 grams deionized water

The Mazuri soft-billed bird diet will first be ground to a powder-like consistency using a UDY Mill (UDY Corp, Ft. Collins, Colorado). In a table-top Kitchenaid mixer, 555 grams of the ground Mazuri diet will be mixed with 25 grams (dry weight) of dessicated earthworms (speed setting of 1 for 5 minutes). Subsequently, 50 mL of deionized water will gradually be added to diet/worm mixture, with the mixer running at a speed setting of 1. The food, water, and dessicated earthworms will then be blended at a speed setting of 2 for 10 minutes. After blending for 10 minutes, the final 370 mls of deionized water will be added and the mixture will be blended on speed 3 until dough is formed. The dough will be kneaded for 5 minutes and then formed into 4.0 gram (wet weight) balls, bagged in ziploc freezer bags, and placed in a freezer until used in the dosing experiments.

Three of the 4.0 gram dough balls (i.e., three separate samples) will be submitted to CAS for analysis of cadmium, chromium, lead, and zinc concentrations, to test for homogeneity of the diet/earthworm mixture.

Three separate batches of food will be prepared for robins in Test Group #3 (one batch for each test soil). Feed balls will be thawed for at least 24 hours before they are fed to

the robins. Feed balls will be rolled in peanut oil prior to being presented to the birds to enhance the palatability of the food.

d. Test Group #4 (Negative Controls):

Test Groups #1, #2, and #3 will each have an associated negative control group (Test Group #4), to control for background exposures to metals (Figure 1). Each of the three negative control dose groups will consist of six robins (for a total of 18 robins; Table 1). The food will be prepared and tested for homogeneity in the same manner as described above for Test Groups #1, #2, and #3, however, the negative control groups will not contain any metals (other than those normally present in the bird laboratory diet for Test Group #1, in the background soil for Test Group #2, or in the stock culture earthworms for Test Group #3).

The negative control group for Test Group #1 will consist of non-dosed food, in the following amounts:

580 grams Mazuri soft-billed bird diet
420 grams deionized water

The negative control group for Test Group #2 will consist of the following:

555 grams Mazuri soft-billed bird diet
25 grams background soil from Flagstaff Mountain, Boulder County, Colorado
420 grams deionized water

The negative control group for Test Group #3 will consist of the following:

555 grams Mazuri soft-billed bird diet
25 grams dessicated earthworms from the stock culture
420 grams deionized water.

4. Pilot Study:

Prior to initiating the experiments outlined above, a pilot study will be conducted to ensure that metal concentrations can be measured in the tissues of the robins when dosed with the DoD soils, and associated metal spikes. The methods implemented in the pilot study will be identical to those described above for the main experiment. The pilot study will utilize one of the three DoD test soils that are planned for the main study. In this manner, metal doses that are equivalent to those used in the main study will be used to ensure that 1) measurable metal uptake can be observed for the test soil dose groups, 2) the concentrations will not cause acute toxicity to the birds, and 3) the robins will not have a taste aversion to soils and/or associated spike solutions containing the

target metals. The different test groups that will be utilized in the pilot study are outlined in Table 1.

The pilot study has been planned to occur in two phases. The difference between the two phases involves how the soluble spikes will be administered: Phase 1 will utilize a single spike solution containing all four target metals, and Phase 2 will utilize single metal spike solutions. If Phase 1 is successful, Phase 2 will not be necessary. However, if Phase 1 is unsuccessful, Phase 2 will need to be implemented, and the main study methodology described above will need to be changed accordingly. If Phase I is successful, the animals that would have been used for Phase 2 will instead be dosed with additional soil doses. These additional soil dose groups will consist of the pilot study soil at a lower dose (e.g., 25% of the initial dose) and two doses (100% and 25%) of a second test soil. These soil doses were selected to provide information on the dose dependency of metal absorption in this animal model, while providing data on a second test soil.

The pilot study has been divided into two phases because past trials with robins in captivity have indicated that the birds do not readily accept feed that has been spiked with multiple metals. Past trials conducted using feed spiked with cadmium, chromium, lead, and zinc (at 1/30th the reported LD-50 for each metal, see Table 3) indicated that the robins exhibit an aversion to the spiked feed. However, it is anticipated that the robins will not exhibit an aversion to the dosed food in the pilot study, because the concentrations of metals will be less than those tested in previous trials (Table 3). Therefore, Pilot Groups #1 (multiple metal spiked control) and #2 (soil treatment) will be tested first (Phase 1), and if the birds do not accept the metal spiked food, then Pilot Groups #3, #4, #5, and #6 (individual metal spiked controls) will be tested (Phase 2). Pilot Group #3 (negative control) will consume non-dosed feed for the duration of the pilot study.

All pilot dose groups will be dosed for 28 days. On the last day of the pilot study (Day 28), all groups of robins that were used will be provided standard "clean" lab food at 1700 hours. At 0800 the following day the robins will be sacrificed. Robins that belonged to the Phase 1 dose groups (Pilot Groups #1, #2 and #3) will be euthanized and skinned. If Phase 2 is implemented, then those birds will be euthanized and skinned as well. The individual robin carcasses will be weighed, skinned, weighed again, and ground in a laboratory meat grinder. The ground carcasses will be freeze-dried and homogenized. A representative subsample will be collected and analyzed for metal content by U.S. EPA Methods 200.8 and 6010. The robin skins and whole-body homogenates will be archived (frozen) for future analysis, if necessary.

The pilot study will include the following dose groups of robins (Table 1):

a. <u>Pilot Group #1 (Positive Control: Multiple Metal Spike):</u>

This group of six robins will be used in Phase 1, and will be dosed with food that has been blended with a metal spike designed to deliver a mass of metals equivalent to the lead, zinc, chromium, and cadmium present in DoD Test Soil #1. The food will be prepared and tested for homogeneity in the same manner as described for the positive control dose groups in the main study (see Section V.A.3.a.).

b. Pilot Group #2 (Soil Treatment):

This group of six robins will be used in Phase 1, and will be dosed with food that has been blended with DoD Test Soil #1. The food will be prepared and tested for homogeneity in the same manner as described for the soil dose groups in the main study (see Section V.A.3.b.).

c. Pilot Group #3 (Negative Control):

This group of six robins will be fed four grams of standard, clean lab food every day for the duration of the study (Phase 1 and Phase 2, if necessary). The food will be prepared and tested for homogeneity in the same manner as described above for the negative control dose groups in the main study (see Section V.A.3.d.).

d. Pilot Group #4 (Positive Control: Lead Spike):

If necessary, this group of six robins will be used in Phase 2, and will be dosed with food that has been blended with a lead spike solution designed to deliver a mass of lead equivalent to that delivered in DoD Test Soil #1. The food will be prepared and tested for homogeneity in the same manner as described above for the positive control dose groups in the main study (see Section V.A.3.a.).

e. Pilot Group #5 (Positive Control: Zinc Spike):

If necessary, this group of six robins will be used in Phase 2, and will be dosed with food that has been blended with a zinc spike solution designed to deliver a mass of zinc equivalent to that delivered in DoD Test Soil #1. The food will be prepared and tested for homogeneity in the same manner as described for the positive control dose groups in the main study (see Section V.A.3.a.).

f. Pilot Group #6 (Positive Control: Chromium Spike):

If necessary, this group of six robins will be used in Phase 2, and will be dosed with food that has been blended with a chromium spike solution designed to deliver a mass of chromium equivalent to that delivered in DoD Test Soil #1. The food will be prepared and tested for homogeneity in the same manner as described for the positive control dose groups in the main study (see Section V.A.3.a.).

g. Pilot Group #7 (Positive Control: Cadmium Spike):

If necessary, this group of six robins will be used in Phase 2, and will be dosed with food that has been blended with a cadmium spike solution designed to deliver a mass of cadmium equivalent to that contained in DoD Test Soil #1. The food will be prepared

and tested for homogeneity in the same manner as described for the positive control dose groups in the main study (see Section V.A.3.a.).

B. Laboratory Animals Required and Justification:

1. Non-Animal Alternatives Considered:

At present, there are no non-animal alternatives that could accomplish the research objectives, because measurement of metals bioavailability is an inherently biological measure. Non-animal alternatives, such as computer modeling or leachability testing would not be acceptable because insufficient information is available to establish whether they could accurately predict biological uptake in the robin.

2. Animal Model and Species Justification:

The avian wildlife receptors for which ecological risk assessment models consistently indicate the greatest level of potential soil exposure are the American robin and the American woodcock. The American robin is the largest, most abundant, and most wide-ranging North American thrush (Sallabanks and James 1999). The diet of the robin changes from primarily soft invertebrates, especially earthworms, in spring and summer to primarily fruit in autumn and winter. Robins forage primarily by hopping along the ground in search of ground-dwelling invertebrates. In the months preceding and during the breeding season, robins feed mainly (greater than 90% volume) on invertebrates and some fruit (U.S. EPA 1993). American robins are prey items for many predatory birds, including Cooper's hawk, northern goshawks, sharp-shinned hawks, and American kestrels (Sallabanks and James 1999). Due to the dietary requirements of the robin during the breeding season, this receptor ingests considerable quantities of soil, both directly and indirectly. It is for this reason that U.S. EPA considers the American robin to be a good sentinel receptor for the avian omnivore guild, and why it is the species selected for this study.

Typically, ecological risk assessments are concerned with the sustainability of wildlife populations, or in the case of threatened and endangered species, the survival and reproductive potential of individuals. In either case, reproductive endpoints for females (e.g., number of young, reproductive success, and age at first reproduction) are among the most relevant measurement endpoints for an assessment, and therefore, only female robins will be used in this bioavailability research. For example, in the Toxicity Reference Value (TRV) data evaluation used to support the derivation of the Ecological Soil Screening Levels (EcoSSLs), U.S. EPA (2000) preferred chronic toxicity data for reproductive endpoints the most, followed by chronic mortality and then growth. Other adverse effects, such as changes in organ function, behavior, neurological function, and biomarkers were considered, but were scored as a lower priority. Therefore, the use of female robins for this research is consistent with current ecological risk assessment practices in the U.S.

Other avian species, aside from robins, were also investigated to serve as possible surrogates, but no other avian species (such as English house sparrows) possess the unique behavioral and physiological attributes of robins, and could serve as an adequate surrogate research species (e.g., omnivorous food habits, with a large proportion of the diet consisting of soil invertebrates). The European starling was also considered, but this species handles iron in a different manner than robins, which could impact metals uptake. The American robin is therefore a unique avian species that is expected to receive much greater metals exposure through direct and indirect soil ingestion than other avian species.

3. Laboratory Animals:

a. Genus & Species: American robin (*Turdus migratorius*), and earthworms (*Eisenia fetida*)

b. Strain/Stock: N/A

c. Source/Vendor: Female American robins will be wild-caught using standard mist-netting techniques. State and federal collection permits have been obtained. Permit numbers: Colorado Division of Wildlife Permit #02-TR821A1, U.S. Fish and Wildlife Collection Permit # MB817084-3. Juvenile earthworms will be obtained from either Carolina Biological Supply Co. or Flowerfield Enterprises and laboratory-bred at Texas A&M University.

d. Age: Only adult female American robins will be used in this study.

e. Weight: Approximately 81 grams.

f. Sex: Female

g. Special Considerations: The animals should be disease-free and not exposed to metals in their diet or living quarters.

h. Other: N/A

4. Total Number of Animals Required:

a. Species A: A total of 199 robins will be needed for this study. Fifty-five robins will be collected for the pilot study (42 are needed, and an additional 13 will be collected to allow for birds that do not acclimate to the laboratory environment), and 144 robins will be needed for the main study.

b. **Species B:** Earthworms

5. **Refinement, Reduction, Replacement:**

a. **Refinement:** N/A

b. **Reduction:**

A pilot study will be conducted to ensure that metal absorption can be measured in the robins, using the actual DoD test soils that would be used in the main study. If detectable metals concentrations are not observed in the robins during the pilot study, it will be necessary to increase the doses, change the analytical methods, or otherwise re-evaluate the study design before the main study commences. In this manner, any logistical or technical difficulties will be resolved with a small number of robins, rather than sacrificing all of the study animals. This approach will minimize the number of animals needed to complete the study.

c. **Replacement:**

Currently ecological risk assessments rely on conservative models that do not take into account the bioavailability of metals to ecological avian receptors. This research will produce data on the bioavailability of metals, which can be used in risk assessment, thereby limiting the need for future animal research, and the collection of wild-caught animals.

C. **Technical Methods:**

1. **Pain:** N/A

a. **USDA (Form 18-3) Pain category:** N/A

(1) **No Pain**

Robins 199 (#) 100% (Column C)

(2) **Alleviated Pain**

0(#) 0% (Column D)

(3) **Unalleviated Pain or Distress**

0(#) 0% (Column E)

b. **Pain Alleviation:** N/A

(1) **Anesthesia/Analgesia/Tranquilization**: N/A

(2) **Paralytics**: N/A

c. **Alternatives to Painful Procedures**: N/A

(1) **Source(s) Searched**: N/A

(2) **Date of Search**: N/A

(3) **Key Words of Search**: N/A

(4) **Results of Search**: N/A

d. **Painful Procedure Justification**: N/A

2. **Prolonged Restraint**: N/A

3. **Surgery**: N/A

a. **Procedure**: N/A

b. **Pre- and Postoperative Provisions**: N/A

c. **Location**: N/A

d. **Multiple Survival Surgery Procedures**: N/A

(1) **Procedures**: N/A

(2) **Scientific Justification**: N/A

4. **Animal Manipulations**: N/A

a. **Injections**: N/A

b. **Biosamples**: N/A – all biosamples will be taken after the animals are euthanized.

c. **Animal Identification**:
Animals will be housed individually, and cage cards and individual bird bands will be used to identify each animal and the study group it belongs to.

d. **Behavioral Studies**: N/A

e. **Other procedures**: N/A

5. **Adjuvants**: N/A

6. **Study Endpoint**:

Study animals will be dosed with metals in food provided by means of metal spike solutions, contaminated soils, or earthworms exposed to contaminated soils. Day 28 will serve as the study endpoint. Whole body metal contents for each of the metals of interest will be collected for each robin, to calculate the relative oral bioavailability of target metals.

If the additional 13 robins that were collected are not utilized in the study, they will be gradually re-acclimated to the outdoors over the course of one week and then released.

7. **Euthanasia**:

Euthanasia will be performed during the study if birds are found injured, moribund or in pain. All robins will be euthanized at the termination of laboratory studies. Euthanasia will be performed via asphyxiation with CO_2 gas. Asphyxiation with CO_2 gas is approved by the Panel of Euthanasia of the American Veterinary Medical Association (AVMA 2000) as an acceptable means of euthanasia. Compressed CO_2 gas in cylinders will serve as the source of carbon dioxide. Jeff Borchert will conduct the euthanasia procedure and the will verify death. The standard operating procedures are listed in Appendix C.

D. **Veterinary Care**:

1. **Husbandry Considerations**:

Genesis maintains an Institutional Animal Care and Use Committee (IACUC) in accordance with the Animal Welfare Regulations (9 CFR Ch. 1). The Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals, Guide for the Care and Use of Laboratory Animals (NRC 1996) and The Guidelines to the Use of Wild Birds in Research approved by the Ornithological Council (Gaunt and Oring 1999) will be followed when completing this study.

American robins (*Turdus migratorius*) will be captured using standard mist-netting techniques. Only female robins will be kept for the study. All other birds will be released. A test room will be reserved exclusively for the robins used in this study. Bedding changes will be performed at least weekly and water will be changed as needed. Temperature within the test room will be maintained at approximately 18-26°C. Strong air currents from heaters or air conditioners will not blow directly onto test animals. Air exchange rates of 10-15 / hour will be maintained. A photoperiod of 12

hours light : 12 hours dark shall be maintained for the duration of the tests. A mean light intensity shall not exceed 37 foot-candles at the cage location. The mean humidity will be maintained at approximately 15-55%. The test room will be kept clean and vermin free. The animals will be observed daily for health.

a. Study Room:

Animals will be maintained at the animal facilities of Genesis Laboratories in Wellington, Colorado.

b. Special Husbandry Provisions:

The robins will be fed daily, using a feeding and fasting regime that is designed to encourage them to consume all of the dosed lab food. Between 0800 and 1000 hours each day, no food will be offered to the robins, causing them to fast for at least two hours prior to receiving dosed lab food. A two-hour fasting period will be sufficient to cause hunger in the robins so that they will consume the dosed lab food, without causing them discomfort. At 1000 hours, each robin, depending on its dose group, will receive a 4-gram feed ball, prepared as described in Section V.A.3. After the robins consume the feed ball, they will be offered standard lab maintenance diet *ad libitum*.

2. Attending Veterinary Care:

The metal doses that will be administered should not cause illness, based on comparison to toxicity reference values for other avian species. However, the animals will be observed daily by the investigators, to determine if the birds are docile or active, and the amount of food eaten will be recorded. No pain is expected during the course of the study. The birds will be observed for signs of distress daily and the veterinarian will be consulted if adverse signs are noticed.

If there are signs of illness, Dr. Tracey Jensen, a consulting small-animal veterinarian with experience in the research environment, will provide veterinary care. She holds a position on the Genesis IACUC committee and advises researchers on: preventive medicine, disease and zoonosis control, surgical procedures, anesthesia and analgesia, assessment of animal well-being and euthanasia. At Genesis, veterinary care is outlined in the Program for Veterinary Care as required by the Animal Welfare Regulations. The veterinarian will evaluate the health status of the animals used prior to exposure to the test substance. This action will be documented in the raw data.

3. Enrichment Strategy: N/A

a. Dogs: N/A

b. Nonhuman Primates: N/A

E. Data Analysis:

1. Data Collection and Analyses:

a. Data to be collected:

Data collected for each robin will include animal code number, body weight at the beginning and end of the 28-day feeding period, total feed consumption per day (grams, wet weight), and total body metal contents (excluding skin and feathers, which will be archived) for each of the metals of interest. Skin and feathers from robins, and whole-body homogenates will be archived (-80 °C) at Genesis for a maximum of two months after the completion of the study.

Data collected for earthworms will include total body metal contents in worms before they are exposed to the test soils (baseline metal contents), and from worms that have been exposed to each test soil on days 14 and 28 (Table 2).

b. Calculating Relative Bioavailability:

For each dose of metals administered to a robin, either in soil, earthworms, or soluble metal spikes, the body burden of each metal in each animal will be calculated as micrograms (ug) of metal/grams (g) of body weight (carcass portion only) at the end of the study. The doses received by the individual animals will be evaluated, and if significant differences in total dose received during the course of study (i.e., mg metal/g body weight) are observed between animals, then body burden (mass) at study termination will be normalized for dose.

Assuming that no obvious outliers are observed for the body burden estimates within each dose group, the individual values will be used to calculate an average body burden for each dose group. A relative absorption factor (RAF) for each metal will then be calculated for each soil, or earthworm, dose group. The RAF will be calculated as the average body burden for a metal from soil (or earthworms) relative to the average body burden of that metal from the soluble spike dose group that produced the biological response (i.e., bodyburden) most similar to that soil dose group. For example, for lead in DoD site soil #1, the RAF would be calculated as follows:

$$RAF_{(soil\ Pb)} = \text{Body burden}_{(soil\ Pb)} / \text{Body burden}_{(lead\ acetate)}$$

These calculations will be performed for each of the four target metals in each of the three DoD test soils, and for the robins dosed with earthworms.

c. Statistics

Statistical analyses will be performed to answer the following questions:

1) What is the whole-body (minus skin and feathers) concentration associated with each dose group in Test Group #1 (robins dosed with soluble metal spikes), and are the concentrations significantly different between the dose groups associated with each test soil?
2) For Test Group #1 (robins dosed with soluble metal spikes), is there a non-linear dose-response across the three dose groups (e.g., 100%, 50%, and 25% of the dose of metals received from each DoD test soil) for each metal? Are the differences in metal accumulation between the three dose groups statistically significant?
3) For Test Group #2 (robins dosed with either contaminated soil, 50% contaminated soil, 25% contaminated soil, or background soil), is there a non-linear dose-response across the three dose groups, for each test soil? Are the differences in metals accumulation between the three dose groups statistically significant?
4) For Test Group #3 (robins dosed with earthworms), is the accumulation of individual metals significantly different between the soil- (Test Group #2) and the earthworm-dosed animals?

An ANOVA model followed by multiple comparison tests will be utilized to assess the significance of the differences in metal absorption between the metal spike doses. An ANOVA model will also be used to assess the significance of the differences in metal absorption between the contaminated soil, 50% contaminated soil, 25% contaminated soil and background soil exposures. A T-test will be used to assess the significance of the differences in metal absorption between the contaminated soil and the earthworm exposures.

These methods assume that concentrations in each exposure group are normally distributed and approximately equal in variability. If these assumptions cannot be met by the data, or by a transformation of the data, then non-parametric methods will be used.

F. Investigator & Technician Qualifications/Training:

Mr. Michael Ruby will serve as the Principal Investigator for this study. Mr. Ruby is a principal scientist with Exponent, and is the principal investigator on the SERDP research project titled, "Development of Extraction Tests for Determining the Bioavailability of Metals in Soil" (CU-1165). He has extensive experience designing and overseeing bioavailability studies in numerous animal models, and recently served on the National Research Council's (NRC's) committee on the bioavailability of contaminants from soils and sediments.

Mr. Richard Poche' at Genesis Labs will serve as the co-investigator on this project. He has over 25 years of experience in wildlife management and toxicology. He has extensive experience in laboratory and field product testing and related environmental studies. He manages Genesis Labs and directs projects adhering to Good Laboratory Practice standards as required under FIFRA and promulgated by the EPA. His research work includes wildlife toxicology and risk assessments, product chemistry and residue studies, analytical method development and validation, various field studies, and animal metabolism.

Mr. Jeff Borchert will act as study director. He has directed and implemented mammalian and avian field and laboratory studies and laboratory studies under FIFRA, OECD, and FDA Guidelines. Mr. Borchert has 5 years of experience as an Animal Health Technician in small veterinary hospitals. He will be responsible for euthanasia of animals, acclimating robins to the laboratory, preparing dosed food, observing animals for signs of distress, and capturing robins in the wild.

In addition to Mr. Borchert, the following individuals will rotate responsibilities involved with maintaining and dosing the animals. Jeff Mach is the ecotoxicology director at Genesis. John Baroch is a senior scientist with 17 years experience in wildlife biology. Karen March is the quality assurance unit manager with 16 years experience in metabolism and residue studies. Dr. Tracey Jensen will act as the attending/consulting veterinarian.

The training and qualifications for all the individuals that will be involved with the robin bioavailability study are listed in their respective curriculum vitas. The CVs are presented in Appendix D.

VI. Biohazard/Safety: Some of the metals included in this study can be toxic. However, the potential for toxicity is low at the concentrations that will be used in this research. However, as a precaution, laboratory personnel will wear gloves when they prepare the spiked foods, feed the robins, clean their cages and euthanize them. Lab personnel will wash their hands thoroughly throughout the day while performing their duties and also following their daily tasks.

VIII. ASSURANCES: The law specifically requires several written assurances from the PI. It states that "research facilities will be held responsible if it is subsequently determined that an experiment is unnecessarily duplicative, and that a good faith review of available sources would have indicated as much."

As the Primary Investigator on this protocol, I acknowledge my responsibilities and provide assurances for the following:

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

B. Duplication of Effort: I have made a reasonable, good faith effort to ensure that this protocol is not an unnecessary duplication of previous experiments.

C. Statistical Assurance: I assure that I have consulted with an individual who is qualified to evaluate the statistical design or strategy of this proposal, and that the "minimum number of animals needed for scientific validity are used."

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

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

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

_____

(Primary Investigator)

G. **Painful Procedures**: N/A

IX. Enclosures:

A. **Literature Searches**: N/A
B. **Pathology Addendum**: N/A
C. **Pain Scoring Guidelines:** N/A
D. **Adjuvant Policy:** NA

E. **<u>Appendix A:</u> Genesis Documentation (USDA Inspection notes, Minutes of Two Most Recent IACUC Meetings, IACUC Approval Letter, Statement of PHS Assurance, Program of Veterinary Care)**

United States Department of Agriculture
Animal and Plant Health Inspection Service

INSPECTION REPORT

GENESIS LABORATORIES, INC.

10122 N.E. FRONTAGE ROAD
WELLINGTON, CO 80549 1195

Customer ID: 1273
Certificate: 84-R-0051

Site: 001
GENESIS LABORATORIES, INC.

Inspection Type: ROUTINE INSPECTION
Date: MAR-26-2002

2.33 (a)(1)
2.33 (b)

ATTENDING VETERINARIAN AND ADEQUATE VETERINARY CARE.

<<<<Each research facility shall employ an attending veterinarian under formal arrangements. In the case of a part-time attending veterinarian or consultant arrangements, the formal arrangements shall include a written program of veterinary care and regularly scheduled visits to the research facility.>>>>

The Program of Veterinary Care (PVC) (APHIS Form 7002) was last updated on 05/01/00. It does not include the Plains Pocket Gophers, which have been housed and used on research protocols since May 2001. The PVC should be updated as needed, or at least reviewed annually by the attending veterinarian and registrant. The PVC will be updated by April 5, 2002.

<<<<Each research facility shall establish and maintain programs of adequate veterinary care that include: .... (2)The use of appropriate methods to prevent, control, diagnose, and treat diseases and injuries , and the availability of emergency, weekend, and holiday care; (3) Daily observations of all animals to assess their health and well-being; and.... a mechanism of direct and frequent communication is required so that timely and accurate information on problems of animal health, behavior, and well-being is conveyed to the attending veterinarian.>>>>

Plains Pocket Gophers were acquired in May 2001 for several studies. From July 12 to July 22, six gophers had died (one was a baby killed by its mother). From Sept. 18 to Oct. 4, five more died. From Dec. 8 to Dec. 10, five more died. None of these animals were being used for an activity at the times of death. No investigation was carried out as to what may have caused their deaths, thus no preventative measures were taken. The Veterinary Inspection Log shows that the attending veteinarian visited the premise on 07/02/01, 08/06/01, 09/06/01, 10/01/01, 11/05/01, 12/03/01, and 01/07/02. There was no communication to the AV about the group deaths of these animals, and no action was taken, i.e. post-mortem exams, evaluation of husbandry practices, etc.

Starting immediately, on animal health issues, the attending veterinarian will be contacted, efforts will be made to determine the cause, and treatment and/or preventative measures will be taken.

3.125 (a)

FACILITIES, GENERAL.

<<<<The indoor and outdoor housing facilities shall be structurally sound and shall be maintained in good repair to protect the animals from injury and to contain the animals.>>>>

In the Peromyscus breeding colony room, Tub 4, containing approximately 15 mice, had heavy rust buildup along the floor of the enclosure, and moderate rust along the interior sides of the enclosure. The rusty metal demonstrates enough deterioration to make adequate sanitization of the tub improbable.

Prepared By: Ruth H. Nelson
RUTH NELSON , USDA, APHIS, Animal Care
Title: VETERINARY MEDICAL OFFICER , Inspector ID: 5028
Date: MAR-26-2002

Received By: K March
KAREN MARCH
Title: IACUC CHAIRPERSON
Date: MAR-27-2002

Mar 12 03 01:59p Genesis Laboratories 970-568-3293 p.6

United States Department of Agriculture
Animal and Plant Health Inspection Service

INSPECTION REPORT

The tub was replaced on March 26, 2002

This inspection was performed on 03/26/02 and 03/27/02.

Non-compliant items previously identified that have been corrected:
Sec. 3.125(c) The storage room was clean and orderly during this inspection.
Sec. 3.130 The water receptacle for the Norway rats was clean and contained clean water.

Inspection was accompanied by the IACUC Chairperson.

Prepared By: Ruth H. Nelson
RUTH NELSON , USDA, APHIS, Animal Care
Title: VETERINARY MEDICAL OFFICER , Inspector ID: 5028
Date: MAR-26-2002

Received By: K March
KAREN MARCH
Title: IACUC CHAIRPERSON
Date: MAR-27-2002

p.7 970-568-3293 Genesis Laboratories

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the form. Send comments regarding this burden estimate or any other aspects of this collection of information, including suggestions for reducing the burden, to USDA, OIRM, Clearance Officer, Room 404-W, Washington, DC 20250. When replying refer to the OMB Number and Form Number in your letter.

The Animal Welfare Regulations, Title 9 Subchapter A, Part II, Subpart C, Section 2.33 and Subpart D, Section 2.40 requires a Program of Veterinary Care.

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

ANIMAL CARE

(Program of Veterinary Care for Research Facilities or Exhibitors/Dealers)

FORM APPROVED OMB NO. 0579-0036

OFFICE USE ONLY

DATE RECEIVED

SECTION I. A PROGRAM OF VETERINARY CARE (PVC) HAS BEEN ESTABLISHED BETWEEN:

A. LICENSEE/REGISTRANT	B. VETERINARIAN
1. NAME: Genesis Laboratories, Inc.	1. NAME: Tracey Jensen, DVM
2. BUSINESS NAME: Genesis Laboratories, Inc.	2. CLINIC: Wellington Veterinary Clinic, PC
3. USDA LICENSE/REGISTRATION NUMBER: 84-R-0051	3. STATE LICENSE NUMBER: CO 6361
4. MAILING ADDRESS: P.O. Box 1195	4. BUSINESS ADDRESS: P.O. Box 1110
5. CITY, STATE AND ZIP CODE: Wellington, CO 80549	5. CITY, STATE AND ZIP CODE: Wellington, CO 80549
6. TELEPHONE NO. (Home): 970-397-2530 / TELEPHONE NO. (Business): 970-568-7059	6. TELEPHONE NO. (Business): 970-568-7387

This is a form that may be used for the Program of Veterinary Care. Also, this form may be used as a guideline for the written Program of Veterinary Care as required.

The attending veterinarian shall establish, maintain and supervise programs of disease control and prevention, pest and parasite control, pre-procedural and post-procedural care, nutrition, euthanasia and adequate veterinary care for all animals on the premises of the licensee/registrant. A written program of adequate veterinary care between the licensee/registrant and the doctor of veterinary medicine shall be established and reviewed on an annual basis. By law, such programs must include regularly scheduled visits to the premises by the veterinarian. Scheduled visits are required to monitor animal health and husbandry.

Pages or blocks which do not apply to the facility should be marked N/A. If space provided is not adequate for a specific topic, additional sheets may be added. Please indicate Section and Item Number.

I have read and completed this Program of Veterinary Care, and understand my responsibilities.

Regularly scheduled visits by the veterinarian will occur at the following frequency: Monthly for all animals contained in the Animal Welfare Act. (minimum annual).

C. SIGNATURE OF LICENSEE/REGISTRANT: [signature] DATE: 4/1/02

D. SIGNATURE OF VETERINARIAN: Tracey Jensen DVM DATE: 4/1/02

APHIS FORM 7002
(JUN 92)

CHECK IF N/A ☒

SECTION II. DOGS AND CATS

A. VACCINATIONS - SPECIFY THE FREQUENCY OF VACCINATION FOR THE FOLLOWING DISEASES

CANINE	JUVENILE	ADULT	FELINE	JUVENILE	ADULT
PARVOVIRUS			PANLEUK		
DISTEMPER			RESP. VIRUSES		
HEPATITIS			RABIES		
LEPTOSPIROSIS			OTHER *(Specify)*		
RABIES					
BORDETELLA					
OTHER *(Specify)*					

B. PARASITE CONTROL PROGRAM - DESCRIBE THE FREQUENCY OF SAMPLING OR TREATMENT FOR THE FOLLOWING:

1 ECTOPARASITES *(Fleas, Ticks, Mites, Lice, Flies)*

2 BLOOD PARASITES *(Heartworm, Babesia, Ehrlichia, Other)*

3 INTESTINAL PARASITES *(Fecals, Deworming)*

C EMERGENCY CARE - DESCRIBE PROVISIONS FOR EMERGENCY, WEEKEND AND HOLIDAY CARE

D. EUTHANASIA

1 SICK, DISEASED, INJURED OR LAME ANIMALS SHALL BE PROVIDED WITH VETERINARY CARE OR EUTHANIZED. EUTHANASIA WILL BE IN ACCORDANCE WITH THE AVMA RECOMMENDATIONS AND WILL BE CARRIED OUT BY THE FOLLOWING:

☐ VETERINARIAN ☐ LICENSEE/REGISTRANT

2 METHOD(S) OF EUTHANASIA

E. ADDITIONAL PROGRAM TOPICS - THE FOLLOWING TOPICS HAVE BEEN DISCUSSED IN THE FORMULATION OF THE PROGRAM OF VETERINARY CARE

☐ Congenital Conditions
☐ Quarantine Conditions
☐ Nutrition
☐ Anthelmintic alternation
☐ Other *(Specify)* _____

☐ Exercise Plan *(Dogs)*
☐ Proper Handling of Biologics
☐ Venereal Diseases
☐ Pest Control and Product Safety
☐ Proper Use of Analgesics and Sedatives

CHECK IF N/A ☐

SECTION III. WILD AND EXOTIC ANIMALS

A. VACCINATIONS - LIST THE DISEASES FOR WHICH VACCINATIONS ARE PERFORMED AND THE FREQUENCY OF VACCINATIONS *(Enter N/A if not applicable)*

CARNIVORES Ferrets will be vaccinated by supplier for Canine Distemper and Rabies. This will be boosted annually.

HOOFED STOCK

PRIMATES

ELEPHANTS

MARINE MAMMALS

OTHER *(Specify)* Wild rats, pocket gophers, Peromyscus, wild house mice (9/24/02) White throated woodrat (9/24/02) Mexican Woodrat Mexican Deer Mice (TST 7/10/02), Golden Mantle Ground Squirrel (9/24/02) Rock Squirrel

B. PARASITE CONTROL PROGRAM - DESCRIBE THE FREQUENCY OF SAMPLING OR TREATMENT FOR THE FOLLOWING

1. ECTOPARASITES *(Fleas, Ticks, Mites, Lice, Flies)* Pyrethrin or permethrin powder will be used at the time of capture and repeated only if needed.

2. BLOOD PARASITES

n/a

3. INTESTINAL PARASITES

n/a

C. EMERGENCY CARE

1. DESCRIBE PROVISIONS FOR EMERGENCY, WEEKEND AND HOLIDAY CARE

On call as needed.

2. DESCRIBE CAPTURE AND RESTRAINT METHOD(S)

Live traps and holding cages, depending on species. Ferret restraint and handling as directed in Biology and Diseases of the Ferret. By James Fox, 2nd edition.

D. EUTHANASIA

1. SICK, DISEASED, INJURED OR LAME ANIMALS SHALL BE PROVIDED WITH VETERINARY CARE OR EUTHANIZED. EUTHANASIA WILL BE IN ACCORDANCE WITH THE AVMA RECOMMENDATIONS AND WILL BE CARRIED OUT BY THE FOLLOWING:

☐ VETERINARIAN ☒ LICENSEE/REGISTRANT

2. METHOD(S) OF EUTHANASIA

Carbon dioxide chamber

E. ADDITIONAL PROGRAM TOPICS - THE FOLLOWING TOPICS HAVE BEEN DISCUSSED IN THE FORMULATION OF THE PROGRAM OF VETERINARY CARE

- ☒ Pest Control and Product Safety
- ☒ Quarantine Procedures
- ☒ Zoonoses
- ☐ Other *(Specify)* _____
- ☐ Environment Enhancement *(Primates)*
- ☐ Water Quality *(Marine Mammals)*
- ☐ Species-specific Behaviors
- ☒ Proper Storage and Handling of Drugs and Biologics
- ☒ Proper Use of Analgesics and Sedatives

F. LIST THE SPECIES SUBJECTED TO TB TESTING, AND THE FREQUENCY OF SUCH TESTS

Not applicable to any of the 4 species listed above.

CHECK IF N/A ☒

SECTION IV. OTHER WARMBLOODED ANIMALS

A. INDICATE SPECIES

B. VACCINATIONS - LIST THE DISEASES FOR WHICH VACCINATIONS ARE PERFORMED AND THE FREQUENCY OF VACCINATIONS
(Enter N/A if not applicable)

C. PARASITE CONTROL PROGRAM - DESCRIBE THE FREQUENCY OF SAMPLING OR TREATMENT FOR THE FOLLOWING

1. ECTOPARASITES *(Fleas, Ticks, Mites, Lice, Flies)*

2. INTERNAL PARASITES *(Helminths, Coccidia, Other)*

D. EMERGENCY CARE - DESCRIBE PROVISIONS FOR EMERGENCY, WEEKEND AND HOLIDAY CARE

E. EUTHANASIA

1. SICK, DISEASED, INJURED OR LAME ANIMALS SHALL BE PROVIDED WITH VETERINARY CARE OR EUTHANIZED. EUTHANASIA WILL BE IN ACCORDANCE WITH THE AVMA RECOMMENDATIONS AND WILL BE CARRIED OUT BY THE FOLLOWING:

☐ VETERINARIAN ☐ LICENSEE/REGISTRANT

2. METHOD(S) OF EUTHANASIA

F. ADDITIONAL PROGRAM TOPICS - THE FOLLOWING TOPICS HAVE BEEN DISCUSSED IN THE FORMULATION OF THE PROGRAM OF VETERINARY CARE

- ☐ Pasteurellosis
- ☐ Pododermatitis
- ☐ Cannibalism
- ☐ Wet Tail
- ☐ Other *(Specify)*
- ☐ Species Separation
- ☐ Malocclusion/Overgrown Incisors
- ☐ Pest Control and Product Safety
- ☐ Handling

U.S. GOVERNMENT PRINTING OFFICE : 1993 O - 339-536

USDA

United States Department of Agriculture
Animal and Plant Health Inspection Service

INSPECTION REPORT

GENESIS LABORATORIES, INC.

10122 N.E. FRONTAGE ROAD
WELLINGTON, CO 80549 1195

Customer ID: 1273
Certificate: 84-R-0051
Site: 001
GENESIS LABORATORIES, INC.
Inspection Type: ROUTINE INSPECTION
Date: AUG-19-2002

3.125 (a)
3.125 (c)
FACILITIES, GENERAL.

<<<<The facility must be constructed of such material and of such strength as appropriate for the animals involved. The indoor and outdoor housing facilities shall be structurally sound and shall be maintained in good repair to protect the animals from injury and to contain the animals.>>>>

The outdoor wooden "condo" for the Norway rats is very soiled and showing signs of deterioration, i.e. wood wearing down and weathered, on the south side. This material is not easily cleaned and not easy to maintain for this species. This will be corrected by pressure washing on a monthly basis and replacing parts in the event of severe deterioration.

<<<<Storage. Supplies of food and bedding shall be stored in facilities which adequatelyprotect such supplies against deterioration, molding, or contamination by vermin.>>>>

Rubbermaid container in bulk feed storage shed containing rolled oats used in rodent feed had a 3-4 inch hole in it next to one of the handles. Food storage containers should be leakproof and pest-proof. This will be corrected by replacing the container by Aug. 20, 2002.

Discussed elaborating on 6 month ACUC program reviews with IACUC chairperson.

Discussed performance of post-mortem exams by non-veterinary personnel.

Discussed use of key words when performing literature search for alternatives to pain and distress.
Gave registrant copy of AC Policy memo #12, Consideration of Alternatives to Painful/Distressful Procedures.

All non-compliant items previously identified have been corrected.

Prepared By: Ruth H. Nelson
RUTH NELSON , USDA, APHIS, Animal Care
Title: VETERINARY MEDICAL OFFICER , Inspector ID: 5028
Date: AUG-19-2002

Received By: Karen March
KAREN MARCH
Title: IACUC CHAIRPERSON
Date: AUG-19-2002

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the form. Send comments regarding this burden estimate or any other aspects of this collection of information, including suggestions for reducing the burden, to USDA, OIRM, Clearance Officer, Room 404-W, Washington, DC 20250. When replying refer to the OMB Number and Form Number in your letter.

The Animal Welfare Regulations, Title 9 Subchapter A, Part II, Subpart C Section 2.33 and Subpart D, Section 2.40 requires a Program of Veterinary Care

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

FORM APPROVED OMB NO. 0579-0036

OFFICE USE ONLY

DATE RECEIVED

ANIMAL CARE

(Program of Veterinary Care for Research Facilities or Exhibitors/Dealers)

SECTION I. A PROGRAM OF VETERINARY CARE (PVC) HAS BEEN ESTABLISHED BETWEEN:

A. LICENSEE/REGISTRANT	B. VETERINARIAN
1. NAME: Genesis Laboratories, Inc.	1. NAME: Tracey Jensen, DVM
2. BUSINESS NAME: Genesis Laboratories, Inc.	2. CLINIC: Wellington Veterinary Clinic, PC
3. USDA LICENSE/REGISTRATION NUMBER: 84-R-0051	3. STATE LICENSE NUMBER: CO 6361
4. MAILING ADDRESS: P.O. Box 1195	4. BUSINESS ADDRESS: P.O. Box 1110
5. CITY, STATE AND ZIP CODE: Wellington, CO 80549	5. CITY, STATE AND ZIP CODE: Wellington, CO 80549
6. TELEPHONE NO. (Home): 970-397-2530 / TELEPHONE NO. (Business): 970-568-7059	6. TELEPHONE NO. (Business): 970-568-7387

This is a form that may be used for the Program of Veterinary Care. Also, this form may be used as a guideline for the written Program of Veterinary Care as required.

The attending veterinarian shall establish, maintain and supervise programs of disease control and prevention, pest and parasite control, pre-procedural and post-procedural care, nutrition, euthanasia and adequate veterinary care for all animals on the premises of the licensee/registrant. A written program of adequate veterinary care between the licensee/registrant and the doctor of veterinary medicine shall be established and reviewed on an annual basis. By law, such programs must include regularly scheduled visits to the premises by the veterinarian. Scheduled visits are required to monitor animal health and husbandry.

Pages or blocks which do not apply to the facility should be marked N/A. If space provided is not adequate for a specific topic, additional sheets may be added. Please indicate Section and Item Number.

I have read and completed this Program of Veterinary Care, and understand my responsibilities.

Regularly scheduled visits by the veterinarian will occur at the following frequency: Monthly for all animals contained in the Animal Welfare Act. (minimum annual).

C. SIGNATURE OF LICENSEE/REGISTRANT	DATE
Richard M Decker	4/1/02
D. SIGNATURE OF VETERINARIAN	**DATE**
Tracey Jensen DVM	4/1/02

APHIS FORM 7002
(JUN 92)

CHECK IF N/A ☒

SECTION II. DOGS AND CATS

A. VACCINATIONS - SPECIFY THE FREQUENCY OF VACCINATION FOR THE FOLLOWING DISEASES

CANINE			FELINE		
	JUVENILE	ADULT		JUVENILE	ADULT
PARVOVIRUS			PANLEUK		
DISTEMPER			RESP VIRUSES		
HEPATITIS			RABIES		
LEPTOSPIROSIS			OTHER *(Specify)*		
RABIES					
BORDETELLA					
OTHER *(Specify)*					

B. PARASITE CONTROL PROGRAM - DESCRIBE THE FREQUENCY OF SAMPLING OR TREATMENT FOR THE FOLLOWING:

1. ECTOPARASITES *(Fleas, Ticks, Mites, Lice, Flies)*

2. BLOOD PARASITES *(Heartworm, Babesia, Ehrlichia, Other)*

3. INTESTINAL PARASITES *(Fecals, Deworming)*

C. EMERGENCY CARE - DESCRIBE PROVISIONS FOR EMERGENCY, WEEKEND AND HOLIDAY CARE

D. EUTHANASIA

1. SICK, DISEASED, INJURED OR LAME ANIMALS SHALL BE PROVIDED WITH VETERINARY CARE OR EUTHANIZED. EUTHANASIA WILL BE IN ACCORDANCE WITH THE AVMA RECOMMENDATIONS AND WILL BE CARRIED OUT BY THE FOLLOWING:

☐ VETERINARIAN ☐ LICENSEE/REGISTRANT

2. METHOD(S) OF EUTHANASIA

E. ADDITIONAL PROGRAM TOPICS - THE FOLLOWING TOPICS HAVE BEEN DISCUSSED IN THE FORMULATION OF THE PROGRAM OF VETERINARY CARE

- ☐ Congenital Conditions
- ☐ Quarantine Conditions
- ☐ Nutrition
- ☐ Antihelmintic alternation
- ☐ Other *(Specify)* _____
- ☐ Exercise Plan *(Dogs)*
- ☐ Proper Handling of Biologics
- ☐ Venereal Diseases
- ☐ Pest Control and Product Safety
- ☐ Proper Use of Analgesics and Sedatives

CHECK IF N/A ☐

SECTION III. WILD AND EXOTIC ANIMALS

A. VACCINATIONS - LIST THE DISEASES FOR WHICH VACCINATIONS ARE PERFORMED AND THE FREQUENCY OF VACCINATIONS *(Enter N/A if not applicable)*

CARNIVORES Ferrets will be vaccinated by supplier for Canine Distemper and Rabies. This will be boosted annually.

HOOFED STOCK

PRIMATES

ELEPHANTS

MARINE MAMMALS

OTHER *(Specify)* Wild rats, pocket gophers, Peromyscus, wild house mice (9/24/02) White-throated woodrat (9/24/02) Mexican Woodrat Guinea Pigs (9/24/02) Golden Mantle Ground Squirrel (9/24/02) Rock Squirrel

B. PARASITE CONTROL PROGRAM - DESCRIBE THE FREQUENCY OF SAMPLING OR TREATMENT FOR THE FOLLOWING

1. ECTOPARASITES *(Fleas, Ticks, Mites, Lice, Flies)* Pyrethrin or permethrin powder will be used at the time of capture and repeated only if needed.

2. BLOOD PARASITES

n/a

3. INTESTINAL PARASITES

n/a

C. EMERGENCY CARE

1. DESCRIBE PROVISIONS FOR EMERGENCY, WEEKEND AND HOLIDAY CARE

On call as needed.

2. DESCRIBE CAPTURE AND RESTRAINT METHOD(S)

Live traps and holding cages, depending on species. Ferret restraint and handling as direct in Biology and Diseases of the Ferret. By James Fox, 2nd edition.

D. EUTHANASIA

1. SICK, DISEASED, INJURED OR LAME ANIMALS SHALL BE PROVIDED WITH VETERINARY CARE OR EUTHANIZED. EUTHANASIA WILL BE IN ACCORDANCE WITH THE AVMA RECOMMENDATIONS AND WILL BE CARRIED OUT BY THE FOLLOWING:

☐ VETERINARIAN ☒ LICENSEE/REGISTRANT

2. METHOD(S) OF EUTHANASIA

Carbon dioxide chamber

E. ADDITIONAL PROGRAM TOPICS - THE FOLLOWING TOPICS HAVE BEEN DISCUSSED IN THE FORMULATION OF THE PROGRAM OF VETERINARY CARE

- ☒ Pest Control and Product Safety
- ☒ Quarantine Procedures
- ☒ Zoonoses
- ☐ Other *(Specify)* _____
- ☐ Environment Enhancement *(Primates)*
- ☐ Water Quality *(Marine Mammals)*
- ☒ Species-specific Behaviors
- ☒ Proper Storage and Handling of Drugs and Biologics
- ☒ Proper Use of Analgesics and Sedatives

F. LIST THE SPECIES SUBJECTED TO TB TESTING, AND THE FREQUENCY OF SUCH TESTS

Not applicable to any of the 4 species listed above.

CHECK IF N/A ☒

SECTION IV. OTHER WARMBLOODED ANIMALS

A. INDICATE SPECIES

B. VACCINATIONS - LIST THE DISEASES FOR WHICH VACCINATIONS ARE PERFORMED AND THE FREQUENCY OF VACCINATIONS
(Enter N/A if not applicable)

C. PARASITE CONTROL PROGRAM - DESCRIBE THE FREQUENCY OF SAMPLING OR TREATMENT FOR THE FOLLOWING

1. ECTOPARASITES *(Fleas, Ticks, Mites, Lice, Flies)*

2. INTERNAL PARASITES *(Helminths, Coccidia, Other)*

D. EMERGENCY CARE - DESCRIBE PROVISIONS FOR EMERGENCY, WEEKEND AND HOLIDAY CARE

E. EUTHANASIA

1. SICK, DISEASED, INJURED OR LAME ANIMALS SHALL BE PROVIDED WITH VETERINARY CARE OR EUTHANIZED. EUTHANASIA WILL BE IN ACCORDANCE WITH THE AVMA RECOMMENDATIONS AND WILL BE CARRIED OUT BY THE FOLLOWING:

☐ VETERINARIAN ☐ LICENSEE/REGISTRANT

2. METHOD(S) OF EUTHANASIA

F. ADDITIONAL PROGRAM TOPICS - THE FOLLOWING TOPICS HAVE BEEN DISCUSSED IN THE FORMULATION OF THE PROGRAM OF VETERINARY CARE

☐ Pasteurellosis
☐ Pododermatitis
☐ Cannibalism
☐ Wet Tail
☐ Other *(Specify)*_____
☐ Species Separation
☐ Malocclusion/Overgrown Incisors
☐ Pest Control and Product Safety
☐ Handling

U.S. GOVERNMENT PRINTING OFFICE : 1993 O - 339-536

Genesis Laboratories, Inc.

MEMORANDUM

To: Rich, John, Jeff M., Jeff B., Chris and Nick
From: Karen
Date: March 27, 2002
cc: Memo file, IACUC files, Dan G., and Tracey J.

RE: USDA Inspection on March 26 and March 27, 2002

During the USDA inspection the following are the findings listed in the Inspection Report.

Item of Concern	Personnel Responsible	Date to Correct By
Program of Veterinary Care (PVC) needs to be updated and include the gophers. Should be done yearly, it was last updated May of 2000.	Rich	April 5, 2002
At thee times 5 to 6 gophers died over a few days to a few weeks time. The veterinary was not contacted, no necropsies were performed, and no assessment as to the why was made. If a number of animals die within a short amount of time, with no explanation, the vet needs to be consulted, animals should be submitted for necropsy, and husbandry practices evaluated.	Rich John Jeff M. Jeff B. Chris Nick	March 27, 2002
One of the tubs in the *Peromyscus* room had heavy rust. The rust made cleaning/sanitizing improbable.	John	March 26, 2002

Other items discussed are listed below with personnel responsible.

1. In protocols that ask the question "Have procedures that cause greater than slight or momentary pain been considered in the planning by consulting with the attending veterinarian?" this should be answered with a clear "Yes" or "No".

   The attending vet does review and approve/disapprove each protocol so the requirement has been fulfilled, however a clear concise answer is more appropriate.

   This should be addressed by each study director with each new protocol from this date (3/27/02) forward.

2. Cans with heavy rust were found in the storage bins for the cans. These cans, used as shelters for mice, must not have heavy rust since it is not probable to sanitize the cans.

   Jeff M. please have the technicians discard cans that fit the above description by 4/5/02.
   Any study directors who are using cans at present should discard any cans with heavy rust by 3/28/02.

Items discussed were

1. Record keeping of the animals obtained and mortality was clear and well documented. One note was to include in the monthly vet inspections the study number with any "Findings Requiring Treatment" to clearly track documentation of actions taken.

2. IACUC inspections and review of protocols was effective and inline with IACUC standards.

3. Reporting of inspection findings and IACUC meetings to the institutional officer was clear and accurate.

Minutes of the IACUC Meeting

Meeting Information

Place: Genesis Laboratories, Inc.
Date: March 21, 2002
Time: 1330 to 1350

Members Present

John Baroch, Genesis Laboratories, Inc., Wellington, CO
Dan Gardinier, Wellington Federated Church, Wellington, CO
Tracey Jensen, D.V.M., Wellington Veterinary Clinic, Wellington, CO
Karen March, Genesis Laboratories, Inc., Wellington, CO

Chairperson Karen March called the meeting to order at 1330.

Items discussed

1. Protocols: None at present expect one within a week or so.
2. Internal/external complaints on animal care and use at Genesis Laboratories, Inc.: None.
3. Review of inspection of animal facilities and study areas:
   J. Baroch and T. Jensen inspected the facilities on 3/20/02. The findings are listed below.

Item	Classification	Recommendation	Correction Timeline
NON-GLP refrigerator in B-1 contains feed and test substances. The freezer contains rat carcasses.	Major	Remove carcasses and test substance. Clean out unit and use only for feed items.	3/28/02
Dirty mops and dirty water in B-1. Unsanitary	Minor	Discard dirty water after use. Put up drying hooks to store mops after use to allow them to dry.	3/28/02
Outdoor rat colony is chewing through platform supports.	Minor	Add more supports before this becomes a problem.	4/10/02
Teklad bags in feed shed do not have receipt and expiration dates.	Minor	Add dates.	3/25/02
Oats in feed storage bag are not in a rodent proof container.	Minor	Place oats in a proper storage container.	3/25/02

4. J. Baroch read minutes from last meeting.
5. New Procedures: None.
6. Review training records of researchers/animal care staff: Reviewed CV of Lauren Schwartz, a new technician at Genesis Laboratories, Inc.
7. Recent inspection by APHIS: None.

Meeting adjourned at 1350.

Minutes prepared by: Karen J March Date: 3-22-02
Karen March

Minutes of the IACUC Meeting (March 21, 2002)

Minutes reviewed by:

Karen March, Chairperson — Date: 3-22-02

John Baroch, Member — Date: 3/22/02

Dan Gardinier, Member — Date: 4-5-02

Tracey Jensen, Member — Date: 4/2/02

Richard Poché, Institutional Officer — Date: 4/10/02

MINUTES OF THE IACUC MEETING HELD SEPTEMBER 12, 2002

Chairman John Baroch called the meeting to order at 1:15 pm. The following members were present:

1. John Baroch, Genesis Laboratories, Inc., Wellington, CO.
2. Jeff Borchert, Genesis Laboratories, Inc., Wellington, CO.
3. Tracey Jensen, D.V.M., Wellington Veterinary Clinic, Wellington, CO.
4. Dan Gardinier, Wellington Federated Church, Wellington, CO.

Items discussed included:

1. *Membership*: Karen March has left the committee to assume other responsibilities. New committee Chair will be John Baroch. Jeff Borchert , who has served on and led the committee in the past, is returning as a member. A memo from the IO (dated 9/10/02) regarding the changing membership was circulated and reviewed.

2. *Protocols*: None to review at this time. Jeff Mach is revising a protocol (02029) which was reviewed by KM, TJ, and John B. John had asked for clarification/revision of a few items. The revised protocol will be re-circulated when available.

   We discussed a deficiency in the past protocols in that we (the committee) were not asking investigators to explicitly address the issue of *distressful* as well as *painful* procedures when writing protocols. Discomfort and distress need to be addressed according to AWA and OLAW regulations. A memo to study directors and principle investigators with guidance on this issue has been issued.

   We discussed the upcoming wild rodent breeding research, which is to be conducted with NIH funding and will require compliance with the OLAW regulations, which differ somewhat from the AWA regulations. We have obtained copies of the 2002 edition of the OLAW (Office of Laboratory Animal Welfare, NIH) IACUC Guidebook. Among other things, the guidebook provides side by side comparisons of AWA and OLAW regulations. The committee asked that each member be provided a copy to aid in reviewing the NIH protocols. Members will also be provided with copies of the APHIS Protocol Review Guide as well.

3. Discussed any complaints from inside or outside the facility regarding animal care at Genesis. No committee members had received any complaints since the last meeting.

4. Minutes of the last meeting were read. We also reviewed items needing correction from last IACUC inspection. All items were corrected within the timeframe recommended by the committee.

5. Reviewed changes in procedures since last meeting. While no formal changes have been made, we have made efforts to better comply with current procedures, particularly in the are of assessing the causes of animal mortality. Patterns of mortality can suggest systematic husbandry problems or disease situations. During the recent APHIS inspection the inspector noted improvements in this respect.

→ ENV BOULDER PATTONENT 00

6. Discussed training records of new AC technicians. There are no new techs. since last meeting.

7 John B. noted that APHIS had granted a waiver of the requirement for a secondary enclosure on the proposed outdoor Peromyscus pen. Construction will proceed, using the same design and materials as the outdoor rat pen.

8. The committee reviewed results of the last two APHIS inspections, both of which occurred since the last semiannual committee meeting last March. Items needing correction were reviewed.

a.) A re-curing problem seems to be in the area of feed storage and management. Specifically, feed bags are not always labeled with expiration dates, damaged storage containers are sometimes being used, and expired items are retained after the expiration date (note: there is no evidence that expired feed has been given to animals, but the possibility exists). A container of oats, which are used to formulate EPA challenge diet, had a hole in the container, and the oats had expired a few days prior to the inspection. The oats and container were disposed on 9-20-02.

b.) Another item which came up again is the use of rusty cans for rodent shelters. Genesis agreed to phase out metal cans entirely and replace with PVC shelters as are now being used for Peromyscus.

c.) The APHIS inspector felt the Norway rat outdoor condo was excessively soiled and should be periodically sanitized. She recommended monthly power-washing. Dr. Jensen (AV) feels the liberal use of water in the pen will facilitate growth of micro-organisms which could have a net detrimental effect on sanitation. The committee agreed that when periodic sanitizing is performed, limiting water use is important. John B. will explore options and put in place a cleaning and documentation procedure in the next 10 days (by 9-23-02).

d.) Inspector encouraged more thorough semi-annual animal care program review and documentation of the review, although the current procedures meet the requirements. Therefore, we followed the APHIS program review guideline. Recommended improvements to the Animal Care program that emerged from the review were:

1.) Put appropriate water bottle changeout schedule and documentation procedure into an SOP if it is not already.

2.) Put appropriate room sanitizing schedule and cage changeout schedule, with a documentation procedure, into SOP(s) for colonies. This is not usually a problem for toxicology studies since most are short term (3-6 weeks).

e.) Inspector reviewed one protocol in which key words used in search for alternative procedures were not listed. This is a requirement that reviewers should always check for.

f.) Inspector encouraged more staff training in necropsy and gross pathology. Dr. Jensen will work with the IACUC to provide periodic training and refreshers for animal carestaff.

EXPONENT → ENV BOULDER 003

9. Inspection of facilities. John Baroch, Tracey Jensen and Dan Gardinier inspected the facility on September 12, 2002. There were no major problems noted. Minor problems noted were :
   a) Gopher carcass (in zip-loc bag) in refrigerator in B-1. **Removed immediately.**
   b) No expiration date on container of Norway rat maintenance diet. **Corrected 9/13/02.**
   c) Particulates in guinea pig water bottles. AC technician (SS) explained that the bottles are replaced weekly, but that the guinea pigs expel food particles into the bottles when drinking. We felt this was an appropriate wash schedule.
   d) Dr. Jensen recommended blocking the north and west sides of the outdoor rat colony condo with plywood over the winter to deflect cold winds.

The meeting was adjourned at 3:05 pm.

Action Items:

No.	Item	Classification	Correction Timeline
1.	Distribute copies of OLAW IACUC Guidebook to Committee Members	N/A	ASAP
2.	Add Whitethroat Woodrat, Golden-mantle ground squirrel, and Rock Squirrel to Veterinary Care Program.	N/A	Before animals acquired
3.	Develop procedure/training to assure feed is properly stored and managed.	Minor	9-30-02
4.	Develop procedure to periodically sanitize rat condo without excessive use of water.	Minor	9-23-02
5.	Conduct periodic training and refresher training for animal care staff in necropsy and gross pathology evaluations	Minor	ASAP (Schedule session this fall)
6.	If not already in SOP's revise or add sections with appropriate water bottle, cage and room sanitation schedules, and documentation procedure. For all studies, and for colonies.	Minor	9-30-02

Minutes prepared by: John Baroch Date: 9/14/02

Minutes reviewed by : John Baroch Date: 9/14/02
John Baroch, Chair

Jeff Borchert, Member Date: 9/20/2

Tracey Jensen, D.V.M., Member Date: 9/20/02

Dan Gardinier, Outside Member Date: 10-11-02

Todd Schmidt, Member Date: 9/20/02

Richard Poché, Institutional Officer Date:

Genesis Labs

Memo

To: Jeff Borchert

From: Karen March, IACUC Chair

CC: IACUC File

Date: 06/27/02

RE: Genesis Protocol No. N02011

Dear Jeff,

This is to inform you that two member of the Genesis Laboratories, Inc. IACUC have completed a review of the Appendix 1, draft protocol "Evaluating the Oral Bioavailability of Metal from Soil and Earthworms to American Robins" received from the sponsor. This protocol **was not** approved.

However, the Genesis Laboratories, Inc. study protocol N02011, Pilot Study Evaluating the Oral Bioavailability of Metals from Soil and Earthworms to American Robins (*Turdus migratorius*) **was** approved. This protocol follows the "Guidelines to the Use of Wild Birds in Research" published by The Ornithological Council.

Thank you,

Karen March

Karen March
IACUC Chair
Genesis Laboratories, Inc.

Vertebrate Animals

Genesis maintains an Institutional Animal Care and Use Committee (IACUC) in accordance with the Animal Welfare Regulations (9 CFR Ch. 1). The Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals, Guide for the Care and Use of Laboratory Animals (NRC 1996) and The Guidelines to the Use of Wild Birds in Research approved by the Ornithological Council (Gaunt and Oring 1999) will be followed when completing this study. Due to recent NIH funding Genesis Laboratories is currently pursing Animal Welfare Assurance from the National Institutes of Health, Office for Protection from Research Risks.

National Research Council. 1996. Guide for the Care and Use of Laboratory Animals. National Academy Press. Washington D.C.

Gaunt, A.S., L.W. Oring. 1999. Guidelines to the Use of Wild Birds in Research. The Ornithological Council Special Publication. Washington, D.C. 57 pgs.

F. Appendix B: Shipping Procedures

Sample Custody, Sample Shipping, and Documentation
Procedures for sample custody, shipping, and documentation must be followed to ensure the proper transfer and documentation of samples collected. Procedures for the careful and consistent documentation of and transfer of samples from the field to the laboratory are outlined herein.

Sample Custody. A stringent, established program of sample chain-of-custody will be followed during sample storage and shipping activities to account for each sample. These procedures outlined herein will be used in conjunction with procedures for field documentation and sample packaging and shipping, as described below. Chain-of-custody record/ sample analysis request forms ensure that samples are traceable from the time of collection through processing and analysis until final disposition.

The chain-of-custody record portion of the form is the most critical because it documents sample possession from the time of collection through the final disposition of the sample. The sample analysis request portion of the form provides information to the laboratory regarding what analyses are to be performed on the samples that are shipped.

The chain-of-custody record/sample analysis request form will be completed before the samples are shipped to the laboratory. Sampling personnel are responsible for the care and custody of the samples until they are shipped. When transferring possession of the samples, the individuals relinquishing and receiving the samples must sign the chain-of-custody record/sample analysis request form(s), indicating the time and date that the transfer occurs. Copies of the forms will be made and kept by Jeff Borchert, and the originals will be included with the samples in the transfer container. The following guidelines will be followed to ensure consistent shipping procedures and to maintain the integrity of the samples:

1. Each chain-of-custody record/sample analysis request form contains a line where the person who relinquishes custody of the samples must sign this form.

2. The chain-of custody record/sample analysis request form should not be signed until the information has been checked for accuracy by the lead sampler. All changes should be made by drawing a single line through the incorrect entry and initialing and dating it. Revised entries should be made in the space below the entries. On the handwritten

chain-of custody record/sample analysis request forms, spaces remaining at the bottom of the page after corrections are made should be marked out with single lines. This procedure will preclude any unauthorized additions.

3. At the bottom of each chain-of custody record/sample analysis request form is a space for the signatures of the persons relinquishing and receiving the samples and the time and date that the transfer occurred. The time that the samples were relinquished should match exactly the time they were received by another party. Under no circumstances should there be any time when custody of the samples is undocumented.

4. If samples are sent by a courier not affiliated with the laboratory, such as Federal Express or UPS, the name of the courier should be entered in the "received by" block. The time of transfer should be as close to the actual drop-off time as possible. After the chain-of custody record/ sample analysis request forms are signed and copied, they should be sealed inside the transfer container.

5. If errors are found after the shipment has left the custody of Genesis, a corrected version of the forms must be made and sent to all relevant parties. Minor errors can be rectified by making the change on a copy of the original with a brief explanation and signature. The person who makes the changes should appear on the chain-of custody record/sample analysis request form as a sampler. Errors in the signature block may require a letter of explanation.

Sample Packaging and Shipping

Specific requirements for sample packaging and shipping must be followed to ensure the proper transfer of samples from the field to the laboratory. Specific equipment or supplies necessary to properly pack and ship environmental samples include the following:

- Dry ice
- Sealable airtight bags (Ziploc)
- Plastic garbage bags
- Coolers

- Bubble wrap
- Fiber reinforced packing tape
- Scissors
- Chain-of-custody seals
- Airbills for overnight shipment
- Chain-of-custody record/sample analysis request forms.

The following steps should be followed to ensure the proper transfer of samples between laboratories:

1. Appropriately document all samples using the proper logbooks and chain-of-custody forms.
2. Samples that will be archived for future possible analysis should be clearly identified on the sample analysis request form.
3. Notify the laboratory contact that samples will be shipped and the estimated arrival time.
4. Clean the outside of all dirty sample containers to remove any residual material that may lead to cross-contamination.
5. Check sample containers against the chain-of-custody record to ensure all samples intended for shipment are accounted for.
6. Store each sample container in a sealable bag that allows the sample label to be read.
7. Choose the appropriate size cooler (or coolers) and line with bubble wrap and a plastic garbage bag.
8. Fill the cooler with the samples. Add enough dry ice or blue ice to keep the samples frozen during overnight transport. After all samples and ice have been added to the cooler, use bubble wrap to fill any empty space to keep the samples from shifting during transport.
9. After all the samples are packed, close the plastic garbage bag and fasten it with a chain-of-custody seal.
10. Store the signed chain-of-custody records/sample analysis request forms in a sealable bag and tape them to the inside of the cooler lid.

11. After the cooler is sufficiently packed to prevent shifting of the containers, close the lid and seal it shut using fiber reinforced packing tape. Also, if the cooler has a drain at the bottom, it should be taped shut.

12. As security against unauthorized handling of the samples, apply one or two chain-of-custody seals across the opening of the cooler lid. Be sure the seals are properly affixed to the cooler so they are not removed during shipment.

13. Label the cooler with destination and return addresses, and add other appropriate stickers, such as "This End Up," "Fragile," and "Handle With Care."

14. If an overnight courier is used, fill out the airbill as required and fasten it to the top of the cooler. The identification number sticker should be taped to the lid, because tracking problems can occur if a sticker is removed during shipment.

15. Samples will be shipped on dry ice to Abbie Spielman at CAS in Kelso, Washington.

Sample Labels—Sample labels are designed to uniquely identify each container that is used for a sample. The labels should be filled out at the time the samples are collected and should consist of the following information:

1. Sample identifier
2. General category of analytes (primarily for identification purposes); the laboratory should follow instructions on the sample analysis request form.
3. Date and time sample is collected
4. Initials of the samplers
5. A unique tag number (preprinted on the tag) consisting of six digits, used to identify individual containers.

The information on each sample label is unique to that sample.

G. Appendix C: Standard Operating Procedures for Euthanasia

GENESIS LABORATORIES, INC.
STANDARD OPERATING PROCEDURE

CHAPTER 19		Laboratory Rodent Studies
SOP AS-9.02		Animal Euthanasia
Effective	9/12/02	Prepared by/Date: 9/11/02
Supersedes	8/31/02	Approved by/Date: 9/12/02

A. PURPOSE

This SOP standardizes the procedure for animal euthanasia and disposal.

B. SCOPE

Personnel at Genesis Labs euthanizing animals and disposing of carcasses must adhere to this SOP for all GLP and NON-GLP studies.

C. PROCEDURE

1. Operation of CO_2 cylinder

a. Make certain the CO_2 bottle contains sufficient gas (check gauge). Before opening the valve, ensure tank is securely fastened to the cart and that the room is properly ventilated.

b. Turn the secondary valve (located on the regulator) counterclockwise until it is loose. This indicates the valve is shut.

c. Turn the primary valve on top of the tank counterclockwise. This will open flow of gas to the regulator. The right gauge (closest to primary valve) will indicate the remaining cylinder pressure.

d. If possible, position the exhaust valve of the euthanasia chamber next to an exhaust vent. To displace the air in the chamber with CO_2, the euthanasia chamber may be opened approximately 0.5 inches.

e. To start the gas flow, slowly turn the secondary valve clockwise. The left gauge indicates the line pressure out of the regulator. When the gas starts to flow, slowly adjust the secondary valve for the desired flow rate, which is 10-20 lbs. for 3-4 minutes. Use the red, inside indicator circle, labeled CO_2, to measure the gas flow. At this point, the air will be replaced by CO_2 and the exhaust valve may be closed.

f. To stop the flow of gas, turn the secondary valve counterclockwise.

g. If the regulator valve develops a coating of frost and the gas flow becomes intermittent, close the valve until the regulator thaws, and then proceed at a lower flow rate.

h. To release the regulator pressure, make sure the primary valve is shut (turn clockwise), then open the secondary valve (turn clockwise) until both gauges indicate zero pressure.

2. Introduction of animals to CO_2 atmosphere.

a. If a large CO_2 chamber is used, fill the chamber with CO_2 and then use proper animal handling techniques to place the animals into the chamber. Add CO_2 as needed by adjusting the flow of CO_2 from the regulator and open the exhaust valve of the euthanasia chamber as needed to ensure death is as painless as possible. When more than one animal is to be euthanized, maintain a continuous flow of CO_2, and leave the exhaust valve open.

If a small chamber is used (i.e. plastic bag) for one animal or smaller animals place the animals in the chamber and immediately fill the chamber with CO_2. Take care to slowly introduce the gas so as not to startle the animal(s).

b. The time required for death is dependent upon the species. CO_2 is heavier than air and most will remain in the euthanasia chamber even when opened, **USE CAUTION WHEN REMOVING ANIMALS TO AVOID BREATHING CO_2.**

c. Death by euthanasia is confirmed by loss of heartbeat and/or no reaction when the area about the eye is touched.

d. After all animals have been euthanized, secure the cylinder and clean the container with Lysol, bleach, or similar disinfectant.

e. Check with the Study Director as to the proper dispose of animal carcasses: incineration, residue analysis, freezer storage, as per SOP AS-3.

3. Other Methods

Other methods for euthanasia may be used if required by the protocol. These methods must be described in the data package and must be considered humane.

H. **<u>Appendix D:</u> Curriculum Vitas for Personnel that will conduct robin bioavailability research (Richard Poche', Jeff Borchert, Jeff Mach, Karen March, John Baroch, Dr. Tracey Jensen)**

April 12, 2002

Richard M. Poché
47594 County Road 13
Wellington, Colorado 80549
970-897-2530

Richard M Poché
CP 4/12/02

EDUCATION

Ph. D. coursework, Wildlife Ecology, U. California, Berkeley
M.S., Wildlife Biology, Texas A&M University
B.A., Education, University of Southwestern Louisiana

Special Studies:

- Wildlife Management, Texas A&M University
- Toxicology, Government Institutes
- Business, Red Rocks Community College
- HPLC - Waters, Albany, NY ; Chicago, IL

Honors and Awards

- Outstanding Young Educator Nominee for State of Louisiana, 1969
- Lions Club Service Award, 1963
- Academic Scholarships to Louisiana State University and University of Southwestern Louisiana, 1965
- Special achievement Award, 1980, U.S. Fish & Wildlife Service
- Outstanding Publication Award, 1984, U.S. Fish & Wildlife Service

PRESENT ACTIVITIES

President of Genesis Laboratories, Inc., Wellington, Colorado. Oversees GLP field studies, laboratory and field product testing, pesticide hazard evaluations, EPA regulatory assistance, analytical services, avian toxicology, and miscellaneous wildlife studies.

PROFESSIONAL EXPERIENCE

Mr. Poché has experience in national parks and wildlife management, having spent four years conducting research in a West African National Park. He has completed studies on big game, small mammal, and bird inventories, census methods, habitat evaluations, management plans, and primate ecology.

Mr. Poché has extensive experience in the area of wildlife toxicology, analytical chemistry, and secondary hazard evaluations of pesticides. Laboratory and field evaluations in different environments were completed in countries such as the U.S., China, Indonesia, Burma, Egypt, Sudan, and Haiti. He has helped set up analytical and toxicology labs in several countries.

Mr. Poché has experience in vertebrate pest management, having established a new research center in Asia. His research has included rodent control, jackal ecology, bird management, and other species in

various parts of the world. In the U.S. he has worked with problems in the urban areas involving raccoons, tree squirrels, woodchucks, skunks (rabies), coyotes, and bats.

He has also performed EIS studies involving slurry pipeline, AC & DC transmission power lines, coal mines, and power plants. He conducted research on endangered species for the Utah Fish & Game, and published a monograph on a rare mammal found in a proposed coal producing area.

1989- Present: President of Genesis Laboratories, Inc.

Consulting laboratory in field of product development and testing: avian toxicology, terrestrial field studies, regulatory assistance, product evaluations, study monitoring, project management, expert testimony, analytical chemistry, and training.

1984-89: Director of Technical Services with Lipha Chemicals, Inc.

Directed research activities for the development of vertebrate pest control products used in the U.S. Activities included coordination of studies in laboratory and field toxicological, environmental fate, efficacy, primary and secondary hazards to wildlife, metabolism , chemistry, residues, and other effects on living organism. As project manager for all research activities, Mr. Poché contracted work with the U.S. Dept. Of the Interior, Washington State University, University of California, Virginia Polytechnic Institute, California Polytechnic Institute, U.S. Dept. Of Agriculture, Cornell University, Louisiana State University, Texas A&M University and private testing laboratories. Served as Quality Control Manager. Established and set up company's first quality control laboratory, including HPLC analysis and u.v. spectrophotometer use. Was trained and assisted in daily analysis of production samples using HPLC and u.v. analysis. Organized labs I New York and Milwaukee. Trained technicians in use of HPLC and other equipment. Involved in tissue residue analysis of several rodenticides in animal tissues. Set up with consulting firm in New York.

He also coordinated all regulatory activities with the EPA and state regulatory agencies. An experimental use permit was obtained to field test a new compound and a section 3 registration was applied for with the EPA. This required four years of coordination and planning.

Projects of major importance included secondary hazard studies using a rodenticide to determine a potential field effects on deer, raptors, coyotes, dogs, snakes, and European ferrets. Results of these projects prevented field use of certain compounds in the U. S.

1981-1984: Senior Scientist with Scimetrics International, Inc.

Owner of a natural resource management consulting firm. Served as Project Manager for integrated pest management, reforestation, field and laboratory pesticidal evaluations, wildlife studies, and vegetation damage prevention projects. Work was completed for the U.S. Forest Service in Idaho-deer prevention to seedlings and pocket gopher control; U.S. Bureau of Indian Affairs in New Mexico and Colorado-reforestation with conifers and deer prevention work; Zeneca-pesticide effects on the environment and wildlife in China, Sudan, Indonesia, and Burma; Velsicol, Inc.- field evaluation of a rodenticide and its potential impact on wildlife; Roncari Industries, Inc.- impact of using rodenticides to control field rats in rice fields in Haiti.

1978-1981: Wildlife Biologist with U.S. Fish & Wildlife Service

Organized and established a vertebrate pest control research project in Bangladesh. He selected staff, designed the laboratory, procured equipment, trained the staff, and managed the project. Mr. Poché organized and completed numerous field studies on animal damage control in agriculture, rat damage in wheat, rodent control in rice fields, laboratory toxicological studies, rodent ecology in deep water rice, jackal ecology and impact in agriculture, bird damage to crops, urban rat studies, simulated rodent damage to rice and wheat, and national survey of wildlife damage problems in agriculture.

Mr. Poché also completed short-term projects in Egypt, Pakistan, Burma, and India relating to vertebrate pest problems in agriculture. Field evaluation of current measures for ground squirrel control in the western U. S. was completed.

1969-72, 1976-77: Peace Corps Volunteer & Associate Director Niger, Africa

Responsible for organizing and executing all phases of national park development in Nigers Park W, including conservation projects and research. Manager of Park W during the 1971-72 season. Surveyed and supervised construction of over 300 km of auto routes through the park. Completed faunal surveys, inventories, census work, vegetation type mapping, and organized anti-poaching programs. As Associate Director of Agriculture & Rural Development, Mr. Poché supervised up to a 120 PC volunteers in agriculture, well construction, wildlife, forestry, fisheries, and pest control. He developed new programs in rodent control, range management, and beekeeping and organized the First West African Wildlife Conference and the First Arid Land Forestry Conference. Mr. Poché served as advisor to the U.S. Delegation to the U.N. Conference on Desertification held in Nairobi, Kenya (1977).

1973-74: Staff Ecologist with Stearns-Roger, Inc.

Project Manager for four environmental impact studies relating to the development of a coal mine, power plants, transmission lines, and coal slurry pipeline (combined budgets of $8 million). Responsible for coordination of aspects of the EIS, including ecology, archeology, geology, hydrology, air quality and meteorology, aquatics, reclamation, and socio-economic studies.

1968-69: Junior High School Teacher with St. Martin Parish, Louisiana

Taught 7th and 8th grade science, math, industrial arts, physical education and reading. Mr. Poché also served as football coach, 4-H Club leader, student counselor, and assistant principal.

MEMBERSHIP IN PROFESSIONAL ORGANIZATIONS

Society of Environmental Toxicology and Chemistry
Society of Quality Assurance, Rocky Mtn. Chapter
National Pest Control Association

TRAINING

Good Laboratory Practice training sessions (5)
Health & Safety (OSHA)
HPLC (Waters-twice)

PUBLICATIONS

Poché, R.M. 1973. Wildlife in urban woodland sites. In: Woodlands Community, by Wallace, McHarg, Roberts and Todd. pp. 54-65.

Poché, R.M. 1973. Niger's threatened Park W. Oryx. 12(2):216-222.

Poché, R.M. 1973. Development of a wildlife monitoring system on the proposed Woodlands city site, Montgomery County, Texas. Texas A&M University, MS thesis.

Poché, R.M. 1974. Notes on the roan antelope (Hippotragus equinus) (Desmarest) in West Africa. J. of Applied Ecology, 2 (3):963-968.

Poché, R.M. 1974. Seasonal distribution and reproduction in Artiodactyla from southwest Niger. 2nd East African Wildlife Symposium. Nairobi, Kenya.

Poché, R.M. And G.L. Baillie. 1974. Notes on the spotted bat (Euderma maculatum) from southwest Utah. Great Basin Naturalist, 34 (4):254-256.

Poché, R.M. 1974. Ecology of the African elephant (Loxodonta a. africana) in Niger. Mammalia, 38:567-580.

Poché, R.M. 1975. The bats of Niger's National Park W, Niger, Africa. Mammalia, 39 (1):39-50.

Poché, R.M. 1975. Notes on reproduction in ^Wfuniscirurs anerythrus^W from Niger. Journal of Mammalogy, 56 (4):700-701.

Poché, R.M. 1975. New record of the spotted bat (Euderma maculatum) from Arizona. Journal of Mammalogy, 56 (4):931-933.

Radovski, F.J. & R.M. Poché 1975. First report of the ectoparasite (Cryptonyssus desultorius, Acari: Mesostigmata: Macronyssidae) associated with the spotted bat. Journal of Medical Entomology, 12 (3):394.

Poché, R.M. 1975. New record of the Plecotus phyllotis from Utah. Great Basin Naturalist, 35 (4):452.

Poché, R.M. 1975. A preliminary census of wild ungulates in Parc National du W du Niger. Nigerian Field, 40 (2):78-88.

Poché, R.M. & G.A. Ruffner. 1975. Roosting behavior of male Euderma maculatum from Utah. Great Basin Naturalist, 35 (1):121-122.

Poché, R.M. & J. Keirans. 1975. First report of a tick, Ornithodoros rossi (Acarina: Argasidae), from the Spotted Bat, ^Weuderma maculatum (Chiroptera: Vespertilionidae). Journal of Medical Entomolgy, 125:503.

Poché, R.M. 1975. Ecology of the spotted bat (Euderma maculatum) from southwest Utah (Abstract). 55th Annual Meeting, American Society of Mammalogist. Missoula, Montana.

Poché, R.M. 1976. Seasonal distribution and reproduction in Artiodactyla from southwest Niger. Nigerian Field, 41 (1):31-40.

Poché, R.M. 1976. Notes on primates in Parc National du W du Niger, West Africa. Mammalia, 40 (2):187-198.

Poché, R.M. 1977. The role of small mammals in national park management. First West African Wildlife Conference. Niamey, Niger. June 20-24.

Poché, R.M. 1977. Study skin preparation. First West African Wildlife Conference. Niamey, Niger. June 20-24.

Poché, R.M. 1977. Comparative food selection in African ungulates. First West African Wildlife Conference. Niamey, Niger. June 20-24.

Poché, R.M. 1977. Elephant management in Africa. First West African Wildlife Conference. Niamey, Niger. June 20-24.

Poché, R.M. 1977. Sampling and monitoring changes in vegetation. First West African Wildlife Conference. Niamey, Niger. June 20-24.

Poché, R.M. 1977. Sources of funding for projects. First West African Wildlife Conference. Niamey, Niger. June 20-24.

Poché, R.M. 1977. The role of the Peace Corps Volunteer in Niger Forestry. Arid Land Forestry Conference. Niamey, Niger. August 15-17.

Poché, R.M. 1977. Financial assistance for forestry projects. Arid Land Forestry Conference. Niamey, Niger. August 15-17.

Ruffner, G.A., Poché, R.M., Meierkord, M., and J.A. Neal. 1979. Winter bat activity over a desert wash in Southwestern Utah. Southwest Naturalist, 24(3):447-453.

Poché, R.M. 1979. Notes on the big free-tailed bat (Tandaria macrotis^W) from Southwest Utah. Mammalia, 43(1):125-126.

Poché, R.M., Sultana, P., Mian, M.Y., and M.E. Hague. 1979. Studies with zinc phosphide on Bandicota bengalensis and Rattus rattus in Bangladesh. Bangladesh Journal of Zoology, 7(2):117-123.

Poché, R.M. 1980. Range extension of Rattus exulans in South Asia. Mammalia, 44(2):272.

Poché, R.M. 1980. Elephant management in Africa. Wildlife Society Bulletin, 8(3):199-207.

Poché, R.M., Hague, M.E. and M.K. Karim. 1980. Bird damage control in sprouting wheat. Bangladesh Journal of Agriculture Research. 5(2):41-46.

Poché, R.M. 1980. Rats. ADAB News. 6:20-21.

Poché, R.M. 1980. Rats in deepwater rice. ADAB News. 8:19-22.

Poché, R.M. and V.Leffler. 1980. Preliminary analysis of rodent problems in Dacca and Comilla. ADAB News. 7:20-22.

Poché, R.M. 1981. Ecology of the spotted bat (Euderma maculatum) in Utah. Utah Division of Wildlife Resources Monograph. 63 pp.

Poché, R.M., Hague, M.E., Mian, M.Y., Sultana, P., and M.A. Karim. 1981. Yield reduction in Bangladesh wheat by simulated rat damage. ^Wjour. Of Tropical Pest Management ^W, 28 (2):242-246.

Poché, R.M., Hague, M.E., Mian, M.Y., and Sultana. P. 1982. Rodent damage and burrowing characteristics in Bangladesh wheat fields. Journal of Wildlife Management, 46(1):139-147.

Poché, R.M. and P. Sultana. Food intake in three Asian Muridae. Mammalia, 46(3):403-405.

Poché, R.M. 1982. Nesokia indica from Bangladesh. Mammalia 46:547-549.

Poché, R.M. and M.Y. Mian. 1986. Effectiveness of four rodenticides in deepwater rice. Zeitschrift Ang. Zool. pp 37-48.

Poché, R.M. and M.Y. Mian. 1985. Comparison of two methods for monitoring population indices of small mammals on seasonal islands in Bangladesh. Acta Theologica. 30(8):161-165.

Poché, R.M., Evans, S.J., Sultana, P., Hague, M.E., Sterner, R., and M.A. Siddique. 1987. Notes on the golden jackal (Canis aureus) in Bangladesh. Mammalia. 51(2):259-270.

Poché, R.M., Sultana, P., Hague, M.E., and M.Y. Mian. Rodent population dynamics in Bangladesh warehouses. Acta in press.

Poché, R.M., Sultana, P., Mian, M.Y., and M.E. Hague. Growth and development in Bandicota indica. Journal of Zoology in press.

Poché, R.M., Sultana, P., and M. Siddique. Rodent damage to rice in exclosures. Mammalia, in press.

Sultana, P., Brooks, J.E. and R.M. Poché. Methods for assessing rat damage to growing wheat in Bangladesh with examples of applications. ASTM, 817:213-238.

Poché, R.M. and R.D. Sharp. 1985. Vole control in the Eastern United States. Second Eastern Wildlife Damage Conference. Raleigh, North Carolina. pp. 138-144.

Baroch, J. and R.M. Poché. 1986. Preliminary field evaluation of a new formulation of Rozol (Chlorophacinone) bait against pocket gophers in Colorado. Seventh Great Plains Wildlife Damage Control Workshop. San Antonio, Texas. pp. 138-144.

Poché, R.M. 1986. Status of Bromadiolone in the United States. Proceedings of the 12th Vertebrate Pest Conference. San Diego, CA. pp. 6-15.

Poché, R.M. 1988. Rodent tissue residue in secondary hazard studies with Bromadiolone. EPPO Bulletin, 18:323-330.

Lechevin, J.C. and R.M. Poché. 1988. Activity of LM-2219 (Difethialone), a new anticoagulant rodenticide, in commercial rodents. Proceedings of 13th Vertebrate Pest Conference. Monterey, CA. pp. 59-63.

Fisher, D.D., Timm, R., Poché, R.M. and S.E. Hyngstrom. 1991. Laboratory syudy on Bromadiolone: effectiveness on prairie dogs and secondary hazards to domestic ferrets. 10th Great Plains Wildlife Damage Control Workshop Proceedings. April 15-18. Lincoln, Nebraska. pp. 70-72.

Hazen, Brent and R.M. Poche. 1992. California ground squrrel field efficacy study using 0.005% chlorophacinone bait. Proc. 15%h Vert. Pest Conf. U.C. Davis. Pp 322-325.

Poché, R.M. 1992. How GLP provisions influence costs of rodenticide field evaluations. 15th Vertebrate Pest Conference. March 3-5, 1992. Newport Beach, CA. pp. 245-248.

Poché, R.M. and P. Furdyna. 1992. A sensitive and specific method for the analysis of p-dichlorobenzene, ziram, and thiram in the animal repellent formulation.

Poché, R.M., D. Fischer, and P. Toll. 1998. Use of radio telemetry to monitor survival of songbirds exposed to the insecticide on golf courses. In L. Brewer & K. Fagerstone (Eds.) Radiotelemetry applications for wildlife toxicology field studies; pp. 85-92. 1993 January 5-8; Pacific Grove. CA Pensacola, FL Society of Environmental Toxicology and Chemistry (SETAC). 224 p.

Askham, L.R. and R.M. Poché. 1992. Biodeterioration of Chlorophacinone in voles, hawks, and an owl. Mammalia, 56:145-150.

Poche, R.M. 1997. Mole cenus techniques. 13th Great Plains Wildlife Damage Control Workshop Proceedings. Nebraska City, NE. Pp. 114-116.

Poche, R.M. 1998. Recent studies with Norway rats. Proc. 18th Vertebr. Pest Conf. (R.O. Baker & A.C. Crabb, Eds.). Univ. of Ca., Davis. pp: 254-261..

Poche, R.M. 1998. Development of a new bird repellent: Flight Control. Proc. 18th Vertebr. Pest Conf. (R.O. Baker & A.C. Crabb, Eds.). Univ. of Ca., Davis. pp: 338-344.

Reichert, P., Devers, P. and R. Poche. 1998. Field test with AQ-9,10 to control Canada geese in Colorado. Proc. 18th Vertebr. Pest Conf. (R.O. Baker & A.C. Crabb, Eds.). Univ. of Ca.,

Davis. pp: 345-349.

Poche, R.M. and J. Mach. 2000. Wildlife primary and secondary toxicity studies with warfarin. Pp 181-196, in J. Johnston Pesticides and Wildlife, ACS Series 771.

Poche, R.M. 2000. Current effective rodent control methods. 55th Annual international Northwestern Conference on Diseases of Nature Communicable to Man.

Poché, R.M. 2002. Recent studies with moles. Vertebrate Pest Conference. Reno, Nevada.

Poche, R.M. 2002. Studies with a new bird repellent. Vertebrate Pest Conference. Reno, Nevada.

RECENT CONFERENCES ATTENDED AND PARTICIPATED IN

1990. Vertebrate Pest Conference. March. Sacramento, CA. SETAC. November. Alexandria, VA. Eastern Wildlife Damage Control Conference. Madison, WI.

1991. Great Plains Wildlife Damage Conference. April. Lincoln, NE. SETAC. Seattle, WA.

1992, 94, 96. 15th, 16th, & 17th Vertebrate Pest Conferences. California..

1993. SETAC avian radio telemetry workshop. Monterey, CA. Society of Toxicology. New Orleans, LA.

1995. Rodent control practices in the U.S. Society of Vector Control Conference. Ft. Collins. Colorado. Update on field rodent control. WRCC Conference. Reno, Nevada.

1996. Great Plains Wildlife Damage Conference. April. Nebraska City, NE.

1997. The Wildlife Society 4th Annual Conference. Snowmass Village, Colorado. Sept. 21-27. Poster: Wildlife Secondary Toxicity Studies Using Warfarin.

1990-02 Society for Environmental Toxicology and Chemistry. Annual Conferences.

1998. 18th Vertebrate Pest Conference. Costa Mesa, Ca. March 2-5.

2000. 55th Annual International Northwest Conference on Diseases of Nature Communicable to man. Fort Collins, Co.

2000. Vertebrate Pest Conference. San Diego, Ca.

2002. Vertebrate Pest Conference. Reno, Nevada.

GENESIS LABORATORIES, INC.

I. EDUCATION

Colorado State University
Ft. Collins, CO Fall 1992-Fall 1994 Bachelor of Animal Science
Science Concentration with Nutrition interest

Los Angeles Pierce College
Woodland Hills, CA Fall 1988-Spring 1992 Associate of Liberal Arts
Pre-Veterinary course work

Graduate Classes: Ecotoxicology, Epidemiology, Statistics

II. TRAINING

High Performance Liquid Chromatography: 2 day training seminar by Waters Corporation, October 1996
Good Laboratory Practice Standards Training: 1day training seminars: March 1997, January 1998, February 2000
Good Laboratory Practices: ½ day training seminar by QA Associates, September 1998
GLP Quality Assurance Unit Responsibilities: ½ day seminar by QA Associates, September 1998
Quality Assurance: The Bigger Picture: 2 day meeting covering GMP, GCP and GLP issues under FIFIRA and FFDCA, January 1999
Society of Quality Assurance: Annual, national 3-day Meeting covering GMP, GCP and GLP issues under FIFIRA and FFDCA, October 1999.
Greater Insights to the GLPs and 21 CFR Part 11: RMSQA sponsored meeting, August 2000.
Safety Seminar: 1 day seminars by NSP & Safety Service, 2/97, 2/98
At Genesis Laboratories: Training sessions covering the following. HPLC, GC, quail husbandry, Egg fumigation, thickness, care, washing and accounting, cannon and mist netting, small mammal trapping, mechanical polar planimeter, avian necropsy.
Adult CPR: October 1998

III. JOB EXPERIENCE

Prior to Genesis Laboratories Inc.

Research Assistant: Agripro Seeds, Berthoud, CO, July 1996-September 1996
Harvested wheat research plots in North Dakota and Colorado, thrashed and prepared thousands of samples for laboratory analysis. Prepared and planted greenhouses for winter generation.

Research Assistant: Metabolic Research Laboratory, Animal Science Department Colorado State University,

July 1994-November 1996. Assisted in the completion of many industry sponsored pesticide, herbicide and antibiotic residue studies involving beef and dairy cattle, sheep, goats, swine and poultry under GLP guidelines. Many studies required the use of radioactively-tagged test articles. Completed two independent research projects. The first tested the rumen degradability of protein supplements on steers and the second evaluated the nutritional status of the Pale-faced Saki monkey population at the Denver Zoo.

Veterinary Technician: McClave Veterinary Hospital, CA and Raintree Animal Hospital, CO. Five years experience. Sterile and aseptic surgical assistance, hematology, parasitology and urine analysis.

At Genesis Laboratories, Inc.

Study Director: Mammalian Toxicology, Epidemiology and Research and Development: January 2000-Present
Oversee the implementation of mammalian studies in the field and laboratory under FIFIRA, OECD and FDA guidelines. Direct the research and development of new products and fields of work as well as the identification of new clientele. Managed bid proposals, allocation of resources and management of staff. As study director, performed avian and mammalian field and laboratory studies. Acting as a Quality Assurance Officer on an as needed basis. Also, implement responsibilities as Alternate Archivist.

Quality Assurance Unit Manager: December 1997-January 2000
Oversee the activities of the QA unit and maintain our laboratory compliance with GLP guidelines under FIFRA and OECD. Responsible for GLP training, maintenance of SOPs, scheduling and assuring adequate QA personnel on studies and assure management that laboratory facilities, equipment, methods, practices and records are in conformance with 40 CFR 160. Acting as Quality Assurance Officer responsibilities include protocol review, study-event inspections, data audits and report reviews.

Institutional Animal Care and Use Committee Chairperson: March 1998-February 2000
Assure our laboratory activities are in compliance with the Animal Welfare Act. Train and advise staff on AWA requirements and animal care techniques and methods. Conduct bi-annual committee meetings and facility audits.

Laboratory Technician: September 1996-December 1997
Various wet and dry chemical techniques on feeds and tissues for Good Laboratory Practice (GLP) studies under FIFRA guidelines. Includes method development and method validation on herbicides, insecticides, fungicides and rodenticides. Projects include evaluation of purity and strength for active ingredients and formulated end products, chemical analysis of biochemical pesticides and residue analysis in tissue and plants.

IV. PROFESSIONAL AND HONORARY SOCIETY MEMBERSHIPS AND AWARDS

Rocky Mountain Regional Chapter of the Society of Quality Assurance (RMSQA), since November 1996

1999 recipient of the Clay Mitchell Scholarship from RMSQA for attendance to the National Society of Quality Assurance Meeting in October 1999

Employee Signature

Initials

2/28/01
Date

CURRICULUM VITAE **JEFF J. MACH** **March 12, 2002**

GENESIS LABORATORIES, INC.

I. EDUCATION

B.S. 1993 - University of Nebraska-Lincoln. **Major:** Forestry, Fisheries, and Wildlife Management. One semester at University of South Australia-Adelaide in the Conservation and Park Management program, with extensive work in field biology (8 weeks of 1 semester) and habitat assessment.

M.S. 1998 - University of Nebraska-Lincoln. **Major:** Natural Resources Sciences. **Thesis:** Warfarin: A Forgotten Rodenticide, Primary and Secondary Effects of a Warfarin Bait for Black-tailed Prairie Dogs

II. TRAINING

Laboratory

Contract Laboratory Inspection. Society of Quality Assurance, 9/21/95.

Good Laboratory Practices Standards. Diane Brodway & John Gillis, EPA, 1/6/95.

Good Laboratory Practices Standards. Dean Hill, Quality Associates Inc., 1/13/98, 9/10/98, 2/18/00.

Quality Assurance. Paula Reichert, Genesis Laboratories, Inc., 10/25/96.

Avian

Snow geese blood sampling techniques and procedures. Pricilla Dressen, DVM, Colorado State University, 2/6/96.

Oral gavage of N. bobwhite, Md. Sayed Ahmed, Genesis Laboratories, Inc., 8/14/95.

Necropsy and tissue retention of snow geese. Terry Spraker, DVM, Colorado State University, 2/21,23/96.

Field radio telemetry (TS1000 receiver) and trapping of pocket gophers. Richard M. Poché, Genesis Laboratories, Inc., 5/10-12/91.

Fumigating N. bobwhite and avian equipment. Chris Gates, Genesis Laboratories, Inc., 1/27/98.

Mallard egg collection procedures. Chris Gates, Genesis Laboratories, Inc., 11/6/98.

N. bobwhite egg collection and storage. Bree A. Medlicott, Genesis Laboratories, Inc., 9/18/98.

N. bobwhite blood sampling techniques. Gregg Howald, Genesis Laboratories, Inc., 2/3/99.

N. bobwhite husbandry. Bree A. Medlicott, Genesis Laboratories, Inc., 3/20/98.

Mammalian

Humane handling and laboratory techniques for the mouse hamster, rabbit, cat and dog; sexing, collect blood, administer injections, handling and restraint. Dale L. Brooks, DVM, PhD; Sharon E. Jahn, BS, AHT; Karen I. Timm, DVM, PhD; and William G. Prote, MBA, DVM. University of California, Davis, 7/21/95.

Operation of bait dispensing probe. Brent Hazen, Wilco Distributors, Inc., 11/3/94.

Small mammal necropsy. Dave Close, DVM., Colorado State University, 3/13/95.

Burrow census and carrot bait census. John Baroch, Genesis Laboratories, Inc., 10/19,21/94.

Visual censusing. John Baroch, Genesis Laboratories, Inc., 5/13/94.

Hantavirus and its prevention. Center for Disease Control video, 5/3/95.

Live-trapping techniques. Richard M. Poché, Genesis Laboratories, Inc. 3/00.

Mammalian blood sampling techniques. Gregg Howald, Jeff Borchert, Genesis Laboratories, Inc., 3/99.

Ocular stick. Richard M. Poché, Genesis Laboratories, Inc. 9/12/00.

Ferret blood draw and intravenous placement. Lora Swoape, Wellington Veterinary Clinic. 9/20/00.

Trapping techniques for the federally threatened species Preble's meadow jumping mouse (*Zapus hudsonius preblei*). Robb Schorr 6/01.

Passive Integrated Transponder (PIT) tag placement in the federally threatened species Preble's meadow jumping mouse (*Zapus hudsonius preblei*). Robb Schorr 6/01.

Aerial survey of marsh vegetation for nutria herbivory (vegetative damage) with GPS. Greg Linscombe, Louisiana Department of Wildlife and Fisheries 3/02.

Chemistry

Lab Safety, Use of balances, Use and cleaning of glassware, and pipeting. Steve Baugh, Genesis Laboratories, Inc., 1/24/96.

Analytical balance. Debbie Whaley, Genesis Laboratories, Inc., 8/18/95.

Sonicator, centrifuge, cyclone mill. Debbie Whaley, Genesis Laboratories, Inc., 9/21/96.

Toploading balance. John Baroch, Genesis Laboratories, Inc., 10/17/94.

High Performance Liquid Chromatography. Jeff Borchert, Genesis Laboratories, Inc., 2/99.

HPLC Tissue analysis for rodenticides (chlorophacinone, diphacinone, warfarin). Genesis Laboratories, Inc., 6/94, 6/95, 12/96.

Gas Chromatograph/Mass Spectrometry. Valerie L. Fuhrman, Genesis Laboratories, Inc., 4/99.

Safety

Radiation Protection. Janet Johnson, Shepard Miller, 4/10/96

Safe driving; Back safety; Lifting; Injury prevention; Stretching; Excercises; Fire extinguishers; Fire safety; Evacuation; Employee access to medical and exposure records; Respirator fitting, use, and testing. Joe Harrelson, NSP & Safety Services, 1/26/96, 4/28/98, 7/14/00.

Adult CPR. Centennial, 10/28/98.

III. JOB EXPERIENCE

Field Technician, University of Nebraska-Lincoln, White-tailed deer telemetry study, DeSoto National Park, Nebraska. 1991.

Field Technician, Genesis Laboratories, Inc., Field efficacy of strychnine bait. Responsibilities included: telemetry, hazard searches, and population censusing. 1991.

Field Technician, Genesis Laboratories, Inc., Field efficacy of turf insecticide and non-target hazard to wildlife. Responsibilities included: telemetry, mist netting of birds, hazard searches, and population censusing. 1992.

Laboratory/Field Crew Leader. Genesis Laboratories, Inc. Responsibilities included: LD_{50}, LC_{50}, reproduction, field efficacy of rodenticide baits and non-target hazard, commensal bait efficacy. 1994-1996.

Study Director – field and laboratory studies (GLP and non-GLP), Genesis Laboratories, Inc. Responsibilities include: APHIS - Animal Welfare Guidelines, FIFRA product registration, product performance, secondary hazard, quality control, ecotoxicology, repellents, palatability testing, bait optimization, pharmacokinetic effects of bait, trap construction, habitat modification, analytical chemistry, human resources, regulatory for pesticides and biopesticides. 1996-current.

Ecotoxicology Director – responsible for the management of avian, mammalian, and field studies (e.g. recommend study director to management for various studies; coordinate study scheduling, reports, meetings, conflict management, performance evaluations). Maintain Study Director positions as well. 2001- current

IV. PUBLICATIONS AND/OR PRESENTATIONS

Mach, J. J., S. R. Mortensen, J. L. Mattsson, and L. G. McFadden. In Press. Avian Reproduction Study: Effect of Brooder Density on Body Weight. *Environmental Toxicology and Chemistry*. **:**-**. Conference Poster Presentation: November 12, 2001.

Mach, J. J. Warfarin: A forgotten rodenticide: primary and secondary effects of a warfarin bait for black-tailed prairie dogs. *Thesis*. University of Nebraska-Lincoln, August, 1998.

Mach, J. J. 1998. Laboratory efficacy study with a warfarin bait to control the black-tailed prairie dog. *In* (Baker and Crabb eds.) Proceedings of the Vertebrate Pest Conference 18:184-190. Topic also presented at conference.

Mach, J. J. and E. Rodriguez. Repellent efficacy of anthraquinone 9,10 on the red-winged blackbird. *Presented at:* Control of rufous-capped blackbirds in rice fields on the plains of Mercosul. August 19-21, 1997. Pelotas, Brazil.

Mach, J. J., and S. E. Hygnstrom, R. M. Poché. 2002. Laboratory efficacy study with six concentrations of warfarin bait for controlling black-tailed prairie dogs. *International Biodeterioration and Biodegradation* 49:157-162.

Mach, J. J., and S. E. Hygnstrom, R. M. Poché. *In Press*. Secondary hazards of warfain-fed black-tailed prairie dogs (*Cynomys ludovicianus*) to domestic ferrets (*Mustela putorius furo*). Submitted for publication – *Mammalia*.

Poché, R. M. and J. J. Mach. 2001. Wildlife primary and secondary toxicity studies with warfarin. Pages 181-196. *In* (Johnston ed.) Pesticides and Wildlife. American Chemical Society, Washington, D.C. 406 pp. Topic also presented at conference.

Mach, J. J. In press. Nutria (*Myocaster coypus*) control in Louisiana. *In* (Schmidt, Salmon, and Marsh eds.) Proceedings of the Vertebrate Pest Conference 20:***-*** Topic also presented at conference.

GENESIS LABORATORIES, INC.

I. EDUCATION

University of Missouri, Columbia Missouri — September, 1979-May 1983
Bachelor of Science, Animal Science

Meramac Community College, St. Louis, Missouri — September 1977-August 1979
General studies

II. TRAINING

Assistant Laboratory Animal Technician-AALAS – October 1987
Introduction to GLP's – June 1991
Laboratory Animal Technician-AALAS – June 1991
Electrical Safety – October 1991
Emergency Response – December 1991
Glassware and Cylinder Handling – December 1991
Radiation Safety – December 1991; May 1994
GLP Training – March 1997; January 1998; September 1998; February 2000
QA Training, FIFRA/TSCA merger and pharmaceutical product registration – August 1998
Adult CPR – October 1998
Respiratory Protection Training – February 1999; February 2000
High Performance Liquid Chromatography Training – March/April 1999
GC/MS Training – May 1999
QA Training, Electronic Data – 1999
Fire Safety Equipment – November 1991; February 2000
Laboratory Safety – 2000
GLPs and 21 CFR Part 11, by RMRCSQA – August 2000
21 CFR Part 11 Training via Internet – May 2001
GLP Training and Facilitator, RMRCSQA – February 2002.

III. JOB EXPERIENCE

Genesis Laboratories, Inc.

Quality Assurance Unit Manager: January 2000 to present. Manage the QAU, maintain the SOP's, assure management that studies, records and equipment are in conformance with the Code of Federal Regulations. Oversee training personnel and adherence of GLP's for FIFRA and OECD. Continue as a Quality Assurance Officer, auditing protocols, critical study phases, raw data, and reports on studies conducted by Genesis Laboratories, Inc. Support Genesis Midwest by writing SOPs, training, and consulting.

Conducted QLP training seminars for outside clients. Facilitator for the Rocky Mountain Region Chapter of SQA training session.

Quality Assurance Officer: January 1998 to December 1999. Quality Assurance Officer on field studies, avian reproduction studies, rodent studies and product chemistry studies. Responsible for auditing all phases of studies, including protocols, study progress, and reports to ensure the integrity of the study.

Study Director: February 1997 to December 1999. Study Director for secondary-hazard studies with birds and mammals, QC and LD50 studies with rodents, and field deer mouse study. Conducted avian reproduction studies.

Institutional Animal Care and Use Committee (IACUC) chairperson: September 2001 to present. Receive and route all protocols concerning animals covered under the Animal Welfare Act. Notify study directors of the status of their studies. Conduct biannual meeting of the IACUC committee.

Institutional Animal Care and Use Committee (IACUC) member: February 1998 to September 2001. Review all protocols concerning animals covered under the Animal Welfare Act. Inspect the animal facilities and ensue that the program for humane care and use of animals is in place and observed.

Archivist: December 1999 to present. Ensure that reports, Quality Assurance, SOP's, and logbooks are properly archived. Revised the archiving system to assure compliance with the GLPs.

ABC Laboratories, Inc.

Scientist: October 1986 to July 1996. Directed studies for regulatory submission. These studies included metabolism and residue studies with domestic animals (hen, goat, dairy and beef cattle) and toxicology (avian, aquatic, and nontarget insects). Wrote protocols for metabolsim, residue, and avian and nontarget insect studies. Setup and conducted the first avian toxicology studies and chronic honeybee studies. Directed a five-member team.

Peace Corps, Costa Rica

Extension Specialist: August 1984 to August 1986. Worked in conjunction with the National Bank of Costa Rica. Introduced and taught farming strategies to dairy farmers in the region. Worked with a woman's group setting up an egg producing operation to increase the family's income.

University of Missouri, Columbia

Dairy Field Hand: 1982 to 1983. Responsible for the daily care, vaccinations, health and records of 100 head of calves. Assisted with herd health and milking. Gave farm talks and informational talks to elementary students.

IV. PROFESSIONAL MEMBERSHIP

Rocky Mountain Regional Chapter of Quality Assurance
International Society of Quality Assurance

V. OTHER ACTIVITIES

Poster at CAS meeting on Chronic Honeybee, August 1996.
Attended the National Society of Quality Assurance meeting, October 1999.
Poster Chairperson for the RMRCSQA, 2000-2002.
Attended the National Society of Quality Assurance meeting, October 2001.

Karen L. March — Initials — 25 Feb 02 Date

2/28/02

GENESIS LABORATORIES, INC.

I. EDUCATION

Fort Lewis College, Durango, Colorado 1972-1977 B.S.
Biology: Natural History Concentration.

Colorado State University, Fort Collins, Colorado 1982-1985
Graduate School in Zoology:
Agricultural Pesticides, Vertebrate Pest Management, Population Dynamics, Genetics, Statistics, Computer Science.

II. TRAINING

EPA Licensed Restricted Use Pesticide Applicator.
California Certified Restricted Use Pesticide Applicator
GLP Regulations for Vertebrate Pesticide Registration. Denver Wildlife Research Center, August, 1993.
Avian Necropsy Techniques. Paul Ragone, D.V.M. August, 1993.
Field Studies Workshop. Society of Quality Assurance. Fresno, CA. March, 1994.
GLP's: A Common Sense Approach. Diane Bradway, Chief, USEPA Compliance Section. March, 1994.
Basic First Aid and Adult C.P.R. Training, August, 1994.
How to Supervise People Seminar. 0.6 Continuing Education Credits, January, 1995.
Small Mammal Necropsy. Dave Close, D.V.M. March, 1995.
Preventing Hantavirus Disease (Video). Centers for Disease Control.
Animal Care Laboratory Techniques (Video). University of California, Davis., June, 1997.
Contract Laboratory Inspections. Rocky Mountain Chapter, Society of Quality Assurance, September, 1995.
Managing Multiple Projects Seminar. 0.6 Continuing Education Credits, October, 1995.
Gel Permeation Chromatography for Tissue Residue Analysis. New York State Department of Hazardous Waste, November, 1995.
Avian Blood Sampling Techniques. Colorado State University Veterinary Teaching Hospital, Pricilla Dressen, D.V.M., Instructor. February, 1996.
Avian Necropsy and Tissue Retention. Colorado State University Veterinary Teaching Hospital, Terry Spraker, D.V.M., Instructor. February, 1996.
Radiation Safety Short Course. Shepherd Miller Associates, April, 1996.
Rodent Diseases. National Wildlife Research Center, July, 1996.
Cannon Net Operation. Colorado Division of Wildlife, November, 1996.
Basic GLP's Workshop. McCann Associates, Inc. John McCann, Instructor. March, 1997.
GLP Standards. Quality Associates, Inc. Dean Hill, Instructor. January, 1998.
HPLC Methods Short Course. Genesis Laboratories, Inc. February, 1998.

III. JOB EXPERIENCE

A. Rodent Control Biologist Peace Corps/Niger Department of Plant Protection. Niger, West Africa. 1977-1979

Rodent Pest Ecology Research
Rodenticide Field Trials
Extension Agent Training

B. Field Trial Manager Great Plains Seeds and Research, Inc. Bozeman, MT. 1985-1987

Wheat Seed Research and Development

C. Vertebrate Pest Control Services Wildlife Pest Management Bozeman, MT 1985-1989

Rodenticide Product Development and Evaluation
Training Programs for Pest Control Operators
Commercial Agricultural Vertebrate Pest Control

D. Field Ecologist Genesis Laboratories, Inc. Richmond, WI 1990-1993

Field Efficacy of Rodenticides
Field Monitoring of Avian Toxicity of Insecticides - Radio Telemetry, Carcass Searches
GLP Compliance (EPA)

E. Senior Scientist/Study Director Genesis Laboratories, Inc. Fort Collins, CO 1993-Present

Design, Conduct, Report Pesticide Assessment Studies:
Avian and Mammalian Laboratory Toxicology - LD50, LC50, Avian Reproduction, Avian and Mammalian Secondary Toxicity
Laboratory and Field Efficacy of Rodenticides
Crop Residues
Write SOP's
GLP Compliance (EPA)
Chair, Genesis Laboratories' Institutional Animal Care and Use Committee

IV. PROFESSIONAL AFFILIATIONS

Chairman: USDA Western Coordinating Committee –95: Vertebrate Pests of Forests, Agriculture and Public Lands. Past Secretary (2000) and Vice-Chairman (2001). Member 1995-present.

Southwest Regional Director: National Animal Damage Control Association 2000-present

Member: The Wildlife Society: 1995 - Present.
Wildlife Society, Wildlife Toxicology Working Group
Wildlife Society, Wildlife Damage Management Working Group

Member: Rocky Mountain Chapter, Society of Quality Assurance 1993 - 1999.

V. PUBLICATIONS, PRESENTATIONS

PUBLICATIONS

Baroch, J. A. 1998. An overview of recent ground squirrel bait registration research supported by the California Bait Surcharge Program. *In* Proc. 18th Vertebr. Pest Conf. (A. C. Crabb, ed.) Univ. of California, Davis. (*In press*). Platform presentation at conference.

Baroch, J. A. 1996. Field efficacy of diphacinone grain baits used to control the California ground squirrel. *In* Proc. 17th Vertebr. Pest Conf. (A. C. Crabb, ed.) Univ. of California, Davis. Platform presentation at conference.

Baroch, J. A., and R. M. Poche. **1985.** Preliminary field evaluation of a new formulation of Rozol (Chlorophacinone) bait against pocket gophers in Colorado. *In* Seventh Great Plains Wildlife Damage Control Workshop Proceedings, San Antonio, TX, pp. 138-144.

PROFESSIONAL AND ACADEMIC RESEARCH REPORTS:

Baroch, J. A. , and M. Hafner. 2002. Chapter 1. Biology and Natural History of the Nutria, with Special Reference to Nutria in Louisiana. Pp. 3-89. *In* Nutria in Louisiana. Genesis Laboratories, Inc. A report prepared under contract for the Louisiana Department of Wildlife and Fisheries. 160 pp.

Baroch, J. A. 1989. The biology and control of ground squirrel pests. Chempar, in-house report. 108 pp.

Baroch, J. A. 1985. An ecogenetic positive feedback model of animal population irruptions. A two year laboratory study of *Drosophila* population genetics. Masters Thesis, Colorado State University, Ft. Collins, CO. 68 pp.

Baroch, J. A. and D. Lewis. 1979. Rodent Pest Control in Niger. A summary of two years of field research into the biology and control of agricultural rodent pests in Niger, West Africa, with recommendations for improved extension programs. 77 pp. (*French*).

POSTER PRESENTATION

Secondary Hazard Study Using Chlorophacinone-Killed Laboratory Rats Fed to Black-billed Magpies (*Pica pica*) **J. A. Baroch.** Presented at Wildlife Society Conference, Snowmass, CO. Sept., 1997.

Signature _____ Initials _____ Date _____

Richard M. Poché _____ Date _____

ISJ 8/15/01

Tracey Smith-Jensen, D.V.M.

9133 N. County Rd. 5
Wellington, CO 80549
Home: (970) 568-0402
Business: (303) 315-8795

Objective

To practice in a progressive small animal hospital that focuses on a teamwork approach to provide client oriented, high quality medicine.

Education

May 1996 — Doctor of Veterinary Medicine
Colorado State University, Ft. Collins, Colorado

May 1988 — Bachelor of Science - Biology
Mesa State College, Grand Junction, Colorado

Clinical Experience

1/97-5/98 — Associate Veterinarian. Mesa Veterinary Hospital, PC Golden, CO. AAHA certified, full service small hospital. Responsible for evening and weekend scheduled appointments and emergency calls.

Research Experience

5/94- present — NIH Postdoctoral Research Fellowship. University of Colorado Health Sciences Center. Department of Neurology. Laboratory of Dr. Donald Gilden. Construction of cDNA libraries and development of nucleotide probing strategies to identify and characterized the IgG response present in the cDNA libraries constructed from brain tissue of Multiple Sclerosis and subacute sclerosing panencephalitis patients.

8/93-5/94 — Research Associate (part-time). Colorado State University, Fort Collins, CO. Department of Pathology. Laboratory of Dr. Gerald Callahan. Technical support for the cloning and expression of MHC class II genes in canine osteosarcoma cell lines.

8/91-8/92 — Research Assistant (full time). National Jewish Medical and Research Center, Denver, CO. Department of Pediatrics, Division of Basic Sciences. Laboratory of Dr. John Cambier. Responsibilities included expansion of transgenic mouse colony and cloning of IgM receptor mutants for use in signal transduction studies.

Publications

Owens, G.P., Kraus, H.K., Burgoon, M.P., Smith, T.D., Devlin, M.E., Gilden, D.H. Restricted Use of VH4 Germline Segments in an Acute Multiple Sclerosis Brain. Annals of Neurology. 1998;43:236-243

References:

American Society for Testing and Materials (ASTM). 1998. E 1676-97, Standard guide for conducting laboratory soil toxicity or bioaccumulation tests with the lumbricid earthworm *Eisenia fetida*.

AVMA, 2000. Report of the AVMA Panel on Euthanasia. JAVMA 218(5): 669-696.

Edwards, C.A. 1984. Report of the second stage in development of a standardized laboratory method for assessing the toxicity of chemical substances to earthworms, Report EUR 9360 EN, Commission of the European Communities, Brussels, Belgium.

Edwards, C.A., and P.J. Bohlen. 1992. The effects of toxic chemicals on earthworms. Rev. Environ. Contam. Toxicol. 125:23-99.

Fitzpatrick, L.C., J.F. Muratti-Ortiz, B.J. Venables, and A.J. Goven. 1996. Comparative toxicity in earthworms *Eisenia fetida* and *Lumbricus* terrestris exposed to cadmium nitrate using artificial soil and filter paper protocols. Bull. Environ. Contam. Toxicol. 57:63-68.

Gaunt, A.S., L.W. Oring. 1999. Guidelines to the Use of Wild Birds in Research. The Ornithological Council Special Publication. Washington, D.C. 57 pgs.

Goats, G.C., and C.A. Edwards. 1988. The protection of field toxicity of chemicals to earthworm by laboratory methods. Earthworms in Waste and Environmental Management, C.A. (Edwards and E.F. Neuhauser, eds.), SPB Academic Publishing, The Hague, The Netherlands, pp. 283-294.

Hartenstein, R., E.F. Neuhauser, and D.L. Kaplan. 1979. Reproductive potential of the earthworm *Esenia fetida*, *Oecologia (Berl.)* 43:329-340.

Hartenstein, R., E.F. Neuhauser, and A. Narahara. 1981. Effects of heavy metal and other elemental additives to activated sludge on growth of *Eisenia fetida*. Journal of Environmental Quality 10:372-376.

Haque, A., and W. Ebing. 1983. Toxicity determination of pesticides to earthworms in the soil substrate. Journal of Plant Disease and Protection 90:395-408.

Marquenie, J.M., and J.W. Simmers. 1988. A method to assess potential bioavailability of contaminants, *SPB Academic Publishing*, pp. 367-375.

National Research Council. 1996. Guide for the Care and Use of Laboratory Animals. National Academy Press. Washington D.C.

Neuhauser, E.F., R. Hartenstein, and D.L. Kaplan. 1980. Growth of the earthworm *Eisenia fetida* in relation to population density and food rationing. Oikos 15:93-98.

Sallabanks, R. and F.C. James. 1999. American robin (Turdus migratorius). In Poole, A. and F. Gill (eds.). The Birds of North America, #462, Philadelphia.

Stafford, E.A., S.P. McGrath, V. Cosimini, M. Fearnhead. 1987. Earthworms as indicators of heavy metal bioavailability, Report of Rothamsted Experimental Station for 1986, Part 1. Harpenden, Herts, United Kingdom, p. 93.

U.S. Environmental Protection Agency. 1993. Wildlife exposure factors handbook; Volume I & II. EPA 600-R-93-187.

U.S. Environmental Protection Agency. 2000. Ecological soil screening level guidance. Washington, D.C., July 10, 2000.

Exponent™

INTERNAL MEMORANDUM

TO: Mike Ruby, Yvette Lowney, Dr. Evelyn Tiffany-Castiglioni

FROM: Johanna Salatas

DATE: January 25, 2002

CONTRACT: 8601191.001 0301 0102 R575

SUBJECT: Selecting an appropriate avian receptor for SERDP bioavailability research

---

The American robin (*Turdus migratorius*) and American woodcock (*Scolopax minor*) are the two avian receptors that consistently indicate the greatest level of potential soil exposure according to exposure models. Therefore, the SERDP research needs to ensure that the avian species selected for our laboratory studies accurately represents the exposures that may be incurred by these target receptors. Since determining that the quail was not an adequate surrogate for the target species, we have discussed using sparrows, starlings, or robins. Below is discussion of several issues regarding the suitability of each of these species for our research.

The House Sparrow (*Passer domesticus*) has been considered as a surrogate research receptor for the robin and woodcock due to its widespread availability and the fact that it is easy to capture. However, the diet and foraging strategies of the sparrow, and the associated gastrointestinal physiology, are not comparable to those of the robin and woodcock, making the sparrow a potentially inappropriate surrogate for the study.

Significant Species Differences

Diet

The house sparrow typically consumes grains and seeds (Lowther and Cink 1992). In the spring and summer, only 9% of the house sparrow diet consists of invertebrates. In contrast, the woodcock consumes 80% invertebrates by volume, with earthworms predominating in the diet (Keppie and Whiting 1994). Similarly, in the spring and summer, the robin is generally considered a ground feeder on terrestrial invertebrates, primarily soft invertebrates such as earthworms (Sallabanks and James 1999). The sparrow is probably not as efficient at digesting invertebrates and associated soil as the woodcock and robin.

Foraging Strategies

The foraging strategies of the house sparrow include foraging on the ground for seeds, and perching on stems to reach seed heads (Lowther and Cink 1992). Although they do consume invertebrates, these prey are typically captured by aerial fly-catching or by gleaning insects from the bark of trees. The sparrow therefore does not typically consume soil invertebrates, whereas the woodcock and starling do. The food choice and foraging behavior differs greatly from that of the robin and woodcock, which probe the ground with their bills when foraging for earthworms. The robin displays a bill pouncing behavior when capturing earthworms, thrusting its bill quickly into the ground (Sallabanks and James 1999). Because both the robin and woodcock are exposed to the soil through their probing behaviors and diet choices, whereas the sparrow gleans seeds from the ground and insects from the air, the sparrow is unlikely to be exposed to soil in quantities similar to the robin and woodcock.

Gastro-Intestinal Physiology

As part of an effort to understand what might control bioavailability among different animals, Menzie-Cura and Associates compared the gastrointestinal physiology of several birds and mammals. According to their document (Menzie-Cura 2000), omnivorous birds such as the robin and woodcock rely on particle retention, pH (acid digestion), intestinal surface area, transit time, and microbial digestion to aid with digestion, whereas granivorous birds such as sparrow-like birds have complicated digestive systems, rely on heavily muscled gizzard and grit to assist in digesting very tough, fibrous foods, and do not rely on gastric acids to help digest. This is important because the acidity of the gastric fluid is known to be the most important factor controlling the oral bioavailability of lead in mammals. In the Menzie-Cura (2000) document, the authors also state, “The physical, chemical, and biological features of wildlife digestive systems vary such that caution should be exercised when extrapolating among species.”

The differences in the foraging methods, dietary needs, and physiology of the house sparrow when compared to the robin and woodcock suggest potential criticisms by study reviewers, and suggest that the sparrow will not be a good surrogate species for assessing the bioavailability of metals to robins and woodcocks.

Appropriateness of the Starling as a Surrogate

The starling is an adaptable bird with a broad diet (Perrins 1996). Between 41% and 73% of the annual diet of the starling consists of animal matter. In the fall and winter, the starling switches from an invertebrate diet to a fruit diet (Cabe 1993), similar to the American robin. The head and bill musculature of starlings is well adapted to foraging for invertebrate prey in soil and short vegetation (Cabe 1993). Foraging behavior is similar to that of the woodcock and robin, because the starling also inserts its bill into the ground while hunting, allowing the bird to dig for soil invertebrates. The diet and foraging behavior of the starling suggest many similarities to the robin and woodcock, and the omnivorous aspect of their diet composition also implies that the starling may serve as an adequate surrogate for robin or woodcock bioavailability studies. It is

important to know that one complication with using the starling is the difficulty with sexing the animals. The plumage of male and female starlings is nearly identical, and they usually have to be transported to the laboratory to be sexed. This necessitates capturing extra animals to ensure an adequate number of a single sex.

Schedule/Permit Issues for Wild Birds

For most avian species, mist netting is not allowed during the height of the breeding season; therefore, birds will need to be collected during February and March, or we must wait until July. It is important to note that if American robins are selected for the SERDP research, permits will be required. Also, Genesis Laboratories, in Wellington, Colorado, could assist us with catching either starlings or robins, if necessary. They would be able to catch robins and ship them to Texas for a fee of $34,207 (they would trap 200 birds to account for mortality from stress during transport). Alternatively, Genesis could perform all of the capturing, housing, and dosing aspects of the study for $44,812. The contact at the lab, Jeff Borchert, mentioned that it would take approximately 30 days to obtain a permit for catching robins, because they already have other permits in place.

Conclusion

In summary, the House Sparrow (*Passer domesticus*) does not appear to be a good surrogate for American robin and woodcock for the purpose of conducting a metals bioavailability study from soil. This is due to differences in diet, foraging strategies, and digestive physiology and chemistry. In contrast, the European starling (*Sturnus vulgaris*) does appear to be an appropriate surrogate for American robin and woodcock. However, if conducting the study using starlings is no more expensive than conducting the study using American robins, then the robin would be the preferred test species, given that they are the ultimate ecological receptor of concern.

References

Keppie, D.M. and R.M. Whiting, Jr. 1994. American woodcock (Scolopax minor). In Birds of North America, No. 100 (A. Poole and F. Gill, Eds.). Philadelphia: The Academy of Natural Sciences, Washington D.C.: The American Ornithologists' Union.

Sallabanks, R. and F.C. James. 1999. American robin (Turdus migratorius). In Birds of North America, No. 462 (A. Poole and F. Gill, Eds.). Philadelphia: The Academy of Natural Sciences, Washington D.C.: The American Ornithologists' Union.

Cabe, P.R. 1993. European starling (Sturnus vulgaris). In Birds of North America, No. 48 (A. Poole and F. Gill, Eds.). Philadelphia: The Academy of Natural Sciences, Washington D.C.: The American Ornithologists' Union.

Lowther, P.E., and C.L. Cink. 1992. House sparrow (Passer domesticus). In Birds of North America, No. 12 (A. Poole and F. Gill, Eds.). Philadelphia: The Academy of Natural Sciences, Washington D.C.: The American Ornithologists' Union.

Perrins, C.M. 1996. The Illustrated Encyclopedia of Birds, Definitive Reference to Birds of the World. Barnes and Noble Books, New York.

Menzie-Cura and Associates, Inc. 2000. Development of a Framework to Address Bioavailability Issues in Wildlife. Draft. March 31, 2000.

Exponent®

INTERNAL MEMORANDUM

TO: Mike Ruby, Yvette Lowney, Rob Pastorok

FROM: Johanna Salatas

DATE: August 29, 2002

CONTRACT: 8601191.002.0401

SUBJECT: Pilot Study for Evaluating the Oral Bioavailability of Metals from Soils and Earthworms to American Robins (*Turdus migratorius*)

INTRODUCTION

The objective of the pilot study was to determine how to best assess the bioavailability of heavy metals to American robins (*Turdus migratorius*) prior to initiating the full-scale study. Specifically the pilot study was conducted to determine how to effectively dose the robins in captivity, and to ensure that metal absorption can be measured in the tissues of the robins when dosed with DoD soils and associated metal spikes. The study was performed from May 14, 2002-August 19, 2002 at the Genesis Laboratories facility in Wellington, Colorado. This memorandum describes the study that was initiated, the problems encountered, and the results obtained.

OBJECTIVES

As originally designed, the avian pilot study was going to incorporate the following three groups of birds:

Pilot Group #1 (Positive Control):
Six robins were to be dosed with food that was blended with a metals spike solution designed to deliver a mass of metals equivalent to the concentrations of the six metals of concern in one of three DoD test soils. The metals of concern included: lead, zinc, chromium, cadmium, mercury and selenium.

Pilot Group #2 (Treatment Group):
Six robins were to be dosed with food that was blended with DoD test soil. The metals of concern and concentrations were intended to be identical to those used in the spike solution for Pilot Group #1.

Pilot Group #3 (Negative Control):

Three robins were to be fed clean lab food throughout the study.

However, early on in the study it was discovered that the robins did not readily accept the spike solutions in their diet, and therefore the objectives of the study switched to determine the best way to dose the birds. Specifically it was important to determine which metals were aversive to the birds, and/or how many metals the birds could tolerate in their dosed food. A full description of the problems encountered during the study and different methods that were used to encourage the birds to accept food containing spike solutions are included in the results section of this memo.

METHODS

Capture, Housing, Laboratory Environment

Sixteen American robins (*Turdus migratorius*) were wild caught from Larimer and Weld Counties, Colorado, using mist nests and Potter traps between May 13 and June 10, 2002. Birds were captured near tree rows on agricultural land, on golf courses and in cemeteries. State and federal permits were obtained prior to collection (Colorado Division of Wildlife Permit #02-TR821A1, U.S. Fish and Wildlife Collection Permit # MB817084-3). Sixteen adult female robins were retained for the study, and all other birds were released. Sexing was based on plumage using guidelines from National Geographic Society (1987), Pearson (1936), Peterson, R.T. (1990), Sallanbanks and James (1999), Sibley (2000), Terres (1991), Tyler (1949) and Udvardy (1977). Each bird was uniquely identified with a numbered leg band prior to the initiation of acclimation. The initial average body weight of the female robins used in the study was approximately 79 grams, ranging from 63-93 grams. On June 26, 2002, a veterinarian evaluated the birds for health status. All birds used in the study appeared healthy.

The birds were held in captivity for 69 to 97 days (depending on when birds were captured). During acclimation and holding, food and water was provided *ad libitum*. The maintenance diet used throughout the study included Mazuri Zulife Soft-Billed Diet 5M12 and deionized water. Water cups were disinfected every day using either Lysol or placed in a dishwasher. Feed cups were disinfected at least once per week using the same methods. Feed and water cups were made of PVC plastic. The diet was stored in rodent proof containers and the temperature and humidity of the feed storage area was monitored and recorded. Samples of the Mazuri diet were submitted for analysis.

Test animals were housed individually in 45.5 cm^3 galvanized steel cages coated with a latex sealer (SealTech water-based waterproofing sealer, Ace Hardware Corp, Oak Brook, IL) to prevent leaching of metals and subsequent exposure to the birds. Cages were equipped with a PVC or wood perch. Beneath each row of pens a galvanized steel tray containing absorbent material was used to collect waste. The pens were cleaned twice per week by removing the woodchips underneath the cages and replacing them with fresh ones.

Temperature and humidity were maintained monitored throughout the study. Adequate ventilation was maintained using an exhaust system at 10-15 air exchanges per hour. Full spectrum lighting was provided in the test rooms with a photoperiod of 16L:8D for the duration of the study.

Spike Solution Preparation

Exponent initially provided Genesis Labs with a spike solution consisting of the six target metals, including cadmium, chromium, lead, zinc, mercury and selenium. Although the spike solution was going to be prepared to match the metal concentrations present in a DoD test soil, it was decided to prepare the spike solution at $1/30^{th}$ of the LD-50 for each target metal. This was decided because in the mammalian counterpart of this study, $1/30^{th}$ of the mammalian LD-50 was the metal spike dose that was tolerated by the shrews.

The six-metal spike solution was prepared on 21 June 2002. First, one 25 ml volumetric flask was cleaned with 10% HCl and rinsed with deionized water. Each metal compound was added to the clean 25 mL volumetric flask that contained 50 µL HNO_3 (0.2%) preservative. The target masses of each metal compound and the actual mass of compounds added to the 25 ml volumetric flask are presented in Table 1. The spike solution was prepared based on the assumption that 4 mls of spike solution would be mixed into bird food to create 1000 grams of food, further described below.

Test Diet Preparation

Pilot Group #1 (Positive Control):
On 21 June 2002, the six-metal spike solution described above was shipped from Exponent to Genesis Labs. Genesis received the spike solution on 24 June 2002. The following ingredients were used to construct 1000 grams of robin test diet:

580 grams Mazuri soft-billed bird diet
4 grams pre-mixed spike solution
416 grams deionized water

The Mazuri soft-billed bird diet was first ground into a powder like consistency using a UDY Mill (UDY Corp, Ft. Collins, CO). In a table-top Kitchenaid mixer, 580 grams of the ground-up Mazuri diet was added. Based on the concentrations of the metals spike solution, the amount required (4 mls/1000 grams of test food) was first added to 46 mL of deionized water and added to the Mazuri diet in the mixer. Subsequently, another 50 mL of deionized water were gradually added to the mixer. The food, water and spike solution was blended on speed 2 for 10 minutes. After blending for 10 minutes, the final 320 mls of deionized water were added and the mixture was blended on speed 3 until dough was formed. The dough was kneaded for 5 minutes and then formed into 4.0 g (wet weight) balls, bagged in zip-lock freezer bags and placed in a freezer until used in the dosing experiments.

Pilot Group #2 (Treatment Group):
Initially the pilot study was planned to incorporate Pilot Group #2, which would have been dosed with soil in the test diet as opposed to spike solution. That diet was going to be prepared in a similar manner to the diet prepared for Pilot Group #1, above, except a portion of DoD test soil was going to be used in lieu of the entire amount of Mazuri soft-billed bird diet. Although this aspect of the pilot study was not carried out, the ingredients would have included the following:

430 grams Mazuri soft-billed bird diet
150 grams soil

420 grams deionized water

[Note: The amount of soil added to the diet (150 grams dry weight) would have represented 15% of the robin diet. This value will probably be increased to 20% for the main study.]

Pilot Group #3 (Negative Control):
For Pilot Group #3, the control diet was prepared in a similar manner to the diet prepared for Pilot Group #1, except only the following ingredients were used.

580 grams Mazuri soft-billed bird diet
420 grams deionized water

Method of Administration

Prior to test substance exposure, all birds were acclimated to non-dosed feed balls so that they would readily accept the balls as part of their diet. At the beginning of the light cycle period, just prior to when the lights are set to come on, the maintenance food (Mazuri diet) was removed from the cage. The birds were fasted for 1-2 hours. One 4-gram feed ball (either dosed with spike solution if birds were part of Pilot Group #1, or not dosed if birds belonged to Pilot Group #3) was placed in each cage, in a feed cup surrounded by a few pieces of maintenance diet kibble. The weight of the feed ball was determined before placement into the cages. The feed balls remained in cages for four hours at which time the maintenance diet was placed back in the cages.

Parameters Investigated

Body weights were measured for each test animal at the beginning of acclimation and on the day the diets were first offered. Feed ball consumption was recorded every day a feed ball was offered during the study. Successful consumption of the feed ball was noted as either not consumed, ¼ consumed, ½ consumed, ¾ consumed, or fully consumed. Mortality, moribundity and signs and symptoms of intoxication were recorded by making observations of each group once daily.

Results

During the study, the mean minimum and maximum daily temperatures were 19°C (±1°C) and 26°C (±2°C), respectively. Temperatures ranged from 16-29 ^{o}C. The mean minimum daily relative humidity was 36% (±9%), and the mean maximum daily relative humidity was 58% (±9%). Humidity ranged from 22-72%. No test-substance related mortality, moribundity, or signs of intoxication were observed in the vehicle control or treatment group. Occasionally, birds were noted to have minor abrasions unrelated to the test substance. On July 13, pen number 8 (bird # 333) which was intended to belong to Pilot Group #3 (Negative Control Group) was noted to have ataxia and tremors. The bird was euthanized two days later and replaced with pen #9 (bird # 334). The average body weight of the birds during the study was 79 grams when initially captured.

Experimental Working Groups

After the robins were fasted for 1-2 hours, food balls were then presented to the birds, in their feed cups, surrounded by a few pieces of maintenance kibble. Birds were checked often during exposure. If the feed balls were found elsewhere in the cage or if they had fallen through the cage into the bedding, they were reformed and again placed into the cages. Birds were acclimated for at least 10 days prior to test substance exposure. All birds successfully ate non-dosed 4 g feed balls prior to the onset of the dosing experiments.

Pilot Group #1 (Positive Control).
Initially the positive control group was exposed to full strength feed balls that were formulated as described above. Full strength feed balls were presented to the six robins in Pilot Group #1 for 8 days (26 June to 7 July 2002). Out of a possible 48 feed balls, only 18 were partially or wholly consumed. The amount of food consumed by each bird ranged from 4 to 20 grams of food during the 8-day period. Due to the sporadic, inconsistent, and minimal consumption of the feed balls, it was inferred that the full strength spiked diet was unpalatable to the birds. The birds would not consume feed balls, even if not dosed with spike solution, implying that the robins had developed an aversion to the appearance of the feed balls (Figure 1).

Pilot Groups #1a and #1b: Reduced Dosages. The robins used in Pilot Group #1 were divided into two groups (Pilot Groups #1a and #1b) after it was discovered that they would not accept a full-strength spike in their diet (Figure 1). On 11 July 2002, Genesis began offering food dosed with ½ the full-strength spike solution to three of the birds in Pilot Group #1a. Despite the reduced concentration in the feed balls, the birds did not reliably consume the feed balls (Figure 1). From 12 July to 16 July 2002, Genesis then offered the other three birds (Pilot Group #1b) feed balls dosed with ¼ of the spike solution, and the other three birds from Pilot Group #1a were offered 1/8 of the spike solution. The birds again did not consume the feed balls. On 18 July 2002 the birds in Pilot Groups #1a and #1b were offered non-dosed feed balls, but did not consume the feed balls, again implying that the robins had developed an aversion to the appearance of the feed balls. From 18 July to 11 August 2002, Genesis tried to re-condition the birds to accept non-dosed feed balls as part of their diet. This was accomplished best by applying peanut oil to the feed balls. Sunflower oil was also used but the peanut oil was more palatable to the robins.

Pilot Group #3: ¼ Dosage.
Because the robins in Pilot Group #1 did not accept feed balls whether they were full-strength, ½, 1/4th, or 1/8 dosages, it was next decided to determine if birds that were previously not exposed to the spike solution would accept feed balls if they only contained ¼ the full-strength six-metal spike solution.

On 18 July 2002, the three birds that were going to constitute Pilot Group #3 were offered feed balls dosed at ¼ the full strength dosage (Figure 1). These birds were already acclimated to the feed ball diet, but did not have previous contact with any feed balls that were dosed. Only two of the three birds consumed the food, and those two birds each only consumed 50% of their feed balls. Therefore these three birds consumed between 0 to 2 grams of dosed food. Subsequently, these three birds were placed on non-dosed feed balls again, which they readily accepted.

Pilot Group #3: Nitric Acid.

The next objective was to determine if the nitric acid solution (the preservative used in the spike solution) caused an aversion to the birds. From 22 July to 24 July 2002, the three robins in Pilot Group #3 were offered feed balls that contained either ¼ nitric acid or full strength nitric acid (Figure 1). The birds did not show any aversion to the nitric acid augmented feed balls, and therefore nitric acid was ruled out as the aversive agent in the diet.

Final Treatment Groups

The final aspect of the pilot study involved using all 15 robins that were initially intended to comprise Pilot Groups #1, #2, and #3. It was necessary to shift directions away from testing the effectiveness of dosing soil to the robins because it was imperative that we discover how to get the robins to consume the spike solutions. Because the robins showed aversion to diets containing a combination of all six metals, but were not offended by solution containing the nitric acid preservatives, it was hypothesized that multiple metals in the diet were causing an aversion to the diet.

To better explore the food aversion phenomenon, four individual spike solutions were prepared by Exponent and sent to Genesis. Prior to the onset of the pilot study, lead, zinc, chromium and cadmium were determined to be the priority metals of interest, in that order. Therefore, at this stage, mercury and selenium were dropped as target metals.

On 24 July 2002, the four individual spike solutions were prepared in the same manner as the initial six-metal spike solution was prepared. Four 25 ml volumetric flasks were cleaned with 10% HCl and rinsed with deionized water. The target masses of each metal compound and the actual mass of compounds added to the 25 ml volumetric flask are presented in Table 1. The spike solution was prepared based on the assumption that 4 mls of spike solution would be mixed into bird food to create 1000 grams of food.

By 12 August 2002, all 15 robins were again acclimated to consuming non-dosed feed balls. The 15 robins were divided into five groups, each containing three birds. The names of the five groups were: Control, Cadmium/Combination, Lead, Chromium, and Zinc. Three of the birds that were not previously exposed to dosed feed balls were used as the control group, and therefore continued to consume only non-dosed feed balls. Another group of three birds that were not previously exposed to dosed feed balls were assigned to the lead spike solution group. The remaining nine birds that previously belonged to either Pilot Group #1 or Pilot Group #3 were randomly assigned to the zinc, chromium and cadmium spike solution groups. The individual spikes were administered from 12 to 18 August 2002.

All birds were exposed to feed balls for seven days, except for the group of birds that belonged to cadmium spike solution group. The cadmium group of birds consumed cadmium-specific feed balls for three days. On the fourth day those birds were switched over to feed balls that contained all four metals, and consumed feed balls spiked with the multiple spike for the remaining four days.

The robins were successful at consuming feed balls containing individual spike solutions. The results are presented Table 2, and can be summarized as follows:

- Robins in the lead spike solution group consumed 21 of 21 (100%) lead spiked feed balls.

- Robins in the chromium feed balls consumed 19.25 of 21 (91.6%) chromium spiked feed balls.
- Robins in the zinc spike solution group consumed 19 of 21 (90.5%) feed balls.
- Robins in the cadmium spike group consumed 9 of 9 (100%) cadmium spiked feed balls for three days. However, when the birds were switched to the combination feed balls (containing all four metals), they only partially consumed 8 of 12 (58%) combination feed balls.

At the end of the study, the three robins from the control group and the three robins from the combination dose group were euthanized via CO_2 asphyxiation. Each bird was skinned and individually double-bagged and labeled with the treatment group and bird number. Bagged birds were placed on dry ice in a cooler and immediately sent, via overnight delivery, to Abbie Spielmann, Columbia Analytical Services, Inc., Kelso, WA. The analytical results are presented in Table 1.

Maintenance diet samples (Mazuri diet) were collected and frozen immediately after collection and maintained frozen until shipped. The samples were shipped with dry ice by overnight freight to Abbie Spielman, Columbia Analytical Services, Inc., Kelso, WA. The analytical results are presented in Table 1.

Discussion

The pilot study, as it was initially designed, could not be carried out because the robins did not readily consume feed balls dosed with the six-metal spike solution. It was imperative that the birds be able to consume the spike solution, because that type of dose group was intended to serve as the positive control for the full-scale study. The robins had an aversion to the dosed food at full-strength, ½, ¼, and 1/8 of the full-strength spike (Figure 1; Table 2). This was true for birds that were initially exposed to full-strength dosages, as well as for birds that were not previously exposed to dosed food (Figure 1; Table 2). The aversion was likely a result of the combination of metals, because when metals were dosed individually, the food was readily eaten. Also, the aversion was not an artefact of the nitric acid preservative contained in the spike solution, because birds readily ate food that contained the preservative without metals.

When it was realized that the pilot study would have to shift from mirroring the full-scale study, the fifteen robins that were in the laboratory were used to test whether the robins had an aversion to any of the target metals, including lead, zinc, chromium, and cadmium (Figure 1). Mercury and selenium were dropped from the full-scale study during the course of the pilot study because those metals are of the least concern at DoD sites. When dosed with individual metal spike solutions, the birds did not seem to have an aversion to the metals (Table 2). Lead was accepted in the diet most readily, followed by cadmium, chromium and zinc (Table 2).

The birds that were individually dosed with cadmium were switched to feed balls that were dosed with a combination of the four target metals after three days of testing (Figure 1). Those birds failed to consume the combination spike. The results from this pilot study indicate that when four or more metals are mixed together in a spike solution at a concentration equal to $1/30^{th}$ the LD-50, the food becomes unpalatable to the birds (Table 2). It is unknown if two or three metals mixed together in a spike solution would also be unpalatable to the birds. However, it is certain that individual metals can be dosed successfully in feed balls for a period of seven days.

The pilot study has failed to answer many questions that are necessary to make decisions about the full-scale study. For example, the dosages used in the pilot study were equivalent to 1/30th of the avian LD-50's for each metal. During the full-scale study, it is likely that metals present in the DoD test soils will contain concentrations higher than 1/30th of the LD-50. This could pose a problem, not only in terms of unpalatability issues, but could result in death. It will be necessary to know if the concentrations of metals in soil will pose danger to the birds, especially because more than one metal may be present, and the metals will be dosed for 28 days. Also, if more than one metal is present, it will be difficult to dose the birds because they will likely have an aversion to more than one metal in spiked food, as they did in the pilot study.

The results of the pilot study indicate that the spike solutions may not have been adequately mixed together with the food, so they are not very reliable dosing vehicles. This is evident from data presented in Table 1, where it is reported that the calculated metal concentrations in the spiked food are lower than the metal concentrations actually measured by CAS in the food samples. For example, the cadmium concentration that supposed to be present in the feed balls (according to calculations) was equal to 211 mg/kg (dry weight), but the cadmium concentration actually measured was equal to 173 mg/kg (dry weight). Zinc was present in the feed balls at significantly higher calculations than what was expected, probably because the baseline food was naturally high in zinc (92.7 mg/kg dry weight). These results indicate that the in the future studies conducted with robins, it will be necessary to 1) try a different method of preparing the feed balls, and 2) account for the high amount of zinc present in the baseline food.

The current method for preparing food (discussed above in Methods) can be altered to possibly allow more thorough homogenization of the spike solution with the food. It would be ideal to first thoroughly mix all of the spike solution with the de-ionized water, and then gradually add the dry food to the liquids. The food may also need to be mixed for a much longer period of time, to ensure that that liquid is being adequately distributed throughout the dough. At least three samples of each batch of food should be submitted for analysis to ensure that the food is adequately homogenized with the metal spike solutions and soil when the full-scale study is initiated.

Approximately half of the birds used in this study were exposed to 0.8 grams of soil mixed into individual feed balls (i.e., 20% of the 4 gram feed ball was composed of soil). The soil was obtained from outside the Genesis laboratory facility, and had not been analyzed for any metals or other contaminants. Genesis did not keep notes on how much the birds consumed when offered soil-augmented feed balls, but the personnel recall that the birds readily accepted soil augmented feed balls into the diet. The offering of soil-dosed feed balls occurred prior to the onset of the pilot study, and therefore the robins did not yet develop any aversions to spiked food.

Some analytical data for metals contained in the robin tissues were obtained during the pilot study. The three control birds that were in captivity for 2.5 months and fed a clean diet contained undetectable concentrations of chromium in their tissue, but detectable concentrations of cadmium, lead, and zinc (Table 1). The three birds that were initially fed cadmium spike feed balls, but were subsequently switched to a combination diet, show metal concentrations similar to those witnessed in birds that consumed the control diet, with the exception of cadmium, which is higher (Table 1). The cadmium is likely higher because it was dosed for a period of seven

days in those birds, as opposed to four days, like the other three metals. In the full-scale study, it is anticipated that the birds will be exposed to metals for 28 days. Based on the short-term nature of the pilot work, it is still unknown if all four metals will be detectable in avian tissues after 28 days of dosing.

Running head:

Oral Bioavailability of Metals in Soil to the Least Shrew

Corresponding author:

Yvette Lowney

Exponent, Inc.

4940 Pearl East Circle, Suite 300

Boulder, Colorado 80301

Telephone 720.406.8127

Fax 303.444.7528

e-mail lowneyy@exponent.com

Word count: 6218

Oral Bioavailability of Metals in Soil to the Least Shrew

M.V. Ruby[1], Y.W. Lowney[1], C. Trinh[3], J.H. Salatas[2], L. Ziccardi[1], M. Edwards[2], R.A. Pastorok[4], and N.A. Darmani [3]

[1] Exponent, Boulder, Colorado USA

[2] Exponent, Bellevue, Washington USA

[3] Department of Pharmacology, Kirksville College of Osteopathic Medicine, Kirksville, Missouri USA

[4] Integral Consulting, Inc., Woodinville, WA

Abstract

Small mammals such as shrews are among the wildlife receptors for which ecological risk assessment models consistently indicate the greatest level of potential exposure to metals in soil. These small mammals receive much of their soil exposure from direct soil ingestion during foraging and preening activities, or from consumption of soil-laden earthworms. In assessing risks to these receptors from soil contamination, the standard method is to assume that contaminants have a relative bioavailability of 100% (i.e., the efficiency of metals absorption from ingested soil is equal to that which occurred in the laboratory tests conducted to determine toxicity thresholds). However, a growing body of research indicates that many chemicals—including metals—are less bioavailable from ingested soil than from soluble forms (i.e., the forms typically used in laboratory toxicity tests), when dosed in a similar manner. This research was conducted to determine the relative oral bioavailability in least shrew (*Cryptotis parva*) of arsenic, cadmium, chromium, and lead from four soils. Each soil was dosed at three different concentrations, as were soluble metals spiked into food to dose-match each soil dose group. Results indicate that the relative bioavailability of arsenic, cadmium, and lead ranged from 7% to 49%, 13% to 81%, and 21% to 60%, respectively. Chromium(III) was not absorbed from soil, even at very high doses, and Cr(VI) was absorbed to a slight extent from a soil that was spiked with a high concentration of Cr(VI).

Key words: shrew, metals, soil, oral bioavailability

Introduction

Limited research has been conducted on the bioavailability of metals from soil to mammalian wildlife. Given the lack of information on this topic, ecological risk assessments generally assume that metals in soil are equally bioavailable as the soluble salt forms typically used in toxicity studies, potentially resulting in overestimates of exposures and related risk. For example, the U.S. Environmental Protection Agency's (EPA's) guidance for developing ecological soil screening levels assumes that the bioavailability of contaminants in soil is equal to the bioavailability of the contaminant in the laboratory study used to establish the toxicity reference value [1]. This assumption is consistent with ecological risk assessment guidance under Superfund [2]. However, a growing body of research indicates that many chemicals—including metals—are less bioavailable from ingested soil than from the soluble forms that are typically used in laboratory toxicity tests [3, 4] (Framework for inorganic metals risk assessment (external review draft), U.S. EPA: http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=88903).

The mammalian wildlife receptors for which ecological risk assessment models consistently indicate the greatest level of potential soil exposure are the short-tailed shrew and the cottontail rabbit, due to incidental soil ingestion during normal activities of burrowing, eating, and grooming. Due to the dietary requirements of the shrew, this receptor ingests considerable quantities of soil, both directly and indirectly (in earthworms, which constitute a large part of their diet). It is partly for this reason that EPA considers the short-tailed shrew to be a good sentinel receptor for the mammalian insectivore guild [1], and why this species was selected for the current study. It is also important to note that the short-tailed shrew is the mammalian receptor that yields the lowest ecological soil

screening levels (eco-SSLs) for most of the metals that have been evaluated in the eco-SSL process to date, and thus will be important in setting soil screening levels for ecological receptors.

To provide data regarding the bioavailability of metals to this sentinel species, this research project was designed to measure the absorption of metals by shrews after soil ingestion, relative to the absorption of the soluble forms of these metals that have been used in toxicity studies. It was the intent of this research to develop an in vivo model for measuring the relative bioavailability of metals in soil to shrews, and to produce data that can be used in ecological risk assessments.

Because this research was funded by the Strategic Environmental Research and Development Program (SERDP), the focus was on metals that occur in soils at Department of Defense (DoD) facilities. As a precursor to this research, metal concentration data for a wide variety of DoD facilities were screened against established regulatory criteria for ecological endpoints. The metals that most frequently exceeded ecological screening criteria, in order, are lead (Pb), cadmium (Cd), mercury (Hg), zinc (Zn), arsenic (As), and chromium (Cr) [5]. In addition, EPA toxicologists and project managers were interviewed in each EPA Region regarding their perceptions of which metals were driving risks and cleanup decisions at DoD sites. Although the majority of EPA personnel interviewed indicated that human health risk, rather than ecological endpoints, generally drives risk-based remedial decision-making, the data indicated that ecological screening thresholds were exceeded more often, and to a greater extent, than human health standards [5].

Animal Model

Shrews are small (generally <3 inches long), insectivorous mammals that inhabit round, underground nests and maintain underground runways, usually in the top 10 cm of soil, in most regions of the United States [6]. Shrews are also prey for many animals, including hawks, owls, weasels, foxes, and skunks [7].

Short-tailed shrews are not generally used for laboratory research, because they must be wild-caught, may have diseases, and often do not adjust well to captivity. No established colonies of short-tailed shrew were available for this research. Therefore, the least shrew, which is already adapted to a laboratory environment, was selected as a surrogate. The diet of both species consists primarily of insects and earthworms, but they also ingest plant matter (mostly roots) at times [6, 8]. In addition, the least shrew is a close relative of the short-tailed shrew, and has similar metabolic and food consumption rates. For these reasons, the least shrew was deemed an appropriate surrogate species for the short-tailed shrew. Only female shrews were used, because the oral bioavailability of lead in other small mammals (e.g., rats) has been observed to be dependent on the sex of the animals [9], and because females are considered to be more ecologically sensitive than male shrews.

Target Metals

As mentioned, the metals identified as important for this research were lead, cadmium, mercury, zinc, arsenic, and chromium. An attempt was made to identify and collect soils from sites that contained this suite of metals at concentrations that would yield measurable post-dosing concentrations in the shrew, but would not be toxic in the sub-acute dosing

periods that were used in this study. No soils were found that contained concentrations of mercury that would allow for measurement of oral absorption. In addition, the cat food that constitutes the basal diet of the least shrew colony used in this study is quite high in zinc concentration (approx. 250 mg/kg, dry weight) (several specialty cat foods were evaluated in an attempt to find one lower in zinc concentration, without success). As a result, the elevated background zinc concentrations in shrew precluded the ability to measure zinc absorption from soil in this study. Thus, the target metals for this study became lead, cadmium, arsenic, and chromium.

Study Design

In these experiments, the relative bioavailability from the test soils was assessed by comparing the absorption of each target metal after soil ingestion to the absorption of soluble forms of the metals. Both the test soils and the aqueous mixtures of soluble metal salts (referred to as “reference mixtures”) were mixed with basal diet and dosed to groups of shrews for 28 days. Three dose levels of each test soil or reference material were given to assess whether any dose-response relationship existed for metal absorption. For a given test soil, the maximum daily mass that could safely be given (estimated from toxicity data and the metal concentrations in each soil) was established and is referred to as the “100% soil dose” (this ranged from 0.01 to 0.20 g soil/day for the four soils tested, depending on the metal concentrations they contained). The other two soil doses were given at one-half and one-fourth of the initial soil dose (referred to as the “50% and 25% soil doses”). Doses of the target metals given as the reference mixtures were matched, to the extent practicable, to those delivered in the three doses for each soil. However, because each shrew consumed the dosed food *ad libitum*, doses received by individual shrews varied.

After the 28-day dosing period, the shrews were terminated, and the body burden of the target metals was determined. The relative bioavailability was then calculated from the concentrations of a target metal in the soil-dosed animals, relative to the concentrations in the reference-dosed animals.

Materials and Methods

Soil Collection and Characterization

Four soils containing the mixture of target metals (Pb, Cd, As, Cr) were used in this research. These included soils from the Naval Weapons Air Station located in Point Mugu, California (hereafter referred to as DoD-PM soil), a mixture of soils from Dugway Proving Grounds and Picatinny Arsenal (referred to as the DoD-DP soil), an orchard soil from Washington State (Orchard soil), and a soil collected in the vicinity of a smelter in Colorado (Smelter soil). Because none of the test soils contained detectable quantities of Cr(VI), the Smelter soil, which contained only 19 mg/kg chromium, was spiked with soluble Cr(VI) to achieve a soil concentration of 1,835 mg/kg chromium [1,355 mg/kg measured as Cr(VI)].

All soils were air-dried and sieved to <2 mm, and then to <500 µm. This particle size was selected for study, because it represents a soil fraction that earthworms, a prey item for shrews, may ingest [10], and that will cling to earthworms when removed from a moist (20% water content) sandy loam soil, based on measurements made in Exponent's laboratory. Both the soil particles within and on the exterior of earthworms may be ingested by shrews, when feeding on earthworms. In addition, this particle size is

representative of that which small mammals are likely to ingest during grooming due to electrostatic adherence of soil to fur.

Each bulk soil sample (<2 mm) was analyzed for particle size distribution (sand, silt, clay), and the fine soil fraction (<500-µm size) was analyzed for the target metals (Pb, Cd, As, Cr), hexavalent chromium concentration, pH, total organic carbon (TOC), total inorganic carbon (TIC), cation exchange capacity (CEC), and extractable concentrations of iron oxides/hydroxides (method of Mehra and Jackson [11]). The <500-μm size fraction was used to prepare all of the soil doses. Each soil was heated to 80 °C for 6 hours prior to mixing with the shrew feed batches to eliminate organisms that could be harmful to the shrews.

Care and Treatment of Least Shrews

Least shrews (*Cryptotis parva*) were bred and maintained at the animal facilities of the Kirksville College of Osteopathic Medicine, Kirksville, Missouri. Only mature female shrews (4–5 g), more than 30 days old, were used for the study. The shrews were kept on a 14:10-hour light-dark cycle at a room temperature of 21±1 °C in individual open-top clear polycarbonate cages (20×18×21 cm) lined with heated dry loam soil and wood chips [12]. A wooden nest box (5.5×5.5×9 cm) containing dry grass, a food bowl, and a lick water bottle was placed in each cage. The soil, wood chips, and grass were tested for target metals, and found to contain negligible concentrations. Before starting the experiment, shrews were acclimated to the containment room and fed standard “clean” diet for one week. Shrews were weighed at the beginning and conclusion of each experiment.

At 0800 hours on the day following the last day of dosing, the animals were euthanized by inhalation of carbon dioxide, and the carcasses were frozen.

Feed Preparation and Dosing Regime

Each batch of shrew feed was prepared in the following manner: 182 g (dry weight) of Laboratory Feline Diet (manufactured by PMI Nutrition International) was mixed with 203 mL of distilled de-ionized water and 156 g (wet weight) of Cozy Kitten Chicken and Fish Dinner (manufactured by Heinz Pet Products). Each feed batch was mixed thoroughly with a stainless steel, food-grade blender. For the soil exposure groups, the appropriate mass of soil was used in place of an equivalent mass of the dry feline diet (soil in the feed ranged from 0.31% to 6.2%, by weight). For the reference material (i.e., soluble spike) groups, 2–8 mL of the appropriate spiking solution was used in place of an equivalent volume of distilled de-ionized water. Each feed batch was sufficient to feed a dose group of 12 shrews for 14 days, after which a new batch of feed was prepared.

A 3-g grab sample of feed was collected for metals analysis each time a new feed batch was prepared. In addition, triplicate 3-g grab samples were collected from 22 feed batches to check for homogeneity of target metals concentrations in the dosed feed.

The shrews were fed 3.2 g of dosed feed once daily at 0800 hours. If any dosed feed remained the next morning at 0800 hours, it was removed, weighed, and archived. When feed was provided each day, an estimate was made of the mass of any feed spilled by each shrew. This estimate was recorded, and the spilled feed was removed from the cage. Distilled de-ionized water for drinking was provided *ad libitum*. On the last day of the study (Day 28), all groups of shrews were provided standard “clean” diet at 1700 hours.

Dose Groups

A target of 8 surviving animals per dose group was set for this study. Due to shrew mortality rates, achieving this target required starting with an excess number of shrews—a number of 12 was selected as the starting value (in a few of the early dose groups, extra animals were added during the first week, and subsequently dosed for 30 days, if the mortality rate within that group was particularly high). As a result of variable shrew mortality rates in the different dose groups, the number of shrews varied from 6 to 11 animals after 28 days of dosing (Table 1). For all dose groups with 8 or fewer animals surviving for the entire dosing period, all of the surviving animals were analyzed. For dose groups with more than 8 surviving animals, 8 animals were selected at random for analysis.

Negative Controls

For each of the different test soils, which were evaluated during separate dosing trials, a negative-control dose group was included and were fed standard shrew diet.

Dosing of Soils

Each soil exposure group consumed feed augmented with a contaminated soil, or with two lesser amounts (50% or 25% of the initial mass) of that contaminated soil. Thus, for each soil there were three doses, representing a four-fold change in dose levels for that soil. The full soil doses (referred to as 100% contaminated soil) ranged from 0.01 to 0.2 g/day, depending on the mixture of metals concentrations contained in each test soil (0.2 g soil/day represented 6.25% soil in feed; a pilot study had indicated that the shrew would accept up to 12% soil in diet before palatability problems occurred).

Reference Mixtures

Each reference mixture, or soluble spike, dose group received a mixture of metals matched to the mixture present in each of the four test soils (i.e., the soluble spikes were designed to deliver the same dose of metals as those from the test soils). The metal spike solution consisted of de-ionized water adjusted to pH 2.0 with nitric acid, with the following metal salts added at appropriate concentrations: lead acetate trihydrate, sodium arsenate heptahydrate, cadmium chloride (anhydrous), and for chromium, proportions of chromium as chromium(III) acetate hydroxide and chromium(VI) oxide to match the proportion of chromium in these two redox states in the soil. The reference mixture matched to the Orchard soil did not contain Cd or Cr, because there was not enough Cd or Cr in this soil to yield detectable amounts of these metals in the post-dosing shrew tissues. Although the Smelter soil was spiked with 1,355 mg/kg Cr(VI), the reference mixture matched to the Smelter soil did not contain Cr. This occurred because addition of Cr(VI) to the reference solution at even a fraction of the concentration required to match the dose from spiked soil caused a precipitate to form in the reference solution. Rather than dose the shrews with this uncharacterized precipitate phase, Cr was eliminated from the reference material associated with the Smelter soil. As a result, none of the shrews dosed with reference mixtures received doses of Cr(VI).

<u>Sample Analyses</u>

The frozen shrew carcasses were shipped to Columbia Analytical Services (CAS) in Kelso, Washington, for analysis. At CAS, entire individual shrew carcasses were ground in a laboratory meat grinder, freeze-dried, and homogenized. A representative subsample of the homogenate was digested and analyzed for concentrations of lead, cadmium, arsenic,

and chromium by inductively coupled plasma/mass spectroscopy (ICP/MS). The 3-g samples of standard diet and dosed feeds were also digested and analyzed for target metals by ICP/MS.

Data Handling and Relative Bioavailability Calculations

For each shrew, the body burden of each target metal was measured in micrograms (µg) of metal per kilogram (kg) of body weight, in the freeze-dried samples. The individual dry-weight measurements were converted to wet-weight concentration values using the % solids measurements made on each sample, and these data were used to calculate average tissue metal concentrations for each dose group. Metal concentrations in the samples of standard diet and dosed feeds were also corrected to wet weight, and were used to calculate actual doses received by each animal (as µg/kg-day), based on mass of feed consumed and average body weight over the 28-day dosing period. These data were used to calculate average doses received by each dose group. In working with the shrew tissue and feed concentrations, one-half the method reporting limit (MRL) was used for metals concentrations that were reported as non-detect.

In biological assays, it is not uncommon to find individual measured responses that are atypical of animals in the same dose group. Evaluation of shrew tissue data graphs indicated that all potential outliers were high relative to mean tissue concentrations for each dose group. The shrew tissue data were then tested for outliers by applying Dixon's outlier test [13] with $\alpha = 0.01$, and testing the highest value against the other tissue concentrations in each dose group. Based on this analysis, data from 12 samples were

identified as outliers (two each for arsenic, chromium, and lead, and six for cadmium); these data points were not used in calculating relative bioavailability values.

Regression methods were used to estimate the relative oral bioavailability of the target metals from the four test soils relative to their respective reference materials. A single simultaneous regression model was used to estimate the slope of the dose-response for each test material while restricting the intercept to be equal to the response from the control animals for each round of dosing. This is appropriate because, at zero dose, both of the test substrates (soil and reference material) should yield the same response [14]. As is typical with animal data of this type, the variability in the response increases with increasing dose—a property known as heteroscedasticity. Because heteroscedasticity of the data is contrary to the assumption of equal variance, which is required for a linear regression to be applicable, each dose group was weighted by the inverse of the predicted variance for that dose group (average dose was assumed for each member of a dose group). The predicted variance was estimated from the variance as a function of the magnitude of the response data, and is considered a more robust measure of variance than the dose group's specific measured variance, because it is less affected by individual measurements. Weighting by the inverse of the predicted variance gives less weight to the more variable data points and achieves homogeneous variability across all dose groups. A simultaneous linear regression model was then fit to the weighted data for each combination of soil/reference material (as described by Draper and Smith [15]). The relative bioavailability for each metal in each soil was then estimated as the ratio of the slope of the regression for the soil versus that for the reference material**.** As described by Finney [14], Fieller's Theorem may be used to calculate the uncertainty range around the ratio of two

model coefficients, and this approach was used to estimate the uncertainty in the relative bioavailability estimates, as represented by the upper and lower 95th percentiles and the standard error.

Results

Soil Characterization

Metals concentrations and soil characterization data for the test substrates are presented in Table 2. The test soils ranged in texture from sand to sandy loam, with pH and TOC ranging from 5.9 to 8.0 and 0.75% to 3.26%, respectively. Arsenic concentrations ranged over a five-fold difference (60 to 331 mg/kg), while cadmium (2.4 to 1,755 mg/kg), chromium (36 to 8.362 mg/kg), and lead (257 to 2640 mg/kg) covered approximately 3, 2, and 1 order of magnitude in concentration ranges, respectively.

Animal Results

Average doses delivered to each dose group of shrews are reported in Table 3 (calculated from metal concentrations measured in each batch of feed and average shrew body weight during the study). Actual doses delivered were consistent with the four-fold target difference between the low and high doses for each of the metals, in each of the four test soils, except in cases where the dosed feed concentrations were very close to the standard diet concentrations. The ratios of metal doses delivered as soil in feed, relative to doses delivered as the matched reference mixture in feed (Table 3), generally ranged from 0.85 to 1.15, with a few values outside this range (0.73 and 1.37 represented the absolute limits of the range). These results indicate that the dose range for metals in each soil and reference

mixture were approximately four-fold, and that similar metal doses were delivered in both the soil-dosed feed and the matched reference materials.

During the course of the study, triplicate analyses for the target metals were conducted on 22 samples of feed. Based on wet-weight feed metals concentrations, the coefficient of variation (CV) for these triplicate analyses ranged from 1% to 21% (average of 4.9%) for arsenic, 1% to 24% (average of 8.6%) for cadmium, 1% to 24% (average of 7.5%) for chromium, and 1% to 27% (average of 6.4%) for lead, for triplicates in which no samples were non-detects. These data indicate that the feed dosed to the shrews was relatively homogeneous and provided consistent doses to the shrews.

High shrew mortality was observed in the first dosing trial (43%), and decreased as the study progressed (8% during final dosing trial, which is similar to that observed in the shrew colony as a whole on a monthly basis; Table 1). With the exception of the 100% exposure group for the Smelter soil, mortality rates were as high for the control groups fed standard diet alone as they were for dose groups fed soil or reference mixtures (Table 1), indicating that mortality was not due to metals exposure. It is believed that the elevated mortality rates early on were due to insufficient food and the effects of isolation. A pilot study had indicated that 3.2 g of feed per day was a good amount for this study, because the average shrew would consume most, but not all, of this feed mass during each 24-hour period. However, when the elevated mortality rates were observed during the first dosing trial, 10% of the de-ionized water in the feed mixture was replaced with an equal weight of dry cat food, and this appeared to increase shrew survival rates. In addition, the shrew colony was normally bred and kept in groups of 6 to 12 individuals, but this study required

shrews to be housed individually so that doses ingested by each shrew could be monitored. This isolation appears to have contributed to the increased mortality rate during the first dosing as well, because when shrews were gradually acclimatized to living in individual cages, the mortality rate decreased. These observations demonstrate the delicate nature of this shrew animal model, because the animals can expire with slight changes in diet or social organization.

As expected, based on the understanding that these animals were underfed, all of the dose groups during the DoD-PM dosing trial (the first one conducted), when mortality rates were at their highest, experienced a decrease in body weight during the 28-day dosing period (Table 4). This was followed by increases in body weight during the DoD-DP dosing trial, consistent with the decline in mortality rates. However, during the last (Smelter soil) dosing trial, body weights declined to an even greater extent than during the DoD-PM dosing trial, even though mortality rates had decreased to levels seen in the overall shrew colony. The Smelter soil delivered the greatest doses of arsenic, cadmium, and chromium, and the second-highest dose of lead, suggesting that one, or some combination, of these metals may have been responsible for the decreases in body weight during the dosing trial with this soil. It should also be noted that shrew mortality was elevated in the 100% Smelter soil dose (36%; Table 1), consistent with the decreases in body weight (–25%; Table 4) in this dose group.

The shrews exhibited a clear dose-response for arsenic, cadmium, and lead in the test soils (insufficient data were available for chromium to make this determination). Figure 1

summarizes all of the dose vs. tissue concentration data for lead in the four test soils that were dosed to the shrews.

Relative Bioavailability

Arsenic

Relative bioavailability of arsenic from soil ranged from 7% to 49% for the three soils in which the regression model yielded significant results (Table 5)—dosing of the DoD-PM soil (82 mg/kg As) and associated spike yielded all non-detect values in the post-dosing shrew tissues. The 7% relative bioavailability value for arsenic in the DoD-DP soil undoubtedly has greater uncertainty associated with it than would be implied by the standard error associated with this value, because many of the tissue arsenic concentrations from the soil-dosed animals were non-detect values, and those that were detects were only slightly greater than the detection limit (Figure 2). As a result, the slope of the dose-response curve for the soil-dosed animals is less certain than that for the reference-material-dosed animals. In contrast, arsenic concentrations in shrew tissues of animals dosed with the Orchard (Figure 3) and Smelter soils were well above detection limits, yielding more robust estimates of relative bioavailability. These results suggest that a dose of approximately 3,500 µg As/kg-day in soil (Table 3) should be considered the lower limit for arsenic in this shrew model.

Cadmium

Relative bioavailability of cadmium from soil ranged from 13 to 81% for the three soils where the regression model yielded significant results (Table 5)—shrew tissue concentrations after dosing of the Orchard soil (2.4 mg/kg Cd) were all non-detect.

Similarly, shrew tissue concentrations after dosing with the DoD-DP were largely non-detects (Figure 4) (analogous to the situation for arsenic in this soil). Thus, the 13% relative bioavailability value for cadmium in this soil is more uncertain than the values for the DoD-PM and Smelter soils, but certainly indicates low absorption of cadmium (and arsenic) from this soil, relative to exposure to soluble salts of these metals. Based on these data, a dose of at least 500 µg Cd/kg-day in soil (Table 3) is required for this shrew model.

Chromium

Chromium(III) does not appear to have been absorbed in the shrew, either from soil or the reference mixtures, regardless of dose level. Doses up to nearly 20,000 µg/kg-day were delivered in the DoD-PM soil and its associated reference mixture (the DoD-PM soil contained 8,362 mg/kg Cr, almost entirely as Cr(III) [Table 2]), and yet no evidence of chromium absorption was observed for either the test soil or its reference mixture (Figure 5). The same was observed with the DoD-DP and Orchard soils, which delivered smaller doses of Cr(III) than the DoD-PM soil.

For the Smelter soil, which was spiked with Cr(VI) and delivered doses up to nearly 90,000 µg-kg/day of Cr (Table 3), uptake into shrew tissue was observed (Figure 6), and tissue response increased with increasing dose. At the intermediate dose of Cr from this soil (42.6 mg/kg-day, or a total dose of 1,192 mg Cr), only a very small fraction (approx. 7.4×10^{-6}) of the chromium dose mixed with soil was found in shrew tissues (average of 0.0089 mg Cr/shrew). In the absence of a soluble reference dose, it is not possible to calculate a relative bioavailability value for Cr(VI) from the Smelter soil.

Lead

Relative lead bioavailability from soils ranged from 21% to 60% (Table 5), with detectable lead concentrations in tissue from all four soils tested. The analytical results from the Orchard soil were anomalous, in that tissue lead concentrations from the soil exceeded those from the associated reference mixture (initial relative bioavailability estimate of 129%), suggesting that soil lead was more readily absorbed than the lead acetate spike. Because it seemed very unlikely that this could actually occur, and because the dose-response from the lead reference doses associated with the other three soils tested yielded consistent results that contradicted results for lead from the Orchard reference mixture, the average dose-response from the lead acetate given during the DoD-PM, DoD-DP, and Smelter soil trials was used to calculate the relative bioavailability of lead from the Orchard soil. The anomalous behavior of lead in the reference material associated with the Orchard soil may have been due to formation of a sparingly soluble precipitate in the reference solution, because the lead concentration in the Orchard reference material was greater than in any of the others. This would explain how lead absorption from the Orchard soil could have exceeded that from the reference material.

Based on these results, a minimum lead dose from soil of approximately 300 µg/kg-day is required for use of the shrew model.

Effect of Soil Parameters on Metals Bioavailability

The relative bioavailability values from this study (Table 5) were compared to the soil parameters and soil metal concentrations (Table 2) to assess whether a particular soil variable appeared to control the relative bioavailability of any target metal. This analysis

was conducted both graphically and using Pearson's correlation at $\alpha = 0.05$. The only significant correlations were for the relative bioavailability of cadmium, which was inversely correlated with both CEC and DCB extractable iron concentrations of the test soils. In addition, the graphed data suggested that relative lead bioavailability increased with increasing soil lead concentration. However, with only a few data points per metal, it is not possible to have a high level of confidence in these correlations.

Discussion

The research described herein involved the development of a novel animal model for assessing the relative bioavailability of metals from soil. The shrew model differs from existing animal models for estimating metals bioavailability from soil (e.g., rats, swine, and monkeys) in that they have very high metabolic and food consumption rates, and are quite fragile. As observed during this study, high mortality occurs with minor changes in diet or habitat. Despite the difficulties in working with this model, this study demonstrates that it is possible to obtain reliable estimates of relative metals bioavailability from the shrew.

Based on the study results, it is clear that arsenic, cadmium, and lead are absorbed to varying extents from different soils in this shrew model, and that site-specific (or soil-specific) factors affect the relative absorption of the metals. Chromium(III) was not absorbed in a detectable manner, and soluble chromium(VI) spiked into soil was absorbed to a limited extent, the degree of which cannot be quantified from the study results.

The relative bioavailability values for arsenic, cadmium, and lead in soil are generally consistent with those observed in other animal models. For example, relative arsenic bioavailability from 13 soils has been evaluated in a juvenile swine model, resulting in values ranging from near 0 to 52% [16]. In addition, five Florida soils were evaluated in a Cebus monkey model, yielding relative bioavailability estimates for arsenic of 10% to 25% [17]. The range of relative arsenic bioavailability values observed in this study (7% to 49%) falls nicely within that observed in juvenile swine, and is somewhat greater than that observed in Cebus monkeys.

The DoD-PM soil used in this study was also evaluated for relative cadmium bioavailability in the juvenile swine model, yielding an average relative bioavailability estimate of 78%, based on measurement of both kidney and liver endpoints [18]. Given that the DoD-PM soil yielded a relative cadmium bioavailability of 81% in the shrew, these two animal models appear to be yielding similar results for cadmium.

The juvenile swine model mentioned above was developed as a surrogate for absorption in human children, and has been used extensively to evaluate relative lead bioavailability in soil. Data from the swine model indicate a broad range of relative bioavailability results for lead in soil (19% to 90% [19]). This is generally consistent with results observed in the shrew model, wherein relative lead bioavailability ranged from 21% to 67%.

Given the consistent results obtained from this shrew model, and the fact that these results are comparable to results from established animal models, the shrew model appears to be a useful tool for assessing metals uptake from soil into shrew and other small mammals, and for improving the accuracy of ecological risk assessment.

Acknowledgements

The authors thank the Strategic Environmental Research & Development Program (SERDP) for funding this research, and Mr. Steven Larson of the U.S. Army Corps of Engineers for assistance in obtaining soils. In addition, the authors are grateful to Dr. Gerry Henningsen (H&H Scientific Services) for helping with the conceptual design of this study, and to Dr. William Brattin (Syracuse Research Corporation) for assistance with data analysis methods.

References

1. U.S. EPA. 2003. Guidance for developing ecological soil screening levels. U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response, Washington, DC.

2. U.S. EPA. 1997. Ecological risk assessment guidance for Superfund: Process for designing and conducting ecological risk assessments. Interim Final. U.S. Environmental Protection Agency, Environmental Response Team, Edison, NJ.

3. NRC. 2003. *Bioavailability of Contaminants in Soils and Sediments. Processes, Tools, and Applications*. The National Academy Press, Washington, DC.

4. Kelly ME, Brauning SE, Schoof RA, Ruby MV. 2002. Assessing oral bioavailability of metals in soil. Battelle Press, Columbus, Ohio.

5. Salatas JH, Lowney YW, Pastorok RA, Nelson RR, Ruby MV. 2004. Metals that drive health-based remedial decisions for soils at U.S. Department of Defense sites. *Human Ecol Risk Assess* 10(6):1117–1128.

6. U.S. EPA. 1993. Wildlife exposure factors handbook, vol I & II. EPA 600-R-93-187. U.S. Environmental Protection Agency, Washington, DC.

7. Churchfield S. 1990. *The Natural History of Shrews*. Comstock Publishing Associates, Ithaca, NY.

8. Brueske CC, Barrett GW. 1991. Dietary heavy metal uptake by the least shrew, *Cryptotis parva*. *Bull Environ Contam Toxicol* 47:845–849.

9. Freeman GB, Johnson JD, Killinger JM, Liao SC, Feder PI, Davis AO, Ruby MV, Chaney RL, Lovre SC, Bergstrom PD. 1992. Relative bioavailability of lead from mining waste soil in rats. *Fund Appl Toxicol* 19:388–398.

10. Edwards CA, Bohlen PJ. 1992. The effects of toxic chemicals on earthworms. *Rev Environ Contam Toxicol* 125:23–99.

11. Mehra OP, Jackson ML. 1960. Iron oxide removal from soils and clays by a dithionite-citrate system buffered with sodium citrate. *Clay Minerals* 7:317–327.

12. Darmani NA. 1998. Serotonin 5-HT_3 receptor antagonists prevent cisplatin-induced emesis in Cryptotis parva: A new experimental model of emesis. *J Neural Transmission* 105:1143–1154.

13. Dixon WJ. 1953. Processing data for outliers. *Biometrics* 9:74–89.

14. Finney DJ. 1978. *Statistical Methods in Biological Assay* (3rd Edition). Charles Griffin and Co., London.

15. Draper NR., Smith H. 1998. *Applied Regression Analysis* (3rd Edition). John Wiley & Sons, New York.

16. Casteel SW, Brown LD, Dunsmore ME. 1997. Relative bioavailability of arsenic in mining wastes. Document Control Number 4500-88-AORH. Veterinary Medical Diagnostic Laboratory, College of Veterinary Medicine, University of Missouri, Columbia, MO.

17. Roberts SM, Weimar WR, Vinson JRT, Munson JW, Bergeron RJ. 2002. Measurement of arsenic bioavailability in soil using a primate model. *Toxicol Sci* 67:303–310.

18. Schoof RA, Casteel SW, Evans TJ, Ruby MV, Lowney YW, Cardenas A. 2005. Soil cadmium bioavailability as a function of mineralogy and soil characteristics. *Environ Sci Technol* (in press). Rick: This isn't really in press, it's actually just about to get submitted (I think, you'd have to check with Yvette). Not sure what to do on this one.

19. Ruby MV, Davis A, Link TE, Schoof R, Chaney RL, Freeman GB, Bergstrom P. 1993. Development of an *in vitro* screening test to evaluate the *in vivo* bioaccessibility of ingested mine-waste lead. *Environ Sci Technol* 27(13):2870–2877.

List of Tables

Table 1. Shrew mortality rates

Table 2. Characterization data for soils dosed to shrews

Table 3. Average doses (µg/kg-day) received by each dose group

Table 4. Shrew body-weight changes

Table 5. Summary of relative bioavailability estimates

List of Figures

Figure 1. Dose-response for lead in all four test soils dosed to shrew

Figure 2. Dose-response graph for arsenic in the DoD-DP soil

Figure 3. Dose-response graph for arsenic in the Orchard soil

Figure 4. Dose-response graph for cadmium in the DoD-DP soil

Figure 5. Dose-response graph for chromium in the DoD-PM soil

Figure 6. Dose-response graph for chromium in the Smelter soil

Table 1. Shrew mortality rates

	DoD-PM			DoD-DP			Orchard			Smelter		
	Initial n	Final n	Mortality Rate	Initial n	Final n	Mortality Rate	Initial n	Final n	Mortality Rate	Initial n	Final n	Mortality Rate
Standard Diet	19	6	68%	16	8	50%	12	11	8%	11	11	0%
Soil Exposure 25%	12	9	25%	12	10	17%	12	9	25%	11	10	9%
Reference Material 25%	12	7	42%	12	9	25%	12	10	17%	11	11	0%
Soil Exposure 50%	13	8	38%	12	11	8%	12	9	25%	11	11	0%
Reference Material 50%	12	10	17%	12	10	17%	12	10	17%	11	11	0%
Soil Exposure 100%	17	7	59%	12	7	42%	12	11	8%	11	7	36%
Reference Material 100%	12	8	33%	12	12	0%	12	11	8%	11	10	9%
Total	97	55	43%	88	67	24%	84	71	15%	77	71	8%

Table 2. Characterization data for soils dosed to shrews

Chemical	Units	DoD-PM	DoD-DP	Orchard	Smelter
Conventionals					
pH	s.u.	7.6	8.0	5.9	7.5
Total organic carbon	%	0.75	3.26	2.98	2.09
Total inorganic carbon	%	0.53	1.02	1.27	0.05 *U*
Cation exchange capacity	meq/100g	46.2	71.3	74.0	52.3
DCB extractable iron	mg/kg	3,830	12,240	5,630	5,110
Particle Size Distribution					
Coarse sand (425 – 2,000 µm)	%	42.3	43.1	28.0	19.8
Medium sand (250 – 425 µm)	%	30.3	12.1	17.9	13.8
Fine sand (75 – 250 µm)	%	21.9	28.3	25.2	35.7
Silt (4 – 75 µm)	%	2.2	15.4	26.9	27.5
Clay (< 4 µm)	%	3.3	1.1	2.0	3.2
Inorganics					
Arsenic	mg/kg	82	60	284	331
Cadmium	mg/kg	1,755	14	2.4	423
Chromium	mg/kg	8,362	79	36	1850
Chromium, hexavalent	mg/kg	1.1	0.5 *U*	0.5 *U*	1,355
Iron	mg/kg	9,330	20,900	22,300	15,800
Lead	mg/kg	569	257	2640	585
Manganese	mg/kg	78	498	394	479
Mercury	mg/kg	0.85	11.3	0.04	7.4
Nickel	mg/kg	1,870	41	16	13
Phosphorus	mg/kg	1,710	1,560	887	673
Zinc	mg/kg	706	356	286	1,200

Table 3. Average doses (µg/kg-day) received by each dose group

	Standard Diet	25% Doses		50% Doses		100% Doses		Ratio of Soil:Reference Material		
		Soil	Reference Material	Soil	Reference Material	Soil	Reference Material	25% Doses	50% Doses	100% Doses
DoD-PM										
Arsenic	150	169	212	214	228	308	367	0.80	0.93	0.84
Cadmium	20	1,003	994	1,949	1,865	3,856	3,671	1.01	1.04	1.05
Chromium	595	5,291	5,380	11,025	10,510	18,458	19,873	0.98	1.05	0.93
Lead	73	319	366	726	688	1,252	1,346	0.87	1.05	0.93
DoD-DP										
Arsenic	127	768	628	1,536	1,224	3,061	2,227	1.22	1.26	1.37
Cadmium	17	106	117	248	224	455	435	0.91	1.11	1.04
Chromium	506	1,375	1,272	2,371	1,947	3,965	3,363	1.08	1.22	1.18
Lead	62	2,390	2,218	4,979	4,731	10,266	9,224	1.08	1.05	1.11
Orchard										
Arsenic	97	1,586	1,808	3,525	3,484	6,236	6,199	0.88	1.01	1.01
Cadmium	16	17	36	20	60	20	87	-- [b]	-- [b]	-- [b]
Chromium	518	669	518	1,022	514	1,351	477	-- [b]	-- [b]	-- [b]
Lead	76	12,472	13,792	30,251	29,834	59,056	56,846	0.90	1.01	1.04
Smelter										
Arsenic	173	5,042	5,083	7,790	10,702	16,919	18,263	0.99	0.73	0.93
Cadmium	20	4,220	4,319	8,084	9,284	17,174	15,977	0.98	0.87	1.07
Chromium	564	20,127	603	42,556	627	89,738	504	-- [a]	-- [a]	-- [a]
Lead	56	5,817	6,344	11,898	13,702	25,077	23,288	0.92	0.87	1.08

[a] Ratio not relevant because the soil was spiked with 1,835 mg/kg Cr, but the reference material was not spiked with Cr because it caused a precipitate to form (possibly $PbCrO_4$) in the reference solution.

[b] Ratio not relevant because Cd and Cr were not added to the reference material matched to the Orchard soil, because there was insufficient Cd and Cr in the Orchard soil to be detectable post-dosing in shrew tissue.

Table 4. Shrew body-weight changes

	DoD-PM			DoD-DP			Orchard			Smelter		
	Initial (g)	Final (g)	Percent change	Initial (g)	Final (g)	Percent change	Initial (g)	Final (g)	Percent change	Initial (g)	Final (g)	Percent change
Standard Diet	4.5	4.0	-11%	4.5	5.3	18%	4.7	4.4	-6%	4.7	4.6	-2%
Soil Exposure 25%	5.0	4.4	-12%	5.1	5.9	16%	4.9	4.8	-2%	5.0	4.5	-10%
Reference Material 25%	4.6	4.2	-9%	4.6	5.4	17%	4.6	4.6	0%	5.2	4.9	-6%
Soil Exposure 50%	4.7	3.8	-19%	4.4	5.4	23%	4.4	4.3	-2%	4.8	4.2	-13%
Reference Material 50%	4.2	4.1	-2%	4.6	4.9	7%	4.4	4.5	2%	4.8	4.2	-13%
Soil Exposure 100%	4.3	4.1	-5%	4.4	4.7	7%	4.7	4.5	-4%	5.1	3.8	-25%
Reference Material 100%	4.7	4.5	-4%	4.5	5.0	11%	5.0	4.9	-2%	5.7	4.8	-16%
Average	4.6	4.2	-9%	4.6	5.2	14%	4.7	4.6	-2%	5.0	4.4	-12%

Table 5. Summary of relative bioavailability estimates

	DoD-PM	DoD-DP	Orchard	Smelter
Arsenic				
RBA	not significant	0.07	0.49	0.31
outliers	none	2	none	none
Lower bound	--	-0.10	0.33	0.20
Upper bound	--	0.21	0.65	0.45
Standard Error	--	0.09	0.10	0.08
p-value (adj. R-sq.)	0.76 (-2.7%)	<0.001 (62%)	<0.001 (58%)	<0.001 (59%)
Cadmium				
RBA	0.81	0.13	not significant	0.66
outliers	1	none	2	3
Lower bound	0.65	-0.003	--	0.57
Upper bound	0.99	0.24	--	0.76
Standard Error	0.10	0.07	--	0.05
p-value (adj. R-sq.)	<0.001 (78%)	<0.001 (80%)	0.34 (0.34%)	<0.001 (90%)
Chromium				
RBA	not significant	1.9	not significant	-0.02
outliers	none	1	none	1
Lower bound	--	--	--	--
Upper bound	--	--	--	--
Standard Error	--	--	--	--
p-value (adj. R-sq.)	0.76 (-1.7%)	0.028 (7.9%)	0.47 (-0.88%)	<0.001 (72%)
Lead				
RBA	0.21	0.34	0.60[a]	0.51
outliers	none	none	1	1
Lower bound	--	0.26	0.53	0.40
Upper bound	--	0.42	0.69	0.62
Standard Error	--	0.05	0.05	0.06
p-value (adj.R-sq.)	<0.001 (35%)	<0.001 (85%)	<0.001 (80%)	<0.001 (79%)

-- Fieller's theorem does not apply or provides uncertain results so no standard error was calculated

[a] - As described in text, relative bioavailability of lead in Orchard soil was calculated using the average lead acetate dose response from the other three dosing trials (I.e., those for DoD-PM, DoD-DP, and Smelter soils).

Figure 1. Dose-response for lead in all four test soils dosed to shrew

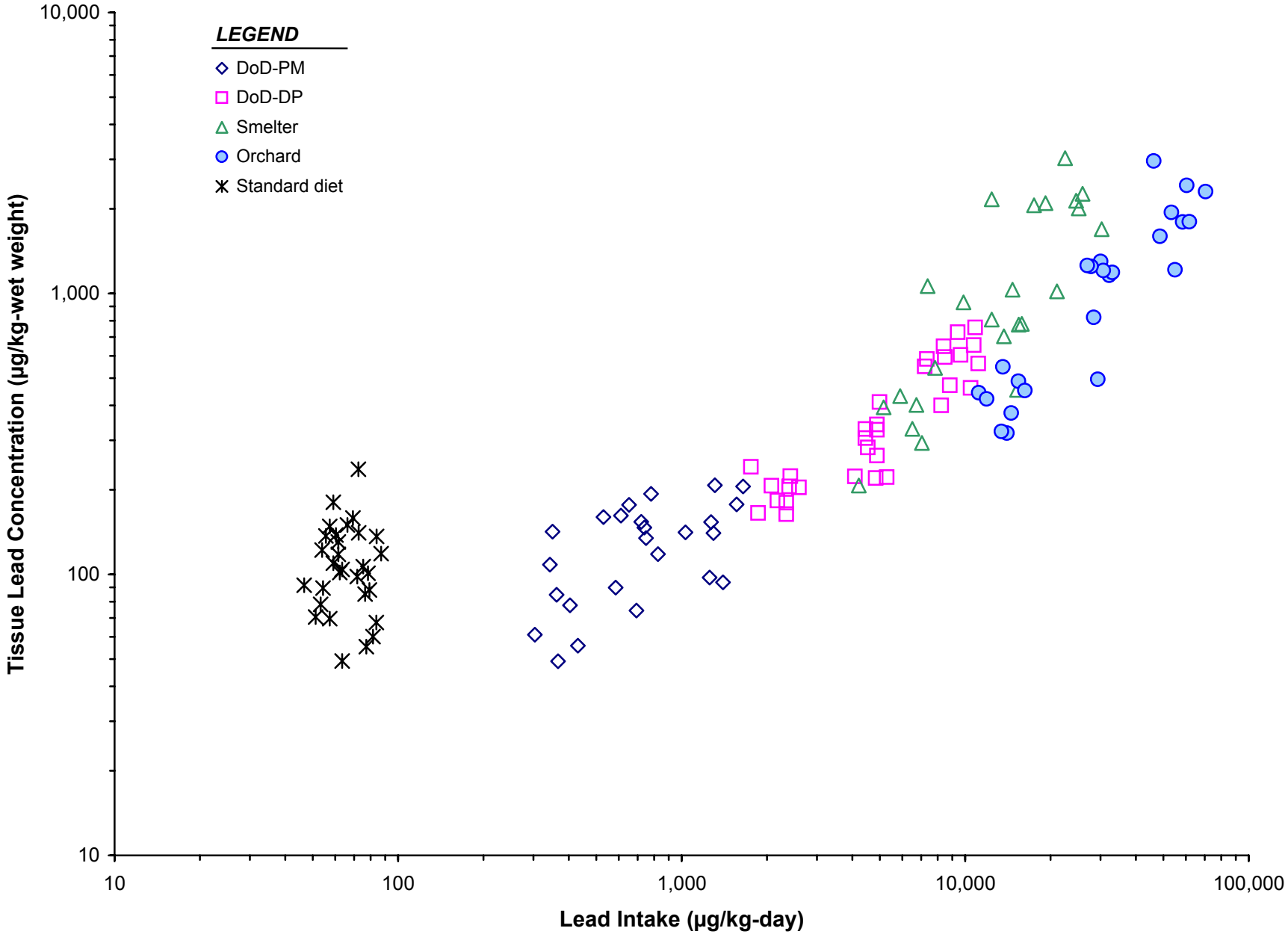


Figure 2. Dose-response graph for arsenic in the DoD-DP soil

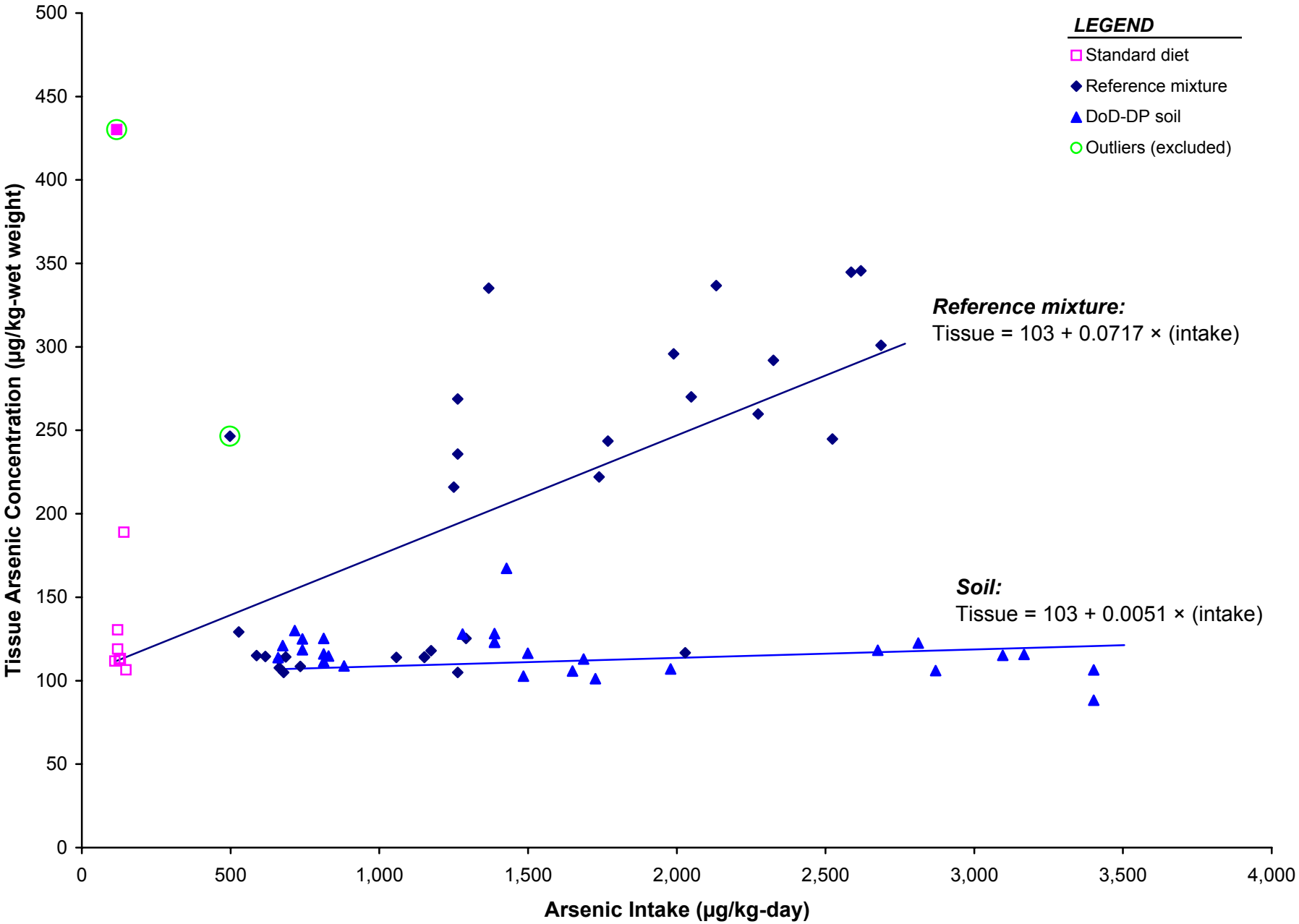


Figure 3. Dose-response graph for arsenic in the Orchard soil

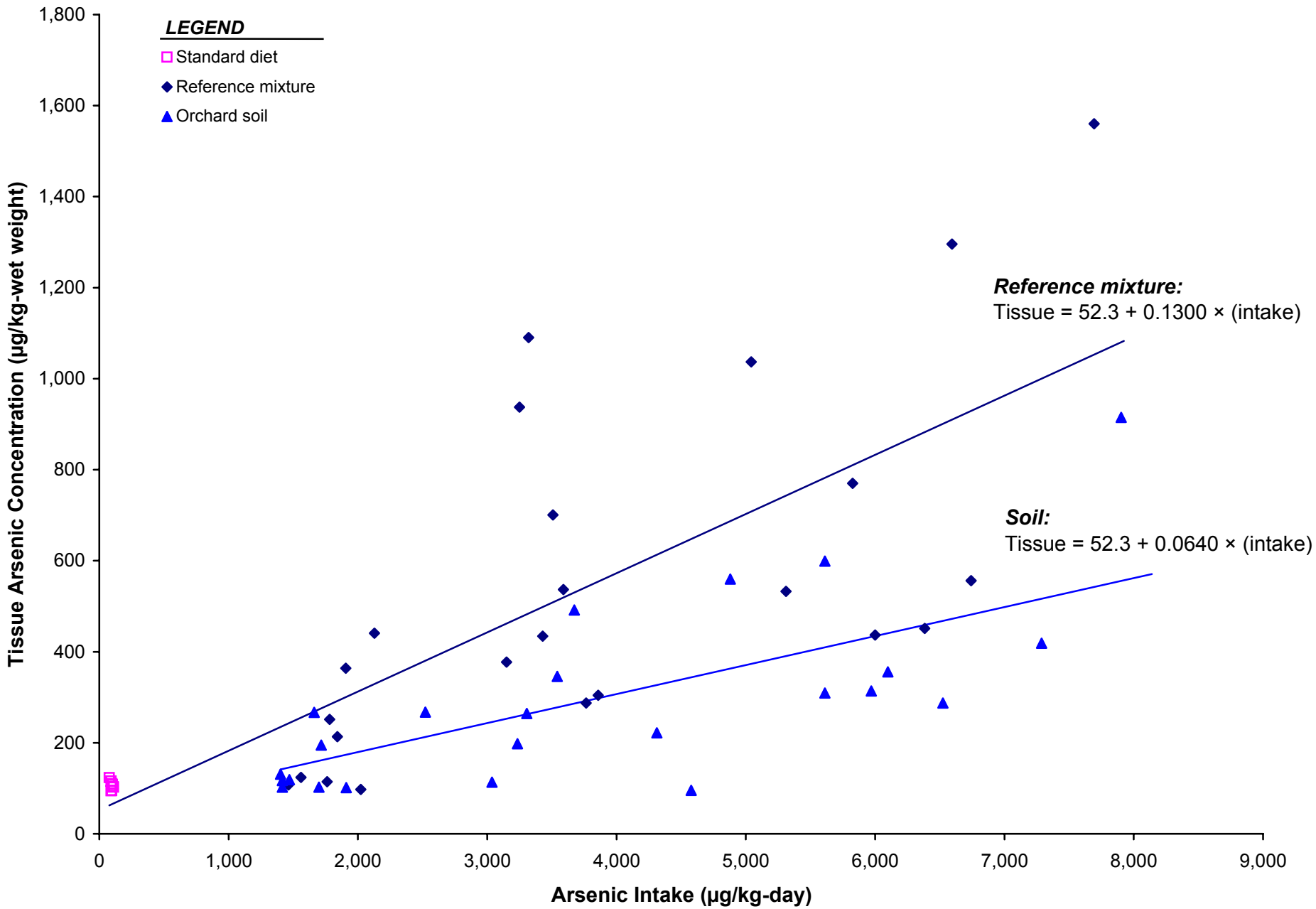


Figure 4. Dose-response graph for cadmium in the DoD-DP soil

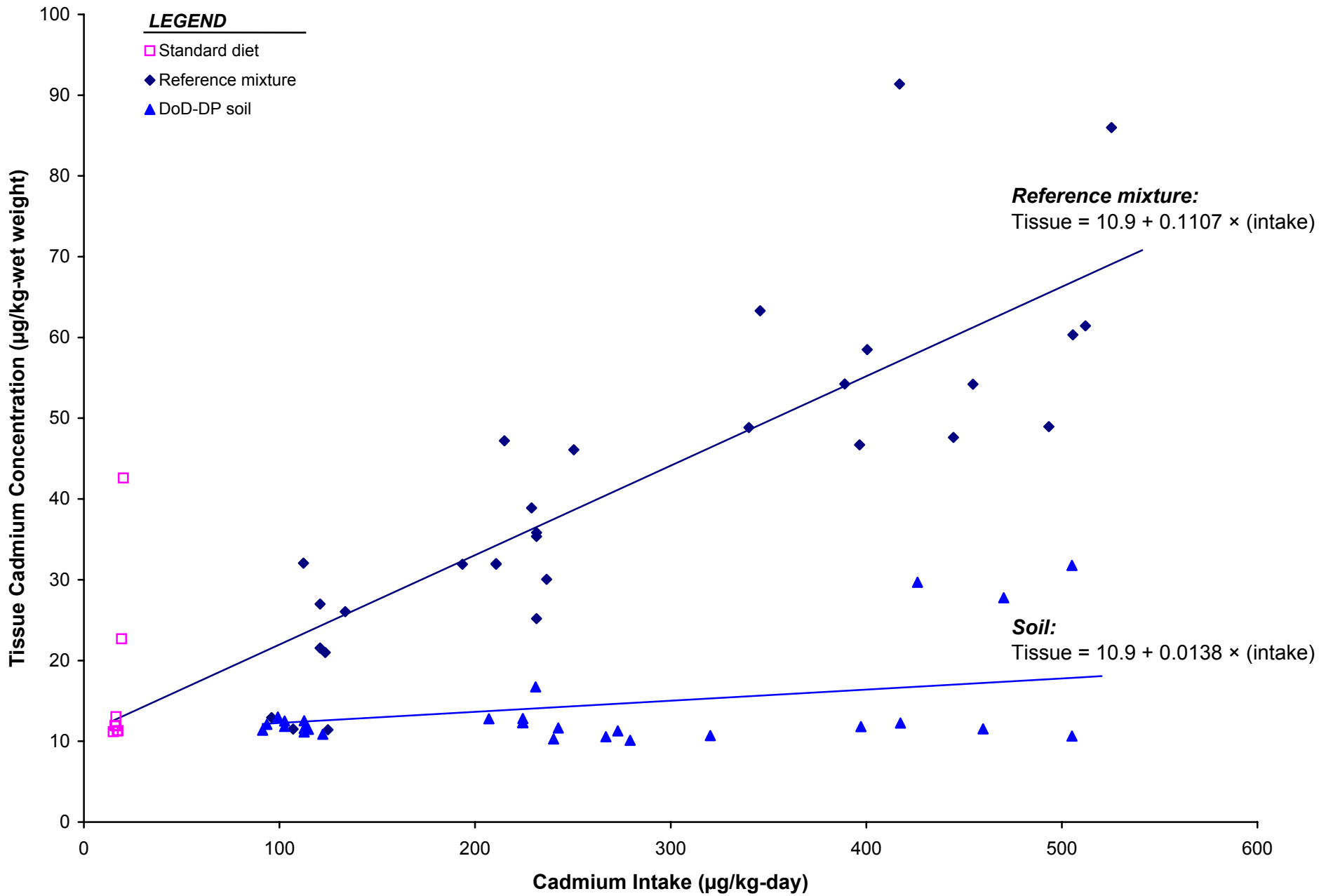


Figure 5. Dose-response for chromium in the DoD-PM soil

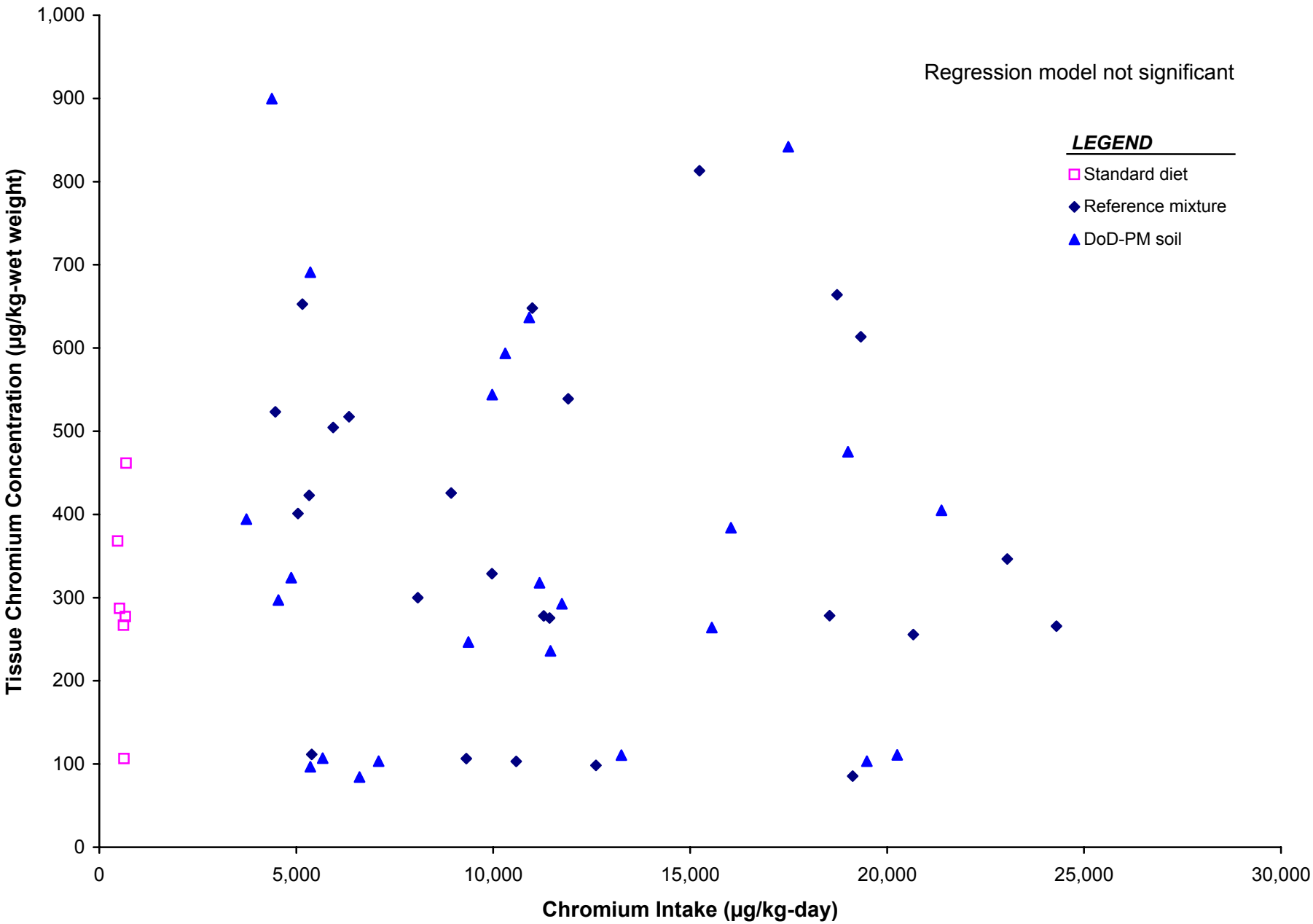
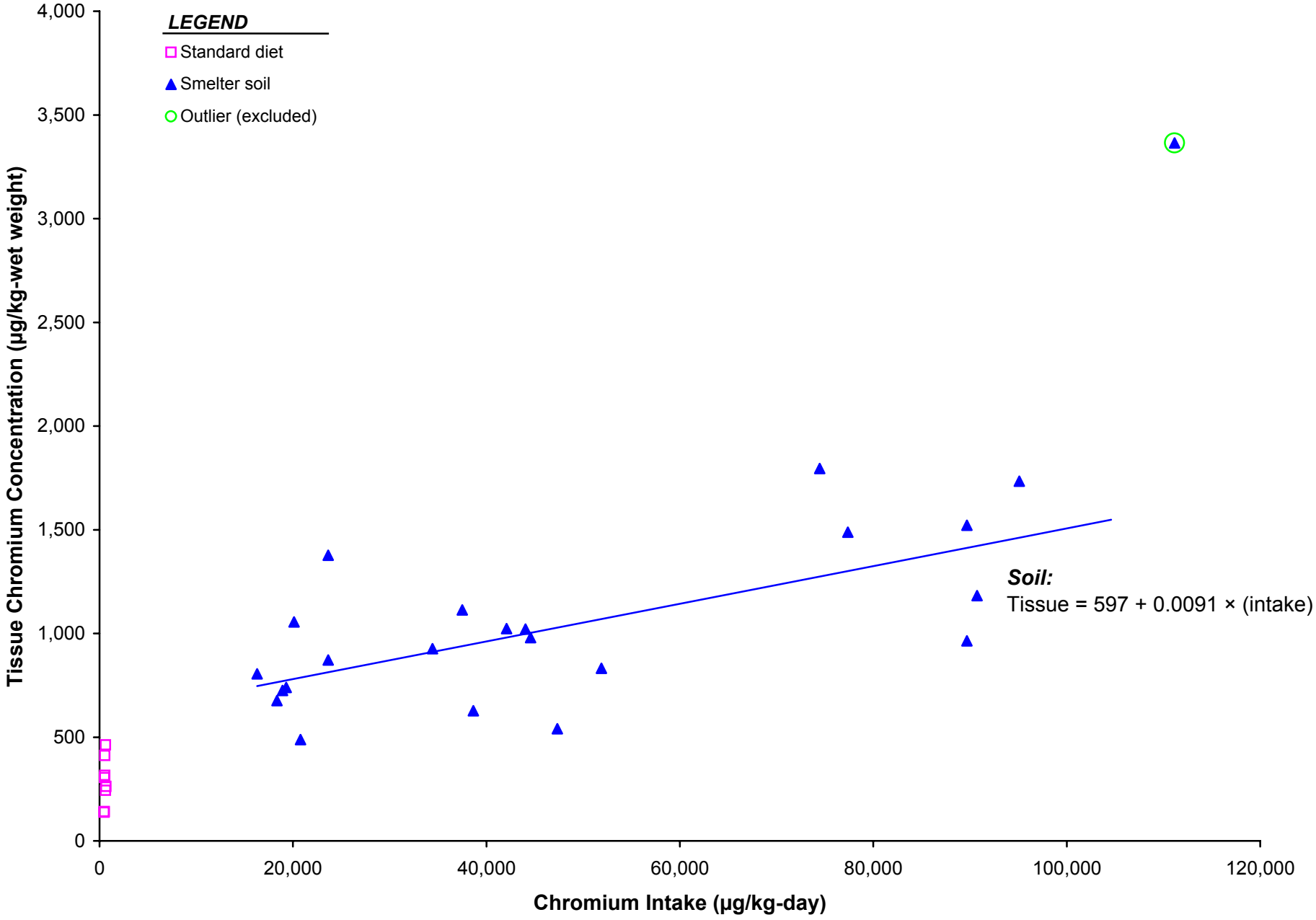


Figure 6. Dose-response for chromium in the Smelter soil



Supplemental Materials for Section 7

Solubility/Bioavailability Research Consortium

Standard Operating Procedure:

In Vitro Method for Determination of Lead and Arsenic Bioaccessibility

Doc. No.: 8601102.001 0601 1099 RN01

Contents

	Page
1. Introduction	1
1.1 Synopsis	1
1.2 Purpose	1
2. Procedure	3
2.1 Sample Preparation	3
2.2 Apparatus and Materials	3
2.2.1 Equipment	3
2.2.2 Standards and Reagents	4
2.3 Leaching Procedure	5
2.4 Calculation of the Bioaccessibility Value	7
2.5 Chain-of-Custody/Good Laboratory Practices	7
2.6 Data Handling and Verification	8
3. Quality Control Procedures	9
3.1 Elements of Quality Assurance and Quality Control (QA/QC)	9
3.2 QA/QC Procedures	10
3.2.1 Laboratory Control Sample (LCS)	10
3.2.2 Reagent Blanks/Bottle Blanks/Blank Spikes	10
4. References	12

Attachment A – Extraction Test Checklist Sheets

1. Introduction

1.1 Synopsis

This SOP describes an *in vitro* laboratory procedure to determine a bioaccessibility value for lead or arsenic (i.e., the fraction that would be soluble in the gastrointestinal tract) for soils and solid waste materials. A recommended quality assurance program to be followed when performing this extraction procedure is also provided.

1.2 Purpose

An increasingly important property of materials/soils found at contaminated sites is the bioavailability of individual contaminants. Bioavailability is the fraction of a contaminant in a particular environmental matrix that is absorbed by an organism via a specific exposure route. Many animal studies have been conducted to experimentally determine the oral bioavailability of individual metals, particularly lead and arsenic. During the period 1989–1997, a juvenile swine model developed by EPA Region VIII was used to predict the relative bioavailability of lead and arsenic in approximately 20 soils/solid materials (Weis and LaVelle 1991; Weis et al. 1994; Casteel et al. 1997a,b). The bioavailability determined was relative to that of a soluble salt (i.e., lead acetate trihydrate or sodium arsenate). The tested materials had a wide range of mineralogy, and produced a range of lead and arsenic bioavailability values. In addition to the swine studies, other animal models (e.g., rats and monkeys) have been used to measure the bioavailability of lead and arsenic from soil.

Several researchers have developed *in vitro* tests to measure the fraction of a chemical solubilized from a soil sample under simulated gastrointestinal conditions. This measurement is referred to as "bioaccessibility" (Ruby et al. 1993). Bioaccessibility is thought to be an important determinant of bioavailability, and several groups have sought

to compare bioaccessibility determined in the laboratory to bioavailability determined in animal studies (Imber 1993; Ruby et al. 1996; Medlin 1997; Rodriguez et al. 1999). The *in vitro* tests consist of an aqueous fluid, into which soils containing lead and arsenic are introduced. The solution then solubilizes the soil under simulated gastric conditions. Once this procedure is complete, the solution is analyzed for lead and/or arsenic concentration. The mass of lead and/or arsenic found in the aqueous phase, as defined by filtration at the 0.45-μm pore size, is compared to the mass introduced into the test. The fraction liberated into the aqueous phase is defined as the bioaccessible fraction of lead or arsenic in that soil. To date, for lead-bearing soils tested in the EPA swine studies, this *in vitro* method has correlated well with relative bioavailability values.

2. Procedure

2.1 Sample Preparation

All soil/material samples should be prepared for testing by oven drying (<40 °C) and sieving to <250 μm. The <250-μm size fraction is used because this particle size is representative of that which adheres to children's hands. Subsamples for testing in this procedure should be obtained using a sample splitter.

2.2 Apparatus and Materials

2.2.1 Equipment

The main piece of equipment required for this procedure consists of a Toxicity Characteristic Leaching Procedure (TCLP) extractor motor that has been modified to drive a flywheel. This flywheel in turn drives a Plexiglass block situated inside a temperature-controlled water bath. The Plexiglass block contains ten 5-cm holes with stainless steel screw clamps, each of which is designed to hold a 125-mL wide-mouth high-density polyethylene (HDPE) bottle (see Figure 1). The water bath must be filled such that the extraction bottles are immersed. Temperature in the water bath is maintained at 37±2 °C using an immersion circulator heater (for example, Fisher Scientific Model 730). Additional equipment for this method includes typical laboratory supplies and reagents, as described in the following sections.

The 125-mL HDPE bottles must have an air-tight screw-cap seal (for example, Fisher Scientific 125-mL wide-mouth HDPE Cat. No. 02-893-5C), and care must be taken to ensure that the bottles do not leak during the extraction procedure.

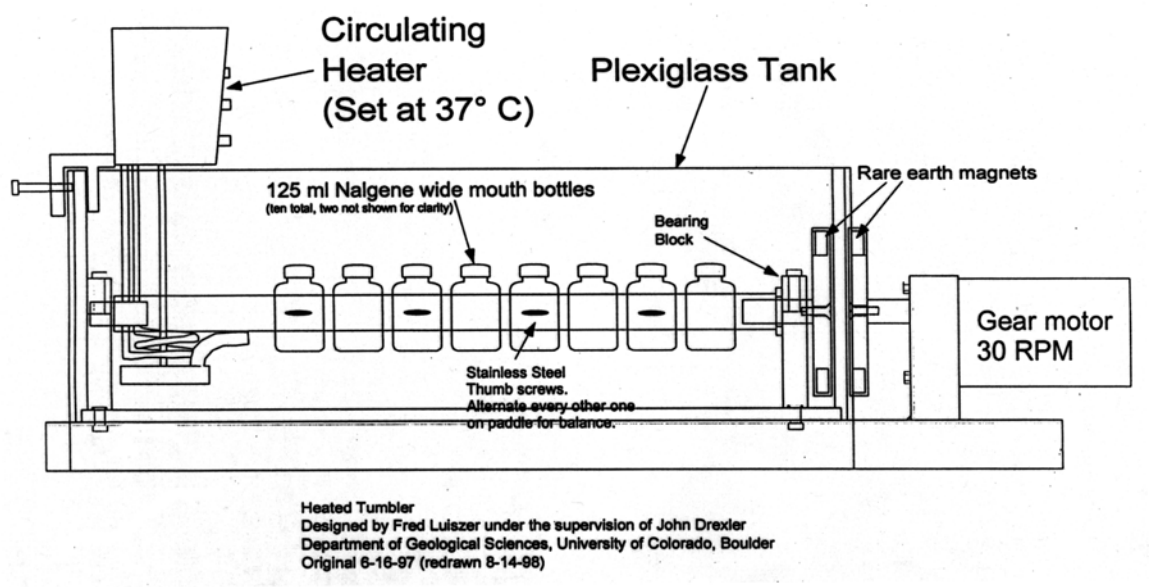


Figure 1. Extraction device for performing the SBRC *in vitro* extraction

2.2.2 Standards and Reagents

The leaching procedure for this method uses a buffered extraction fluid at a pH of 1.5. The extraction fluid is prepared as described below.

The extraction fluid should be prepared using ASTM Type II deionized (DI) water. To 1.9 L of DI water, add 60.06 g glycine (free base, Sigma Ultra or equivalent). Place the mixture in a water bath at 37 °C until the extraction fluid reaches 37 °C. Standardize the pH meter using temperature compensation at 37 °C or buffers maintained at 37 °C in the water bath. Add concentrated hydrochloric acid (12.1 N, Trace Metal grade) until the solution pH reaches a value of 1.50 ±0.05 (approximately 120 mL). Bring the solution to a final volume of 2 L (0.4 M glycine).

Cleanliness of all reagents and equipment used to prepare and/or store the extraction fluid is essential. All glassware and equipment used to prepare standards and reagents must be properly cleaned, acid washed, and finally, rinsed with DI water prior to use. All

reagents must be free of lead and arsenic, and the final fluid should be tested to confirm that lead and arsenic concentrations are less than 25 and 5 μg/L, respectively.

2.3 Leaching Procedure

Measure 100 ±0.5 mL of the extraction fluid, using a graduated cylinder, and transfer to a 125-mL wide-mouth HDPE bottle. Add 1.00 ±0.05 g of test substrate (<250 μm) to the bottle, ensuring that static electricity does not cause soil particles to adhere to the lip or outside threads of the bottle. If necessary, use an antistatic brush to eliminate static electricity prior to adding the soil. Record the volume of solution and mass of soil added to the bottle on the extraction test checklist (see Attachment A for example checklists). Hand-tighten each bottle top, and shake/invert to ensure that no leakage occurs, and that no soil is caked on the bottom of the bottle.

Place the bottle into the modified TCLP extractor, making sure each bottle is secure and the lid(s) are tightly fastened. Fill the extractor with 125-mL bottles containing test materials or Quality Control samples.

The temperature of the water bath must be 37±2 °C. Record the temperature of the water bath at the beginning and end of each extraction batch on the appropriate extraction test checklist sheet (see Attachment A).

Rotate the extractor end over end at 30±2 rpm for 1 hour. Record start time of rotation.

When extraction (rotation) is complete, immediately remove bottles, wipe them dry, and place them upright on the bench top.

Draw extract directly from reaction vessel into a disposable 20-cc syringe with a Luer-Lok attachment. Attach a 0.45-μm cellulose acetate disk filter (25 mm diameter) to the syringe, and filter the extract into a clean 15-mL polypropylene centrifuge tube or other

appropriate sample vial for analysis. Store filtered sample(s) in a refrigerator at 4 °C until they are analyzed.

Record the time that the extract is filtered (i.e., extraction is stopped). If the total elapsed time is greater than 1 hour 30 minutes, the test must be repeated.

Measure and record the pH of fluid remaining in the extraction bottle. If the fluid pH is not within ±0.5 pH units of the starting pH, the test must be discarded and the sample reanalyzed as follows.

If the pH has dropped by 0.5 or more pH units, the test will be re-run in an identical fashion. If the second test also results in a decrease in pH of greater than 0.5 s.u., the pH will be recorded, and the extract filtered for analysis. If the pH has increased by 0.5 or more units, the test must be repeated, but the extractor must be stopped at specific intervals and the pH manually adjusted down to pH 1.5 with dropwise addition of HCl (adjustments at 5, 10, 15, and 30 minutes into the extraction, and upon final removal from the water bath [60 minutes]). Samples with rising pH values must be run in a separate extraction, and must not be combined with samples being extracted by the standard method (continuous extraction).

Extracts are to be analyzed for lead and arsenic concentration using analytical procedures taken from the U.S. EPA publication, *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods. SW-846*. (current revisions). Inductively coupled plasma (ICP) analysis, method 6010B (December 1996 revision) will be the method of choice. This method should be adequate for determination of lead concentrations in sample extracts, at a project-required detection limit (PRDL) of 100 μg/L. The PRDL of 20 μg/L for arsenic may be too low for ICP analysis for some samples. For extracts that have arsenic concentrations less than five times the PRDL (e.g., <100 μg/L arsenic), analysis by ICP-hydride generation (method 7061A, July 1992 revision) or ICP-MS (method 6020, September 1994 revision) will be required.

2.4 Calculation of the Bioaccessibility Value

A split of each solid material (<250 μm) that has been subjected to this extraction procedure should be analyzed for total lead and/or arsenic concentration using analytical procedures taken from the U.S. EPA publication, *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods. SW-846*. (current revisions). The solid material should be acid digested according to method 3050A (July 1992 revision) or method 3051 (microwave-assisted digestion, September 1994 revision), and the digestate analyzed for lead and/or arsenic concentration by ICP analysis (method 6010B). For samples that have arsenic concentrations below ICP detection limits, analysis by ICP-hydride generation (method 7061A, July 1992 revision) or ICP-MS (method 6020, September 1994 revision) will be required.

The bioaccessibility of lead or arsenic is calculated in the following manner:

$$Bioaccessibility\ value = \frac{(concentration\ in\ in\ vitro\ extract, mg/L)\,(0.1L)}{(concentration\ in\ solid, mg/kg)\ (0.001\ kg)} \times 100$$

2.5 Chain-of-Custody/Good Laboratory Practices

All laboratories that use this SOP should receive test materials with chain-of-custody documentation. When materials are received, each laboratory will maintain and record custody of samples at all times. All laboratories that perform this procedure should follow good laboratory practices as defined in 40 CFR Part 792 to the extent practical and possible.

2.6 Data Handling and Verification

All sample and fluid preparation calculations and operations should be recorded in bound and numbered laboratory notebooks, and on extraction test checklist sheets. Each page must be dated and initialed by the person who performs any operations. Extraction and filtration times must be recorded, along with pH measurements, adjustments, and buffer preparation. Copies of the extraction test checklist sheets should accompany the data package.

3. Quality Control Procedures

3.1 Elements of Quality Assurance and Quality Control (QA/QC)

A standard method for the *in vitro* extraction of soils/solid materials, and the calculation of an associated bioaccessibility value, are specified above. Associated QC procedures to ensure production of high-quality data are as follows (see Table 1 for summary of QC procedures, frequency, and control limits):

- Reagent blank—Extraction fluid analyzed once per batch.
- Bottle blank—Extraction fluid only run through the complete extraction procedure at a frequency of no less than 1 per 20 samples or one per extraction batch, whichever is more frequent.
- Blank spikes—Extraction fluid spiked at 10 mg/L lead and/or 1 mg/L arsenic and run through the extraction procedure at a frequency of no less than every 20 samples or one per extraction batch, whichever is more frequent. Blank spikes should be prepared using traceable 1,000-mg/L lead and arsenic standards in 2 percent nitric acid.
- Duplicate—duplicate extractions are required at a frequency of 1 for every 10 samples. At least one duplicate must be performed on each day that extractions are conducted.
- Standard Reference Material (SRM)—National Institute of Standards and Technology (NIST) material 2711 (Montana Soil) should be used as a laboratory control sample (LCS).

Control limits for these QC samples are delineated in Table 1, and in the following discussion.

Table 1. Summary of QC samples, frequency of analysis, and control limits

QC Sample	Minimum Frequency of Analysis	Control Limits
Reagent Blank	Once per batch (min. 5%)	<25 μg/L lead <5 μg/L arsenic
Bottle Blank	Once per batch (min. 5%)	<50 μg/L lead <10 μg/L arsenic
Blank Spike	Once per batch (min. 5%)	85–115% recovery
Duplicate	10%	±20% RPD
SRM (NIST 2711)	2%	9.22 ±1.50 mg/L Pb 0.59 ±0.09 mg/L As

3.2 QA/QC Procedures

Specific laboratory procedures and QC steps are described in the analytical methods cited in Section 2.3, and should be followed when using this SOP.

3.2.1 Laboratory Control Sample (LCS)

The NIST SRM 2711 should be used as a laboratory control sample for the *in vitro* extraction procedure. Analysis of 18 blind splits of NIST SRM 2711 (105 mg/kg arsenic and 1,162 mg/kg lead) in four independent laboratories resulted in arithmetic means ± standard deviations of 9.22 ±1.50 mg/L lead and 0.59 ±0.09 mg/L arsenic. This SRM is available from the National Institute of Standards and Technology, Standard Reference Materials Program, Room 204, Building 202, Gaithersburg, Maryland 20899 (301/975-6776).

3.2.2 Reagent Blanks/Bottle Blanks/Blank Spikes

Reagent blanks must not contain more than 5 μg/L arsenic or 25 μg/L lead. Bottle blanks must not contain arsenic and/or lead concentrations greater than 10 and 50 μg/L,

respectively. If either the reagent blank or a bottle blank exceeds these values, contamination of reagents, water, or equipment should be suspected. In this case, the laboratory must investigate possible sources of contamination and mitigate the problem before continuing with sample analysis. Blank spikes should be within 15% of their true value. If recovery of any blank spike is outside this range, possible errors in preparation, contamination, or instrument problems should be suspected. In the case of a blank spike outside specified limits, the problems must be investigated and corrected before continuing sample analysis.

4. References

Casteel, S.W., R.P. Cowart, C.P. Weis, G.M. Henningsen, E. Hoffman, et al. 1997a. Bioavailability of lead in soil from the Smuggler Mountain site of Aspen, Colorado. Fund. Appl. Toxicol. 36:177–187.

Casteel, S.W., L.D. Brown, M.E. Dunsmore, C.P. Weis, G.M.Henningsen, E. Hoffman, W.J. Brattin, and T.L. Hammon. 1997b. Relative bioavailability of arsenic in mining waste. U.S. Environmental Protection Agency, Region VIII, Denver, CO.

Imber, B.D. 1993. Development of a physiologically relevant extraction procedure. Prepared for BC Ministry of Environment, Lands and Parks, Environmental Protection Division, Victoria, BC. CB Research International Corporation, Sidney, BC.

Medlin, E.A. 1997. An *in vitro* method for estimating the relative bioavailability of lead in humans. Masters thesis. Department of Geological Sciences, University of Colorado, Boulder.

Rodriguez, R.R., N.T. Basta, S.W. Casteel, and L.W. Pace. 1999. An in vitro gastrointestinal method to estimate bioavailable arsenic in contaminated soils and solid media. Environ. Sci. Technol. 33(4):642–649.

Ruby, M.W., A. Davis, T.E. Link, R. Schoof, R.L. Chaney, G.B. Freeman, and P. Bergstrom. 1993. Development of an *in vitro* screening test to evaluate the *in vivo* bioaccessibility of ingested mine-waste lead. Environ. Sci. Technol. 27(13):2870–2877.

Ruby, M.W., A. Davis, R. Schoof, S. Eberle, and C.M. Sellstone. 1996. Estimation of lead and arsenic bioavailability using a physiologically based extraction test. Environ. Sci. Technol. 30(2):422–430.

Weis, C.P., and J.M. LaVelle. 1991. Characteristics to consider when choosing an animal model for the study of lead bioavailability. In: Proceedings of the International Symposium on the Bioavailability and Dietary Uptake of Lead. Sci. Technol. Let. 3:113–119.

Weis, C.P., R.H. Poppenga, B.J. Thacker, and G.M. Henningsen. 1994. Design of phamacokinetic and bioavailability studies of lead in an immature swine model. In: Lead in paint, soil, and dust: Health risks, exposure studies, control measures, measurement methods, and quality assurance, ASTM STP 1226, M.E. Beard and S.A. Iske (Eds.). American Society for Testing and Materials, Philadelphia, PA, 19103-1187.

Attachment A:

Extraction Test Checklist Sheets

Extraction Fluid Preparation

Date of Extraction Fluid Preparation:_____ Prepared by:_____

Extraction Fluid Lot #:_____

Component	Lot Number	Fluid Preparation 1L	Fluid Preparation 2L	Acceptance Range	Actual Quantity	Comments
Deionized Water		0.95 L (approx.)	1.9 L (approx.)	---		
Glycine		30.03±0.05 g	60.06±0.05g	---		
HCl [a]		60 mL (approx.)	120 mL (approx.)	---		
Final Volume	---	1 L (Class A, vol.)	2 L (Class A, vol.)	---		
Extraction Fluid pH value (@ 37°C)	---	1.50±0.05	1.50±0.05	1.45–1.55		

[a] Concentrated hydrochloric acid (12.1 N)

INVITRO PROCEDURE REQUIRED PARAMETERS:

Volume of extraction fluid (V) = 100 ±0.5 mL
Mass of test substrate (M) = 1.00 ±0.05 g
Temperature of water bath = 37 ±2 °C
Extraction time = 60 ±5 min

Extractor rotation speed = 30 ±2 rpm
Maximum elapsed time from extraction to filtration = 90 minutes
Maximum pH difference from start to finish (ΔpH)= 0.5 pH units
Spike solution concentrations: As = 1 mg/L; Pb = 10 mg/L

Date of Extraction:_____
Extraction Fluid Lot #:_____
Extracted by:_____

As Spike Solution Lot #:_____
Pb Spike Solution Lot #:_____

EXTRACTION LOG (Page 1 of 2)
[Complete 1 log for every batch of 20 samples]

	Sample Preparation		Extraction								Filtration	
Sample ID	V (mL)	M (g)	Start Time[a]	End Time[a]	Elapsed Time (min)	Start pH	End pH	ΔpH	Start Temp (°C)	End Temp (°C)	Time[a]	Time Elasped from extraction (min)
Acceptance Range	(95.5–100.5)	(0.95–1.05)	---	---	(55–65 min)	---	---	(≤ 0.5)	(35–39)	(35–39)		(Max = 90 min)

EXTRACTION LOG (Page 2 of 2)
[Complete 1 log for every batch of 20 samples]

Sample ID	Sample Preparation		Extraction								Filtration	
	V (mL)	M (g)	Start Time[a]	End Time[a]	Elapsed Time (min)	Start pH	End pH	ΔpH	Start Temp (°C)	End Temp (°C)	Time[a]	Time Elasped from extraction (min)
Acceptance Range	(95.5–100.5)	(0.95–1.05)	---	---	(55–65 min)	---	---	(≤ 0.5)	(35–39)	(35–39)		(Max = 90 min)

[a] 24-hour time scale

NOTES:

Analytical Procedures

QC Requirements:

QC Sample	Minimum Analysis Frequency	Control Limits	Corrective Action[a]
Reagent blank	once per batch (min. 5%)	< 25 µg/L Pb <5 µg/L As	Investigate possible sources of target analytes. Mitigate contamination problem before continuing analysis.
Bottle blank	once per batch (min. 5%)	< 50 µg/L Pb <10 µg/L As	Investigate possible sources of target analytes. Mitigate contamination problem before continuing analysis.
Blank spike	once per batch (min. 5%)	85–115%	Re-extract and reanalyze sample batch
Duplicate	10% (min. once/day)	±20% RPD	Re-homongenize, re-extract and reanalyze

RPD – Relative percent difference
a – Action required if control limits are not met