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Subject:	Fw: Transmittal of the Arsenic RBA Upper bound report technical document and policy memo
Date:	Friday, January 4, 2013 9:16:47 AM
Attachments:	TRW Mineralogical Report Final_508.pdf Arsenic Bioavailability POLICY Memorandum_12"20"12.pdf Arsenic Bioavailability SCIENCE Report_SRC_09"20"12.pdf AS-HI RBA Report Final 06`04`10_508.pdf Barber Orchard Swine RBA 9-18-09_508.pdf Iron King Swine RBA 02-25-10_SRC_508.pdf Mohr Orchard RBA Report_10`28`09_SRC_508.pdf NIST1_As RBA Report Final 3-13-09_508.pdf NIST 2710a Swine RBA 03"22"12.pdf PTX As-V RBA Report_Final 2005_508.pdf Transmittal Memo from Becki Clark to the Regions, dated December 31, 2012.pdf

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Happy New Year! Enjoy some of the cool science I have been working on for many years. I am so excited to bring science to think smarter about risk. I look forward to collaborating on our work in Superfund to address metals in soils issues. All the best in 2013

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Date: 01/04/2013 10:20 AM

Subject: Transmittal of the Arsenic RBA Upper bound report technical document and policy memo

National Superfund Program Managers,

On behalf of Becki Clark , Director of the Assessment and Remediation Division of the Office of Superfund Remediation and Technology Innovation (OSRTI) and the Technical Review Workgroup for Metals and Asbestos, Bioavailability Committee, I am transmitting the attached Transmittal Memorandum, technical report (OSWER Directive 9200.1-113) entitled "Compilation and Review of Data on Relative Bioavailability of Arsenic in Soil", and policy memorandum entitled "Recommendations for Default Value for Relative Bioavailability".

This report identifies and evaluates published literature relevant to estimating a relative bioavailability (RBA) value of arsenic in soil.

Based upon the analysis and external independent peer review, we have reached the following conclusions:

1) Currently available research information suggests that an RBA of arsenic in soils can be expected to be less than 100%.

2) Based upon evaluation of current data sets of arsenic RBAs in the US, the upper percentile of the data set results in a default RBA value of 60%.

3) The default RBA for arsenic in soils should only be used if site-specific assessments for arsenic RBA are not feasible.

The documents are posted on the EPA Technical Review Workgroup for Metals and Asbestos, Bioavailability Committee website located at http://www.epa.gov/superfund/bioavailability/guidance.htm.

Please contact Michele Burgess at (703) 603-9003, if you have questions or concerns.

Thank you

Nancy Jones

Acting Special Assistant

Office of Superfund Remediation and Technology Innovation

U.S. Environmental Protection Agency

703-603-8736

United States
Environmental Protection
Agency

Technical Review Workgroup for Metals and Asbestos: Bioavailability Committee. Mineralogical Report. XAS Data and Linear Combination Fitting Results

Bradley W. Miller and Kirk G. Scheckel

13 August 2012

Introduction

To enhance our understanding and capabilities to protect human health and safeguard the natural environment, the application of molecular-level spectroscopic techniques that are highly sensitive and non-destructive to sample integrity would provide definitive answers to complex environmental questions. One such atomic-level technique, X-ray absorption spectroscopy (XAS), works by bombarding an element of interest with a beam of high-energy particles from a synchrotron radiation source to excite and expel outer-shell electrons of the particular element of interest. When the outer-shell electrons are expelled, they emit an energy called fluorescence that can be measured by computer-controlled detectors. The data collected by the detector yield characteristic spectra that provide information such as oxidation state, number and type of nearest neighboring atoms, coordination environment, and interatomic bond distances. XAS can be used to probe most phases of matter including crystalline or amorphous solids, liquids, and gases thus making XAS one of the most versatile research tools to fully investigate the molecular nature of a wide variety of substances. XAS is an in-situ technique meaning one can analyze samples taken directly from the field without any alterations that may skew true results. This type of research enhances our understanding of the fate and transport of toxic elements in the environment.

X-ray absorption spectroscopy (XAS) has been used in many different studies to examine contaminates such as Pb in soils (Cotter-Howells et al., 1994, 1999; Ryan et al., 2001; Scheckel and Ryan 2004). The use of XAS can determine the speciation of element and quantify via comparison to reference spectra with statistical analyses such as linear combination fitting (LCF) or principle component analysis (PCA) to predict the mineralogical identification of the element (Beauchemin et al., 2002; Scheinost et al., 2002; Scheckel and Ryan, 2004). Speciation refers to its chemical form or species. This includes its redox state and physicochemical characteristics that are relevant to bioavailability. This information can be used in conjunction with additional experiments to predict the reaction of an element of interest in the environment or human body. The speciation and bioavailability of a metal play a significant role in the risk assessment of contaminated media.

This mineralogical report contains the result of XAS analyses with LCF predictions of the As minerals present from nine samples including residential soil, orchard soil, an agricultural soil and mining wastes. XAS analyses have been performed on more than 11 reference arsenic minerals and have been included in this study. The minerals used for the LCF predictions include the As minerals most commonly found under oxidizing and reducing conditions in soil environments and at the sites where the materials were collected.

Materials/ Methods

X-Ray Absorption Spectroscopy

X-ray absorption spectroscopy data were collected on samples from nine sites at the Materials Research Collaborative Access Team 10-BM beamline, Advanced Photon Source (Argonne National Laboratory). All samples were fractured with a mortar and pestle, passed through a 250 µm sieve, pressed into a 1 cm pellet, and mounted on Kapton tape. Data was collected using a 4-element Vortex florescence detector with several layers of aluminum foil shield to suppress florescence from other elements such as iron in the samples. Arsenic concentrations < 20 mg As kg^{-1} were determined to be below the detection limit of the Vortex detector in our experiments. Three As K_{α} (11874 eV) spectra were collected in fluorescence mode at room temperature for every soil with a NaAs(V) reference sample for calibration.

Data analysis was conducted using Athena software (Ravel and Newville 2005). Each replicate scan was calibrated against the NaAs(V) reference (11874 eV), merged, normalized, and converted to k space. Linear combination fitting (LCF) was used to identify the As species in each soil samples. The LCF models were performed using the normalized, derivative, and chi(k) spectra of the soil samples and reference standards. There were 14 reference minerals included in the LCF models (Table 1). The reference minerals include a mix of synthetic and natural minerals received from the Smithsonian National Museum of Natural History. The XAS spectra of the 14 reference minerals are shown in Figure 1. The LCF models predicts the As speciation in each soil as percentages of the reference minerals.

The results of the LCF analyses generate a model with the best fit (indicated by the lowest R-factor and reduced chi square values). The pH and elemental concentration of Method 3051a extractable elements in each sample was consulted when assessing the LCF predictions of As minerals present. In some cases, the LCF model predicted mineral phases unlikely to be the present. If the LCF model predicted As minerals that were not appropriate (e.g. Yukonite was predicted but 0 mg Ca kg^{-1} soils was reported from 3051a extractions and sample pH was very acidic) then the mineral phase is very unlikely to be present. Therefore, LCF models were perform again without the predicted mineral (Yukonite in this example) and the LCF model was repeated.

Results and Discussion

XAS Analyses

The As XAS spectra, both normalized and derivative data, are found in Figure 2. Analyses of the samples collected from surface soil horizons or from mining activities had strong peaks at binding energies around 11875 eV. This demonstrates that As(V) was the dominant As oxidation state in the samples. The best results of the LCF model predictions (indicated by the lowest R- factor and reduced chi square values) that most samples are dominated by arsenate sorbed to ferrihydrite or other iron minerals (Table 2a). The LCF models also predicted the concentrations of As minerals in each sample (Table 2b).

The LCF predicted that most samples contaminated with pesticides were dominated by As(V) sorbed to ferrihydrite (Table 1). Our synthetic As(V) sorbed to ferrihydrite has a strong peak around 11874.5 eV (Figure 1) which corresponds to the peaks in the samples (Figure 2). Many soils in the US are moderately to highly weathered. Therefore, these soils have higher concentrations of secondary minerals like kaolinite (alumina silicate minerals) and Fe-oxi(hydr)oxide precipitates like ferrihydrite. Most of the finely sieved reddish brown or yellowish samples appear to be dominated with Fe-minerals or Al-minerals respectively. Arsenic has a high affinity for Fe minerals. Thus, the LCF prediction that most of the samples that were contaminated with arsenical pesticides are bound to ferrihydrite was expected and is supported by previous research.

The samples collected from or affected by mining sites have more than one As species present and from less common As minerals. Generally, As mineral with arsenite have strong peak at 11871 eV, and As(III)-S bonds are formed around 11867 eV (Figure 2). Sample IKJ 583 with significant concentrations of Pb and S were predicted to have minerals with these elements, Beudantite ($PbFe^{3+}_3(AsO_4)(SO_4)(OH)_6$). Scorodite and orpiment were among the most abundant phases predicted in soils after As(V) sorbed to ferrihydrite (Table 2). The LCF model predicted that the sample Asarco-Ruston contained the mineral Lollingite ($FeAs_2$). This mineral is typically found in highly reducing environments or as an ore component.

Brief Conclusions

All of the samples were collected from oxidized environments, are dominated by the more stable As(V) phases and stable iron minerals. Only the samples from mine operations had reduced As minerals present and at concentrations less than 400 mg kg^{-1} soil. The few samples that had high concentrations

of reduced As minerals, thus have the potential to be oxidized and leach As, were taken directly from or affected by mining activities.

Planned work

New As minerals, are being added to the pool of reference standards used during the LCF modeling (Table 1). These include synthetic yukonite, mimetite ($Pb_5(AsO_4)_3Cl$, an analogue of the pesticide once widely used), As(V) and As(III) adsorbed to synthetic Al minerals will be analyzed in the summer of 2012. If the new binding energies (E_0) of the synthetic minerals falls within 3 eV of the E_0 of the new reference materials, we will repeat the LCF model with the new reference minerals.

Works Cited

Beauchemin S, Hesterberg D, and Beauchemin M. 2002. Principal component analysis approach for modeling sulfur K-XANES spectra of humic acids. Soil Sci Soc Am J 66:83-91.

Cotter-Howells JD, Champness PE, Charnock JM, and Pattrick RAD. 1994. Identification of pyromorphite in mine-waste contaminated soils by ATEM and EXAFS. Eur J Soil Sci 45:393-402.

Cotter-Howells JD, Champness PE, and Charnock JM. 1999. Mineralogy of Pb–P grains in the roots of Agrostis capillaris L-by ATEM and EXAFS. Mineral Mag 63:777-89.

Prietzel, J, Botzaki, A, Tyufekchieva, N, Brettholle, M, Thieme, J, and Klysubun, W. 2011. Sulfur Speciation in Soil by S K-Edge XANES Spectroscopy: Comparison of Spectral Deconvolution and Linear Combination Fitting. Environ Sci Technol 45:2878-2886.

Ryan, JA, Zhang, PC, Hesterberg, D, Chou, J, and Sayers, DE. 2001. Formation of chloropyromorphite in a lead-contaminated soil amended with hydroxyapatite. Environ Sci Technol 35:3798-3803.

Scheckel, KG, and Ryan, JA. 2004. Spectroscopic speciation and quantification of lead in phosphate-amended soils. J Environ Qual 33:1288-1295.

Scheinost AC, Kretzschmar R, and Pfister S. 2002. Combining selective sequential extractions, X-ray absorption spectroscopy, and principal component analysis for quantitative zinc speciation in soil. Environ Sci Technol 36:5021-8.

Figures and Tables

Table 1. List of natural and synthetic As bearing minerals used for linear combination fits (LCF) using XAS normalized and derivative μ(E) spectra as well as chi(k) function to predict As phases in the soil samples. Syn = Synthetic. TBD = To Be Determined at future experiments at Advance Photon Source.

Mineral	Chemical Elements	As Species	Edge (E_0)
Arsenopyrite	FeAsS	As (III)	11865.84
Orpiment Cryst	As2S3	As(III)S	11866.67
Realgar	As4S4	As(III)S	11866.89
Lollingite	FeAs2	As(III)	11867.49
Mackinawite	Fe(Ni)S0.9	As(III)	11867.59
Fougerite	$(Fe^{2+},Mg)_6Fe^{3+}{}_2(OH)18{\bullet}4H_2OAs_3$	As(III)O	11868.42
Arsenolite NMNH 94146	As_2O_3	As(III)	11868.48
As(III) Ferrihydrite	$FeOOH{\bullet}0.4(H_2O)$ As(3)	As(III)O	11868.68
Beudantite NMNH B13898	$PbFe^{3+}{}_3(AsO_4)(SO_4)(OH)_6$	As(V)O	11872.66
Scorodite	$Fe^{3+}AsO_4 \bullet 2H_2O$	As(V)O	11873.11
Sodium Arsenate	NaAs	As(V)O	11874.00
As(V) Ferrihydrite	$FeOOH{\bullet}0.4(H_2O)$ As(5)	As(V)O	11874.61
Yukonite NMNH 6481	$Ca_7Fe^{3+}{}_{12}(AsO_4)_{10}(OH)_{20} \bullet 15H_2O$	As(V)O	11875.69
Yukonite (syn)	$Ca_7Fe^{3+}{}_{12}(AsO_4)_{10}(OH)_{20} \bullet 15H_2O$	As(V)O	TBD
As(V) AlOH (syn)	(AsO_4) - AlOH	As(V)O	TBD
As(V) Kaolinite (syn)	$(AsO_4)Al_2(Si_2O_5)(OH)_4$	As(V)O	TBD
As(V) Montmorillonite (syn)	$(AsO_3)(Na,Ca)_{0.33}(Al,Mg)_2(Si_4O_{10})(OH)_2 \cdot nH_2O$	As(V)O	TBD
As(III) AlOH (syn)	(AsO_3) - AlOH	As(III)O	TBD
As(III) Kaolinite (syn)	$(AsO_3)Al_2(Si_2O_5)(OH)_4$	As(III)O	TBD
As(III) Montmorillonite (syn)	$(AsO_3)(Na,Ca)_{0.33}(Al,Mg)_2(Si_4O_{10})(OH)_2 \cdot nH_2O$	As(III)O	TBD
Mimetite (syn)	$Pb_5(AsO_4)_3Cl$	As(V)O	TBD
Hydroxlmimetite (syn)	$Pb_5(AsO_4)_3OH$	As(V)O	TBD

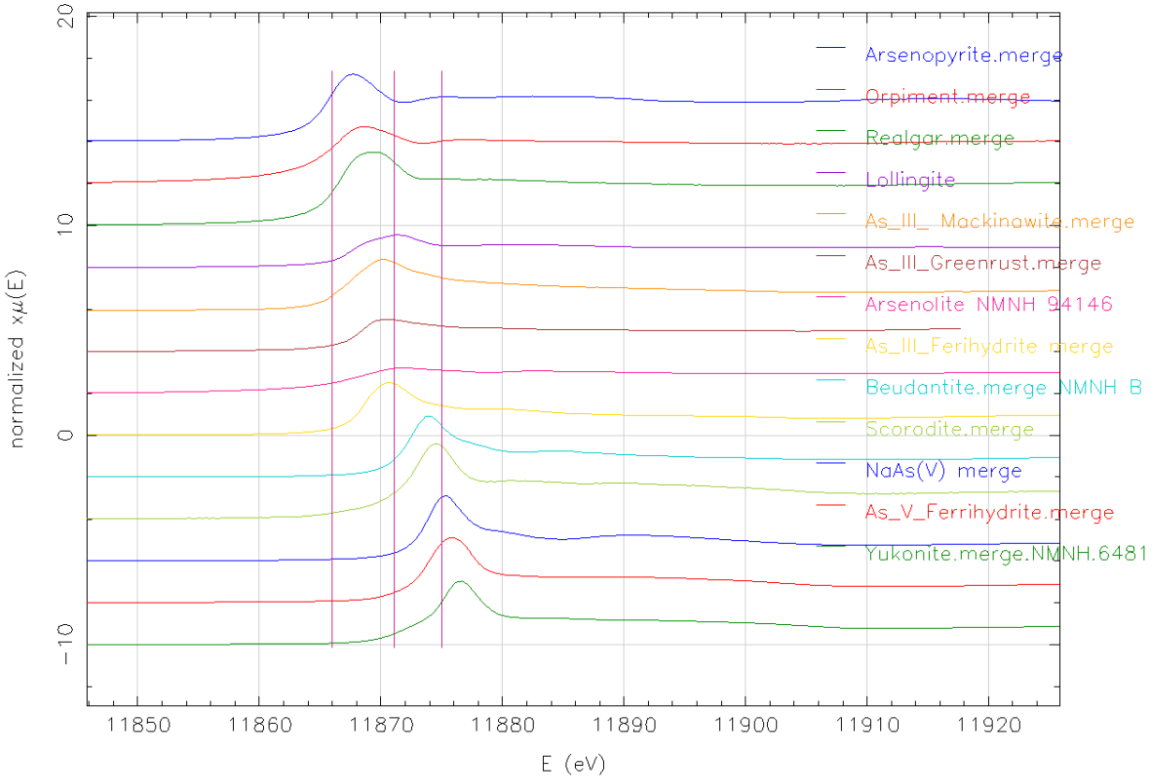
Table 2. Results of linear combination fitting (LCF) models with arsenic source, concentration (3051a extractable), and linear combination fitting (LCF) models. A) Predictions of mineral present (%); B) concentrations of mineral present (mg kg^{-1}).

						LCF Analyses %			
Soil Name	**As Source**	**RBA As**	**As mg/kg***	**R-factor**	**Reduced chi**	**Beudantite**	**As(V) Ferrihydrite**	**Scorodite**	**Lollingite**
Asarco-Ruston	Smelter	Mouse	162	0.0170	0.0220	-	76%	-	24%
Barber Orchard MS1	Pesticide	Mouse	283	0.0355	0.0442	-	100%	-	-
Barber Orchard MS4	Pesticide	Mouse	353	0.0253	0.0302	-	100%	-	-
Barber Orchard MS5	Pesticide	Mouse	391	0.0414	0.0526	-	100%	-	-
Barber Orchard MS8	Pesticide	Mouse	375	0.0335	0.0398	-	100%	-	-
HI-Hilo	Pesticide	Mouse	641	0.0119	0.0172	-	64%	36%	-
HSJ 583	Mining	Swine	249	0.0119	0.0176	-	61%	39%	-
IKJ 583	Mining	Swine	3913	0.0095	0.0145	8%	67%	25%	-
Mohr Orchard	Pesticide	Mouse	332	0.0048	0.0071	-	100%	-	-

						LCF Analyses mg/kg			
Soil Name	**As Source**	**RBA As**	**As mg/kg***	**R-factor**	**Reduced chi**	**Beudantite**	**As(V) Ferrihydrite**	**Scorodite**	**Lollingite**
Asarco-Ruston	Smelter	Mouse	162	0.0170	0.0220	-	123.63	-	38.76
Barber Orchard MS1	Pesticide	Mouse	283	0.0355	0.0442	-	282.81	-	-
Barber Orchard MS4	Pesticide	Mouse	353	0.0253	0.0302	-	352.65	-	-
Barber Orchard MS5	Pesticide	Mouse	391	0.0414	0.0526	-	390.85	-	-
Barber Orchard MS8	Pesticide	Mouse	375	0.0335	0.0398	-	375.27	-	-
HI-Hilo	Pesticide	Mouse	641	0.0119	0.0172	-	409.89	231.13	-
HSJ 583	Mining	Swine	249	0.0119	0.0176	-	152.71	96.58	-
IKJ 583	Mining	Swine	3913	0.0095	0.0145	331.43	2610.05	971.71	-
Mohr Orchard	Pesticide	Mouse	332	0.0048	0.0071	-	331.64	-	-

Figure 1**.** XAS scans of standards used for linear combination fit models. A) Normalized data and B) smoothed derivative of normalized XAS data used for linear combination fits models. Three vertical lines are at 11867, 11871 and 11875 eV.

A



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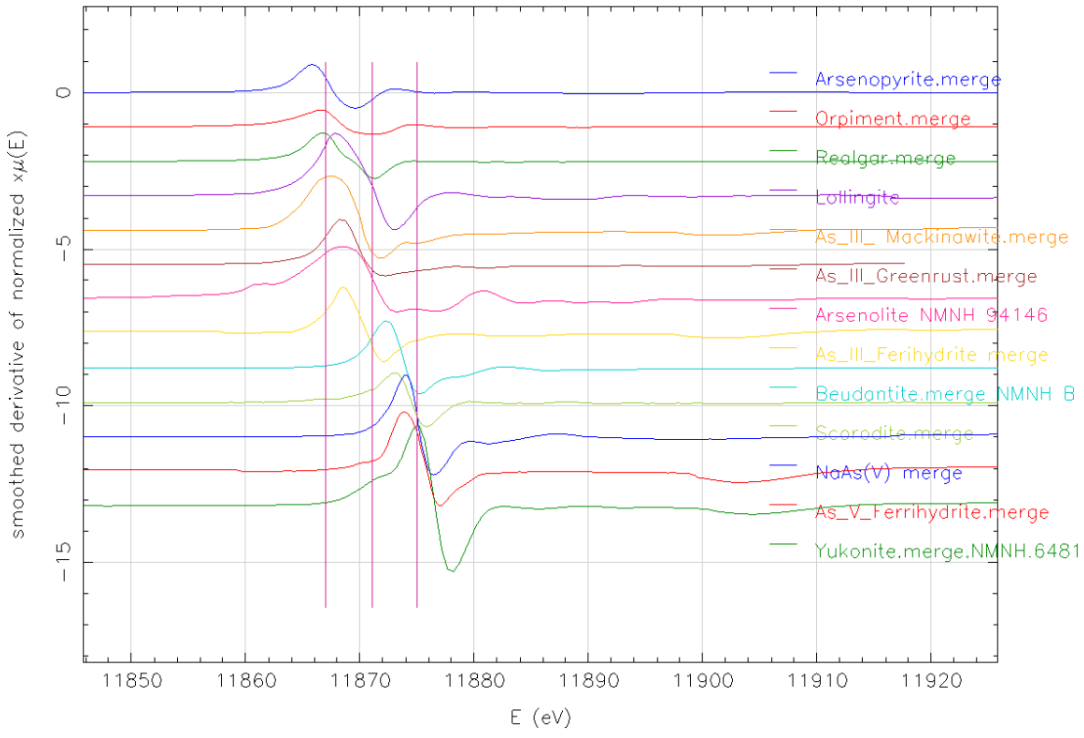
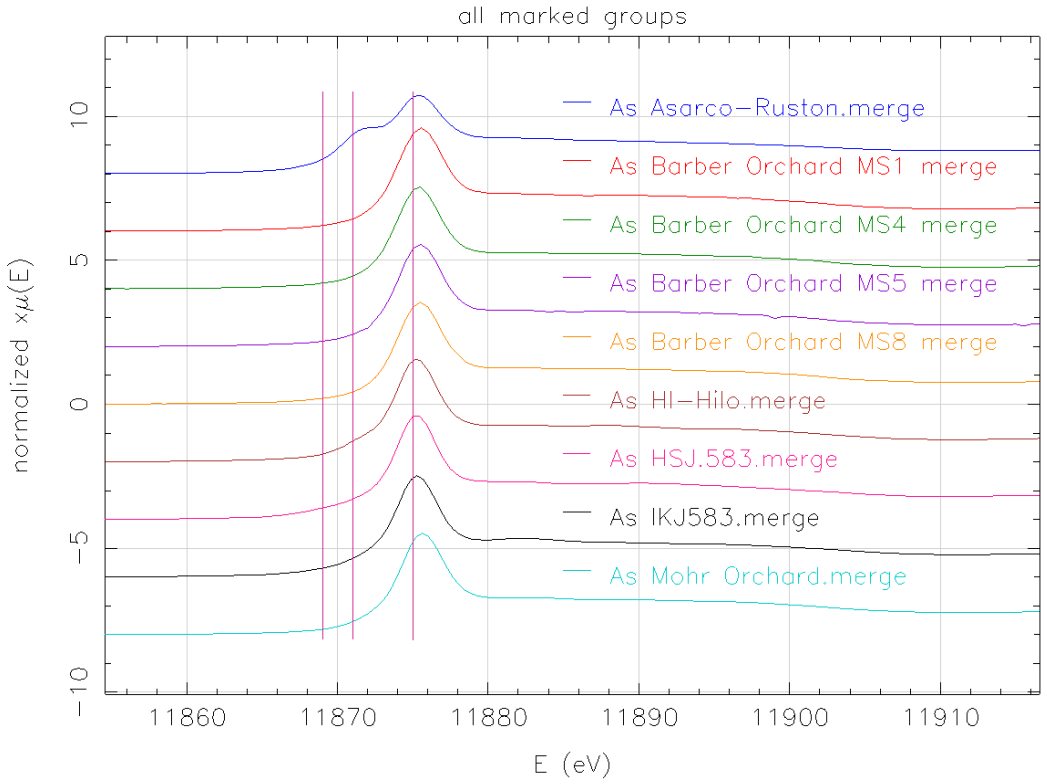
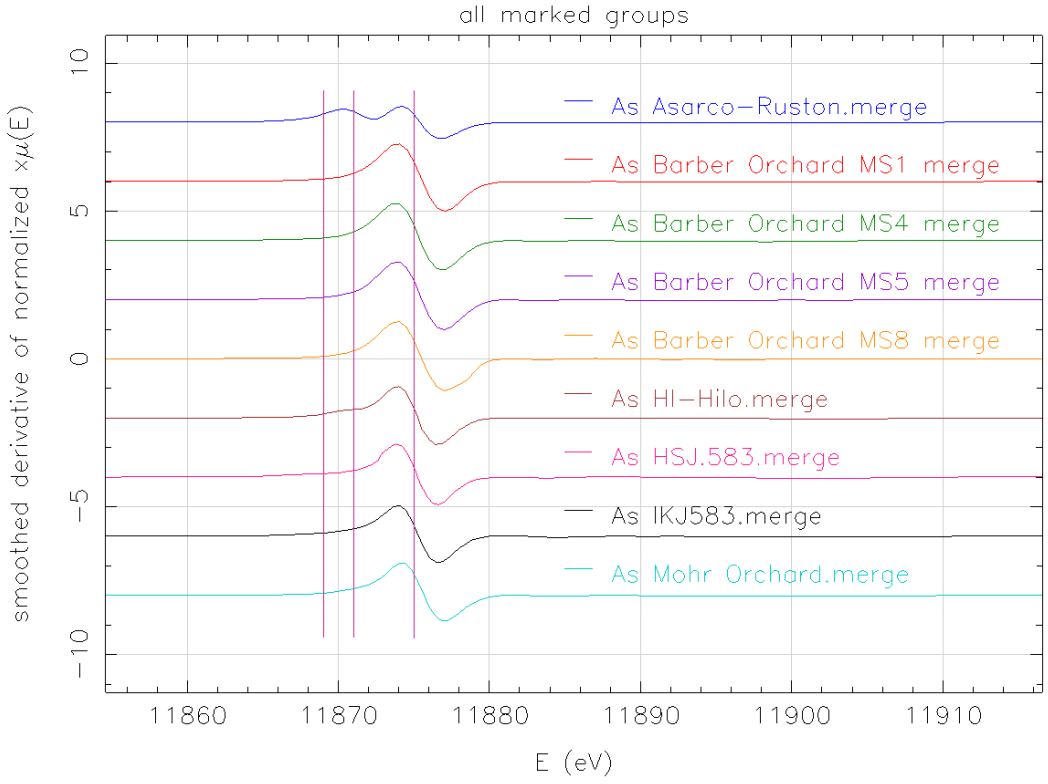


Figure 2**.** A) Normalized XAS data of soils used for linear combination fit models. B) Smoothed derivative of normalized XAS data of standards used for linear combination fits models. Three vertical lines are at 11867, 11871 and 11875 eV.

A





United States
Environmental
Protection Agency

OSWER 9200.1-113

Recommendations for Default Value for Relative Bioavailability of Arsenic in Soil

December 2012

The Risk Assessment Guidance for Superfund (RAGS) Part A (U.S. EPA, 1989), Framework for Metals Risk Assessment (U.S. EPA, 2007a), and Guidance for Evaluating the Bioavailability of Metals in Soils for Use in Human Health Risk Assessment (U.S. EPA, 2007b) discuss using site-specific bioavailability data to make adjustments to exposure estimates in site-specific risk assessments when the medium of exposure in the exposure assessment differs from the medium of exposure associated with the toxicity value (cancer slope factor, reference dose value, etc.). In the absence of reliable site-specific data, the default assumption is that the bioavailability of the contaminant in the exposure medium at the site (e.g., soil, water, etc.) is the same as the bioavailability in the exposure medium used to derive the toxicity value. For arsenic, the toxicity values in EPA's Integrated Risk Information System (IRIS) are based upon exposure to arsenic in water (U.S. EPA, 2012). The current default assumption for assessing risk from arsenic in soil is that the bioavailability of arsenic in soil is the same as the bioavailability of arsenic in water (relative bioavailability [RBA] soil/water = 100%). However, recent bioavailability studies conducted in animal models show that bioavailability of arsenic in soil is typically less than that of highly water soluble forms of arsenic (e.g., sodium arsenate dissolved in water). This suggests that bioavailability of arsenic in soil will typically be less than that of arsenic dissolved in drinking water (i.e., RBA<100%). At sites where this applies, the default assumption of RBA=100% will result in an overestimation of risk.

In an effort to provide a more accurate default RBA value for arsenic in soil, the TRW Bioavailability Committee compiled all available estimates of soil arsenic RBA (U.S. EPA, 2011). The resulting database included 103 RBA estimates: 64 estimates obtained from swine bioassays, 24 estimates obtained from monkey bioassays, and 15 estimates obtained from mouse bioassays. Analyses of these data showed that while soil RBA exhibited substantial variability, all of the RBA estimates were less than 1. The RBA estimates considered in the above analysis are derived from an opportunistic sample of soils and do not represent a statistical sample of soils in any geographic region or source of arsenic contamination. This limits the use of these data for making statistical inference about arsenic RBA in U.S. soils in general. Most of these samples were collected to support remedial investigation and risk assessments of specific sites. Although the data set includes samples from sites impacted by various sources of arsenic contamination (mining and/or smelter operations, pesticide application, and manufacturing/electrical waste, and

volcanic soils with naturally occurring high arsenic levels), the absence of a statistical sampling design limits any inferential value of the data set. For example, sample statistics such as the mean and standard deviation, even for specific categories of arsenic contamination, mineralogy, or soil characteristics, cannot be reliably assumed to represent these categories in general. Nevertheless, the data set has unique value to describe the distribution of arsenic RBA values that have been encountered in soils from various sites of regulatory interest. The empirical distribution of RBA values in this data set suggests that values for arsenic RBA exceeding 60% are relatively uncommon (i.e., <5% of the RBA estimates exceed 60%). Based on this data set, it is reasonable to expect that future RBA estimates exceeding 60% would also be uncommon, if samples were to be drawn from a collection of similar types of sites and soils. This prediction could be further evaluated with additional data collection efforts.

Based on the above considerations, the TRW Bioavailability Committee recommends a default vale for RBA of arsenic in soil based on an upper percentile from the data set of arsenic RBAs reported in U.S. EPA (2011). An RBA value of 60% was selected as the default value and is supported by the analysis of soil arsenic RBA estimates which showed that less than 5% of the RBA estimates exceeded 60%. Selection of a default RBA value that is expected to be in the upper percentile range reduces the likelihood that sites are screened out from further evaluation when, in fact, they may present a significant health risk.

Agency guidance (U.S. EPA, 2007b) recommends that even in cases where sufficient data exist to support default medium-specific absorption factors for a chemical, site-specific data collection may also be important. Important factors that can affect the bioavailability of arsenic in soil can be expected to vary from site to site, or within a given site. These include the chemical forms of the arsenic, as well as the physical and chemical characteristics arsenic-bearing soil particles. Default values for arsenic RBA may not reflect all of these factors (e.g., chemistry, particle size, matrix effects) at any given site. Therefore, site-specific assessments of bioavailability should still be performed where such assessments are deemed feasible and valuable for improving the characterization of risk at the site. Default RBA values generally should not be used when site-specific assessments are performed. In general, the Agency (U.S. EPA, 2007b) recommends that efforts be made to collect data that support site-specific estimates,

rather than relying on the default value recommended in this memorandum which may not accurately represent arsenic RBA at any specific site. Use of the national default in place of site-specific estimates may underestimate or overestimate risk. Where development of site-specific RBA estimates is not feasible (e.g., screening-level assessments), the default value of 60% can be used, recognizing that the default value is an estimate that is not likely to be exceeded at most sites and is preferable to the assumption of an RBA equal to 100%.

REFERENCES

U.S. EPA (U.S. Environmental Protection Agency). (1989) Risk Assessment Guidance for Superfund (RAGS). Volume I. Human Health Evaluation Manual (Part A). U.S. Environmental Protection Agency, Office of Emergency and Remedial Response: Washington, DC. EPA/540/1-89/002. December. Available online at: http://www.epa.gov/swerrims/riskassessment/ragsa/pdf/rags-vol1-pta_complete.pdf.

U.S. EPA (U.S. Environmental Protection Agency). (2007a) Framework for Metals Risk Assessment. U.S. Environmental Protection Agency, Office of the Science Advisor: Washington, DC. EPA 120/R-07/001. Available online at: http://www.epa.gov/raf/metalsframework/pdfs/metals-risk-assessment-final.pdf.

U.S. EPA (U.S. Environmental Protection Agency). (2007b) Guidance for Evaluating the Oral Bioavailability of Metals in Soils for Use in Human Health Risk Assessment. U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response: Washington, DC. OSWER 9285.7-80. Available online at: http://www.epa.gov/superfund/health/contaminants/bioavailability/bio_guidance.pdf

U.S. EPA. (U.S. Environmental Protection Agency). (2011). Relative Bioavailability of Arsenic in Soil. Memorandum from the TRW Lead Committee to Helen Dawson, OSRTI.

U.S. EPA (U.S. Environmental Protection Agency). (2012) Arsenic, inorganic. Integrated Risk Information System (IRIS). U.S. Environmental Protection Agency, National Center for Environmental Assessment: Washington, DC. Available online at: http://www.epa.gov/ncea/iris/subst/0278.htm.

United States Environmental Protection Agency

OSWER 9200.1-113

Compilation and Review of Data on Relative Bioavailability of Arsenic in Soil

December 2012

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ACRONYMS AND ABBREVIATIONS

ABA	Absolute bioavailability
AF_o	Oral absorption fraction
AM	Arithmetic mean
As	Arsenic
AUC	Area-under-the-curve
bw	Body weight
CI	Confidence interval
CTE	Central tendency estimate
D	Dose
FeAs	Iron arsenide
ICP-AES	Inductively coupled plasma-atomic emission spectrometry
ICP-MS	Inductively coupled plasma-mass spectrometry
INAA	Instrumental neutron activation analysis
IRIS	Integrated Risk Information System
IVBA	*In vitro* bioaccessibility
kg	Kilogram
LCL	Lower confidence limit
mg	Milligram
n	Number of data points
NIST	National Institute of Standards and Technology
ppm	Parts per million
QA	Quality assurance
RAGS	Risk Assessment Guidance for Superfund
RBA	Relative bioavailability
RM	Reference material
SD	Standard deviation
SE	Standard error
SRM	Standard reference material
TM	Test material
UCL	Upper confidence limit
UEF	Urinary excretion fraction
µg	Microgram
µm	Micrometer
U.S. EPA	United States Environmental Protection Agency

1.0 INTRODUCTION

1.1 Background

The Risk Assessment Guidance for Superfund (RAGS) Part A (U.S. EPA, 1989), Framework for Metals Risk Assessment (U.S. EPA, 2007b), and Guidance for Evaluating the Bioavailability of Metals in Soils for Use in Human Health Risk Assessment (U.S. EPA, 2007c) discuss using site-specific bioavailability data to make adjustments to exposure estimates or toxicity values in Superfund site-specific risk assessments when the medium of exposure in the exposure assessment differs from the medium of exposure associated with the toxicity value (e.g., cancer slope factor, reference dose value, etc.). In the absence of reliable site-specific data, the default assumption is that the bioavailability of the contaminant is the same in the exposure medium at the site (e.g., soil, water, etc.) as in the exposure medium used to derive the toxicity value. For arsenic, the toxicity values in EPA's Integrated Risk Information System (IRIS) are based upon exposure to arsenic in water (U.S. EPA, 2012). The default assumption for assessing risk from arsenic in soil is that the bioavailability of arsenic in soil is the same as the bioavailability of arsenic dissolved in water. In other words, the relative bioavailability (RBA) of arsenic (all forms) in soil compared to water-soluble arsenic is assumed to be 1. This assumption will result in an overestimate of the true risk if the bioavailability of arsenic in soil is less than that of arsenic in water. The EPA is evaluating the general applicability and potential uncertainties associated with the assumption that the bioavailability of arsenic in soil is the same as that of water-soluble arsenic, and is also evaluating and developing laboratory methods for estimating RBA of soil arsenic. In support of these assessments, EPA is compiling information on bioassays that have been used to measure RBA of arsenic in soil along with estimates of RBA that have been derived from these bioassays. This report summarizes RBA estimates compiled as of September 2011. EPA expects that future data collection efforts will add to this data set and that the analyses in this report would be periodically updated.

1.2 Bioavailability – Definitions

In this report, the term *bioavailability* refers to the fraction or percentage of an ingested dose of arsenic that is absorbed into the systemic circulation. Bioavailability of arsenic in soil can be expressed either in absolute terms (absolute bioavailability) or in relative terms (relative bioavailability):

1. Absolute bioavailability (ABA) is defined as the ratio of the amount of arsenic absorbed to the amount ingested. This ratio is also referred to as the oral absorption fraction (AF_o).

2. Relative bioavailability (RBA) is defined as the ratio of the ABA or AF_o of arsenic present in the soil (test material, TM) to the absolute bioavailability of arsenic in some appropriate reference material (RM, Equation 1):

$$RBA = \frac{ABA_{TM}}{ABA_{RM}} \qquad \text{Eq. (1)}$$

3. Bioaccessibility refers to the physiological solubility of arsenic in the gastrointestinal tract (NRC, 2003). Ingested arsenic must become bioaccessible in the gastrointestinal tract in order to be absorbed. This process may include physical transformation of arsenic-bearing particles (e.g., break down of the particle to expose arsenic to gastrointestinal tract fluids), dissolution of arsenic, and chemical transformation of dissolved arsenic.

For human health risk assessment purposes, relative bioavailability is important because we are most often interested in knowing the extent to which the absolute bioavailability of a chemical increases or decreases in different exposure matrices (e.g., food vs. water vs. soil) or with the physical or chemical form(s) of the chemical to which humans are exposed.

For example, if 100 micrograms (µg) of arsenic dissolved in drinking water were ingested and a total of 50 µg were absorbed, the ABA (or AF_o) would be 50/100 or 0.50 (50%). Likewise, if 100 µg of arsenic contained in soil were ingested and 30 µg were absorbed into the body, the ABA (or AF_o) for arsenic in soil would be 30/100 or 0.30 (30%). The RBA for arsenic in soil, relative to arsenic in water, would be 0.30/0.50 or 0.60 (60%).

The form of arsenic typically used as the reference material in a RBA bioassay is an arsenic compound dissolved in water or a readily soluble form (e.g., sodium arsenate) that is expected to completely dissolve when ingested (i.e., 100% bioaccessible).

2.0 KEY AND RELEVANT STUDIES

A search of the literature was conducted to identify studies in which soil arsenic RBA was estimated from data collected in controlled human clinical studies or from animal bioassays. Studies that reported only bioaccessibility measurements (e.g., *in vitro* extraction of soils) or that attempted to predict arsenic RBA from bioaccessibility measurements were not included in this data compilation for several reasons. Although there is good evidence to suggest that bioaccessibility influences and may be an important determinant of RBA, there is no current consensus on whether or not *in vitro* bioaccessibility measurements can be used to accurately predict soil arsenic RBA. EPA has not identified a validated *in vitro* assay for predicting RBA. Other on-going efforts by EPA are evaluating methods for predicting arsenic RBA from bioaccessibility measurements.

Pertinent studies from the published literature were identified by searching bibliographic databases (i.e., PUBMED, TOXLINE) and other secondary source documents including the cited references of the retrieved literature. The search period for TOXLINE covered 1980 through August 2011 and for PUBMED was comprehensive through August 2011. Reference lists from selected literature were also searched. For additional information or clarification of published data, study authors were contacted as necessary.

Studies were classified as “key” or “relevant” based on considerations of experimental design, the number of different test materials analyzed in each animal species, and the source of test materials. RBA estimates were taken from studies that included a wide variety of bioassay

protocols that reflect methods currently being used to assess arsenic RBA. Requirements for inclusion in the analyses were that:

(1) the study was conducted by or for EPA in which EPA developed the RBA estimates from the raw data using established standard protocols and/or the raw data were available for Quality Assurance (QA) review by the U.S. EPA Bioavailability Committee of the Technical Review Workgroup (e.g., EPA swine and mouse studies); or

(2) the study was conducted by other research groups and results had been subjected to peer review as a requirement for publication. No attempt was made to reanalyze the primary data on which each RBA was based (e.g., to verify the RBA value or to apply the same data reduction methods to the raw data derived from different study protocols).

Evaluation of multiple test materials in each animal species was considered important for characterization of uncertainty and variability in RBA estimates. Studies described in this report assessed RBA of soils that were contaminated *in situ*. Studies of soils that were spiked with arsenic in the laboratory (Juhasz et al., 2008; Konstantinos et al., 2008; Nagar et al., 2009) were not considered based on evidence that RBA of soils spiked with highly bioaccessible sodium arsenate can change as the soil ages (Juhasz et al., 2008). Studies that assessed absolute bioavailability and did not report RBA or provide data for calculation of RBA (i.e., Ellickson et al., 2001) were not considered. As described in Section 2.2 (Key Studies), all "key" studies were conducted in swine, monkey, or mouse; multiple test materials were analyzed using these animal models to estimate arsenic RBA. In "key" studies, a total of 103 RBA estimates for 88 unique test materials were obtained in swine (64 RBA estimates), monkeys (24 RBA estimates), and mice (15 RBA estimates). Among these "key" studies, direct comparisons of swine, monkey, and mouse RBA estimates are available for only 4 test materials and direct comparisons of swine and mice RBA estimates are available for 11 test materials. Data obtained from "key" studies were analyzed to develop summary statistics describing the distribution of RBA values and to explore sources of variability in the RBA values (i.e., using regression analysis). As described in Section 2.3 (Relevant Studies), "relevant" studies analyzed a single test material using a unique animal model (i.e., rabbit). "Relevant" studies provided supportive data, but were not included in the statistical summary.

A single human experimental study of bioavailability of arsenic soil was reported (Stanek et al., 2010). This study was not selected for inclusion in this report as a key or relevant study because of several methodological limitations and uncertainties, which are summarized in Appendix A.

2.1 Methodologies Used in Key and Relevant Studies

A variety of different *in vivo* methods have been utilized for estimating soil arsenic RBA. All of these methods share a common general approach in which biomarkers of arsenic absorption (blood arsenic concentration or urinary arsenic excretion) were measured following a single dose or during a period of repeated dosing with arsenic in soil (the test material) and

following dosing with sodium arsenate (the reference material). The study protocols differ with respect to dose (e.g., mg/kg), dosing frequency, the absorption biomarker measured (blood or urine arsenic), and the computational methods applied to the data for calculating RBA.

In studies that measured urinary arsenic excretion, the absorption dose metric was the urinary excretion fraction (UEF) which is the amount or rate of arsenic excreted in urine (U_{As}) divided by the arsenic dose (D_{As}, Equation 2).

$$UEF = \frac{U_{As}}{D_{As}} \quad \text{Eq. (2)}$$

The RBA was estimated as the ratio of the UEF for arsenic when administered in soil (test material, TM) to that of the reference material (RM; i.e., sodium arsenate, Equation 3).

$$RBA = \frac{UEF_{TM}}{UEF_{RM}} \quad \text{Eq. (3)}$$

In studies in which animals were dosed one time, the UEF was the cumulative amount of arsenic excreted during a defined post-dose observation period (e.g., 4 days) divided by the administered dose. In studies in which doses of arsenic were administered repeatedly to achieve a quasi-steady state, the UEF was the rate of excretion of arsenic (e.g., µg As/day) divided by the dosing rate (e.g., µg As/day). In studies in which arsenic was administered at more than one dose (e.g., 25, 50, or 100 µg As/kg bw/day), the UEF was estimated as the regression slope of the relationship between urinary arsenic excretion and dose.

In studies that relied on blood arsenic concentration for estimating RBA, the absorption dose metric was the time-integrated arsenic blood concentration. This was typically measured as the time-integrated blood concentration of arsenic, referred to in this report and in most of the literature as the area under the curve (AUC) of the arsenic blood concentration-time profile (e.g., estimated using a geometric approximation such as the trapezoid rule). The AUC estimate was divided by the administered dosage, and the RBA was estimated as the ratio of AUC/dose for the test and reference materials (Equation 4).

$$RBA = \frac{AUC_{TM}}{D_{TM}} \div \frac{AUC_{RM}}{D_{RM}} \quad \text{Eq. (4)}$$

If arsenic was administered at more than one dose (mg/kg), the AUC/dose ratio was estimated as the regression slope of the relationship between the blood AUC and dose.

Each of these methods is described in greater detail in the sections that follow.

2.1.1 Single Dose Urinary Excretion Fraction Method

In studies conducted using this method, a one-time oral dose of test material or reference material (sodium arsenate) was administered. Following administration of the arsenic dose, urine was collected for up to 7 days. Relative bioavailability in test materials was calculated as the ratio of the UEFs for the test and reference materials, where the UEF was the cumulative urinary excretion of arsenic divided by the arsenic dose.

2.1.2 Repeated Dose Steady-State Urinary Excretion Fraction Method

In studies conducted using this method, groups of animals typically were dosed with the test material or reference material (sodium arsenate) repeatedly for 10–15 days. At various times during the dosing period, urine samples were collected from each animal and analyzed for arsenic. The RBA of a test material was calculated as the ratio of the UEFs for the test and reference materials. In studies in which a single dose level was administered, UEF was estimated as the cumulative urinary arsenic excretion (e.g., µg As) divided by the dose. In studies in which arsenic was administered at more than one dose level (e.g., 25, 50, or 100 µg As/kg bw/day), UEF was calculated by fitting a regression model to the data on dose and urinary excretion and estimating UEF as the regression slope.

2.1.3 Single Dose Blood-Time Concentration Curve Method

In studies conducted using this method, groups of animals were administered a one-time oral dose of test material or reference material (sodium arsenate) or an intravenous dose of the reference material. Test and reference materials were administered at multiple dose levels. Blood samples were collected at various time points up to 6 days after dosing. For the calculation of RBA, the time-integrated blood arsenic concentration (AUC) and arsenic dose for both the test material and reference material were subjected to regression analysis. RBA was estimated as the ratio of the regression slopes.

2.2 Key Studies

Methods and protocols of key studies are summarized below. Many of these studies estimated RBA for multiple test materials. Sources of uncertainties that were considered in assessing confidence in RBA estimates and making statistical inference regarding arsenic RBA in soils are summarized in Table 1. The identity of the individual test materials, dosing schedules, and dose levels used to assess RBA for each test material are provided in Table 2.

2.2.1 U.S. EPA, 2010

The RBA of arsenic was estimated for several test materials using the steady-state urinary excretion fraction method described in U.S. EPA (2010). These studies were sponsored by U.S. EPA Region 8. Test materials were obtained from various locations throughout the U.S. and included residential and non-residential soils and mining slag. The concentration of arsenic in these test materials ranged from 72 to 1050 ppm. All studies were performed using young, intact male swine (genetically defined Line 26 strain), typically 5 to 7 weeks old, weighing 7 to 12 kg. Groups of animals (usually 4–5 per dose group) were exposed to 1 to 3 dose levels of test material or reference material (sodium arsenate) daily for 12–15 days. Test materials were placed in the center of moistened feed (dough ball) and administered to the animals by hand. Sodium arsenate (reference material) was administered by gavage or intravenous injection. Samples of urine were collected from each animal on several different days during the study (the exact days varied from study to study, with collection periods ranging from 24–48 hours). Urine samples were prepared for analysis using one of two alternative methods referred to as Phase II

(acid digestion) and Phase III (acid digestion and ashing). Arsenic in digested urine samples was measured by hydride generation using atomic absorption spectrometry (limit of detection ~1–2 µg/L). Detailed descriptions of the acid digestion and ashing methodologies are provided in U.S. EPA (2010). The Phase II method yielded a poor recovery of organic metabolites of arsenic, which could result in underestimates of urinary arsenic. However, comparative studies using the same test materials showed that the Phase II and Phase III methods yielded essentially the same RBA estimates. Therefore, RBA estimates using Phase II methods are considered reliable. For the RBA calculation, regression was used to estimate the slope of the relationship between urinary arsenic excretion (e.g., µg/day) and arsenic dose (e.g., µg/day) for both the test and reference materials. The RBA of the test material was calculated as the ratio of the slopes. A total of 24 test materials were evaluated with RBA estimates ranging from 8 to 61%.

2.2.2 Casteel and SRC, 2005

The RBA of arsenic was estimated for one test material using the steady-state urinary excretion fraction method described in U.S. EPA (2010). This study was sponsored by U.S. EPA Region 6. The test material was a soil sample containing 47 ppm arsenic, obtained from a U.S. Superfund site in Palestine, Texas. The study was conducted using Phase III methodology as described in U.S. EPA (2010), with groups of 5 intact male swine (genetically defined Line 26 strain) administered 3 dose levels of test material or reference material (sodium arsenate) daily for 15 days. The estimated RBA of the test material was 15%.

2.2.3 Casteel and SRC, 2009a

The RBA of arsenic was estimated for four test materials using the steady-state urinary excretion fraction method described in U.S. EPA (2010). This study was sponsored by U.S. EPA Office of Superfund Remediation and Technology Innovation. The test materials were soil samples containing 290 to 388 ppm arsenic obtained from a former commercial apple orchard, the Barber Orchard site located near Waynesville, Haywood County, North Carolina. The study was conducted using Phase III methodology as described in U.S. EPA (2010), with groups of 4 intact male swine (genetically defined Line 26 strain) administered 2 to 3 dose levels of test material or reference material (sodium arsenate) daily for 14 days. The RBA of the test materials ranged from 31 to 53%. Arsenic RBA estimates for these four Barber Orchard test materials were also obtained in monkeys (U.S. EPA, 2009; see Section 3.2.8).

2.2.4 Casteel and SRC, 2009b

The RBA of arsenic was estimated for one test material using the steady-state urinary excretion fraction method described in U.S. EPA (2010). This study was sponsored by U.S. EPA Office of Superfund Remediation and Technology Innovation. The test material was a sample of National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 2710. This soil sample, collected in Montana from an area contaminated by mine tailings deposits, contained 626 ppm arsenic. The study was conducted using Phase III methodology as described in U.S. EPA (2010), with groups of 4 intact male swine (genetically defined Line 26 strain) administered 3 dose levels of test material or reference material (sodium arsenate) daily for 14 days. The RBA of the test material was 44%.

2.2.5 Casteel and SRC, 2009c

The RBA of arsenic was estimated for one test material using the steady-state urinary excretion fraction method described in U.S. EPA (2010). This study was sponsored by U.S. EPA Office of Superfund Remediation and Technology Innovation. The test material was a sample of soil from the Mohr Orchard site located in Region 3, Lehigh County, Pennsylvania. The arsenic concentration of the Mohr Orchard soil sample was 340±4.5 mg/kg (mean±SD). The study was conducted using Phase III methodology as described in U.S. EPA (2010), with groups of 4 intact male swine (genetically defined Line 26 strain) administered 3 dose levels of test material or reference material (sodium arsenate) daily for 14 days. The RBA of the test material was 53%.

2.2.6 Casteel and SRC, 2010a

The RBA of arsenic was estimated for two test materials using the steady-state urinary excretion fraction method described in U.S. EPA (2010). This study was sponsored by U.S. EPA Office of Superfund Remediation and Technology Innovation. The test materials were samples of soil from the Iron King Mine – Humboldt Smelter Superfund Site. The soil samples (HSJ583 and IKJ583) were collected from the Chaparral Gulch near a residential area (HSJ583) and a tailings pile (IKJ583). The mean arsenic concentrations of the soil samples were 200 ppm (HSJ583, TM1) and 3957 ppm (IKJ583, TM2). The study was conducted using Phase III methodology as described in U.S. EPA (2010), with groups of 4 intact male swine (genetically defined Line 26 strain) administered 3 dose levels of test material or reference material (sodium arsenate) daily for 14 days. The RBA of the test materials were 60% (TM1) and 19% (TM2).

2.2.7 Casteel and SRC, 2010b

The RBA of arsenic was estimated for two test materials (ASARCO and Hawaii) using the steady-state urinary excretion fraction method described in U.S. EPA (2010). This study was sponsored by U.S. EPA Office of Superfund Remediation and Technology Innovation. The ASARCO material was collected from a former smelter site near Tacoma, Washington. Multiple samples were collected from a stockpile of soil that was removed from residential properties and composited prior to analysis. The Hawaii material was collected from a garden plot used by Kea'au Middle School, located in the town of Kea'au on the island of Hawaii. The garden has high arsenic concentrations attributable to herbicide use between 1920 and 1950 in former sugar mill plantation lands in the area. The soil samples contained 182 ppm (ASARCO) and 769 ppm (Hawaii) arsenic. The study was conducted using Phase III methodology as described in U.S. EPA (2010), with groups of 4 intact male swine (genetically defined Line 26 strain) administered 3 dose levels of test material or reference material (sodium arsenate) daily for 14 days. The RBA of the test materials were 49% (ASARCO) and 33% (Hawaii).

2.2.8 Casteel and SRC, 2010c

The RBA of arsenic was estimated for one test material using the steady-state urinary excretion fraction method described in U.S. EPA (2010). This study was sponsored by U.S. EPA Office of Superfund Remediation and Technology Innovation. The test material was a sample of

NIST SRM 2710a. This soil sample, obtained in Montana from an area contaminated by mine tailings deposits, contained 1540 ppm arsenic. The study was conducted using Phase III methodology as described in U.S. EPA (2010), with groups of 4 intact male swine (genetically defined Line 26 strain) administered 3 dose levels of test material or reference material (sodium arsenate) daily for 14 days. The RBA of the test material was 42%.

2.2.9 Basta et al., 2007; Rodriguez et al., 1999

Rodriguez et al. (1999) estimated the RBA of arsenic in several test materials in juvenile swine using the same steady-state urinary excretion fraction method described in U.S. EPA (2010). Test materials (soils and slags), with arsenic concentrations ranging from 233 to 17,500 ppm, were collected from mining/smelter sites in the western U.S. Studies were performed in young, intact male swine (Line 26 strain), weighing 10–12 kg. Test groups of animals (2–5 per dose group) were administered a single dose level of test material (in a dough ball) and a control group was administered a reference material (sodium arsenate). The animals were dosed daily for 15 days, and urine was collected for five 24-hour periods. For the calculation of RBA, the UEF of arsenic (cumulative urinary excretion/dose) administered in test material and in reference material (sodium arsenate) was calculated, and the RBA was calculated as the ratio of the UEF values. The Rodriguez et al. (1999) report did not include standard deviations (SD), standard errors (SE), or confidence limits (CI) for mean RBA values. Due to concerns regarding recovery of organoarsenical compounds in urine, Basta et al. (2007) re-analyzed urine samples from nine test materials reported in Rodriguez et al. (1999) using the Phase III analytical method (U.S. EPA, 2010). Revised RBA estimates for these nine samples were reported graphically in Basta et al. (2007); numeric values (mean RBA estimates and standard deviations) were provided for this report through a personal communication with Dr. Basta. A total of 14 test materials were evaluated in the Basta et al. (2007) and Rodriguez et al. (1999) studies, with RBA estimates ranging from 4 to 43%.

2.2.10 U.S. EPA, 1996

In a study sponsored by U.S. EPA Region 10, the RBA of arsenic was estimated for two test materials (mining soil and slag collected from the Ruston/North Tacoma Superfund site) using the single dose blood-time concentration curve method. Arsenic concentrations in the test materials were 1600 ppm for the mining soil and 10,100 ppm for the slag. The study was conducted in young, female swine (bred from Hampshire sires and Landrace/Large White/Duroc dams), 6–7 weeks of age, weighing approximately 15 kg. Groups of three animals were administered a single oral dose of test material as an aqueous suspension or single oral or intravenous dose of reference material (sodium arsenate); multiple dose levels of test and reference materials were evaluated. Following administration, blood samples were obtained at various time points from 15 minutes to 144 hours after dosing. Following acid digestion and heat treatment, arsenic was measured by hydride generation using atomic absorption spectrometry (limit of detection = 1 μg/L). Regression models were fit to the data on time-integrated blood arsenic concentration (AUC) and dose, and RBA was calculated as the ratio of slopes for test and reference materials. The study report did not include standard deviations or standard errors, but reported 95% confidence limits. RBA estimates ranged from 42% (slag) to 78% (soil).

2.2.11 Juhasz et al., 2007

Juhasz et al. (2007) estimated the RBA of arsenic in several Australian test materials, with arsenic concentrations ranging from 42 to 1114 ppm, using the single dose blood-time concentration curve method. Test materials were collected from railway corridors, cattle tick dip sites, mining sites, and gossans (areas containing naturally elevated concentrations of arsenic). Groups of 3 female swine (strain: large white; body weight: 20 to 25 kg) were administered single doses of test materials as soil slurries or sodium arsenate by gavage. Blood samples were collected at various times up to 26 hours following dosing. Samples were digested by nitric acid or ammonium hydroxide; arsenic was measured by inductively coupled plasma-mass spectrometry (ICP-MS; limit of detection not reported). Relative bioavailability of arsenic in test materials was determined using the ratio of the time-integrated blood arsenic concentration (AUC) divided by the dose, for the test and reference material. Although Juhasz et al. (2007) did not report RBA estimates for individual test materials, study authors provided means and standard deviations for individual test materials in a personal communication (dated June 18, 2008). A total of 12 test materials were evaluated in this study, with RBA estimates ranging from 7 to 75%.

2.2.12 Roberts et al., 2007

The RBA of arsenic was estimated for several soils (arsenic concentration range: 125 to 1492 ppm) collected from various locations throughout the U.S. (California, Colorado, Florida, Hawaii. Montana, New York, Washington, and Wisconsin) using the single dose urinary excretion fraction method. The study was conducted in young adult male cynomolgus monkeys, weighing 4 to 5 kg. Five animals were administered single doses of test materials (as soil slurry) or reference material (sodium arsenate) by gavage. Each monkey received the test and reference material, with dosing of each material separated by at least 3 weeks. Urine and feces were collected for 4 days after dosing. Urine samples were treated with nitric acid, heat, and hydrogen peroxide; urin arsenic was measured using inductively coupled plasma-atomic emission spectrometry (ICP-AES) (limit of detection = 2.3 µg/L). The relative bioavailability in test materials was determined using the ratio of the UEF for test and reference materials, where UEF was the cumulative urinary arsenic (µg) excretion divided by the arsenic dose (µg). A total of 14 test materials were evaluated in this study, with RBA estimates ranging from 5 to 31%.

2.2.13 U.S. EPA, 2009

The RBA of arsenic was estimated for 4 soils collected from the Barber Orchard site near Waynesville, Haywood County, North Carolina (a former commercial apple orchard, soil arsenic concentration range: 290 to 388 ppm) using the single dose urinary excretion fraction method. Single doses of test materials (as soil slurry) or reference material (sodium arsenate) were administered by gavage to 5 young adult male cynomolgus monkeys, weighing 4 to 5 kg. Each monkey received the test and reference material, with dosing of each material separated by at least 3 weeks. Urine and feces were collected for 4 days after dosing. Urine samples were treated with nitric acid, heat, and hydrogen peroxide; urinary arsenic was measured using ICP-AES (limit of detection = 0.3 µg/L). Relative bioavailability in test materials was determined

using the ratio of the UEF for test and reference materials, where UEF was the cumulative urinary arsenic (µg) excretion divided by the arsenic dose (µg). RBA estimates for the Barber Orchard test materials assayed in this study ranged from 25 to 38%. RBA estimates for these 4 Barber Orchard test materials were also obtained in swine (Casteel and SRC, 2009a; see Section 3.2.3).

2.2.14 Roberts et al., 2002

The RBA of arsenic was estimated for contaminated Florida surface soils (arsenic concentration range: 101 to 743 ppm) using the single dose urinary excretion fraction method. The study was conducted using adult male *Cebus apella* monkeys, weighing 2.5 to 3 kg. Single doses of test materials (as soil slurry) or reference material (sodium arsenate) were administered by gavage to 5 animals. Urine and feces were collected for 4 days after dosing. Urine samples were treated with nitric acid, heat, and hydrogen peroxide; urinary arsenic was measured using ICP-AES (limit of detection = 2.5 µg/L). Relative bioavailability in test materials was determined using the ratio of the UEF for test and reference materials, where UEF was the cumulative urinary arsenic (µg) excretion divided by the arsenic dose (µg). A total of 5 test materials were evaluated in this study, with RBA estimates ranging from 11 to 25%.

2.2.15 Freeman et al., 1995

Freeman et al. (1995) estimated the RBA of arsenic in a single test material (residential soil, arsenic concentration: 410 ppm) using both the single dose urinary excretion fraction and single dose blood-time concentration curve methods in female cynomolgus monkeys (weighing 2 to 3 kg). Three female monkeys were administered single doses of the test material in a capsule by gavage or reference material (sodium arsenate in solution) by gavage or intravenous injection. Each monkey received the test and reference material. Urine was collected for 7 days after dosing, and blood samples were collected at several time points from 15 minutes to 120 hours after dosing. In this study, the ABA of arsenic was calculated for the test and reference materials. For this report, RBA was calculated as the ratio of the reported ABA for the test and reference material.

Freeman et al. (1995) estimated arsenic ABA from both measurements of UEF and time-integrated arsenic blood concentration (AUC). For each, the ABA was calculated as the ratio of the biomarker measured following the oral dose to that measured following an intravenous dose (i.e., 100% absorption, Equations 5 and 6):

$$ABA = \frac{UEF_{TM,oral}}{UEF_{RM,iv}} \quad \text{Eq. (5)}$$

$$ABA = \frac{AUC_{TM,oral}}{D_{TM,oral}} \div \frac{AUC_{RM,iv}}{D_{RM,iv}} \quad \text{Eq. (6)}$$

The arsenic RBA calculated based on the UEF data for the individual animals (n=3) was 20.1% (SD=6.9%), compared to 11.0% (SD=7.7%) based on the blood AUC data. These estimates are not significantly different (paired t-test, p=0.37).

2.2.16 Bradham et al., 2011, 2012

The RBA of arsenic was estimated for contaminated surface soils (arsenic concentration range: 182 to 4495 ppm) using the repeated dose steady state urinary excretion fraction method (Bradham et al., 2011, 2012). Test materials were obtained from various locations throughout the U.S. and included agricultural soils and soils impacted by mining and smelting. Four to six week-old female C57BL/6 mice were fed diets containing the test soil or sodium arsenate. The test soil and sodium arsenate groups typically consisted of 12 mice that were housed in metabolic cages containing 3 mice per cage. The test soil was mixed into the powdered AIN-93G purified rodent diet to achieve a 1% (w/w) soil:diet ratio. Mice received the diets for a period of 10 days during which urine and feces were collected daily. Arsenic concentrations in diet, soil, urine, and feces were determined by Instrumental Neutron Activation Analysis (INAA). Daily arsenic dosages were estimated from measurements of daily diet consumption. Doses ranged from 0.32 to 6.10 mg As/kg bw/day, and soil dose ranged from 1.15 to 1.65 g soil/kg bw/day (over a 10-day period). Arsenic RBA was estimated as the ratio of UEFs for soil arsenic and sodium arsenate treatment groups, where the UEF was the cumulative urinary arsenic (µg) excretion divided by the cumulative arsenic dose (µg). A total of 15 test materials were evaluated in these studies, with RBA estimates ranging from 11 to 52%.

2.3 Relevant Studies

Studies that evaluated soil arsenic RBA bioavailability using a unique animal model (i.e., rabbit) were considered to be "relevant" studies in that they provided supportive data but were not included in the data analysis.

2.3.1 Freeman et al., 1993

Freeman et al. (1993) estimated the RBA of arsenic in a single test material using the single dose urinary excretion fraction method in New Zealand white rabbits. The arsenic concentration of the test material (soil contaminated through smelter activities) was 3900 ppm. Groups of 5 male and 5 female rabbits (9 to 12 weeks old, body weight 2 kg) were administered single oral doses of test material (formulated in a gelatin capsule) or reference material (sodium arsenate solution). Urine was collected for 120 hours after dosing. Urine samples were digested with nitric acid and hydrogen peroxide, and urine arsenic was measured using ICP-MS (limit of detection = 30 µg/L). The RBA of the test material was estimated by calculating the ratio of the UEF values for test and reference materials normalized for dose. This study did not report standard deviations, standard errors, or confidence limits for the mean RBA values of 48%.

3.0 LIMITATIONS OF DATA

The data used to estimate RBA for arsenic in soil materials have the following limitations and uncertainties for making generic prediction of soil arsenic RBA in humans.

Extrapolation of results to humans: The swine and monkey models have been utilized to predict human RBA of arsenic for site risk assessment because the gastric physiology of both animal species is similar to that of humans (U.S. EPA, 2007a) and because of a prior history of

using these models for assessing RBA of other inorganic contaminants (e.g., lead; U.S. EPA, 2007a) and gastrointestinal absorption of drugs (Chiou and Buehler, 2002; Roberts et al., 2007). Although estimates of RBA of arsenic in soil materials in animal models have not been quantitatively compared to estimates made in humans for the same material, this report shows that RBA estimates obtained from swine, monkey, and mouse for the same test materials are sufficiently similar to suggest that large differences in RBA across mammalian species are unlikely. This increases confidence in extrapolating RBA estimates obtained from these assays to humans.

Comparability of estimates from swine, monkey, and mouse assays: When applied to the same test materials, the swine, monkey, and mouse assays yielded remarkably similar RBA estimates for some materials and widely different estimates for other materials (see Section 4.2.1). However, collectively, the differences in the RBA estimates were relatively small. The absolute difference in the RBA estimates (e.g., RBA_{swine} - RBA_{mouse}, RBA_{swine} - RBA_{monkey}) ranged from <1 to 28%, and the average difference was 12%. This magnitude of difference is relatively small in the context of risk assessment, where uncertainties in other parameters in risk calculations can exceed several orders of magnitude. Therefore, from the perspective of use of the assays to support risk assessment, the swine, monkey, and mouse assays appear to yield essentially equivalent information about arsenic RBA.

The reason why the same test materials give different outcomes in the three animal models are discussed in Section 4.4.1.

Single dose vs. steady-state models: Animal models that estimate RBA with steady state dosing have some useful advantages over single dose assays.

(1) Steady state models more closely mimic the status of the human receptor who receives continuous daily exposure to soil.

(2) At steady state, urinary excretion of arsenic will be relatively constant over time, and as a result, urinary arsenic excretion rate and UEF can be estimated by averaging multiple estimates obtained from several urine samples collected over time. By contrast, in a single dose study, UEF must be estimated as the cumulative urinary arsenic excretion. This requires absolute accuracy in sampling urine at each interval of the post-dosing observation period.

(3) Random errors in urine sampling (e.g., completeness of collection) would be expected to have a larger impact on estimates of the cumulative arsenic excretion than on average steady state arsenic excretion.

Single vs. multiple dose level models: Assays that estimate RBA at multiple arsenic dose levels have some useful advantages over single dose level assays.

(1) Potential dependence of UEF on arsenic dose level can be detected and accounted for in the data reduction and estimate of RBA. Thus far, dose dependence of arsenic

UEF has not been demonstrated in swine or monkeys, at least not with the range of arsenic doses examined in reported studies (Roberts et al., 2007; U.S. EPA, 2010).

(2) In multiple dose level studies, UEF can be estimated from regression models of the relationship between excretion and dose (i.e. change in urinary arsenic excretion/change in dose level) This provides a statistical alternative to discrete estimates of UEF based on results obtained at a single dose level.

Test material dose levels: Ideally, animal bioassays should administer test material doses (i.e., mg soil/kg bw/day) that are similar to those expected in the human receptor population. This would reduce uncertainty related to possible dependences of arsenic RBA on test material dose. However, the design of animal RBA assays, particularly detection limits for blood and urinary arsenic and the wide variation in the arsenic concentrations of test materials, has placed constraints on experimental control of both the arsenic dose and test material dose used in each assay. The doses (single doses were administered) of test material in key studies ranged from approximately 0.4 to 3528 mg soil/kg bw in swine, 490 to 2970 mg soil/kg bw in monkeys and 1150 to 1650 mg soil/kg bw in mice. These ranges include values that are substantially higher than typical daily soil ingestion rates in children or adults (U.S. EPA, 2008). The implication of these high test material doses in extrapolating RBA estimates from animals to humans (e.g., effect of the test material dose on RBA) has not been thoroughly investigated.

4.0 SUMMARY OF ARSENIC RBA ESTIMATES

4.1 Summary of Arsenic RBA Estimates

Relative bioavailability estimates for individual test materials evaluated in "key" and "relevant" studies are summarized in Table 2. Summary statistics for RBA estimates from "key" studies are provided in Table 3. "Key" studies consist of 64 RBA estimates based on bioassays in juvenile swine (Basta et al., 2007; Casteel and SRC, 2005, 2009a,b,c, 2010a,b,c; Juhasz et al., 2007; Rodriguez et al., 1999; U.S. EPA, 1996, 2010), 24 RBA estimates based on bioassays in monkeys (Freeman et al., 1995; Roberts et al., 2002, 2007; U.S. EPA, 2009), and 15 RBA estimates based on bioassays in mice (Bradham et al., 2011, 2012). Eleven test materials were evaluated in both swine and mice, and 4 test materials (Barber Orchard soils) were evaluated in swine, monkeys, and mice. Test materials assessed in "key" studies come from sites impacted by various arsenic sources: mining/smelting (n=57); agriculture, including orchards and livestock dipping sites (n=12); other chemical manufacturing/processes, mainly pesticide manufacture (n=9); railway corridors (n=6); and miscellaneous or uncharacterized sites such as volcanic soils (n=1). In developing summary statistics shown in Table 3, two approaches were used:

(1) RBA estimates for materials tested in more than one assay were treated either as independent estimates (where RBA is represented in sample statistics), or

(2) as repeated measurements of the same sample (where the average value for all assays of the same test material is represented in the sample statistics).

The two approaches yield essentially the same values for the summary statistics (n=103 or n=88, see Table 3). For the entire data set (n=103), RBA estimates ranged from 4.1 to 78%, with an arithmetic mean of 31% (±16, SD, 5th–95th percentile range: 7–57%).

Summary statistics shown in Table 3 give equal weight to each of the RBA estimates in the key study data set. However, each RBA estimate represents a mean value for a group of animals, and each mean has an associated uncertainty given by the standard error and confidence limits. If each RBA estimate were to be weighted according to its associated confidence, the resulting distribution of RBA estimates would be a more accurate reflection of the confidence in each RBA estimate. Monte Carlo simulation was used to derive an uncertainty-weighted estimate of the mean and selected percentiles and to derive confidence limits for these empirical parameters. Monte Carlo analysis was conducted as follows.

(1) For each test material, a mean RBA and standard error (SE) were identified.

(2) A distribution for the mean RBA for each test material was defined as

TRUNCATED NORMAL (mean, SE, 0, 100)

where 0 and 100 were the truncation limits and represent the minimum and maximum values possible for RBA, respectively, and SE is the standard error. If the standard deviation (SD) was reported but not a SE, the SE was estimated as SD/n$^{0.5}$, where *n* was the number of animals represented in the mean. If confidence limits were available but not standard errors, the standard error was estimated assuming the standard normal distribution of error and the appropriate value for Z value for the standard normal distribution (i.e., 1.96 for 95% confidence limits). For 95% upper and lower confidence limits (UCL, LCL), the corresponding SE was calculated as follows (Equation 7).

$$SE = \frac{95\%UCL - 95\%LCL}{2 \cdot 1.96} \qquad \text{Eq. (7)}$$

(3) Each iteration of the Monte Carlo simulation consisted of a random selection from the distribution of means from each and every test material (i.e., sampling without replacement). Iteration yielded 10,000 sets of RBA estimates (one per test material).

(4) The mean and 5th, 50th and 95th percentile RBA values were calculated for each iteration of the Monte Carlo, yielding 10,000 realizations of each parameter.

(5) The 2.5th percentile and 97.5th percentile values were calculated from the 10,000 values for each parameter. These were used to represent the 95% confidence intervals on the mean 5th, 50th, and 95th percentile RBA values.

Results of the Monte Carlo analysis are shown in Table 4. The uncertainty-weighted estimates from the Monte Carlo simulation are very similar to the unweighted estimates (see Table 3). For example, the weighted estimate of the 50th percentile (n=103) is 28.5% (unweighted = 29.1%), and the confidence interval is 26–31%. The weighted estimate of the 95th percentile RBA is 58.1% (compared to 56.8% for the unweighted estimate), and the confidence

interval is 53–64%. Truncation of the distributions used in the Monte Carlo analysis had a negligible effect on the weighted parameter estimates and confidence limits. Only one RBA estimate, the Tacoma, WA sample (U.S. EPA, 1996), which had an RBA of 78% (±14 SE) in swine, would have been affected by truncation. A random draw from this distribution would be expected to yield values 2 SE above the mean (106%) at a frequency of approximately 2.5%. However, this had a minimal effect on the weighted estimates and confidence limits for the full data set.

4.2 Factors Influencing RBA Estimates

RBA estimates showed a wide range (i.e., 4.1 to 78%). Variability in RBA estimates may be due to several factors, including differences between animal species, experimental methods and methods of data reduction, arsenic source, arsenic soil concentration and dose, soil characteristics, and arsenic mineralogy. Not all of these factors could be assessed with the available data.

4.2.1 Species Differences

Comparisons of RBA estimates assayed in swine, monkeys, and mice show that arsenic RBA estimates for materials assayed in swine and mice tended to be higher than estimates for test materials assayed in monkeys (see Table 3, Figure 1). The mean RBA estimates for test materials assayed in swine and mice are 34.5% (95% CI: 30.2–38.8, n=64) and 33.5% (95% CI: 27.1–39.8, n=15), respectively, compared to 19.2% (95% CI: 15.8–22.6, n=24) in monkeys. Data from two different species of monkey, cynomolgus (Freeman et al., 1995; Roberts et al., 2007) and *C. apella* (Roberts et al., 2002), are represented in the data set. These data were combined in the summary statistics reported above because comparison of RBA estimates from cynomolgus and *C. apella* bioassays did not show significant differences. The mean RBA values were 19.9% (±9.2 SD, n=19) for cynomolgus and 16.7 (±5.1 SD, n=5) for *C. apella*. However, these estimates correspond to different test materials assayed in the two species. Available data do not allow comparisons of RBA estimates for the same test materials assayed in different monkey species to determine if different species actually yield different RBA values. Given the lack of information on which to distinguish RBA estimates from cynomolgus and *C. apella*, RBA estimates from both monkeys species were combined for comparison of RBA estimates from swine, monkey, and mouse assays (described below).

Differences between RBA estimates from swine, monkey, and mouse assays may also be attributable to:

(1) species difference in RBA;

(2) differences in assay protocols;

(3) differences in data reduction methods used to calculate RBA;

(4) differences in methods used to measure arsenic concentration in soils and biological samples, and

(5) differences in the test materials assayed.

Theoretically, direct comparison of results from different bioassays when applied to the same test materials would provide a test of whether or not differences can be attributed to the test materials, rather than to the bioassay protocols and/or species. Thus far, such direct comparisons between swine, monkey, and mouse assays are available for only 4 test materials, all of which were obtained from the same site (Barber Orchard, Region 4). These data are shown in Table 5 and Figure 2. The sample size (n=4) is too small to make meaningful statistical comparisons. However, based on the 95% confidence limits, the uncertainty bounds on estimates obtained from the three assays show substantial overlap. Furthermore, the 95% confidence limits on the group mean RBA (n=4) also overlap substantially (see Figure 2). Therefore, if these four soil samples were used in a risk assessment to represent the RBA for the Barber Orchard site (it is not unusual to base site-wide RBA estimates on a few samples of *in vivo* RBA estimates), the site-wide RBA estimates from the swine, monkey, and mouse assays would be statistically indistinguishable.

A larger set of comparisons are available for swine and mouse RBA estimates. The data set includes 2 standard reference materials (NIST 2710 and 2710a), the 4 Barber Orchard samples, and 5 soil samples from 4 other sites (see Table 6). Collectively, these comparisons show that the assays yielded similar results for 5 of the materials (95% confidence limits overlap) and dissimilar estimates for 6 of the materials (see Figure 3). In all of the latter cases, the RBA from the mouse bioassay was less than the RBA from the swine assay. Figure 4 shows a scatter plot of RBA estimates in swine and mice for these 11 test materials. The data tend to cluster around the line of identity; however, the linear regression model showed a relatively weak association between the RBA estimates obtained in swine and mice (R^2=0.35, p=0.053). Although different RBA values were obtained from the swine and mouse assays for some test materials, the differences were relatively small. The absolute difference in the RBA estimates (RBA_{swine} - RBA_{mouse}) ranged from ≤1% (NIST 2710 and 2710a) to 28% (Barber Orchard MS-5), and the average difference was 12%. For the 4 Barber Orchard soils, the absolute difference between swine and monkey RBA values (RBA_{swine} - RBA_{monkey}) ranged from 2% (Barber Orchard MS-1) to 28% (Barber Orchard MS-8), and the average difference was 8%; and the absolute difference between monkey and mouse (RBA_{mouse} - RBA_{monkey}) ranged from 7% (Barber Orchard MS-1 and MS 4) to 17% (Barber Orchard MS-5), and the average difference was 10%.

4.2.2 Urinary Excretion Fraction (UEF) Method vs. Blood AUC Method

In theory, we expect RBA estimates based on blood AUC measurements to be equivalent to RBA estimates based on urinary excretion measurements. The underlying assumption for both methods is that arsenic absorbed from the test and reference materials have the same toxicokinetics; and therefore, for both test and reference material, the same fraction of the absorbed dose is expected to appear in blood or urine.

The only direct comparison of the two methods is from Freeman et al. (1995). This study used blood AUC and UEF to estimate arsenic ABA for an oral dose of sodium arsenate and arsenic in soil, using the same three monkeys. These data allow calculation of the RBA for each monkey, for each method, and for the same test material (see Table 7). The RBA estimates based on the two methods were not significantly different based on paired t-test ($p=0.37$) or unpaired t-test ($p=0.20$). As there is no evidence to suggest that the blood AUC method and UEF method would yield different estimates of RBA, and there is no theoretical argument for a difference, RBA estimates obtained from the UEF method and blood AUC method are combined in summary statistics of RBA estimates for the entire data set (see Table 3).

4.2.3 Test Material Arsenic Dose and Concentration

Doses of arsenic varied with test material and study. In general, arsenic doses administered to monkeys were higher than those administered to swine, although the range of doses evaluated in each species overlapped. The range of arsenic doses evaluated in swine was approximately 1.5 to 1540 µg As/kg bw/day, in monkeys approximately 120 to 1330 µg As/kg bw (single dose), and in mice approximately 320–6100 µg As/kg bw/day. It is not possible to evaluate potential effects of arsenic dose on RBA because of the different dosing protocols used in the various studies. In some protocols, repeated doses of arsenic were administered at multiple dose levels, and RBA was derived from the composite data (e.g., Casteel and SRC, 2009a,b,c, 2010a,b,c), whereas other protocols administered repeated doses of arsenic at the same dose level (e.g., Basta et al., 2007; Bradham et al., 2011, 2012; Casteel and SRC, 2009a,b,c, 2010a,b,c; Rodriguez et al., 1999) or administered a single dose of arsenic (e.g., Freeman et al., 1995; Juhasz et al., 2007; Roberts et al., 2002, 2007; U.S. EPA, 1996, 2009). Doses used in these different protocols are not directly comparable. In studies conducted in swine, arsenic urinary excretion rate (µg As/day) was a linear function of arsenic dose for both sodium arsenate (dose range ≤310 µg As/kg bw/day) and test material arsenic (dose range ≤1540 µg As/kg bw/day). This observation suggests that arsenic absorption (based on UEF) was not strongly dependent on arsenic dose (Casteel and SRC, 2009a,b,c, 2010a,b,c; U.S. EPA, 2010). In studies conducted in cynomolgus monkeys, the arsenic UEF was shown to be independent of dose (administered as a single gavage dose) over the dose range 250–1000 µg/kg (Roberts et al., 2007). In mice, arsenic UEF was shown to be independent of dose over a dose range of 580–2600 µg As/kg bw/day (Bradham et al., 2011, 2012).

Arsenic levels in the test materials assayed in swine ranged from 42 to 17,500 mg/kg, in monkeys from 101 to 1492 mg/kg, and in mice from 182 to 4495 mg/kg. The wide range of arsenic concentrations resulted in a similarly wide range of soil doses given to the animals (e.g., lower soil arsenic concentrations required larger doses of soil to be administered to achieve the same arsenic dose). The soil doses ranged from approximately 0.4 to 3528 mg soil/kg bw/day in swine, 490 to 2970 mg soil/kg (single dose) in monkeys, and 1150 to 1650 mg soil/kg bw/day in mice. A direct evaluation of the influence of soil dose on arsenic RBA cannot be made from these data because of the differences in dosing regimens used in the various bioassays. However, a strong dependence of RBA on soil dose would be expected to also result in a dependence on soil arsenic concentration since these two variables would be strongly negatively correlated if soil dose was adjusted to achieve a fixed range of soil arsenic doses. Simple regression analysis of these data indicated a relatively small influence (≤14%) of arsenic level on

RBA, with values for R^2 of 0.10 (p=0.01, n=64) for test materials assayed in swine, 0.14 (p=0.07, n=24) for test materials assayed in monkeys, 0.03 (p=0.51, n=15) for test materials assayed in mice, and 0.06 (p=0.01, n=1036) for swine, monkey, and mice combined.

4.2.4 Explanatory Variables Influencing RBA Estimates in Key Studies

Multivariate regression analyses were conducted using factors found to be significant variables in simple regression analyses (species, iron arsenide [FeAs] sulfate content of arsenic-bearing particles, and arsenic levels in test materials) as explanatory variables. These analyses were restricted to data from swine and monkey studies for which data on arsenic mineralogy were available. Content of FeAs sulfate was examined because it has been shown to be an influential variable on RBA in monkeys (Roberts et al., 2007). The R^2 for the model that included all three variables was 0.38 (p=0.006, n=29); however, only species (i.e., monkey or swine) was significant (p=0.02). When the analysis was restricted to monkeys, the dominant influential variable was relative mass of the FeAs sulfate phase of arsenic-bearing particles (R^2= 0.70, p=0.015, n=10), as reported in Roberts et al. (2007). When the analysis was restricted to swine none of the variables (i.e., arsenic level, FeAs sulfate) were found to be significant predictors of RBA (R^2= 0.05, p=0.68, n=19).

Based on these analyses, the dominant influential variable on RBA in this data set appears to be species (i.e., whether the test material was assayed in monkeys or swine) and for test materials assayed in monkeys, the relative mass of the FeAs sulfate phase of arsenic-bearing particles. As previously noted, an explanation for the difference between RBA estimates from monkey and swine assays is not apparent from these analyses.

Other factors, not explored in this analysis, may contribute to the unexplained variability in the arsenic RBA estimates. Approximately 62% of the RBA estimates are based on an R^2 value of 0.38 for the model that included species, FeAs sulfate content of arsenic-bearing particles, and arsenic levels in test materials. Likely candidates are arsenic mineralogy (chemical composition and morphology of the arsenic-bearing particles) and soil characteristics, which together may determine arsenic bioaccessibility and/or absorption of bioaccessible arsenic.

4.3 Uncertainties in Use of Compiled RBA Estimates for Prediction of Arsenic RBA

Table 1 summarizes sources of uncertainties to be considered in assessing confidence in RBA estimates and making statistical inference regarding arsenic RBA in soils. These include the following.

- **Adequacy of Approach:**
    - Confidence in predictions of arsenic RBA in humans based on animal bioassays has not been assessed. This would require measuring RBA of the same soils in both humans and animal models.
    - When applied to the same test materials (see results for Barber Orchard soil samples in Table 5), the swine, monkey, and mouse assays yielded remarkably similar RBA

estimates for some materials and widely different estimates for other materials. However, collectively, the differences in the RBA estimates were relatively small. The average absolute difference in the RBA estimates for assays conducted on the same test materials ranged from <1 to 28%, and the average differences were 8, 12, and 10% for RBA_{swine} - RBA_{monkey}, RBA_{swine} - RBA_{mouse}, and RBA_{mouse} - RBA_{monkey}, respectively. When the three assays were applied to multiple samples from the same site (i.e., 4 samples from the Barber Orchard site), 95% confidence limits on the site-wide mean RBA values overlapped substantially, suggesting that for these samples, assays in the 3 species provided site-wide estimates of RBA that were statistically indistinguishable. The reason why the same test materials give different RBA outcomes for some of the Barber Orchard samples tested in the three animal models is not apparent from available data and could be related to one or more factors (as described in Section 4.7.1):

(1) animal species differences in arsenic absorption;

(2) differences in assay protocols;

(3) differences in data reduction methods used to calculate RBA; and

(4) differences in methods used to measure arsenic concentration in soils and biological samples.

- Experimental protocols of RBA bioassays differ (e.g., multiple dose levels vs. single dose level, repeated dosing vs. single dose), and each protocol may have different sources and magnitudes of measurement error.

- The arsenic dose range for test materials administered in the bioassays includes values that are substantially higher than typical daily soil ingestion rates in children or adults. The implication of these high test material doses in extrapolating RBA estimates from animal bioassays to humans (e.g., the effect of test material dose on RBA) has not been thoroughly investigated; however, based on measurements of urinary arsenic, the absorption fraction does not appear to be strongly dependent on dose.

- **Representativeness:** The RBA estimates considered in this analysis are derived from an opportunistic sample of soils and do not represent a statistical sample of soils in any geographic region (e.g., U.S.) or source of arsenic contamination. The samples were collected because of regulatory interest in specific sites. Although the data set includes samples from sites impacted by various sources of arsenic contamination (e.g., mining/smelting, agricultural, chemical/pesticide manufacturing facilities, and railway corridors), the dominant arsenic sources in the data set are mining and smelting (54 of 88 test materials). The absence of a statistical sampling design limits confidence in statistical inference based on the data set. For example, sample statistics such as the mean and standard deviation, even for specific categories of arsenic contamination, mineralogy, or soil characteristics, cannot be assumed to represent these categories in

general. Nevertheless, the data set does describe the distribution of RBA values that have been encountered in soils from various sites of regulatory interest. The empirical distribution of RBA values in this data set suggests that values for arsenic RBA exceeding 60% are relatively uncommon (i.e., less than 5% of the estimates exceed 60% RBA). Based on this experience, it is reasonable to expect that future RBA estimates exceeding 60% would also be uncommon if samples were to be drawn from a collection of similar types of sites and soils. This prediction could be further evaluated with additional data collection efforts and may be of value for informing assumptions about soil arsenic RBA at sites where RBA estimates have not yet been made (e.g., screening level assessments).

- **Variability of Test Material RBA Estimates:** Multivariate regression models used to explore the contribution of bioassay and soil variables to variability in RBA estimates yielded R^2 values ≤38%. Therefore, these models could explain no more than 38% of the variability observed in the RBA estimates, most of which was attributed to bioassay species. The relatively low explanatory power of the models explored in this analysis precludes their use in making predictions about RBA of arsenic in soil. It is likely that more informative regression models (or other variance models) could be developed that account for test material variables not considered in this analysis (e.g., arsenic mineralogy and soil characteristics). These variables are currently being explored as part of on-going EPA research. In addition to variables related to the soil test materials, other variables are likely to have contributed to the unexplained variability in the RBA estimates. These include the bioassay methods (e.g., dosing regimens), biomarkers used to estimate absorption (e.g., urine and blood), methods used to measure arsenic in soil and in biological samples, measurement error (e.g., doses administered, urinary arsenic excretion, and blood arsenic concentrations), and differences in data reduction methods. It is expected that differences in experimental design and protocol, data reduction methods, and measurement error contribute to variability in the RBA estimates. The above variables may explain differences in RBA estimates for some test materials that have been assayed in swine, monkey, and mouse. This complicates analyses of the impacts of other variables (e.g., arsenic mineralogy and soil characteristics) on RBA.

- **Interindividual Variability in RBA:** The RBA estimates for each test material represent mean values derived from experiments made on groups of animals. Estimates of interindividual variability in RBA were not possible for all studies and study designs. Interindividual variability in UEF for the test and reference material groups were accounted for in the calculation of group mean RBA estimates in the swine and mouse studies; however, the statistical design of the studies does not yield an estimate of interindividual variability in RBA, although it does provide an estimate of uncertainty in the RBA represented by the confidence limits. The monkey studies used a repeated measures design in which each animal received the soil and reference materials. This design allowed estimation of a group mean and standard deviation for RBA for each study, representing the interindividual variability in the RBA for each test material. Coefficients of variation (SD/mean) for the 20 RBA estimates derived from monkey bioassays ranged from 0.11 to 0.80 (mean 0.38 ± 0.17 SD). This outcome suggests that interindividual variability in RBA in monkeys that received the same test material varies

across test materials and/or studies. Numerous other factors may contribute to interindividual variability in arsenic RBA, including diet, nutrition, and age. Since these variables were controlled in the animal bioassays, interindividual variability observed in the animal bioassays is presumably dominated by contributions from the test material and physiological variables that affect bioaccessibility and absorption of arsenic. However, in human populations, interindividual variability in diet/nutrition, disease states, and other factors may also contribute to variability in RBA.

- **Intraindividual Variability in RBA:** This analysis did not attempt to estimate intraindividual variability in RBA. The RBA studies compiled in this review did not provide data on intraindividual variability, which would have required repeated measurements of RBA in the same animals. As noted above, the controlled conditions of the bioassays would have eliminated variables that may contribute to intraindividual variability in RBA estimates in humans. Variables that may contribute to intraindividual variability in arsenic RBA include age, diet/nutrition, disease states, etc.

- **Relevance of Soil Arsenic Concentrations Tested:** Arsenic RBA was not significantly correlated with arsenic concentration (<100 to 17,500 mg kg^{-1}). Nevertheless, RBA estimates at sites that have arsenic concentrations well below or above the risk-based decision level may not influence cleanup decisions.

- **Data Collection Period and Relevance of Soil Aging to Arsenic RBA:** RBA estimates in this report cannot represent temporal changes in soil characteristics (e.g., changes in soil composition or arsenic speciation) at the sites that might alter RBA. Bioavailability of arsenic in soil may change over time. Although direct evidence for this for *in situ* contaminated soils is not available, studies of laboratory-contaminated soils suggest that changes over time in certain soils can be substantial. Juhasz et al. (2008) found that RBA decreased from 100 to 25% in 3 months and then remained constant for the next 9 months following addition of sodium arsenate to a soil containing a high iron content (99,671 mg Fe/kg soil). Arsenic RBA remained approximately 100% in a similarly spiked soil that contained lower iron content (7980 mg/kg). The predominant arsenic phase in the high iron content soil was associated with iron oxides. Although this study was limited to soils spiked in the laboratory with sodium arsenate, it suggests the possibility that arsenic RBA may change over time and that the magnitude of the change may depend on soil characteristics. Studies in which arsenic RBA is measured repeatedly over time, in a variety of soils, would be needed to determine the relevance of this observation to arsenic-contaminated sites. On-going EPA research is attempting to evaluate the long-term stability of arsenic bioaccessibility of soils contaminated *in situ*.

- **Extrapolation to Humans:** Studies comparing arsenic RBA in humans and animals for the same soils are not available and are not likely to be undertaken. This limitation introduces uncertainty into predictions of arsenic RBA in humans based on results from animal bioassay studies; however, it should not preclude making extrapolations of animal bioassay data to humans. EPA currently recommends use of a swine RBA assay (or an *in vitro* bioaccessibility (IVBA) assay that was validated with a swine assay) for predicting site-specific lead RBA in human health risk assessments (U.S. EPA,

2007a,b,c). As noted previously, when applied to the same test materials, RBA estimates based on the swine, monkey, and mouse assays yielded remarkably similar RBA estimates for some materials and collectively, the differences in the RBA estimates were relatively small. The similarity of RBA estimates based on assays in three mammalian species increases confidence in extrapolation of these results to humans.

- **Quality Assurance:** For some studies, information on quality assurance/quality control was limited or absent.

5.0 REFERENCES

Basta, N.T., Foster, J.N., Dayton, E.A., Rodriguez, R.R., and Casteel, S.W. (2007) The effect of dosing vehicle on arsenic bioaccessibility in smelter-contaminated soils. J. Environ. Sci. Heath Part A 42: 1275–1281.

Bradham, K.D., Scheckel. K.G., Nelson, C.M., Seales, P.E., Lee, G.E., Hughes, M.F., Miller, B.W., Yeow, A., Gilmore, T., Harper, S., Thomas, D.J. (2011) Relative Bioavailability and Bioaccessibility and Speciation of Arsenic in Contaminated Soils. Environ. Health Perspect. 119(11): 1629–1634.

Bradham et al. (2012) Assessing performance of the mouse assay of bioavailability of arsenic. (manuscript in preparation)

Casteel and SRC. (2005) Relative Bioavailability of Arsenic and Vanadium in Soil from a Superfund Site in Palestine, Texas. Prepared by University of Missouri, Columbia and SRC. Prepared for U.S. Environmental Protection Agency, Office of Superfund Remediation Technology Innovation. Prepared by University of Missouri, Columbia and SRC.

Casteel and SRC. (2009a) Relative Bioavailability of Arsenic in Barber Orchard Soils. Prepared for U.S. Environmental Protection Agency, Office of Superfund Remediation Technology Innovation. Prepared by University of Missouri, Columbia and SRC.

Casteel and SRC. (2009b) Relative Bioavailability of Arsenic in NIST SRM 2710 (Montana Soil). Prepared for U.S. Environmental Protection Agency, Office of Superfund Remediation Technology Innovation. Prepared by University of Missouri, Columbia and SRC.

Casteel and SRC. (2009c) Relative Bioavailability of Arsenic in a Mohr Orchard Soil. Prepared for U.S. Environmental Protection Agency, Office of Superfund Remediation Technology Innovation. Prepared by University of Missouri, Columbia and SRC.

Casteel and SRC. (2010a) Relative Bioavailability of Arsenic in an Iron King Soil. Prepared for U.S. Environmental Protection Agency, Office of Superfund Remediation Technology Innovation. Prepared by University of Missouri, Columbia and SRC.

Casteel and SRC. (2010b) Relative Bioavailability of Arsenic in an ASARCO and Hawaiian soil. Prepared for U.S. Environmental Protection Agency, Office of Superfund Remediation Technology Innovation. Prepared by University of Missouri, Columbia and SRC.

Casteel and SRC. (2010c) Relative Bioavailability of Arsenic in NIST SRM 2710a (Montana Soil). Prepared for U.S. Environmental Protection Agency, Office of Superfund Remediation Technology Innovation. Prepared by University of Missouri, Columbia and SRC.

Chiou, W.L. and Buehler, P.W. (2002) Comparison of oral absorption and bioavailability of drugs between monkey and human. Pharm. Res. 19(6): 868–874.

Ellickson, K.M., Meeker, R.J., Gallo, M.A., Buckley, B.T., Lioy, P.J. (2001) Oral bioavailability of lead and arsenic from a NIST standard reference soil material. Arch. Environ. Contam. Toxicol. 40(1): 128–135.

Freeman, G.B., Johnson, J.D., Killinger, J.M., Liao, S.C., Davis, A.O., Ruby, M.V., Chaney, R.L., Lovre, S.C., and Bergstrom, P.D. (1993) Bioavailability of arsenic in soil impacted by smelter activities following oral administration in rabbits. Fundam. Appl. Toxicol. 21(1): 83–88.

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. Fundam. Appl. Toxicol. 28(2): 215–222.

Juhasz, A.L., Smith, E., Weber, J., Rees, M., Rofe, A., Kuchel, T., Sansom, L., and Naidu, R. (2007) Comparison of *in vivo* and *in vitro* methodologies for the assessment of arsenic bioavailability in contaminated soils. Chemosphere 69(6): 961–966.

Juhasz, A.L., Smith, E., Weber, J., Naidu, R., Rees, M., Rofe, A., Kuchel, T., and Sansom, L. (2008) Effect of aging on *in vivo* arsenic bioavailability in two dissimilar soils. Chemosphere 71(10): 2180–2186.

Konstantinos, C.M., Makris, S.Q., Nagar, R., Sarkar, D., Datta, R., and Sylvia, L. (2008) *In vitro* model improves the prediction of soil arsenic bioavailability: Worst-case scenario. Environ. Sci. Technol. 42: 6278–6284.

Nagar, R., Sarkar, D., Konstantinos C.M., Datta, R., and Sylvia, V.L. (2009) Bioavailability and bioaccessibility of arsenic in a soil amended with drinking-water treatment residuals. Arch. Environ. Contam. Toxicol. 57: 755–766.

NRC (National Research Council). 2003. Bioavailability of Contaminants in Soils and Sediments: Processes, Tools, and Applications. National Academies Press: Washington, DC. http://www.nap.edu/openbook/0309086256/html/.

Roberts, S.M., Weimar, W.R., Vinson, J.R., Munson, J.W., and Bergeron, R.J. (2002) Measurement of arsenic bioavailability in soil using a primate model. Toxicol. Sci. 67(2): 303–310.

Roberts, S.M., Munson, J.W., Lowney, Y.W., and Ruby, M.V. (2007) Relative oral bioavailability of arsenic from contaminated soils measured in the cynomolgus monkey. Toxicol. Sci. 95(1): 281–288. (Erratum for Table 3 of the report, correcting the columns headings for the NYPF samples, was provided as a personal communication from the co-authors S. Roberts and Y. Lowney on 09/24/2010.)

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

Stanek, E.J., Calabrese, E.J., Barnes, R.M., Danku, J.M.C., Zhou, Y., Kostecki, P.T., Zillioux, E. (2010) Bioavailability of arsenic in soil: Pilot study results and design considerations. Hum. Exper. Toxicol. 29(11): 945–960.

U.S. EPA (U.S. Environmental Protection Agency). (1989) Risk Assessment Guidance for Superfund (RAGS). Volume I. Human Health Evaluation Manual (Part A). U.S. Environmental Protection Agency, Office of Emergency and Remedial Response: Washington, DC. EPA/540/1-89/002. December. Available online at: http://www.epa.gov/swerrims/riskassessment/ragsa/pdf/rags-vol1-pta_complete.pdf.

U.S. EPA (U.S. Environmental Protection Agency). (1996) Bioavailability of Arsenic and Lead in Environmental Substrates. U.S. Environmental Protection Agency, Region 10: Seattle, WA. EPA910/R-96-002. February. Available online at: http://yosemite.epa.gov/r10/OMP.NSF/webpage/Bioavailability+of+Arsenic+and+Lead+in+Environmental+Substrates/$FILE/bio-arsenic.pdf.

U.S. EPA (U.S. Environmental Protection Agency). (2007a) Estimation of Relative Bioavailability of Lead in Soil and Soil-Like Materials by *In Vivo* and *In Vitro* Methods. U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response: Washington, DC. OSWER 9285.7-77. Available online at: http://www.epa.gov/superfund/health/contaminants/bioavailability/lead_tsd_main.pdf.

U.S. EPA (U.S. Environmental Protection Agency). (2007b) Framework for Metals Risk Assessment. U.S. Environmental Protection Agency, Office of the Science Advisor: Washington, DC. EPA 120/R-07/001. March. Available online at: http://www.epa.gov/raf/metalsframework/pdfs/metals-risk-assessment-final.pdf.

U.S. EPA (U.S. Environmental Protection Agency). (2007c) Guidance for Evaluating the Oral Bioavailability of Metals in Soils for Use in Human Health Risk Assessment. U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response: Washington, DC. OSWER 9285.7-80. May. Available online at: http://www.epa.gov/superfund/health/contaminants/bioavailability/bio_guidance.pdf.

U.S. EPA (U.S. Environmental Protection Agency). (2008) Child-Specific Exposure Factors Handbook. U.S. Environmental Protection Agency, National Center for Environmental Assessment, Office of Research and Development: Washington, DC. EPA/600/R-06/096F. Available online at: http://ofmpub.epa.gov/eims/eimscomm.getfile?p_download_id=484738.

U.S. EPA (U.S. Environmental Protection Agency). (2009) Relative Bioavailability of Arsenic from Soil Barber Orchard Superfund Site Waynesville, North Carolina. Prepared for U.S. Environmental Protection Agency, Region 4 by Center for Environmental & Human Toxicology, University of Florida. (available through U.S. EPA Region 4 Administrative Record Index for the Barber Orchard (Explanation of Significant Differences) NCSDN0406989).

U.S. EPA (U.S. Environmental Protection Agency). (2010) Relative Bioavailability of Arsenic in Soils at 11 Superfund Sites Using an *In Vivo* Juvenile Swine Method. U.S. Environmental Protection Agency. Available online at: http://epa.gov/superfund/bioavailability/pdfs/as_in_vivo_rba_main.pdf.

U.S. EPA (U.S. Environmental Protection Agency). (2012) Arsenic, inorganic. Integrated Risk Information System (IRIS). U.S. Environmental Protection Agency, National Center for Environmental Assessment: Washington, DC. Available online at: http://www.epa.gov/ncea/iris/subst/0278.htm.

Table 1. Confidence in Arsenic RBA Estimates		
General Assessment Factors	**Rationale**	**Rating**
Soundness		
Adequacy of Approach	Methodologies included several limitations: (1) Estimates of RBA of arsenic in soil materials in humans have not been reported. The monkey and swine models have been utilized for predicting RBA of arsenic in humans because the gastric physiology of both animal species share many similarities to that of humans and because of a prior history of use of the models for assessing RBA of other inorganic contaminants (e.g., lead) and gastrointestinal absorption of drugs. Estimates of RBA of arsenic in soil materials in animal models cannot be quantitatively compared to estimates made in humans, as estimates in humans are not available for these test materials. (2) Reported estimates of RBA for arsenic in soil materials obtained from monkey assays are significantly lower than reported estimates obtained from swine or mouse assays. The mechanism for the different outcomes from the two assays is not apparent and could be related to several factors (e.g., species differences, protocol differences, test material differences). (3) Experimental protocols utilizing a steady-state design with multiple dose levels may introduce less error than experimental protocols using a steady-state design with a single dose level or a single dose (i.e., non steady-state) design. (4) Variations in the design of animal RBA assays, in particular, different detection limits for blood and urinary arsenic and wide variations in arsenic concentrations of test materials, has placed constraints on experimental control of both the arsenic dose and test material dose used in each assay. Therefore, the dose range for test materials administered in the animal bioassays includes values that are substantially higher than typical daily soil ingestion rates in children or adults. The implication of these high test material doses in extrapolating RBA estimates from monkey and swine assays to humans has not been thoroughly investigated (e.g., effect of test material dose on RBA).	Medium
Bias	Numerous sources of measurement error exist. Studies utilizing multiple dose levels and dosing regimens to achieve steady-state are more likely to have less measurement error in the critical parameter (i.e., UEF). The upper bound estimate may be biased by sample selection bias (samples dominated by mining/smelter sources).	

Table 1. Confidence in Arsenic RBA Estimates		
General Assessment Factors	**Rationale**	**Rating**
Applicability and Utility		
Default Value of Interest	All "key" and "relevant" studies focus on the relative bioavailability of arsenic.	Medium
Representativeness	The RBA estimates considered in this analysis do not represent a statistical sample of soils in any geographic region (e.g., U.S.). Although not a statistical sample of soils, nearly all samples were collected at hazardous waste sites. These included test materials collected from mining and/or smelter operations, pesticides (orchards), and manufacturing/electrical waste. Therefore, the samples may provide adequate representation of soils at sites of the highest regulatory interest or concern.	
Currency	Test materials assayed reflect recent conditions (samples collected over ≤10–15 years).	
Data Collection Period	Test materials assayed represent a cross-sectional sample of soils. However, RBA estimates of those test materials cannot assess temporal change in soil characteristics (e.g., changes in soil composition or arsenic speciation) at the sites and potential related changes in RBA estimates of those materials.	
Clarity and Completeness		
Accessibility	Observations for individual data on which RBA estimates were based are available in the published literature or online.	Low
Reproducibility	Reproducibility has not been evaluated across methodologies.	
Quality Assurance	For some studies, information on quality assurance/quality control was limited or absent.	
Variability and Uncertainty		
Variability in Estimates	The sample of test materials is not a statistical sample of soils. Therefore, variability in arsenic RBA for soils in general or for any subset of characteristics of the test materials (e.g., arsenic mineralogy, soil characteristics) cannot be inferred from the variability represented in the data set.	Low
Minimal Uncertainty	Estimates of the mean and percentiles for RBAs of test material sample are reasonably certain; however, the representativeness of the sample for making statistical inference about arsenic RBA estimates for soils in general, or about soils at specific sites is uncertain.	
Evaluation and Review		
Peer Review	The animal bioassays used in all studies either appeared in peer reviewed journals or the study was conducted by or for EPA in which EPA developed the RBA estimates from the raw data using established standard protocols and/or the raw data were available for QA review by the U.S. EPA Bioavailability Committee of the Technical Review Workgroup (e.g., EPA swine studies); or, the study was conducted by other research groups and results had been subjected to peer review as a requirement for publication.	Medium

Table 1. Confidence in Arsenic RBA Estimates		
General Assessment Factors	**Rationale**	**Rating**
Number and Agreement of Studies	Application of similar assay methodologies produced highly variable estimates of arsenic RBA. However, these differences may reflect differences in test material characteristics, differences in assay protocols, or differences in species (monkeys, swine, mouse). Direct comparisons of swine, monkey, and mouse RBA estimates are available for only 4 test materials and direct comparisons of swine and mouse RBA estimates are available for 11 test materials. Based on this limited comparison, the magnitude of difference between RBA estimates derived from swine, monkey, and mouse assays is relatively small in the context of risk assessment, where uncertainties in other parameters in risk calculations can exceed several orders of magnitude. Therefore, from the perspective of use of the assays to support risk assessment, the swine, monkey, and mouse assays appear to yield essentially equivalent information about arsenic RBA.	Medium
Overall Rating		Medium

Table 2. Key and Relevant Study Results				
Test Material	**Species**	**Method/Dose**	**RBA (%)**	**Reference**
Key Studies				
Source: Bingham Creek Channel soil (sieved to <250 µm) Type: Mining/smelting As concentration: 149 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 5, 20, or 50 µg As/kg bw/day; 5 animals/group Test material dose: 15.8 µg As/kg bw/day (106.0 mg soil/kg bw/day); 5 animals/group	39±8 Mean±SE	U.S. EPA, 2010
Source: Murray smelter slag (sieved to <250 µm) Type: Mining/smelting As concentration: 695 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 5, 20, or 50 µg As/kg bw/day; 5 males/group Test material dose: 13.4 µg As/kg bw/day (19.2 mg soil/kg bw/day); 5 animals/group	55±10 Mean±SE	U.S. EPA, 2010
Source: Butte soil, composite soil waste rock dumps (sieved to <250 µm) Type: Mining/smelting As concentration: 234 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 5, 20, or 50 µg As/kg bw/day; 5 animals/group Test material dose: 6.3 µg As/kg bw/day (26.2 mg soil/kg bw/day); 5 animals/group	9±3 Mean±SE	U.S. EPA, 2010
Source: Midvale slag, composite sample Midvale smelter slag pile (sieved to <250 µm) Type: Mining/smelting As concentration: 591 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 5, 20, or 50 µg As/kg bw/day; 5 animals/group Test material dose: 16.8 µg As/kg bw/day (28.5 mg soil/kg bw/day); 5 animals/group	23±4 Mean±SE	U.S. EPA, 2010
Source: California Gulch Phase I residential soil, composite residential soil, Leadville, CO (sieved to <250 µm) Type: Mining/smelting As concentration: 203 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 5, 20, or 50 µg As/kg bw/day; 5 animals/group Test material dose: 6.1 µg As/kg bw/day (30.0 mg soil/kg bw/day); 5 animals/group	8±3 Mean±SE	U.S. EPA, 2010
Source: California Gulch Fe/Mn PbO, composite soil, Leadville, CO (sieved to <250 µm) Type: Mining/smelting As concentration: 110 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 5, 20, or 50 µg As/kg bw/day; 5 animals/group Test material dose: 5.7 µg As/kg bw/day (52.1 mg soil/kg bw/day); 5 animals/group	57±12 Mean±SE	U.S. EPA, 2010
Source: Palmerton Location 2, composite soil, Palmerton, PA (sieved to <250 µm) Type: Mining/smelting As concentration: 110 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 5, 20, or 50 µg As/kg bw/day; 5 animals/group Test material dose: 7.7 µg As/kg bw/day (70.0 mg soil/kg bw/day); 5 animals/group	49±10 Mean±SE	U.S. EPA, 2010

Table 2. Key and Relevant Study Results				
Test Material	**Species**	**Method/Dose**	**RBA (%)**	**Reference**
Source: Palmerton Location 4, composite soil, Palmerton, PA (sieved to <250 µm) Type: Mining/smelting As concentration: 134 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 5, 20, or 50 µg As/kg bw/day; 5 animals/group Test material dose: 14.0 µg As/kg bw/day (104.7 mg soil/kg bw/day); 5 animals/group	61±11 Mean±SE	U.S. EPA, 2010
Source: California Gulch AV slag, Leadville, CO (sieved to <250 µm) Type: Mining/smelting As concentration: 1050 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 5, 20, or 50 µg As/kg bw/day; 2 animals/group Test material dose: 22.3 µg As/kg bw/day (21.2 mg soil/kg bw/day); 2 animals/group	18±2 Mean±SE	U.S. EPA, 2010
Source: Murray Smelter Soil, composite (sieved to <250 µm) Type: Mining/smelting As concentration: 310 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 5, 20, or 50 µg As/kg bw/day; 5 animals/group Test material dose: 65.4 µg As/kg bw/day (211.0 mg soil/kg bw/day); 5 animals/group	33±5 Mean±SE	U.S. EPA, 2010
Source: Clark Fork Tailings, MT (sieved to <250 µm) Type: Mining/smelting As concentration: 181 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 20 or 50 µg As/kg bw/day; 4 animals/group Test material dose: 10.0 or 25 µg As/kg bw/day (55.2 or 138.1 mg soil/kg bw/day); 4 animals/group	51±6 Mean±SE	U.S. EPA, 2010
Source: Sample TM1 Vasquez Boulevard and I-70, composite residential, Denver CO (sieved to <250 µm) Type: Mining/smelting As concentration: 312 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 50 or 125 µg As/kg bw/day; 4 animals/group Test material dose: 37.0 or 92.5 µg As/kg bw/day (59.2 or 148.1 mg soil/kg bw/day); 4 animals/group	40±4 Mean±SE	U.S. EPA, 2010
Source: Sample TM2 Vasquez Boulevard and I-70, composite residential, Denver CO (sieved to <250 µm) Type: Mining/smelting As concentration: 983 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 50 or 125 µg As/kg bw/day; 4 animals/group Test material dose: 33.9 or 84.7 µg As/kg bw/day (17.2 or 43.1 mg soil/kg bw/day); 4 animals/group	42±4 Mean±SE	U.S. EPA, 2010

Table 2. Key and Relevant Study Results				
Test Material	**Species**	**Method/Dose**	**RBA (%)**	**Reference**
Source: Sample TM3 Vasquez Boulevard and I-70, composite residential, Denver CO (sieved to <250 µm) Type: Mining/smelting As concentration: 390 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 50 or 125 µg As/kg bw/day; 4 animals/group Test material dose: 27.5 or 68.7 µg As/kg bw/day (35.2 or 88.0 mg soil/kg bw/day); 4 animals/group	37±3 Mean±SE	U.S. EPA, 2010
Source: Sample TM4 Vasquez Boulevard and I-70, composite residential, Denver, CO (sieved to <250 µm) Type: Mining/smelting As concentration: 813 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 50 or 125 µg As/kg bw/day; 4 animals/group Test material dose: 37.4 or 93.5 µg As/kg bw/day (22.9 or 57.5 mg soil/kg bw/day); 4 animals/group	24±2 Mean±SE	U.S. EPA, 2010
Source: Sample TM5 Vasquez Boulevard and I-70, composite residential, Denver, CO (sieved to <250 µm) Type: Mining/smelting As concentration: 368 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 50 or 125 µg As/kg bw/day; 4 animals/group Test material dose: 41.1 or 102.7 µg As/kg bw/day (55.8 or 139.5 mg soil/kg bw/day); 4 animals/group	21±2 Mean±SE	U.S. EPA, 2010
Source: Sample TM6 Vasquez Boulevard and I-70, composite residential, Denver, CO (sieved to <250 µm) Type: Mining/smelting As concentration: 516 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 50 or 125 µg As/kg bw/day; 4 animals/group Test material dose: 32.4 or 81.0 µg As/kg bw/day (31.4 or 78.5 mg soil/kg bw/day); 4 animals/group	24±3 Mean±SE	U.S. EPA, 2010
Source: Butte TM1, composite waste rock dumps (U.S. EPA Sample #8-37926) (sieved to <250 µm) Type: Mining/smelting As concentration: 234 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 34, 59, or 94 µg As/kg bw/day; 4 animals/group Test material dose: 30.4, 60.5, or 92.0 µg As/kg bw/day (130.0, 258.5, or 393.2 mg soil/kg bw/day); 4 animals/group	18±3 Mean±SE	U.S. EPA, 2010
Source: Butte TM2, composite (U.S. EPA Sample #BPSOU-0501-ASBIO) (sieved to <250 µm) Type: Mining/smelting As concentration: 367 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 34, 59, or 94 µg As/kg bw/day; 4 animals/dose Test material dose: 25.7, 62.5, or 92.6 µg As/kg bw/day (70.0, 170.3, or 252.3 mg soil/kg bw/day); 4 animals/dose	24±2 Mean±SE	U.S. EPA, 2010

Table 2. Key and Relevant Study Results				
Test Material	**Species**	**Method/Dose**	**RBA (%)**	**Reference**
Source: Aberjona River sediment composite TM1 (fine sieved, but no information was reported on size) Type: Mining/smelting As concentration: 676 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 30, 60, or 90 µg As/kg bw/day; 4 animals/dose Test material dose: 18.3, 40.2, or 46.9 µg As/kg bw/day (27.1, 59.5, or 73.3 mg soil/kg bw/day); 4 animals/dose	38±2 Mean±SE	U.S. EPA, 2010
Source: Aberjona River sediment composite TM2 (fine sieved, but no information was reported on size) Type: Mining/smelting As concentration: 313 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 30, 60, or 90 µg As/kg bw/day; 4 animals/group Test material dose: 18.8, 35.9, or 61.9 µg As/kg bw/day (60.1, 114.7, or 197.8 mg soil/kg bw/day); 4 animals/group	52±2 Mean±SE	U.S. EPA, 2010
Source: Soil sample (TM1) American Canal, El Paso County, TX (sieved to <250 µm) Type: Mining/smelting As concentration: 74 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 25 or 50 µg As/kg bw/day; 5 animals/group Test material dose: 40, 80, or 160 µg As/kg bw/day (540.5, 1081.1, or 2162.2 mg soil/kg bw/day); 5 animals/group	44±3 Mean±SE	U.S. EPA, 2010
Source: Soil sample (TM2) American Canal, El Paso County, TX (sieved to <250 µm) Type: Mining/smelting As concentration: 73 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 25 or 50 µg As/kg bw/day; 5 animals/group Test material dose: 40, 80, or 160 µg As/kg bw/day (547.9, 1095.9, or 2191.8 mg soil/kg bw/day); 5 animals/group	37±3 Mean±SE	U.S. EPA, 2010
Source: Utility pole soil, Conley, GA (sieved to <250 µm) Type: Pesticide application As concentration: 320 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 30 or 60 µg As/kg bw/day; 5 animals/group Test material dose: 46.5 or 91.0 µg As/kg bw/day (145.3 or 284.4 mg soil/kg bw/day); 5 animals/group	47±3 Mean±SE	U.S. EPA, 2010
Source: Soil, Superfund site, Palestine, TX (sieved to <250 µm) Type: Mining/smelting As concentration: 47 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 30, 60, or 121 µg As/kg bw/day; 5 animals/group Test material dose: 42.6, 84.8, or 165.8 µg As/kg bw/day (906.4, 1804.3, or 3527.7 mg soil/kg bw/day); 5 animals/group	15±1.1 Mean±SE	Casteel and SRC, 2005

Table 2. Key and Relevant Study Results				
Test Material	**Species**	**Method/Dose**	**RBA (%)**	**Reference**
Source: Barber Orchard NC, sample MS-1 (sieved to <250 µm) Type: Agriculture As concentration: 290 mg/kg soil	Swine (Line 26, male, immature, 6–7 weeks old)	**Steady-state urinary excretion fraction method** Reference material dose: 32.0, 55.7, or 125.2 µg As/kg bw/day; 4 animals/group Test material dose: 72.9 or 145.7 µg As/kg bw/day (251.0 or 502.4 mg soil/kg bw/day); 4 animals/group	31±4.0 Mean±SE	Casteel and SRC, 2009a
Source: Barber Orchard NC, sample MS-4 (sieved to <250 µm) Type: Agriculture As concentration: 388 mg.kg soil	Swine (Line 26, male, immature, 6–7 weeks old)	**Steady-state urinary excretion fraction method** Reference material dose: 25.4, 53.6, or 104.6 µg As/kg bw/day; 4 animals/group Test material dose: 52.6, 77.3, or 144.4 µg As/kg bw/day (135.6, 199.2, or 372.2 mg soil/kg bw/day); 4 animals/group	41±1.8 Mean±SE	Casteel and SRC, 2009a
Source: Barber Orchard NC, sample MS-5 (sieved to <250 µm) Type: Agriculture As concentration: 382 mg/kg soil	Swine (Line 26, male, immature, 6–7 weeks old)	**Steady-state urinary excretion fraction method** Reference material dose: 29.7 or 57.3 µg As/kg bw/day; 4 animals/group Test material dose: 46.0, 71.0, or 138.9 µg As/kg bw/day (120.4, 185.8, or 363.6 mg soil/kg bw/day); 4 animals/group	49±4.7 Mean±SE	Casteel and SRC, 2009a
Source: Barber Orchard NC, sample MS-8 (sieved to <250 µm) Type: Agriculture As concentration: 364 mg/kg soil	Swine (Line 26, male, immature, 6–7 weeks old)	**Steady-state urinary excretion fraction method** Reference material dose: 25.4, 53.6, or 104.6 µg As/kg bw/day; 4 animals/group Test material dose: 44.6, 72.0, or 155.0 µg As/kg bw/day (122.5, 197.8, or 425.8 mg soil/kg bw/day); 4 animals/group	53±2.3 Mean±SE	Casteel and SRC, 2009a
Source: NIST SRM 2710 (sieved to 74 µm) Type: Mining/smelting As concentration: 626±38 mg/kg soil	Swine (Line 26, male, immature, 6–7 weeks old, ~9–10 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 24.1, 47.5, or 95.9 µg As/kg bw/day; 4 animals/group Test material dose: 58.2 or 114.5 µg As/kg bw/day (93.0 or 182.9 mg soil/kg bw/day); 4 animals/group	44±2.3 Mean±SE	Casteel and SRC, 2009b
Source: Mohr Orchard PA sample (sieved to <250 µm) Type: Agriculture As concentration: 340 mg/kg soil	Swine (Line 26, male, immature, 6–7 weeks old, ~9–10 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 29, 62, or 130 µg As/kg bw/day; 4 animals/group Test material dose: 52, 72, or 153 µg As/kg bw/day (153, 212, or 450 mg soil/kg bw/day); 4 animals/group	53 (51–57; 90% CI)	Casteel and SRC, 2009c

Table 2. Key and Relevant Study Results				
Test Material	**Species**	**Method/Dose**	**RBA (%)**	**Reference**
Source: Iron King, AZ soil sample TM1 (sieved to <250 µm) Type: Mining/smelting As concentration: 200±5.3 mg/kg soil	Swine (Line 26, male, immature, 6–7 weeks old, ~9–10 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 25, 50, or 100 µg As/kg bw/day; 4 animals/group Test material dose: 40, 60, or 120 µg As/kg bw/day (200, 300, or 600 mg soil/kg bw/day); 4 animals/group	60±2.7 Mean±SE	Casteel and SRC, 2010a
Source: Iron King, AZ soil sample TM2 (sieved to <250 µm) Type: Mining/smelting As concentration: 3957±332.7 mg/kg soil	Swine (Line 26, male, immature, 6–7 weeks old, ~9–10 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 25, 50, or 100 µg As/kg bw/day; 4 animals/group Test material dose: 116, 175, or 349 µg As/kg bw/day (29, 44, or 88 mg soil/kg bw/day); 4 animals/group	19±1.0 Mean±SE	Casteel and SRC, 2010a
Source: ASARCO soil sample (sieved to <250 µm) Type: Mining/smelting As concentration: 181.9±6.3 mg/kg soil	Swine (Line 26, male, immature, 6–7 weeks old, ~9–10 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 25, 50, or 100 µg As/kg bw/day; 4 animals/group Test material dose: 40, 60, or 120 µg As/kg bw/day (220, 330, or 660 mg soil/kg bw/day); 4 animals/group	49±2.5 Mean±SE	Casteel and SRC, 2010b
Source: Hawaiian soil sample (sieved to <250 µm) Type: Agriculture As concentration: 768.85±32.3 mg/kg soil	Swine (Line 26, male, immature, 6–7 weeks old, ~9–10 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 25, 50, or 100 µg As/kg bw/day; 4 animals/group Test material dose: 40, 60, 120 µg As/kg bw/day (80, 120, or 240 mg soil/kg bw/day); 4 animals/group	33±1.7 Mean±SE	Casteel and SRC, 2010b
Source: NIST SRM 2710a (sieved to <74 µm) Type: Mining/smelting As concentration: 1540±100 mg/kg soil	Swine (Line 26, male, immature, 6–7 weeks old, ~9–10 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 26, 52, or 105 µg As/kg bw/day; 4 animals/group Test material dose: 41, 62, or 121 µg As/kg bw/day (27, 40, or 79 mg soil/kg bw/day); 4 animals/group	42±1.4 Mean±SE	Casteel and SRC, 2010c
Source: Mining smelter soil (sample #1) (sieved to <250 µm) Type: Mining/smelting As concentration: 11,300 mg/kg soil	Swine (Line 26, male, 10–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: not reported; 5 animals/group Test material dose: 70.6 µg As/kg/day (6.25 mg soil/kg/day); 5 animals/group	8.6±6.9 Mean±SD	Basta et al., 2007

Table 2. Key and Relevant Study Results				
Test Material	**Species**	**Method/Dose**	**RBA (%)**	**Reference**
Source: Mining smelter soil (sample #2) (sieved to <250 µm) Type: Mining/smelting As concentration: 17,500 mg/kg soil	Swine (Line 26, male, 10–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: not reported; 5 animals/group Test material dose: 109 µg As/kg bw/day (6.25 mg soil/kg bw/day); 5 animals/group	4.1±2.1 Mean±SD	Basta et al., 2007
Source: Mining smelter soil (sample #3) (sieved to <250 µm) Type: Mining/smelting As concentration: 13,500 mg/kg soil	Swine (Line 26, male, 10–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: not reported; 4 animals/group Test material dose: 84.4 µg As/kg bw/day (6.25 mg soil/kg bw/day); 4 animals/group	7.9±4.3 Mean±SD	Basta et al., 2007
Source: Mining smelter soil (sample #4) (sieved to <250 µm) Type: Mining/smelting As concentration: 11,500 mg/kg soil	Swine (Line 26, male, 10–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: not reported; 5 animals/group Test material dose: 71.9 µg As/kg bw/day (6.25 mg soil/kg bw/day); 5 animals/group	22.8±4.6 Mean±SD	Basta et al., 2007
Source: Mining smelter soil (sample #6) (sieved to <250 µm) Type: Mining/smelting As concentration: 405 mg/kg soil	Swine (Line 26, male, 10–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: not reported; 2 animals/group Test material dose: 2.5 µg As/kg bw/day (6.25 mg soil/kg bw/day); 2 animals/group	38.7±15.3 Mean±SD	Basta et al., 2007
Source: Mining smelter soil (sample #7) (sieved to <250 µm) Type: Mining/smelting As concentration: 450 mg/kg soil	Swine (Line 26, male, 10–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: not reported; 4 animals/group Test material dose: 2.8 µg As/kg bw/day (6.25 mg soil/kg bw/day); 4 animals/group	43.0±23.8 Mean±SD	Basta et al., 2007
Source: Mining smelter soil (sample #8) (sieved to <250 µm) Type: Mining/smelting As concentration: 1180 mg/kg soil	Swine (Line 26, male, 10–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: not reported; 4 animals/group Test material dose: 7.4 µg As/kg bw/day (6.25 mg soil/kg bw/day); 4 animals/group	39.1±15.5 Mean±SD	Basta et al., 2007

Table 2. Key and Relevant Study Results				
Test Material	**Species**	**Method/Dose**	**RBA (%)**	**Reference**
Source: Mining smelter soil (sample #9) (sieved to <250 µm) Type: Mining/smelting As concentration: 5020 mg/kg soil	Swine (Line 26, male, 10–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: not reported; 5 animals/group Test material dose: 31.4 µg As/kg bw/day (6.25 mg soil/kg/day); 5 animals/group	32.9±7.4 Mean±SD	Basta et al., 2007
Source: Mining smelter soil (sample #10) (sieved to <250 µm) Type: Mining/smelting As concentration: 4650 mg/kg soil	Swine (Line 26, male, 10–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: not reported; 4 animals/group Test material dose: 29.1 µg As/kg bw/day (6.25 mg soil/kg bw/day); 4 animals/group	21.9±5.6 Mean±SD	Basta et al., 2007
Source: Mining smelter soil (sample #11) (sieved to <250 µm) Type: Mining/smelting As concentration: 331 mg/kg soil	Swine (Line 26, male, 10–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: not reported; 5 animals/group Test material dose: 2.2 µg As/kg bw/day (6.25 mg soil/kg bw/day); 5 animals/group	6.2 Mean (SE or SD not reported)	Rodriguez et al., 1999
Source: Mining smelter soil (sample #12) (sieved to <250 µm) Type: Mining/smelting As concentration: 233 mg/kg soil	Swine (Line 26, male, 10–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: not reported; 5 animals/group Test material dose: 1.5 µg As/kg bw/day (6.25 mg soil/kg bw/day); 5 animals/group	42.8 Mean (SE or SD not reported)	Rodriguez et al., 1999
Source: Mining smelter soil (sample #13) (sieved to <250 µm) Type: Mining/smelting As concentration: 799 mg/kg soil	Swine (Line 26, male, 10–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: not reported; 5 animals/group Test material dose: 5.0 µg As/kg bw/day (6.25 mg soil/kg bw/day); 5 animals/group	29.1 Mean (SE or SD not reported)	Rodriguez et al., 1999
Source: Mining smelter soil (sample #14) (sieved to <250 µm) Type: Mining/smelting As concentration: 1460 mg/kg soil	Swine (Line 26, male, 10–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: not reported; 5 animals/group Test material dose: 9.1 µg As/kg bw/day (6.25 mg soil/kg bw/day); 5 animals/group	18.7 Mean (SE or SD not reported)	Rodriguez et al., 1999

Table 2. Key and Relevant Study Results				
Test Material	**Species**	**Method/Dose**	**RBA (%)**	**Reference**
Source: Mining smelter soil (sample #15) (sieved to <250 µm) Type: Mining/smelting As concentration: 401 mg/kg soil	Swine (Line 26, male, 10–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: not reported; 5 animals/group Test material dose: 2.5 µg As/kg bw/day (6.25 mg soil/kg bw/day); 5 animals/group	36.5 Mean (SE or SD not reported)	Rodriguez et al., 1999
Source: Smelter composite soil Ruston/North Tacoma Superfund site (no information available on particle size of test material) Type: Mining/smelting As concentration: 1600 mg/kg soil	Swine (sires: Hampshire hybrid; dams: crossbred Landrace/Large White/Duroc, immature, ~6–7 weeks old, ~15 kg)	**Single dose blood-time concentration curve method** Reference material dose: 10, 110, or 310 µg As/kg bw; 3 animals/group Test material dose: 40, 100, 160, or 240 µg As/kg bw (25, 62.5, 100, or 150 mg soil/kg bw); 3 animals/group	78 Mean (SE or SD not reported)	U.S. EPA, 1996
Source: Smelter composite slag Ruston/North Tacoma Superfund site (no information available on particle size of test material) Type: Mining/smelting As concentration: 10,100 mg/kg soil	Swine (sires: Hampshire hybrid; dams: crossbred Landrace/Large White/Duroc, immature, ~6–7 weeks old, ~15 kg)	**Single dose blood-time concentration curve method** Reference material dose: 10, 110, or 310 µg As/kg bw; 3 animals/group Test material dose: 610, 1010, or 1540 µg As/kg bw (60.4, 100, or 152.5 mg soil/kg bw); 3 animals/group	42 Mean (SE or SD not reported)	U.S. EPA, 1996
Source: Australian railway corridor soil (sample #2) (sieved to <250 µm) Type: Railway corridor As concentration: 267 mg/kg soil	Swine (large white, female, 20–25 kg)	**Single dose blood-time concentration curve method** Reference material dose: 100 µg As/kg bw; 3 animals/group Test material dose: 119 to 297 µg As/kg bw (0.4 to 1.1 mg soil/kg bw); 3 animals/group	67.4±32.2 Mean±SD	Juhasz et al., 2007
Source: Australian railway corridor soil (sample #4) (sieved to <250 µm) Type: Railway corridor As concentration: 42 mg/kg soil	Swine (large white, female, 20–25 kg)	**Single dose blood-time concentration curve method** Reference material dose: 100 µg As/kg bw; 3 animals/group Test material dose: 19 to 47 µg As/kg bw (0.4 to 1.1 mg soil/kg bw); 3 animals/group	41.6±11.5 Mean±SD	Juhasz et al., 2007
Source: Australian railway corridor soil (sample #5) (sieved to <250 µm) Type: Railway corridor As concentration: 1114 mg/kg soil	Swine (large white, female, 20–25 kg)	**Single dose blood-time concentration curve method** Reference material dose: 100 µg As/kg bw; 3 animals/group Test material dose: 495 to 1238 µg As/kg bw (0.4 to 1.1 mg soil/kg bw); 3 animals/group	20.0±16.5 Mean±SD	Juhasz et al., 2007

Table 2. Key and Relevant Study Results				
Test Material	**Species**	**Method/Dose**	**RBA (%)**	**Reference**
Source: Australian railway corridor soil (sample #10) (sieved to <250 µm) Type: Railway corridor As concentration: 257 mg/kg soil	Swine (large white, female, 20–25 kg)	**Single dose blood-time concentration curve method** Reference material dose: 100 µg As/kg bw; 3 animals/group Test material dose: 114 to 285 µg As/kg bw (0.4 to 1.1 mg soil/kg bw); 3 animals/group	11.2±4.7 Mean±SD	Juhasz et al., 2007
Source: Australian railway corridor soil (sample #16) (sieved to <250 µm) Type: Railway corridor As concentration: 751 mg/kg soil	Swine (large white, female, 20–25 kg)	**Single dose blood-time concentration curve method** Reference material dose: 100 µg As/kg bw; 3 animals/group Test material dose: 334 to 834 µg As/kg bw (0.4 to 1.1 mg soil/kg bw); 3 animals/group	22.5±3.8 Mean±SD	Juhasz et al., 2007
Source: Australian railway corridor soil (sample #18) (sieved to <250 µm) Type: Railway corridor As concentration: 91 mg/kg soil	Swine (large white, female, 20–25 kg)	**Single dose blood-time concentration curve method** Reference material dose: 100 µg As/kg bw; 3 animals/group Test material dose: 40 to 101 µg As/kg bw (0.4 to 1.1 mg soil/kg bw); 3 animals/group	74.7±11.2 Mean±SD	Juhasz et al., 2007
Source: Australian cattle tick dip soil (sample #24) (sieved to <250 µm) Type: Agriculture As concentration: 713 mg/kg soil	Swine (large white, female, 20–25 kg)	**Single dose blood-time concentration curve method** Reference material dose: 100 µg As/kg bw; 3 animals/group Test material dose: 317 to 792 µg As/kg bw (0.4 to 1.1 mg soil/kg bw); 3 animals/group	33.0±17.0 Mean±SD	Juhasz et al., 2007
Source: Australian cattle tick dip soil (sample #27) (sieved to <250 µm) Type: Agriculture As concentration: 228 mg/kg soil	Swine (large white, female, 20–25 kg)	**Single dose blood-time concentration curve method** Reference material dose: 100 µg As/kg bw; 3 animals/group Test material dose: 100 to 250 µg As/kg bw (0.4 to 1.1 mg soil/kg bw); 3 animals/group	49.9±11.0 Mean±SD	Juhasz et al., 2007
Source: Australian mine site (sample #33) Type: Mining/smelting (sieved to <250 µm) As concentration: 807 mg/kg soil	Swine (large white, female, 20–25 kg)	**Single dose blood-time concentration curve method** Reference material dose: 100 µg As/kg bw; 3 animals/group Test material dose: 359 to 897 µg As/kg bw (0.4 to 1.1 mg soil/kg bw); 3 animals/group	40.8±7.4 Mean±SD	Juhasz et al., 2007
Source: Australian mine site (sample #34) (sieved to <250 µm) Type: Mining/smelting As concentration: 577 mg/kg soil	Swine (large white, female, 20–25 kg)	**Single dose blood-time concentration curve method** Reference material dose: 100 µg As/kg bw; 3 animals/group Test material dose: 248 to 619 µg As/kg bw (0.4 to 1.1 mg soil/kg bw); 3 animals/group	6.9±5.0 Mean±SD	Juhasz et al., 2007

Table 2. Key and Relevant Study Results				
Test Material	**Species**	**Method/Dose**	**RBA (%)**	**Reference**
Source: Australian gossan soil (sample #44) (sieved to <250 µm) Type: Mining/smelting As concentration: 190 mg/kg soil	Swine (large white, female, 20–25 kg)	**Single dose blood-time concentration curve method** Reference material dose: 100 µg As/kg bw; 3 animals/group Test material dose: 84 to 211 µg As/kg bw (0.4 to 1.1 mg soil/kg bw); 3 animals/group	16.4±9.1 Mean±SD	Juhasz et al., 2007
Source: Australian gossan soil (sample #45) (sieved to <250 µm) Type: Mining/smelting As concentration: 88 mg/kg soil	Swine (large white, female, 20–25 kg)	**Single dose blood-time concentration curve method** Reference material dose: 100 µg As/kg bw; 3 animals/group Test material dose: 39 to 98 µg As/kg bw (0.4 to 1.1 mg soil/kg bw); 3 animals/group	12.1±8.5 Mean±SD	Juhasz et al., 2007
Source: Montana smelter soil (sieved to <250 µm) Type: Mining/smelting As concentration: 650 mg/kg soil	Cynomolgus monkeys (male, 4–5 kg)	**Single dose urinary excretion fraction method** Reference material dose: 250, 500, or 1000 µg As/kg bw; 5 animals/group Test material dose: 650 µg As/kg bw (1000 mg soil/kg bw); 5 animals/group	13±5 Mean±SD	Roberts et al., 2007
Source: Wisconsin smelter soil (sieved to <250 µm) Type: Mining/smelting As concentration: 1412 mg/kg soil	Cynomolgus monkeys (male, 4–5 kg)	**Single dose urinary excretion fraction method** Reference material dose: 250, 500, or 1000 µg As/kg bw; 5 animals/group Test material dose: 1330 µg As/kg bw (942 mg soil/kg bw); 5 animals/group	13±7 Mean±SD	Roberts et al., 2007
Source: Florida cattle dip site (sieved to <250 µm) Type: Agriculture As concentration: 189 mg/kg soil	Cynomolgus monkeys (male, 4–5 kg)	**Single dose urinary excretion fraction method** Reference material dose: 250, 500, or 1000 µg As/kg bw; 5 animals/group Test material dose: 180 µg As/kg bw (952 mg soil/kg bw); 5 animals/group	31±4 Mean±SD	Roberts et al., 2007
Source: California mine tailings (sieved to <250 µm) Type: Mining/smelting As concentration: 300 mg/kg soil	Cynomolgus monkeys (male, 4–5 kg)	**Single dose urinary excretion fraction method** Reference material dose: 250, 500, or 1000 µg As/kg bw; 5 animals/group Test material dose: 300 µg As/kg bw (1000 mg soil/kg bw); 5 animals/group	19±2 Mean±SD	Roberts et al., 2007
Source: Washington orchard soil (sieved to <250 µm) Type: Agriculture As concentration: 301 mg/kg soil	Cynomolgus monkeys (male, 4–5 kg)	**Single dose urinary excretion fraction method** Reference material dose: 250, 500, or 1000 µg As/kg bw; 5 animals/group Test material dose: 300 µg As/kg bw (997 mg soil/kg bw); 5 animals/group	24±9 Mean±SD	Roberts et al., 2007

Table 2. Key and Relevant Study Results				
Test Material	**Species**	**Method/Dose**	**RBA (%)**	**Reference**
Source: New York orchard soil (sieved to <250 µm) Type: Agriculture As concentration: 125 mg/kg soil	Cynomolgus monkeys (male, 4–5 kg)	**Single dose urinary excretion fraction method** Reference material dose: 250, 500, or 1000 µg As/kg bw; 5 animals/group Test material dose: 120 µg As/kg bw (960 mg soil/kg bw); 5 animals/group	15±8 Mean±SD	Roberts et al., 2007
Source: Colorado smelter soil (sieved to <250 µm) Type: Mining/smelting As concentration: 394 mg/kg soil	Cynomolgus monkeys (male, 4–5 kg)	**Single dose urinary excretion fraction method** Reference material dose: 250, 500, or 1000 µg As/kg bw; 5 animals/group Test material dose: 400 µg As/kg bw (1015 mg soil/kg bw); 5 animals/group	18±6 Mean±SD	Roberts et al., 2007
Source: Colorado smelter (sieved to <250 µm) Type: Mining/smelting As concentration: 1230 mg/kg soil	Cynomolgus monkeys (male, 4–5 kg)	**Single dose urinary excretion fraction method** Reference material dose: 250, 500, or 1000 µg As/kg bw; 5 animals/group Test material dose: 1000 µg As/kg bw (813 mg soil/kg bw); 5 animals/group	17±8 Mean±SD	Roberts et al., 2007
Source: Colorado smelter soil (sieved to <250 µm) Type: Mining/smelting As concentration: 1492 mg/kg soil	Cynomolgus monkeys (male, 4–5 kg)	**Single dose urinary excretion fraction method** Reference material dose: 250, 500, or 1000 µg As/kg bw; 5 animals/group Test material dose: 1000 µg As/kg bw (670 mg soil/kg bw); 5 animals/group	5±4 Mean±SD	Roberts et al., 2007
Source: Florida chemical plant soil (sieved to <250 µm) Type: Chemical manufacturing As concentration: 268 mg/kg soil	Cynomolgus monkeys (male, 4–5 kg)	**Single dose urinary excretion fraction method** Reference material dose: 250, 500, or 1000 µg As/kg bw; 5 animals/group Test material dose: 340 µg As/kg bw (1269 mg soil/kg bw); 5 animals/group	7±3 Mean±SD	Roberts et al., 2007
Source: New York pesticide facility soil #1 (sieved to <250 µm) Type: Chemical manufacturing As concentration: 1000 mg/kg soil[a]	Cynomolgus monkeys (male, 4–5 kg)	**Single dose urinary excretion fraction method** Reference material dose: 250, 500, or 1000 µg As/kg bw; 5 animals/group Test material dose: 990 µg As/kg bw (2920 mg soil/kg bw); 5 animals/group	19±5 Mean±SD	Roberts et al., 2007
Source: New York pesticide facility soil #2 (sieved to <250 µm) Type: Chemical manufacturing As concentration: 339 mg/kg soil[a]	Cynomolgus monkeys (male, 4–5 kg)	**Single dose urinary excretion fraction method** Reference material dose: 250, 500, or 1000 µg As/kg bw; 5 animals/group Test material dose: 300 µg As/kg bw (549 mg soil/kg bw); 5 animals/group	28±10 Mean±SD	Roberts et al., 2007

Table 2. Key and Relevant Study Results				
Test Material	**Species**	**Method/Dose**	**RBA (%)**	**Reference**
Source: New York pesticide facility soil #3 (sieved to <250 µm) Type: Chemical manufacturing As concentration: 546 mg/kg soil[a]	Cynomolgus monkeys (male, 4–5 kg)	**Single dose urinary excretion fraction method** Reference material dose: 250, 500, or 1000 µg As/kg bw; 5 animals/group Test material dose: 490 µg As/kg bw (490 mg soil/kg bw); 5 animals/group	20±10 Mean±SD	Roberts et al., 2007
Source: Hawaiian volcanic soil (sieved to <250 µm) Type: Volcanic As concentration: 724 mg/kg soil	Cynomolgus monkeys (male, 4–5 kg)	**Single dose urinary excretion fraction method** Reference material dose: 250, 500, or 1000 µg As/kg bw; 5 animals/group Test material dose: 730 µg As/kg bw (1008 mg soil/kg bw); 5 animals/group	5±1 Mean±SD	Roberts et al., 2007
Source: Barber Orchard NC, sample MS-1 (sieved to <250 µm) Type: Agriculture As concentration: 290 mg/kg soil	Cynomolgus monkeys (adult male, 4–5 kg)	**Single dose urinary excretion fraction method** Reference material dose: 300 and 500 µg As/kg bw; 5 animals/group Test material dose: 290 µg As/kg bw (1000 mg soil/kg bw); 5 animals/group	33±5 Mean±SE	U.S. EPA, 2009
Source: Barber Orchard NC, sample MS-4 (sieved to <250 µm) Type: Agriculture As concentration: 388 mg/kg soil	Cynomolgus monkeys (adult male, 4–5 kg)	**Single dose urinary excretion fraction method** Reference material dose: 300 and 500 µg As/kg bw; 5 animals/group Test material dose: 388 µg As/kg bw (1000 mg soil/kg bw); 5 animals/group	28±3 Mean±SE	U.S. EPA, 2009
Source: Barber Orchard NC, sample MS-5 (sieved to <250 µm) Type: Agriculture As concentration: 382 mg/kg soil	Cynomolgus monkeys (adult male, 4–5 kg)	**Single dose urinary excretion fraction method** Reference material dose: 300 and 500 µg As/kg bw; 5 animals/group Test material dose: 382 µg As/kg bw (1000 mg soil/kg bw); 5 animals/group	38±7 Mean±SE	U.S. EPA, 2009
Source: Barber Orchard NC, sample MS-8 (sieved to <250 µm) Type: Agriculture As concentration: 364 mg/kg soil	Cynomolgus monkeys (adult male, 4–5 kg)	**Single dose urinary excretion fraction method** Reference material dose: 300 and 500 µg As/kg bw; 5 animals/group Test material dose: 364 µg As/kg bw (1000 mg soil/kg bw); 5 animals/group	25±5 Mean±SE	U.S. EPA, 2009
Source: Florida electrical substation soil (sieved to <250 µm) Type: Other manufacturing As concentration: 312 mg/kg soil	*Cebus apella* monkeys (adult male, 2.5–3.0 kg)	**Single dose urinary excretion fraction method** Reference material dose: 1000 µg As/kg bw; 5 animals/group Test material dose: 500 µg As/kg bw (1602 mg soil/kg bw); 5 animals/group	14.6±5.1 Mean±SD	Roberts et al., 2002

Table 2. Key and Relevant Study Results				
Test Material	**Species**	**Method/Dose**	**RBA (%)**	**Reference**
Source: Cattle dip site soil (sieved to <250 µm) Type: Agriculture As concentration: 189 mg/kg soil	*Cebus apella* monkeys (adult male, 2.5–3.0 kg)	**Single dose urinary excretion fraction method** Reference material dose: 1000 µg As/kg bw; 5 animals/group Test material dose: 500 µg As/kg bw (2646 mg soil/kg bw); 5 animals/group	24.7±3.2 Mean±SD	Roberts et al., 2002
Source: Florida pesticide site #1 soil (sieved to <250 µm) Type: Chemical manufacturing As concentration: 743 mg/kg soil	*Cebus apella* monkeys (adult male, 2.5–3.0 kg)	**Single dose urinary excretion fraction method** Reference material dose: 1000 µg As/kg bw; 5 animals/group Test material dose: 1000 µg As/kg bw (1346 mg soil/kg bw); 5 animals/group	10.7±4.9 Mean±SD	Roberts et al., 2002
Source: Wood preservative site #2 soil (sieved to <250 µm) Type: Chemical manufacturing As concentration: 101 mg/kg soil	*Cebus apella* monkeys (adult male, 2.5–3.0 kg)	**Single dose urinary excretion fraction method** Reference material dose: 1000 µg As/kg bw; 5 animals/group Test material dose: 300 µg As/kg bw (2970 mg soil/kg bw); 5 animals/group	16.3±6.5 Mean±SD	Roberts et al., 2002
Source: Pesticide site soil (sieved to <250 µm) Type: Chemical manufacturing As concentration: 329 mg/kg soil	*Cebus apella* monkeys (adult male, 2.5–3.0 kg)	**Single dose urinary excretion fraction method** Reference material dose: 1000 µg As/kg bw; 5 animals/group Test material dose: 500 µg As/kg bw (1520 mg soil/kg bw); 5 animals/group	17.0±10.0 Mean±SD	Roberts et al., 2002
Source: Composite residential soil, Anaconada, MT (sieved to <250 µm) Type: Mining/smelting As concentration: 410 mg/kg soil	Cynomolgus monkeys (adult female, 2–3 kg)	**Single dose urinary excretion fraction method** Reference material dose: 620 µg As/kg bw; 3 animals/group Test material dose: 620 µg As/kg bw (1500 mg soil/kg bw); 3 animals/group	20.1 Mean (SE or SD not reported)	Freeman et al., 1995
Source: NIST SRM 2710 (sieved to 74 µm) Type: Mining/smelting As concentration: 601 mg/kg soil (INAA)	C57BL/6 mice (female, 6 weeks, 15–20 g)	**Steady-state urinary excretion fraction method** Reference material dose: 820–1160 µg As/kg bw/day Test material dose: 650–1020 µg As/kg bw/day (1150–1420 mg soil/kg bw/day)	42.9 (40.5–45.4) Mean (95% CI)	Bradham et al., 2011, 2012
Source: NIST SRM 2710a (sieved to <74 µm) Type: Mining/smelting As concentration: 1513 mg/kg soil (INAA)	C57BL/6 mice (female, 6 weeks, 15–20 g)	**Steady-state urinary excretion fraction method** Reference material dose: 820–1160 µg As/kg bw/day Test material dose: 580–2360 µg As/kg bw/day (1460–1490 mg soil/kg bw/day)	42.1 (39.8–44.4) Mean (95% CI)	Bradham et al., 2011, 2012

Table 2. Key and Relevant Study Results				
Test Material	**Species**	**Method/Dose**	**RBA (%)**	**Reference**
Source: Iron King, AZ soil sample TM1 (sieved to <250 µm) Type: Mining/smelting As concentration: 280 mg/kg soil (INAA)	C57BL/6 mice (female, 6 weeks, 15–20 g)	**Steady-state urinary excretion fraction method** Reference material dose: 820–1160 µg As/kg bw/day Test material dose: 390 µg As/kg bw/day (1490 mg soil/kg bw/day)	39.9 (36.2–43.8) Mean (95% CI)	Bradham et al., 2011, 2012
Source: Iron King, AZ soil sample TM2 (sieved to <250 µm) Type: Mining/smelting As concentration: 4495 mg/kg soil (INAA)	C57BL/6 mice (female, 6 weeks, 15–20 g)	**Steady-state urinary excretion fraction method** Reference material dose: 820–1160 µg As/kg bw/day Test material dose: 6100 µg As/kg bw/day (1430 mg soil/kg bw/day)	14.5 (11.2–17.8) Mean (95% CI)	Bradham et al., 2011, 2012
Source: ASARCO soil sample (sieved to <250 µm) Type: Mining/smelting As concentration: 182 mg/kg soil (INAA)	C57BL/6 mice (female, 6 weeks, 15–20 g)	**Steady-state urinary excretion fraction method** Reference material dose: 820–1160 µg As/kg bw/day Test material dose: 320 µg As/kg bw/day (1460 mg soil/kg bw/day)	26.7 (22.8–30.7) Mean (95% CI)	Bradham et al., 2011, 2012
Source: Sample TM2 Vasquez Boulevard and I-70, composite residential, Denver CO (sieved to <250 µm) Type: Mining/smelting As concentration: 990 mg/kg soil (INAA)	C57BL/6 mice (female, 6 weeks, 15–20 g)	**Steady-state urinary excretion fraction method** Reference material dose: 820–1160 µg As/kg bw/day Test material dose: 1580 µg As/kg bw/day (1450 mg soil/kg bw/day)	48.7 (43.4–54.2) Mean (95% CI)	Bradham et al., 2011, 2012
Source: Sample TM4 Vasquez Boulevard and I-70, composite residential, Denver, CO (sieved to <250 µm) Type: Mining/smelting As concentration: 829 mg/kg soil (INAA)	C57BL/6 mice (female, 6 weeks, 15–20 g)	**Steady-state urinary excretion fraction method** Reference material dose: 820–1160 µg As/kg bw/day Test material dose: 1190 µg As/kg bw/day (1400 mg soil/kg bw/day)	49.7 (45.0–54.5) Mean (95% CI)	Bradham et al., 2011, 2012
Source: Sample TM5 Vasquez Boulevard and I-70, composite residential, Denver, CO (sieved to <250 µm) Type: Mining/smelting As concentration: 379 mg/kg soil (INAA)	C57BL/6 mice (female, 6 weeks, 15–20 g)	**Steady-state urinary excretion fraction method** Reference material dose: 820–1160 µg As/kg bw/day Test material dose: 520 µg As/kg bw/day (1580 mg soil/kg bw/day)	51.6 (47.0–56.3) Mean (95% CI)	Bradham et al., 2011, 2012

Table 2. Key and Relevant Study Results				
Test Material	**Species**	**Method/Dose**	**RBA (%)**	**Reference**
Source: Midvale slag, composite sample Midvale smelter slag pile (sieved to <250 µm) Type: Mining/smelting As concentration: 837 mg/kg soil (INAA)	C57BL/6 mice (female, 6 weeks, 15–20 g)	**Steady-state urinary excretion fraction method** Reference material dose: 820–1160 µg As/kg bw/day Test material dose: 1040 µg As/kg bw/day (1650 mg soil/kg bw/day)	11.2 (10.6–11.8) Mean (95% CI)	Bradham et al., 2011, 2012
Source: Hawaiian soil sample (sieved to <250 µm) Type: Agriculture As concentration: 769 mg/kg soil (INAA)	C57BL/6 mice (female, 6 weeks, 15–20 g)	**Steady-state urinary excretion fraction method** Reference material dose: 820–1160 µg As/kg bw/day Test material dose: 1100 µg As/kg bw/day (1500 mg soil/kg bw/day)	24.0 (20.9–27.2) Mean (95% CI)	Bradham et al., 2011, 2012
Source: Barber Orchard NC, sample MS-1 (sieved to <250 µm) Type: Agriculture As concentration: 322 mg/kg soil (INAA)	C57BL/6 mice (female, 6 weeks, 15–20 g)	**Steady-state urinary excretion fraction method** Reference material dose: 820–1160 µg As/kg bw/day Test material dose: 470 µg As/kg bw/day (1470 mg soil/kg bw/day)	26.3 (23.4–29.4) Mean (95% CI)	Bradham et al., 2011, 2012
Source: Barber Orchard NC, sample MS-4 (sieved to <250 µm) Type: Agriculture As concentration: 387 mg/kg soil (INAA)	C57BL/6 mice (female, 6 weeks, 15–20 g)	**Steady-state urinary excretion fraction method** Reference material dose: 820–1160 µg As/kg bw/day Test material dose: 600 µg As/kg bw/day (1480 mg soil/kg bw/day)	35.2 (30.9–39.6) Mean (95% CI)	Bradham et al., 2011, 2012
Source: Barber Orchard NC, sample MS-5 (sieved to <250 µm) Type: Agriculture As concentration: 467 mg/kg soil (INAA)	C57BL/6 mice (female, 6 weeks, 15–20 g)	**Steady-state urinary excretion fraction method** Reference material dose: 820–1160 µg As/kg bw/day Test material dose: 630 µg As/kg bw/day (1370 mg soil/kg bw/day)	20.9 (15.9–26.0) Mean (95% CI)	Bradham et al., 2011, 2012
Source: Barber Orchard NC, sample MS-8 (sieved to <250 µm) Type: Agriculture As concentration: 396 mg/kg soil (INAA)	C57BL/6 mice (female, 6 weeks, 15–20 g)	**Steady-state urinary excretion fraction method** Reference material dose: 820–1160 µg As/kg bw/day Test material dose: 640 µg As/kg bw/day (1510 mg soil/kg bw/day)	35.0 (31.2–38.9) Mean (95% CI)	Bradham et al., 2011, 2012

Table 2. Key and Relevant Study Results				
Test Material	**Species**	**Method/Dose**	**RBA (%)**	**Reference**
Source: Mohr Orchard PA sample (sieved to <250 µm) Type: Agriculture As concentration: 340 mg/kg soil (INAA)	C57BL/6 mice (female, 6 weeks, 15–20 g)	**Steady-state urinary excretion fraction method** Reference material dose: 820–1160 µg As/kg bw/day Test material dose: 500 µg As/kg bw/day (1440 mg soil/kg bw/day)	33.2 (27.7–38.7) Mean (95% CI)	Bradham et al., 2011, 2012
Relevant Studies				
Source: Residential soil, Anaconda, MT (test material particle size 19 µm) Type: Mining/smelting As concentration: 3900 mg/kg soil	Rabbit (New Zealand white rabbits male and female; 9–12 weeks old, ~2 kg)	**Single dose urinary excretion fraction method** Reference material dose: 1950 µg As/kg bw; 5 animals/sex/group Test material dose: 780, 1970, or 3900 µg As/kg bw (200, 500, or 1000 mg soil/kg bw); 5 animals/sex/group	48.2 Mean (SE or SD not reported)	Freeman et al., 1993

[a] Arsenic concentrations based on personal communication from the co-authors S. Roberts and Y. Lowney (09/24/2010) which corrects an error in column headings in Table 3 of Roberts et al. (2007); reported values: NYPF1=339 ppm, NYPF2=546 ppm, and NYPF3=1000 ppm)

Table 3. Summary Statistics for RBA (%) Estimates Based on Key Studies					
Parameter	**Swine**	**Monkeys**	**Mice**	**All Species[a]**	**All Species[b]**
N[c]	64	24	15	103	88
AM	34.5	19.2	33.5	30.8	29.9
SD	17.5	8.6	12.6	16.4	16.8
SE	2.2	1.7	3.3	1.6	1.8
95LCL[d]	30.2	15.8	27.1	27.6	26.4
95UCL[d]	38.8	22.6	39.8	34.0	33.4
MIN	4.1	5.0	11.2	4.1	4.1
5th %	7.9	5.3	13.5	7.1	6.9
10th %	9.7	8.1	17.0	10.8	8.9
25th %	20.8	14.2	25.2	18.0	16.9
50th %	37.0	18.5	35.0	29.1	28.3
75th %	44.8	24.8	42.5	42.0	42.0
90th %	54.4	30.1	49.3	51.5	50.3
95th %	60.9	32.7	50.2	56.8	56.3
MAX	78.0	38.0	51.6	78.0	78.0
SKEW	0.21	0.29	-0.24	0.47	0.55
KURT	-0.42	-0.21	-0.92	-0.23	-0.14

[a] Each RBA estimate for materials evaluated in more than one assay is given equal weight.
[b] RBA estimates for materials evaluated in more than on assay are represented by the average of values from all assays. These include the following test materials: Barber Orchard MS-1, -4, -5, and -8 (swine, monkey, and mouse); and Iron King TM1 and TM2, Ruston/ASARCO, Hawaii, Mohr Orchard, NIST 2710 and NIST 2710A (swine and mouse).
[c] Number of RBA estimates.
[c] Number of RBA estimates.
[d] Assumes central limit and Z=1.96 for standard normal

AM, arithmetic mean; KURT, kurtosis; LCL, lower confidence limit on the mean; MAX, maximum; MIN, minimum; SD, standard deviation; SE, standard error; UCL, upper confidence limit on the mean; 5th %, 5th percentile

Table 4. Weighted RBA Summary Statistics and Confidence Limits[a]			
Parameter	**CTE**	**95% LCL**	**95% UCL**
AM	30.8	29.8	31.7
5th %	6.6	5.1	8.3
50th %	28.5	26.2	31.0
95th %	58.1	53.3	64.0

[a] Weighted for uncertainty (SE of mean, based on Monte Carlo analysis of all RBA estimates from swine, monkey, and mouse studies [n=103]).

AM, arithmetic mean; CTE, central tendency estimate; LCL, lower confidence limit; UCL, upper confidence limit

Table 5. RBA Estimates for Barber Orchard Soils Administered to Mice, Monkeys, and Swine				
	RBA % (95% Confidence Limits)			
Species	**MS-1 (290 ppm)[a]**	**MS-4 (388 ppm)[a]**	**MS-5 (382 ppm)[a]**	**MS-8 (364 ppm)[a]**
Mice	26 (23–29)	35 (31–40)	21 (16–26)	35 (31–39)
Monkey	33 (23–43)[b]	28 (22–34)[b]	38 (24–52)[b]	25 (15–35)[b]
Swine	31 (24–40)	41 (37–44)	49 (40–59)	53 (48–57)

[a] Test material number (As concentration): arsenic concentration measured on sieved (250 µm) fractions.
[b] Estimated as SE x 1.96 (Z=1.96 for standard normal), where SE values were reported in U.S. EPA, 2009.

Table 6. Comparison Between RBA Estimates Based on Mice and Swine Bioassays		
Test Materials	**RBA % (95% Confidence Limits)**	
	Mice	**Swine**
Iron King HSJ-583	40 (36–44)	60 (55–66)[a]
Iron King IKJ-583	14 (11–18)	19 (17–20)
Ruston ASARCO	27 (23–31)	49 (44–54)[a]
Hawaii	24 (21–27)	33 (30–36)[a]
Barber Orchard MS-1	26 (23–29)	31 (24–40)
Barber Orchard MS-4	35 (31–40)	41 (37–44)
Barber Orchard MS-5	21 (16–26)	49 (40–59)[a]
Barber Orchard MS-8	35 (31–39)	53 (48–57)[a]
Mohr Orchard	33 (28–39)	53 (50–57)[a]
NIST 2710	43 (40–45)	44 (40–49)
NIST 2710A	42 (40–44)	42 (39–45)

[a] Confidence limits do not overlap.

Table 7. Comparison Between RBA Estimates Based on UEF and Blood AUC in Monkeys		
Monkey Number	**RBA based on UEF**	**RBA based on Blood AUC**
30–544	27.7	6.1
20–784	18.6	6.9
30–537	14.1	19.9
Mean	20.1	11.0
SD	6.9	7.7

Based on Freeman et al. (1995). RBA estimates based on the two methods are not significantly different based on paired t-test ($p=0.37$) or unpaired t-test ($p=0.20$).
AUC, area under the blood concentration – time curve; UEF, urinary excretion fraction

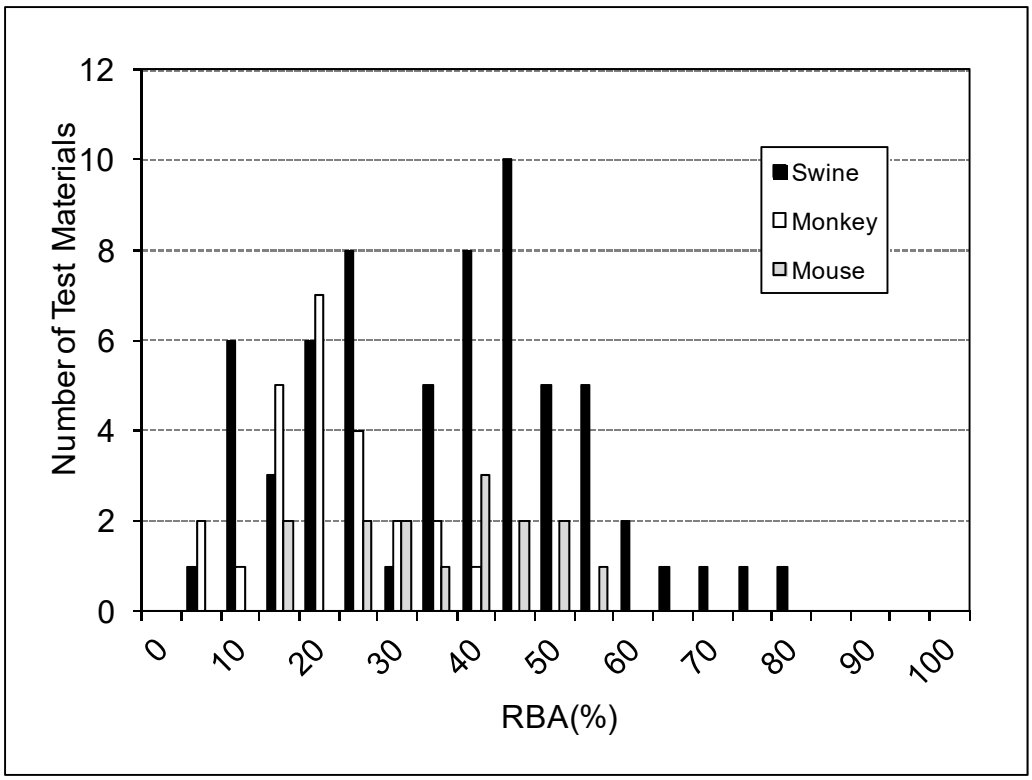

Figure 1. Distribution of RBA Values for Materials Assayed in Swine, Monkey, and Mouse.

The mean RBA value for test materials assayed in monkeys is 19.2% (95% CI: 15.8–22.6, n=24); the mean for test materials assayed in swine is 34.5% (95% CI: 30.2–38.8, n=64); the mean for test materials assayed in mice is 33.5% (95% CI: 27.1–39.8, n=15).

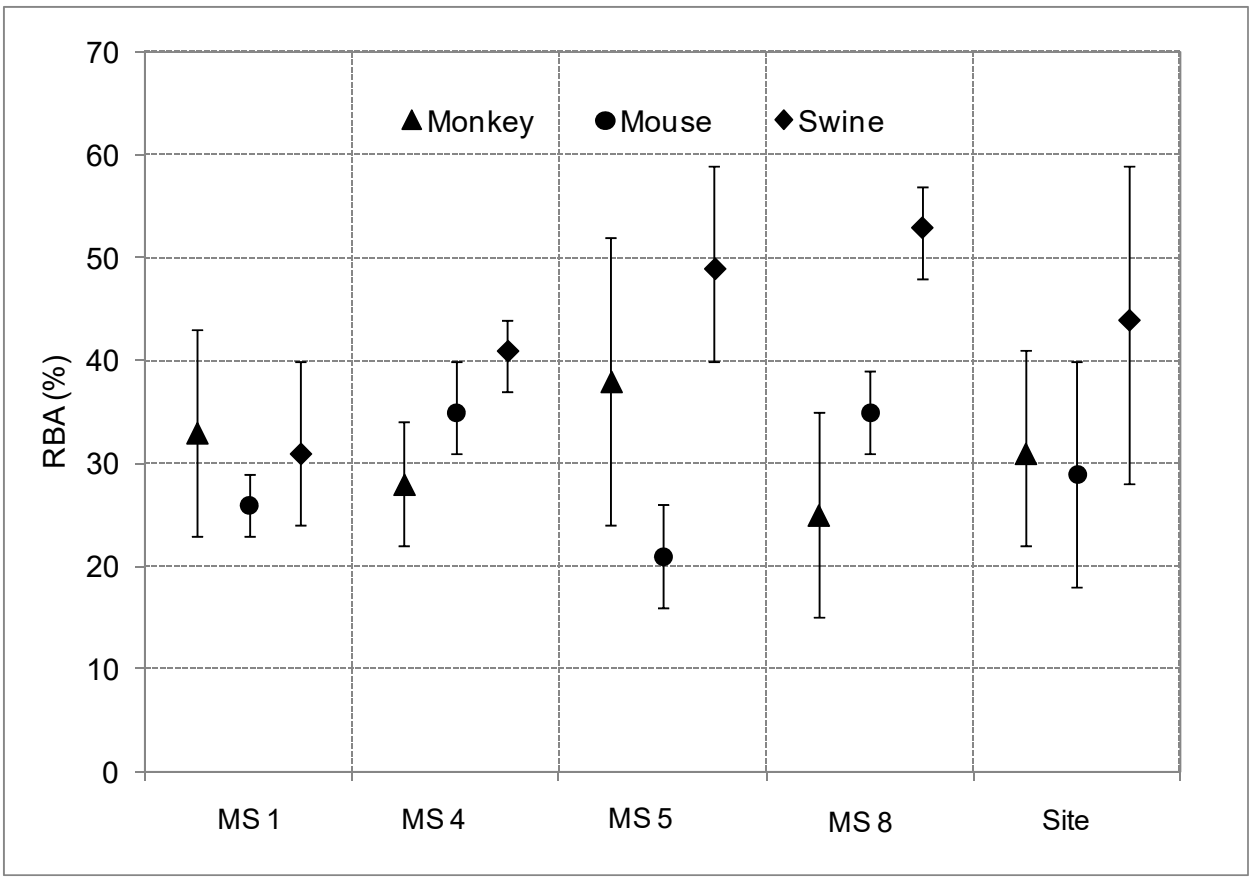

Figure 2. Comparison Between Arsenic RBA Estimates from Swine, Monkey, and Mouse Bioassays of Four Soil Samples from the Barber Orchard Site.

Shown are mean and 95% confidence limits. The values shown for "site" are the means for all four soil samples.

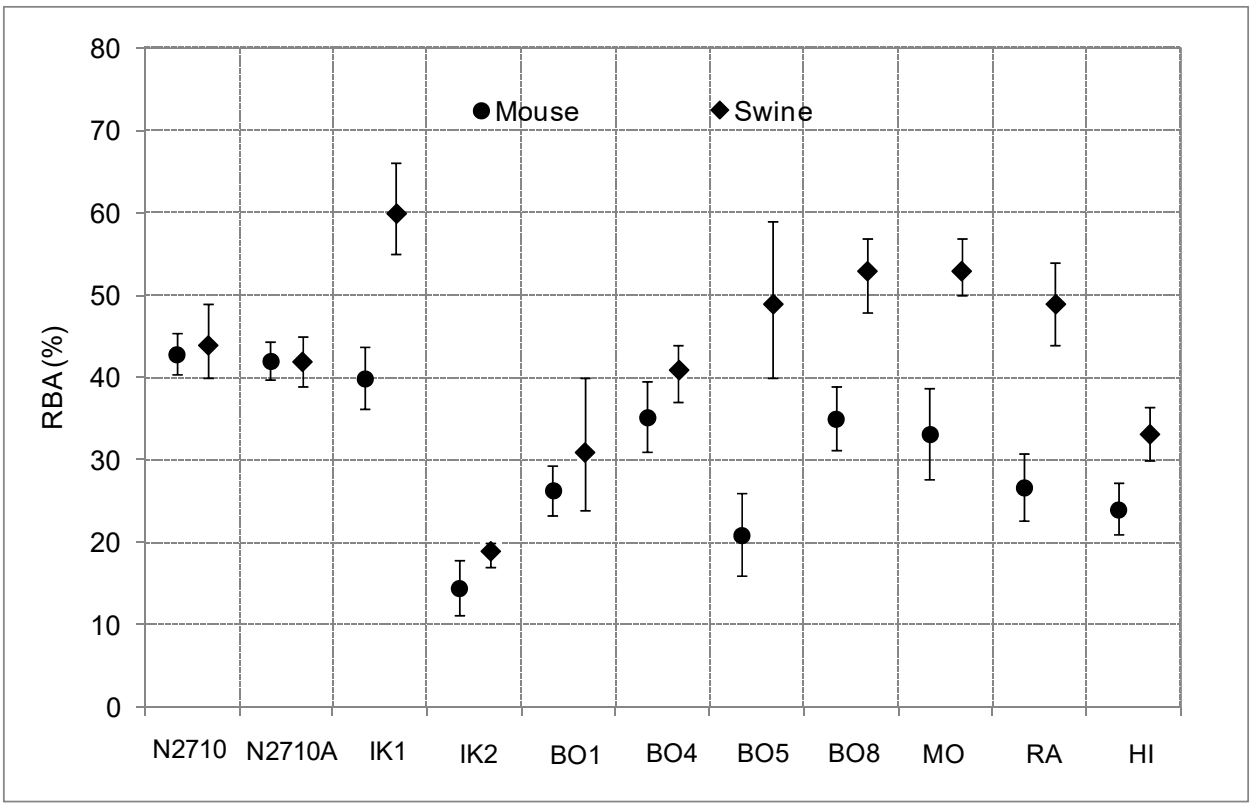

Figure 3. Comparison Between Arsenic RBA Estimates from Swine or Mouse Bioassays of 11 Test Materials.

Shown are mean and 95% confidence limits. The values shown for "site" are the means for all four soil samples.

BO, Barber Orchard; HI, Hawaii; IK, Iron King; MO, Mohr Orchard; N, NIST; RA, Ruston-ASARCO

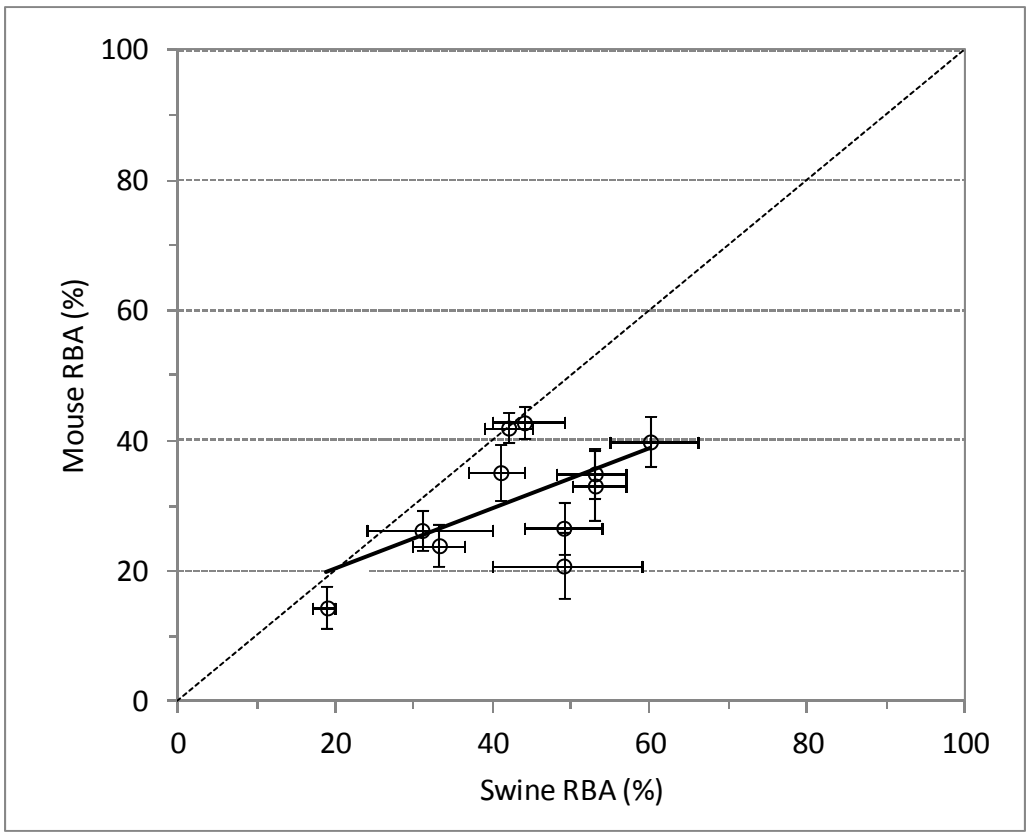

Figure 4. Relationship Between Arsenic RBA Estimates Based on Mouse and Swine Bioassays Applied to 11 Test Materials.

Error bars for mice are 95% confidence limits. Solid line is the linear regression model (R^2=0.35, p=0.053).The mouse and swine RBA estimates are not significantly correlated (Pearson r=0.60, p=0.053; Spearman r=0.42, p=0.19).

APPENDIX A: Summary Description of Human Arsenic Bioavailability Study (Stanek et al., 2010)

A single human experimental study of bioavailability of arsenic in soil was reported (Stanek et al., 2010). This study was not used selected for inclusion in this report as a key or relevant study because of several methodological limitations and uncertainties, which are briefly summarized below. Stanek et al. (2010) utilized a mass balance approach to estimate absolute bioavailability of arsenic in food and soil in a small group of human subjects (n=13 subjects including 7 females and 6 males, age 26–53 years). The study consisted of two phases conducted approximately 2–3 years apart, with partial overlap of subjects in both phases. Phase 1 of the study estimated absolute bioavailability of arsenic in food and included 11 subjects (6 females and 5 males, age 26–53 years). Daily complete urine and fecal samples, and duplicate diet samples were collected from each subject for a period of 7 consecutive days. For each subject, for each day, absolute bioavailability of ingested arsenic was calculated as follows (Equation A-1):

$$ABA_{food} = \frac{As_{food} - As_{fecal}}{As_{food}} \qquad \text{Eq. (A-1)}$$

where ABA is absolute bioavailability and As_{food} and As_{fecal} are the rate of intake of arsenic in food and rate of excretion of arsenic in feces (µg/day), respectively.

Phase 2 estimated the absolute bioavailability of arsenic in soil and included 11 subjects, 9 of whom participated in Phase 1. Subjects were asked to avoid eating seafood, rice, mushrooms, spinach, or grape juice (foods typically having high levels of arsenic) for 4 days preceding the 7-day observation period. On day 2 of the observation period, each subject ingested a gelatin capsule containing 111.7 µg As in 0.636 g of soil. The soil was obtained from a cattle dip site (see Roberts et al., 2007). Absolute bioavailability of arsenic in soil was calculated as follows (Equation A-2):

$$ABA_{soil} = \frac{As_{fecal} - As_{food} \cdot (1 - ABA_{food})}{As_{soil}} \qquad \text{Eq. (A-2)}$$

The above calculation utilizes the estimate of the absolute bioavailability of arsenic in food to calculate the amount of fecal arsenic attributable to food in Phase 2. The difference between total fecal arsenic and fecal arsenic attributed to food was attributed to the soil dose. Bioavailable arsenic from the soil dose was calculated as the difference between the soil arsenic dose and fecal arsenic attributed to the soil dose.

Stanek et al. (2010) reported estimates of 87.5% (95% CI: 81.2, 93.8) and 89.7% (95% CI: 83.4, 96.0) for absolute arsenic bioavailability in food, based on Phase 1 and Phase 2 respectively. The estimate for absolute bioavailability of arsenic in soil was 48.7% (95% CI: 36.2, 61.3). The estimate for bioavailability of arsenic from soil relative to food was 54.5% (48.7%/89.7%).

Several important uncertainties attend these above estimates of bioavailability, which precluded the using the estimates in the calculation of soil RBA for the upper bound estimate for soil RBA:

- Stanek et al. (2010) does not provide an estimate of the RBA for arsenic in soil relative to that of a completely bioaccessible form of arsenic (e.g., to sodium arsenate). The ratio of the absolute bioavailability of arsenic in soil to that of arsenic in food, reported in Stanek et al. (2010), is not directly comparable to RBAs based on key studies described in this report (e.g., soil RBA relative to sodium arsenate).

- The two study phases were separated by ~2.5 years and, although there was substantial overlap among subjects in both phases, individual subjects could not serve as their own measures for absolute bioavailability of dietary arsenic in the calculation of absolute bioavailability of soil arsenic.

- Sample collection (duplicate diets, feces, and urine) appears to have been unsupervised and was performed by individual subjects outside of a clinical research center where adherence to sampling protocols could have been assured.

- No attempt was made to control dietary arsenic intake, other than the 4-day voluntary "arsenic suppression" diet that preceded Phase 2. As a result, intra- and inter-subject variability in dietary intakes was substantial (e.g., maximum/minimum arsenic intake ratio in Phase 1 ranged from 6 to 84). This magnitude of variability in dietary arsenic intakes during the study is likely to have contributed substantial dietary noise to the estimation the fraction of fecal arsenic attributed to the soil dose in Phase 2.

- The recovery of arsenic from a duplicate diet spiked with a known amount of soil arsenic was reported to have been 78.9% and no explanation is given for the low recovery. The resulting uncertainty in the dietary and soil arsenic doses contributes to uncertainty in the corresponding bioavailability estimates for food and soil. The magnitude of the error in the bioavailability estimates attributable to error in the arsenic dose estimates depends on whether or not the low arsenic recovery represents arsenic in soil, and/or arsenic in food, and/or arsenic in soil added to food. Therefore, without an understanding of the recovery problem, or of the reproducibility of recovery, the magnitude of the error cannot be reliably determined. Based on data reported in the Appendix to Stanek et al. (2010), the estimates of soil RBA may have ranged from 40 to 60%, depending on the assignment of the recovery error to food, soil, or both media.

RELATIVE BIOAVAILABILITY OF ARSENIC IN AN ASARCO AND A HAWAII SOIL

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EXECUTIVE SUMMARY

A study using juvenile swine as test animals was performed to measure the gastrointestinal absorption of arsenic from an ASARCO and a Hawaii soil sample. The ASARCO material was collected from a stockpile of soil from a former smelter site near Tacoma, Washington. The Hawaii material was collected from a school garden located near Kea'au town, Hawaii that had been impacted by arsenic associated with herbicide use in former sugar mill plantation land. The arsenic concentrations (mean ± SD) of the ASARCO and Hawaii soil samples are 181.9 ± 6.3 and 500 ± 43.3 mg/kg, respectively.

The relative oral bioavailability of arsenic was assessed by comparing the absorption of arsenic from ASARCO and Hawaii soil samples ("test materials") to that of sodium arsenate. Groups of four swine were given oral doses of sodium arsenate or a test material twice a day for 14 days. Groups of three non-treated swine served as a control.

The amount of arsenic absorbed by each animal was evaluated by measuring the amount of arsenic excreted in the urine (collected over 48-hour periods beginning on days 6, 9, and 12). The urinary excretion fraction (UEF) is the ratio of the amount excreted per 48 hours divided by the dose given per 48 hours. UEF was calculated for the test materials and the sodium arsenate using simultaneous weighted linear regression. The relative bioavailability (RBA) of arsenic in each test material compared to sodium arsenate was calculated as follows:

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

Estimated RBA values (mean and 90% confidence interval) are shown below:

Estimated RBA for Asarco and Hawaii Soils

Measurement Interval	Estimated RBA (90% Confidence Interval)	
	Test Material 1 (ASARCO)	Test Material 2 (Hawaii)
Days 6/7	0.52 (0.44–0.61)	0.52 (0.44–0.61)
Days 9/10	0.49 (0.43–0.56)	0.48 (0.42–0.55)
Days 12/13	0.46 (0.39–0.54)	0.51 (0.43–0.60)
All Days	**0.49 (0.45–0.53)**	**0.51 (0.46–0.55)**

The best fit point estimate RBA of arsenic in an ASARCO and Hawaii soil sample observed was 49 and 51%, respectively.

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ACRONYMS AND ABBREVIATIONS

ABA	Absolute bioavailability
AF_o	Oral absorption fraction
As^{+3}	Trivalent inorganic arsenic
As^{+5}	Pentavalent inorganic arsenic
cm	Centimeter
DMA	Dimethyl arsenic
D	Ingested dose
g	Gram
GLP	Good Laboratory Practices
INAA	Instrumental Neutron Activation Analysis
kg	Kilogram
K_u	Fraction of absorbed arsenic which is excreted in urine
mL	Milliliter
mm	Millimeter
MMA	Monomethyl arsenic
N	Number of data points
NaAs	Sodium arsenate
NIST	National Institute of Standards and Technology
NRC	National Research Council
ORD	Office of Research and Development
PE	Performance evaluation
QC	Quality control
RBA	Relative bioavailability
ref	Reference material
RfD	Reference dose
RPD	Relative percent difference
SD	Standard deviation
SF	Slope factor
SRM	Standard reference material
TM	Test material
UEF	Urinary excretion fraction
USEPA	United States Environmental Protection Agency
µg	Microgram
µm	Micrometer
°C	Degrees Celsius

1.0 INTRODUCTION

1.1 Overview of Bioavailability

Reliable analysis of the potential hazard to humans from ingestion of a chemical depends upon accurate information on a number of key parameters, including the concentration of the chemical in environmental media (e.g., soil, dust, water, food, air, paint), intake rates of each medium, and the rate and extent of absorption ("bioavailability") of the chemical by the body from each ingested medium. The amount of a chemical that actually enters the body from an ingested medium depends on the physical-chemical properties of the chemical and of the medium. For example, some metals in soil may exist, at least in part, as poorly water-soluble minerals, and may also exist inside particles of inert matrix such as rock or slag of variable size, shape, and association. These chemical and physical properties may influence (usually decrease) the absorption (bioavailability) of the metals when ingested. Thus, equal ingested doses of different forms of a chemical in different media may not be of equal health concern.

Bioavailability of a chemical in a particular medium may be expressed either in absolute terms (absolute bioavailability) or in relative terms (relative bioavailability):

Absolute bioavailability (ABA) is the ratio of the amount of the chemical absorbed to the amount ingested:

$$ABA = \frac{Absorbed\ Dose}{Ingested\ Dose}$$

This ratio is also referred to as the oral absorption fraction (AF_o).

Relative bioavailability (RBA) is the ratio of the AF_o of the chemical present in some test material ("*test*") to the AF_o of the chemical in an appropriate reference material such as sodium arsenate (e.g., either the chemical dissolved in water or a solid form that is expected to fully dissolve in the stomach) ("*ref*"):

$$RBA(test\ vs\ ref) = \frac{AF_o(test)}{AF_o(ref)}$$

For example, if 100 micrograms (μg) of a chemical dissolved in drinking water were ingested and a total of 50 μg were absorbed into the body, the AF_o would be 50/100, or 0.50 (50%). Likewise, if 100 μg of the same chemical contained in soil were ingested and 30 μg were absorbed into the body, the AF_o for this chemical in soil would be 30/100, or 0.30 (30%). If the chemical dissolved in water were used as the frame of reference for describing the relative bioavailability of the same chemical in soil, the RBA would be 0.30/0.50, or 0.60 (60%).

For additional discussion about the concept and application of bioavailability, see Gibaldi and Perrier (1982), Goodman et al. (1990), and/or Klaassen et al. (1996).

1.2 Using RBA Data to Improve Risk Calculations

When reliable data are available on the RBA of a chemical in a site medium (e.g., soil), the information can be used to improve the accuracy of exposure and risk calculations at that site. RBA data can be used to adjust default oral toxicity values (reference dose and slope factor) to account for differences in absorption between the chemical ingested as a soluble form of arsenic and the chemical ingested in site media, assuming the toxicity factors are also based on a readily soluble form of the chemical. For non-cancer effects, the default reference dose ($RfD_{default}$) can be adjusted ($RfD_{adjusted}$) as follows:

$$RfD_{adjusted} = \frac{RfD_{default}}{RBA}$$

For potential carcinogenic effects, the default slope factor ($SF_{default}$) can be adjusted ($SF_{adjusted}$) as follows:

$$SF_{adjusted} = SF_{default} \cdot RBA$$

Alternatively, it is also acceptable to adjust the dose (rather than the toxicity factors) as follows:

$$Dose_{adjusted} = Dose_{default} \cdot RBA$$

This dose adjustment is mathematically equivalent to adjusting the toxicity factors as described above.

1.3 Purpose of this Study

The objective of this study was to use juvenile swine as a test system in order to determine the RBA of arsenic in an ASARCO and a Hawaii soil sample compared to a soluble form of arsenic (sodium arsenate).

2.0 STUDY DESIGN

The test and reference materials were administered to groups of four juvenile swine at three different dose levels for 14 days. The study included a non-treated group of three animals to serve as a control for determining background arsenic levels. Study details are presented in Table 2-1. All doses were administered orally. The study was performed as nearly as possible within the spirit and guidelines of Good Laboratory Practices (GLP: 40 CFR 792).

Table 2-1. Study Design and Dosing Information

Group	Group Name Abbreviation	Dose Material Administered	Number of Swine in Group	Arsenic Dose		
				Target (µg/kg BW-day)	Actual [a] (µg/kg BW-day)	Actual [b] (µg-day)
1	NaAs	Sodium arsenate	4	25	25	339
2	NaAs	Sodium arsenate	4	50	50	678
3	NaAs	Sodium arsenate	4	100	100	1354
4	TM1	ASARCO	4	40	40	542
5	TM1	ASARCO	4	60	60	813
6	TM1	ASARCO	4	120	120	1625
7	TM2	Hawaii	4	40	40	542
8	TM2	Hawaii	4	60	60	813
9	TM2	Hawaii	4	120	120	1625
10	Control	Negative control	3	0	0	0

[a] Calculated as the administered daily dose divided by the measured or extrapolated daily body weight, averaged over days 0–14 for each animal and each group.
[b] Calculated as the mass of soil or sodium arsenate solution administered times the concentration of the soil or sodium arsenate solution.
Doses were administered in two equal portions given at 8:00 AM and 3:00 PM each day. Doses were held constant based on the expected mean weight during the exposure interval (14 days).

2.1 Test Materials

2.1.1 Sample Description

The ASARCO material was collected from a former copper smelter site near Tacoma, Washington that operated from 1890 through 1985. In addition to copper, the site produced arsenic trioxide, lead, sulfuric acid, and precious metals at various times during its operation. Multiple samples were collected from a stockpile of soil that was removed from residential properties near the site. The samples were composited prior to analysis.

The Hawaii material was collected from a garden plot used by Kea'au Middle School, located in the town of Kea'au on the island of Hawaii, approximately nine miles southwest of the City of Hilo. The garden has high arsenic concentrations attributable to herbicide use between 1920 and 1950 in a former sugar mill plantation land in the area. An area of approximately 0.5 by 0.5 in dimension was loosened by pick and shovel to a depth of approximately 30 cm. Rocks large than 5 cm in diameter were removed by tilling or by hand picking. The remaining soil was slightly mixed by tilling in place, then shoveled into a 5-gallon poly container and sealed for transport to EPA in field moist condition. All field tools were cleaned prior to sampling.

2.1.2 Sample Preparation and Analysis

2.1.2.1 Hawaii

Hawaii samples were shipped to USEPA's Office of Research and Development (ORD) for sample processing, which was conducted by Dr. Kirk Scheckel and Ben Miller. The samples were oven dried at 105°C. After drying, the soils were passed through a Gilson automatic Porta-

Sieve. Soil aggregates in the fine earth fraction (<2 mm and >250 µm) were ground using a mortar and pestle and then were mixed and further ground using a Thunderbird 20 quart commercial mixer (model ARM-02). The ground soil then passed through the 250 µm sieve. Soil that passed through the 250 µm sieve was homogenized using a customized machine consisting of a rotating V-shaped Plexiglas compartment with motorized tines rotating within the Plexiglas compartment. The soil was mixed in the homogenizer until it reached a uniform color and texture. Once dried, sieved, and homogenized, the soils were stored in plastic bags until analysis.

The Hawaii soil arsenic concentration was determined by instrumental neutron activation analysis (INAA). Three replicates of the Hawaii soil were analyzed and the arsenic concentration was 500 ± 43.3 mg/kg (mean ± SD). X-ray absorption spectroscopy was conducted on the test material to characterize the arsenic mineralogy (Miller and Scheckel, 2012).

2.1.2.2 ASARCO

ASARCO samples were collected by USEPA from a stockpile of soil removed from residential properties. Using a large mesh stainless steel sieve, the samples were field sieved to remove large rocks or plant material. The samples were then placed in 2.5-gallon plastic buckets and shipped to USEPA's ORD for sample processing, which was conducted by Dr. Karen Bradham (ORD, Research Triangle Park, North Carolina). After the sample weights were recorded, the soils were combined, blended, and spread out in drying trays. The trays containing the soil were placed in an air-drying oven and dried for approximately 5 days at <40ºC and sample weights were collected subsequent to air-drying. The soil was then added to a vibrating 2 mm stainless steel sieve screen to remove any large chunks of aggregated soil. Material remaining on the screen was deaggregated using a gloved hand and rescreened. A small portion of the <2 mm sieve fraction of soil was retained for subsequent analyses. The remainder of the soil was then screened to <250 µm to maximize the quantity of soil for bioavailability studies. The soil was passed through a riffler five times and aliquots were collected in pre-cleaned 250 mL high-density polyethylene bottles. Dr. Bradham provided samples (via chain of custody) to Dr. David Thomas (USEPA, ORD) for INAA at North Carolina State University's Nuclear Reactor Program.

The ASARCO soil arsenic concentration was determined by INAA. An aliquot of the ASARCO soil was analyzed in duplicate and the arsenic concentration was 181.9 ± 6.3 mg/kg (mean ± SD). X-ray absorption spectroscopy was conducted on the test material to characterize the arsenic mineralogy (Miller and Scheckel, 2012).

2.2 Experimental Animals

Juvenile swine were selected for use because they are considered to be a good physiological model for gastrointestinal absorption in children (Weis and LaVelle, 1991; Casteel et al., 1996). The animals were intact males of the Pig Improvement Corporation genetically defined Line 26, and were purchased from Chinn Farms, Clarence, Missouri.

The number of animals purchased for the study was several more than required by the protocol. These animals were purchased at an age of about 5–6 weeks (weaning occurs at age 3 weeks) and housed in individual stainless steel cages. The animals were then held under quarantine for one week to observe their health before beginning exposure to dosing materials. Each animal was examined by a certified veterinary clinician (swine specialist) and any animals that appeared to be in poor health during this quarantine period were excluded from the study. To minimize weight variations among animals and groups, extra animals most different in body weight (either heavier or lighter) five days prior to exposure (day 5) were also excluded from the study. The remaining animals were assigned to dose groups at random (group assignments are presented in Appendix A).

When exposure began (day 0), the animals were about 6–7 weeks old. The animals were weighed at the beginning of the study and every three days during the course of the study. In each study, the rate of weight gain was comparable in all dosing groups. Body weight data are presented in Appendix B.

All animals were examined daily by an attending veterinarian while on study and were subjected to detailed examination at necropsy by a certified veterinary pathologist in order to assess overall animal health.

2.3 Diet

Animals received from the supplier were weaned onto standard pig chow (made at the University of Missouri Animal Science Feed Mill). The feed was nutritionally complete and met all requirements of the National Institutes of Health-National Research Council (NRC, 1988). The ingredients of the feed are presented in Table 2-2. Arsenic concentration in a randomly selected feed sample measured 0.2 μg/g.

Prior to the start of dosing and throughout the dosing period, every animal was given a daily amount of feed equal to 4.0% of the mean body weight of all animals on study. Feed amounts were adjusted every three days, when animals were weighed. Feed was administered in two equal portions, at 11:00 AM and 5:00 PM daily.

Drinking water was provided *ad libitum* via self-activated watering nozzles within each cage. Arsenic concentration of five water samples from randomly selected drinking water nozzles were ≤1 μg/L.

Table 2-2. Typical Feed Composition

Purina TestDiet® 5TXP: Porcine Grower Purified Diet with Low Lead [a]

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

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

[a] This special purified diet was originally developed for lead RBA studies.
[b] Based on the latest ingredient analysis information. Since nutrient composition of natural ingredients varies, analysis will differ accordingly. Nutrients expressed as percent of ration on an arsenic fed basis except where otherwise indicated.
[c] Energy (kcal/gm) – sum of decimal fractions of protein, fat, and carbohydrate × 4, 9, and 4 kcal/g, respectively.

2.4 Dosing

Animals were exposed to dosing materials (sodium arsenate or sieved test material) for 14 days, with the dose for each day being administered in two equal portions beginning at 8:00 AM and 3:00 PM (two hours before feeding). Swine were dosed two hours before feeding to ensure that they were in a semi-fasted state. To facilitate dose administration, dosing materials were placed in a small depression in a ball of dough consisting of moistened feed (typically about 5 g) and the dough was pinched shut. This was then placed in the feeder at dosing time.

Target arsenic doses (expressed as µg of arsenic per kg of body weight per day) for animals in each group were determined in the study design (see Table 2-1). The daily mass of arsenic administered (either as sodium arsenate or as sieved test material) to animals in each group was calculated by multiplying the target dose (µg/kg-day) for that group by the anticipated average weight of the animals (kg) over the course of the study:

$$Mass\ (\mu g / day) = Dose\ (\mu g / kg - day) \cdot Average\ Body\ Weight\ (kg)$$

The average body weight expected during the course of the study was estimated by measuring the average body weight of all animals one day before the study began, and then assuming an average weight gain of 0.5 kg/day during the study. After completion of the study, the true mean body weight was calculated using the actual body weights (measured every three days during the study), and the resulting true mean body weight was used to calculate the actual dose achieved. Any missed or late doses were recorded and the actual doses adjusted accordingly. Actual doses (µg arsenic per day) for each group are shown in Table 2-1.

2.5 Collection and Preservation of Urine Samples

Samples of urine were collected from each animal for 48-hour periods on days 6 to 7 (U-1), 9 to 10 (U-2), and 12 to 13 (U-3) of the study. Collection began at 9:00 AM and ended 48 hours later. The urine was collected in a plastic bucket placed beneath each cage, which was emptied into a plastic storage bottle. Aluminum screens were placed under the cages to minimize contamination with feces or spilled food. Due to the length of the collection period, collection containers were emptied periodically (typically twice daily) into a separate plastic bottles to ensure that there was no loss of sample due to overflow.

At the end of each collection period, the total urine volume for each animal was measured (see Appendix C) and three 60-mL portions were removed and acidified with 0.6 mL concentrated nitric acid. All samples were refrigerated. Two of the aliquots were archived and one aliquot was sent for arsenic analysis. Refrigeration was maintained until arsenic analysis.

2.6 Arsenic Analysis

Urine samples were assigned random chain-of-custody tag numbers and submitted to the analytical laboratory for analysis in a blind fashion. The samples were analyzed for arsenic by L. E. T., Inc., (Columbia, Missouri). In brief, 25-mL samples of urine were digested by

refluxing and then heating to dryness in the presence of magnesium nitrate and concentrated nitric acid. Following magnesium nitrate digestion, samples were transferred to a muffle furnace and ashed at 500°C. The digested and ashed residue was dissolved in hydrochloric acid and analyzed by the hydride generation technique using a Perkin Elmer 3100 atomic absorption spectrometer. This method has established that each of the different forms of arsenic that may occur in urine, including trivalent inorganic arsenic (As^{+3}), pentavalent inorganic arsenic (As^{+5}), monomethyl arsenic (MMA), and dimethyl arsenic (DMA) are all recovered with high efficiency.

Analytical results for the urine samples are presented in Appendix C.

2.7 Quality Control

A number of quality control (QC) steps were taken during this project to evaluate the accuracy of the analytical procedures. The results for QC samples are presented in Appendix D and are summarized below.

Blind Duplicates (Sample Preparation Replicates)

A random selection of about 10% of all urine samples generated during the study were prepared for laboratory analysis in duplicate and submitted to the laboratory in a blind fashion. Results are shown in Appendix D (see Table D-1 and Figure D-1). Results were similar between duplicate pairs.

Spike Recovery

During analysis, one feed and water sample and every tenth urine sample were spiked with known amounts of arsenic (sodium arsenate) and the recovery of the added arsenic was measured. Results (see Table D-2) show that mean arsenic concentrations recovered from spiked samples were within 10% of expected concentrations.

Laboratory Duplicates

During analysis, every tenth sample was analyzed in duplicate. Duplicate results for urine samples (see Table D-3) typically agreed within 10% relative percent difference (RPD).

Laboratory Control Standards

National Institute of Technology (NIST) standard reference materials (SRMs), for which certified concentrations of specific analytes has been established, were tested periodically during sample analysis. Recovery of arsenic from these standards was within acceptable ranges (see Table D-4).

Performance Evaluation Samples

A number of Performance Evaluation (PE) samples (urine samples of known arsenic concentration) were submitted to the laboratory in a blind fashion. The PE samples included

varying concentrations (20, 100, or 400 µg/L) each of four different types of arsenic (As^{+3}, As^{+5}, MMA, and DMA). The results for the PE samples are shown in Appendix D (see Table D-5 and Figure D-2). All sample results were close to the expected values, indicating that there was good recovery of the arsenic in all cases.

Blanks

Laboratory blank samples were run along with each batch of samples at a rate of about 10%. Blanks never yielded a measurable level of arsenic (all results <1 µg/L). Results are shown in Table D-6.

Summary of QC Results

Based on the results of all of the QC samples and steps described above, it is concluded that the analytical results are of sufficient quality for derivation of reliable estimates of arsenic absorption from the test materials.

3.0 DATA ANALYSIS

3.1 Overview

Figure 3-1 shows a conceptual model for the toxicokinetic fate of ingested arsenic. Key points of this model are as follows:

- In most animals (including humans), absorbed arsenic is excreted mainly in the urine over the course of several days. Thus, the urinary excretion fraction (UEF), defined as the amount excreted in the urine divided by the amount given, is usually a reasonable approximation of the AF_o or ABA. However, this ratio will underestimate total absorption, because some absorbed arsenic is excreted in the feces via the bile, and some absorbed arsenic enters tissue compartments (e.g., skin, hair) from which it is cleared very slowly or not at all. Thus, the UEF should not be equated with the absolute absorption fraction.

- The RBA of two orally administered materials (i.e., a test material and reference material) can be calculated from the ratio of the UEF of the two materials. This calculation is independent of the extent of tissue binding and of biliary excretion:

$$RBA(test\ vs\ ref) = \frac{AF_o(test)}{AF_o(ref)} = \frac{D \cdot AF_o(test) \cdot K_u}{D \cdot AF_o(ref) \cdot K_u} = \frac{UEF(test)}{UEF(ref)}$$

where:

D = ingested dose (μg)

K_u = fraction of absorbed arsenic that is excreted in the urine

Based on the conceptual model above, the basic method used to estimate the RBA of arsenic in a particular test material compared to arsenic in a reference material (sodium arsenate) is as follows:

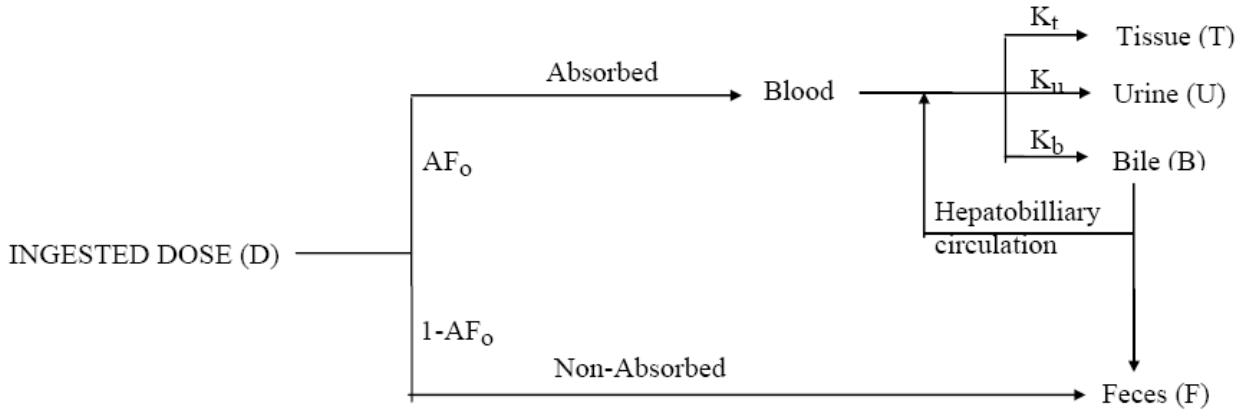
1. Plot the amount of arsenic excreted in the urine (μg per 48 hours) as a function of the administered amount of arsenic (μg per 48 hours), both for reference material and for test material.

2. Find the best fit linear regression line through each data set. The slope of each line (μg per 48 hours excreted per μg per 48 hours ingested) is the best estimate of the UEF for each material.

3. Calculate RBA for each test material as the ratio of the UEF for test material compared to UEF for reference material:

$$RBA(test\ vs\ ref) = \frac{UEF(test)}{UEF(ref)}$$

Figure 3-1. Conceptual Model for Arsenic Toxicokinetics



where:

AF_o = Oral Absorption Fraction

K_t = Fraction of absorbed arsenic which is retained in tissues

K_u = Fraction of absorbed arsenic which is excreted in urine

K_b = Fraction of absorbed arsenic which is excreted in the bile

BASIC EQUATIONS:

Amount in Urine

$$U_{oral} = D \bullet AF_o \bullet K_u$$

Urinary Excretion Fraction (UEF)

$$UEF_{oral} = \frac{U_{oral}}{D_{oral}} = AF_o \bullet K_u$$

Relative Bioavailability

$$RBA_{(x\ vs.\ y)} = \frac{UEF_{x,oral}}{UEF_{y,oral}} = \frac{AF_o(x) \bullet K_u}{AF_o(y) \bullet K_u} = \frac{AF_o(x)}{AF_o(y)}$$

3.2 Data Fitting

A detailed description of the data-fitting methods and rationale and the methods used to quantify uncertainty in the arsenic RBA estimates for a test material are summarized below. All data fitting was performed in Microsoft Excel® using matrix functions.

Simultaneous Regression

The techniques used to derive linear regression fits to the dose-response data are based on the methods recommended by Finney (1978). As noted by Finney (1978), when the data to be analyzed consist of two dose-response curves (the reference material and the test material), it is obvious that both curves must have the same intercept, since there is no difference between the curves when the dose is zero. This requirement is achieved by combining the two dose response equations into one and solving for the parameters simultaneously, as follows:

Separate Models:

$$\mu_r(i) = a + b_r \cdot x_r(i)$$

$$\mu_t(i) = a + b_t \cdot x_t(i)$$

Combined Model

$$\mu(i) = a + b_r \cdot x_r(i) + b_t \cdot x_t(i)$$

where $\mu(i)$ indicates the expected mean response of animals exposed at dose $x(i)$, and the subscripts r and t refer to reference and test material, respectively. The coefficients of this combined model are derived using multivariate regression, with the understanding that the combined data set is restricted to cases in which one (or both) of x_r and x_t are zero (Finney, 1978). When a study consists of a reference group and two test materials, as is the case for this study, the same approach is used, except that all three curves are fit simultaneously:

$$\mu(i) = a + b_r \cdot x_r(i) + b_{t1} \cdot x_{t1}(i) + b_{t2} \cdot x_{t2}(i)$$

Weighted Regression

Regression analysis based on ordinary least squares assumes that the variance of the responses is independent of the dose and/or the response (Draper and Smith, 1998). It has previously been shown that this assumption is generally not satisfied in swine-based RBA studies, where there is a tendency toward increasing variance in response as a function of increasing dose (heteroscedasticity) (USEPA, 2007). One method for dealing with heteroscedasticity is through the use of weighted least squares regression (Draper and Smith, 1998). In this approach, each observation in a group of animals is assigned a weight that is inversely proportional to the variance of the response in that group:

$$w_i = \frac{1}{\sigma_i^2}$$

where:

w_i = weight assigned to all data points in dose group *i*

σ_i^2 = variance of responses in animals in dose group *i*

When the distributions of responses at each dose level are normal, weighted regression is equivalent to the maximum likelihood method.

There are several alternative strategies for assigning weights. The method used in this study estimates the value of σ_i^2 using an "external" variance model based on an analysis of the relationship between variance and mean response using data consolidated across many different swine-based arsenic RBA studies. The data used to derive the variance model are shown in Figure 3-2. As seen, log-variance increases as an approximately linear function of log-mean response:

$$\ln(s_i^2) = k1 + k2 \cdot \ln(\bar{y}_i)$$

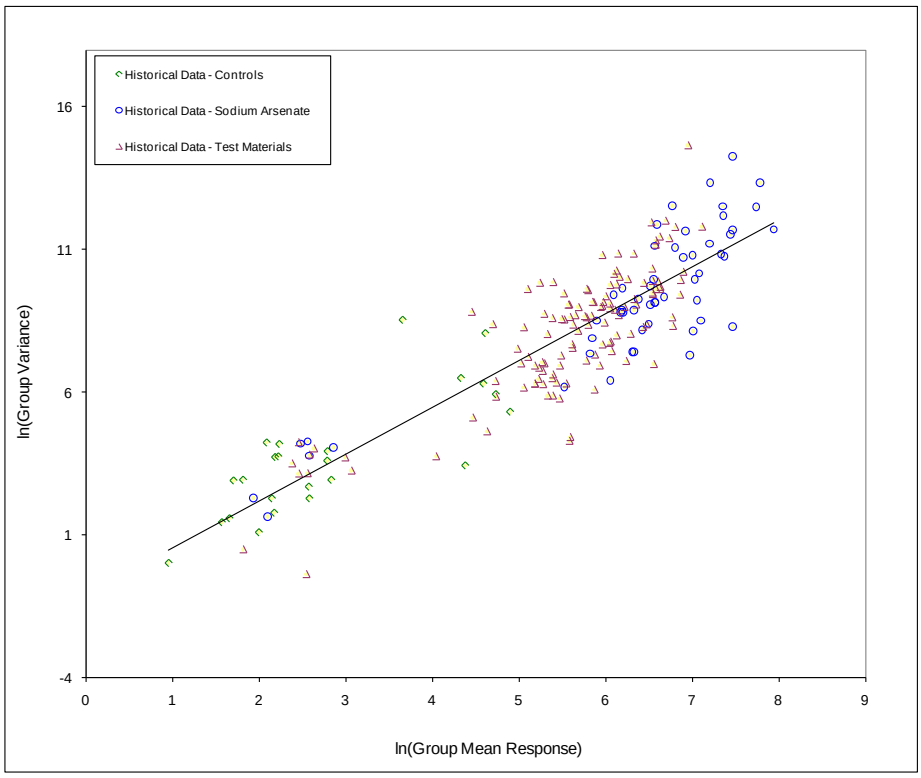
where:

si2 = observed variance of responses of animals in dose group i

$\bar{y}$i = mean observed response of animals in dose group i

Based on these data, values of *k*1 and *k*2 were derived using ordinary least squares minimization. The resulting values were -1.10 for *k*1 and 1.64 for *k*2.

Figure 3-2. Urinary Arsenic Variance Model



Goodness of Fit

The goodness-of-fit of each dose-response model was assessed using the F test statistic and the adjusted coefficient of multiple determination (Adj R^2) as described by Draper and Smith (1998). A fit is considered acceptable if the p-value is less than 0.05.

Assessment of Outliers

In biological assays, it is not uncommon to note the occurrence of individual measured responses that appear atypical compared to the responses from other animals in the same dose group. In these types of studies, responses that yield standardized weighted residuals greater than 3.5 or less than -3.5 are considered to be potential outliers (Canavos, 1984). Such a data point was not encountered in the data set for this study.

3.3 Calculation of RBA Estimates

The arsenic RBA values were calculated as the ratio of the slope term for the test material data set (b_t) and the reference material data set (b_r):

$$RBA = \frac{b_t}{b_r}$$

The uncertainty range about the RBA ratio was calculated using Fieller's Theorem as described by Finney (1978).

4.0 RESULTS

4.1 Clinical Signs

The doses of arsenic administered in this study are below a level that is expected to cause toxicological responses in swine. No clinical signs of arsenic-induced toxicity were noted in any of the animals used in the studies.

4.2 Dosing Deviations

There was one missed dose (Swine #733) on day 1 of the study. This was noted during the study but the calculated dose amounts for days 6/7, 9/10, and 12/13 were not affected by this deviation.

4.3 Background Arsenic Excretion

Measured values for urinary arsenic excretion for control animals from days 6 to 13 are shown in Table 4-1. Urinary arsenic concentration (mean ± SD) was 50.3 ± 31.5 µg/L. The values shown are representative of endogenous background levels in food and water and support the view that the animals were not exposed to any significant exogenous sources of arsenic throughout the study.

Table 4-1. Background Urinary Arsenic

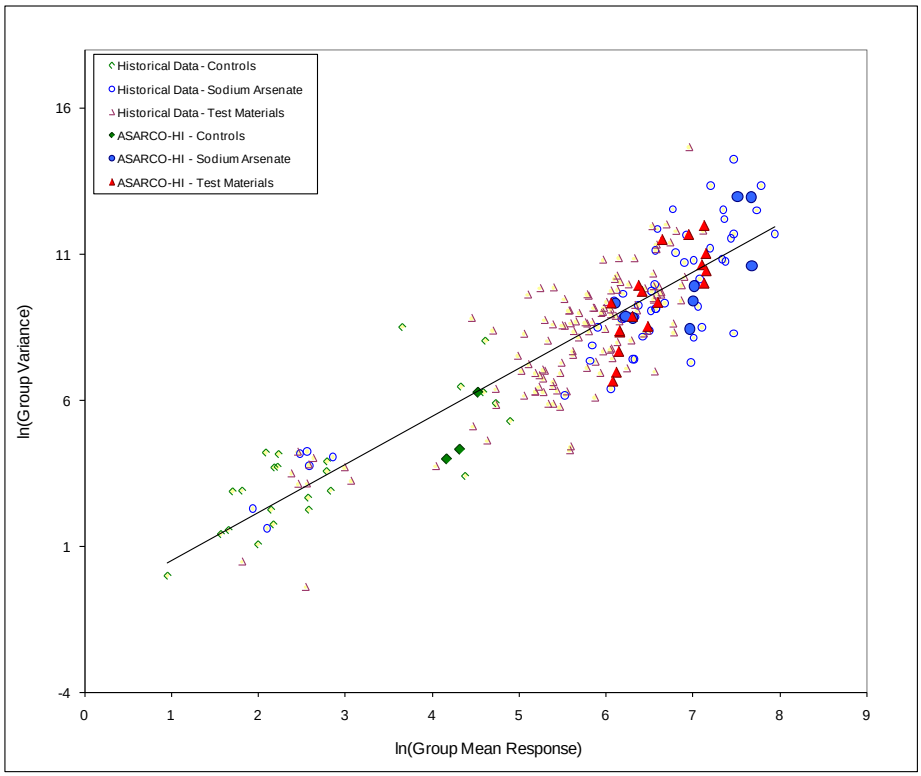
Swine Number	Urine Collection Period (days)	As Dose (µg per collection period)	As Concentration in Urine (µg/L)	Urine Volume (mL)	Total As Excreted (µg/48 hrs)
703	6/7	0	120	600	72
727	6/7	0	34	1680	57
729	6/7	0	56	1140	64
703	9/10	0	65	1260	82
727	9/10	0	23	3360	77
729	9/10	0	55	1180	65
703	12/13	0	37	2340	87
727	12/13	0	10	11760	118
729	12/13	0	53	1360	72

4.4 Urinary Arsenic Variance

As discussed in Section 3.2, the urinary arsenic dose-response data are analyzed using weighted least squares regression and the weights are assigned using an "external" variance model. To ensure that the variance model was valid, the variance values from each of dose groups were

superimposed on the historic data set (see Figure 4-1). As shown, the variance of the urinary arsenic data from this study are consistent with the data used to generate the variance model.

Figure 4-1. ASARCO and Hawaii Data Compared to Urinary Arsenic Variance Model



4.5 Dose-Response Modeling

The dose-response data for arsenic in urine were modeled using all of the data, and no outliers were identified. Modeling results are shown in Figures 4-2 through 4-5.

All of the dose-response curves were approximately linear, with the slope of the best-fit straight line being equal to the best estimate of the UEF. The resulting slopes (UEF estimates) for the final fittings of the test material and corresponding reference material are shown in Table 4-2.

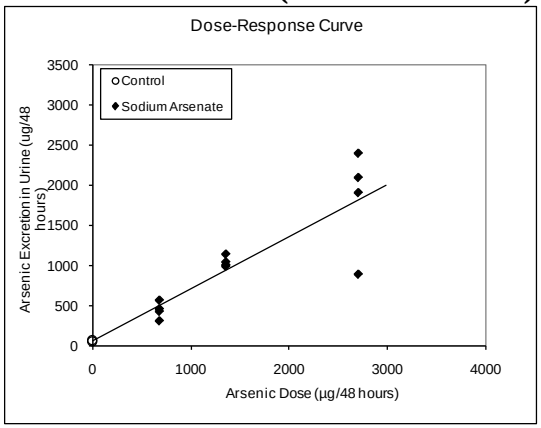
Table 4-2. Urine Excretion Fraction (UEF) Estimates

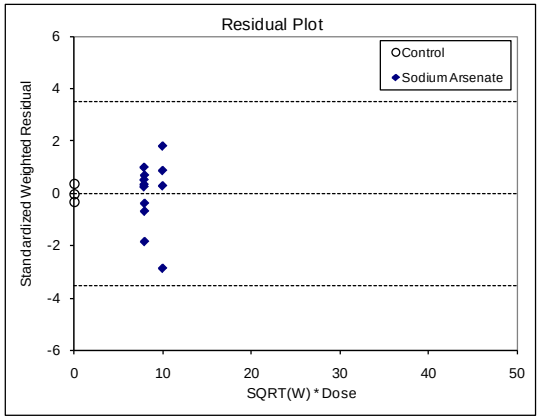
Urine Collection Period (days)	Outliers Excluded	Slopes (UEF Estimates)		
		b_r	b_{t1}	b_{t2}
Days 6/7	0	0.65	0.34	0.34
Days 9/10	0	0.73	0.36	0.35
Days 12/13	0	0.74	0.34	0.38
All Days	0	0.70	0.34	0.36

b_r = slope for reference material dose-response
b_{t1} = slope for test material 1 dose-response
b_{t2} = slope for test material 2 dose-response

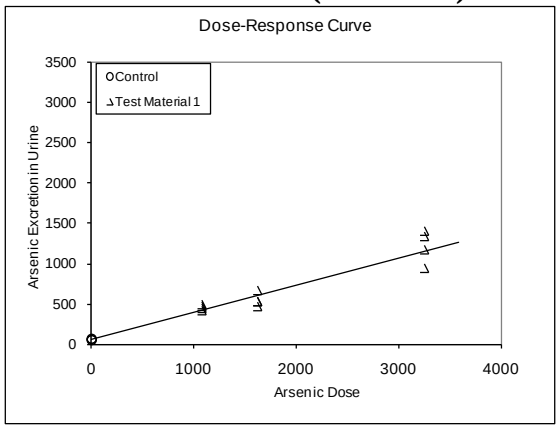
Figure 4-2. ASARCO and Hawaii Urinary Excretion of Arsenic: Days 6/7

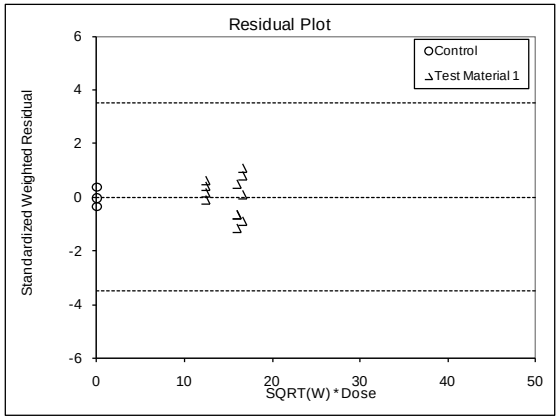
Reference Material (Sodium Arsenate)



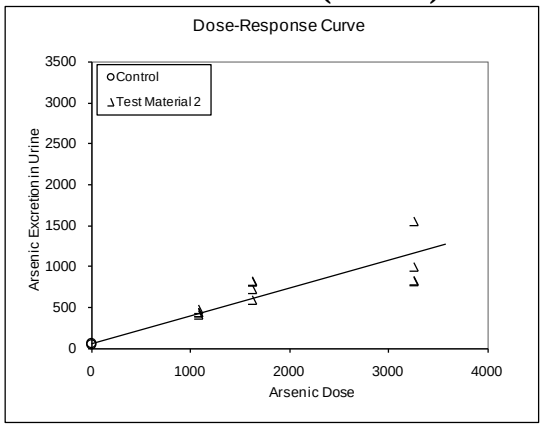


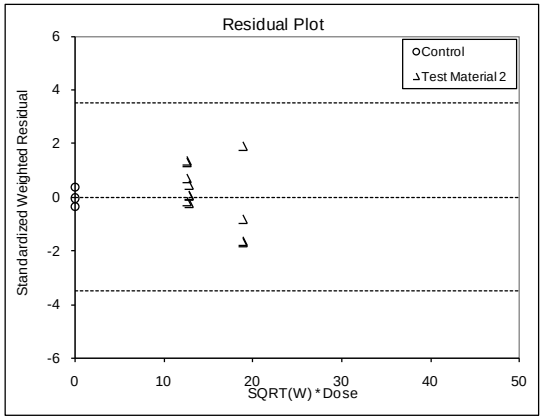
Test Material 1 (ASARCO)





Test Material 2 (Hawaii)





Summary of Fitting [a]

Parameter	Estimate	Standard Error
a	64.3	12.2
b_r	0.65	0.04
b_{t1}	0.34	0.02
b_{t2}	0.34	0.02
Covariance (b_r,b_{t1})	0.0620	–
Covariance (b_r,b_{t2})	0.0584	–
Degrees of Freedom	36	–

[a] $y = a + b_r*x_r + b_{t1}*x_{t1} + b_{t2}*x_{t2}$
where r = Reference Material, t1 = Test Material 1, and t2 = Test Material 2

ANOVA

Source	SSE	DF	MSE
Fit	825.64	3	275.21
Error	53.96	35	1.54
Total	879.60	38	23.15

Statistic	Estimate
F	178.506
P	<0.001
Adjusted R^2	0.9334

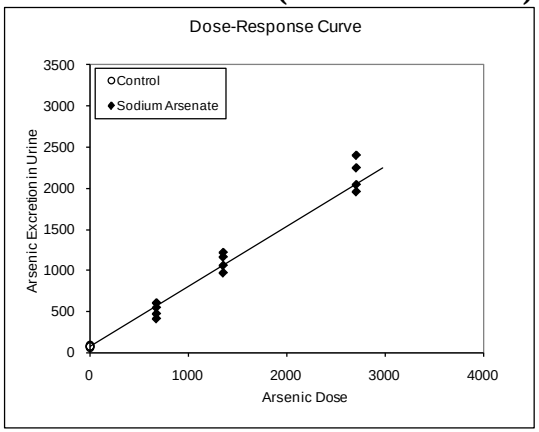
RBA and Uncertainty

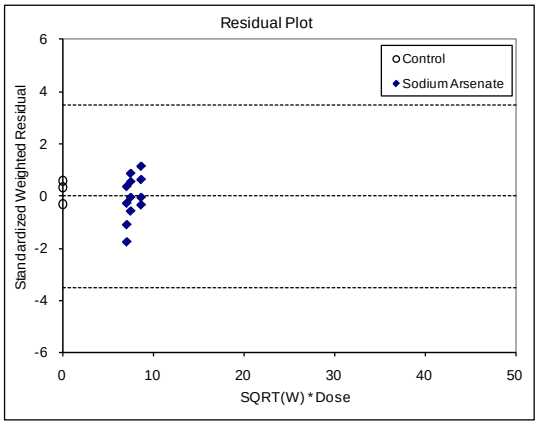
	Test Material 1	Test Material 2
RBA	0.52	0.52
Lower bound [b]	0.44	0.44
Upper bound [b]	0.61	0.61
Standard Error	0.049	0.050

[b] 90% confidence interval as calculated using Fieller's theorem

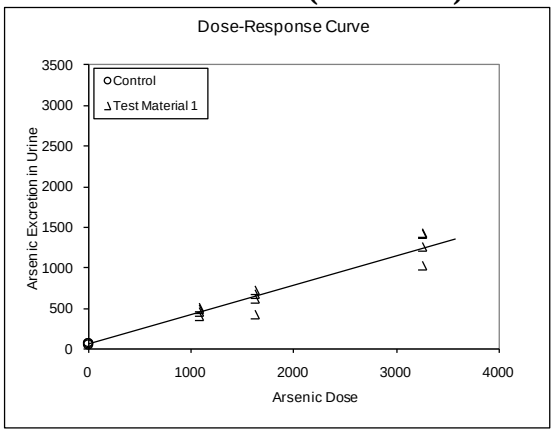
Figure 4-3. ASARCO and Hawaii Urinary Excretion of Arsenic: Days 9/10

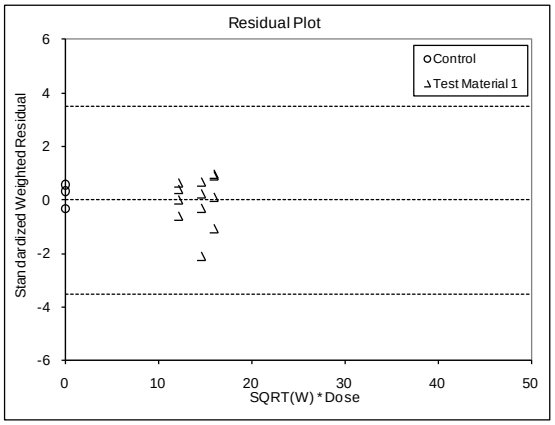
Reference Material (Sodium Arsenate)



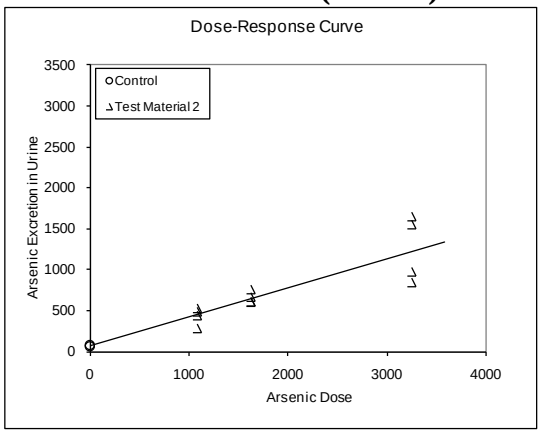


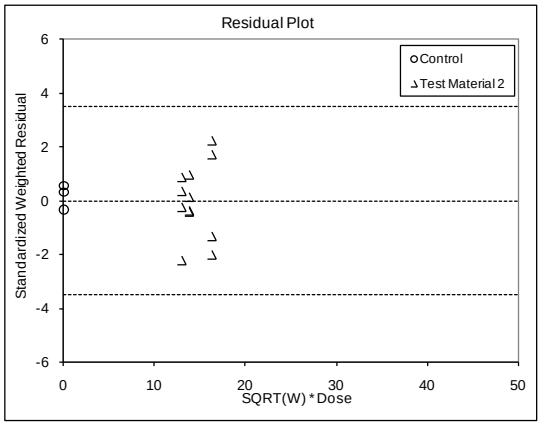
Test Material 1 (ASARCO)





Test Material 2 (Hawaii)





Summary of Fitting [a]

Parameter	Estimate	Standard Error
a	71.0	11.0
b_r	0.73	0.04
b_{t1}	0.36	0.02
b_{t2}	0.35	0.02
Covariance (b_r,b_{t1})	0.0684	–
Covariance (b_r,b_{t2})	0.0698	–
Degrees of Freedom	36	–

[a] $y = a + b_r*x_r + b_{t1}*x_{t1} + b_{t2}*x_{t2}$
where r = Reference Material, t1 = Test Material 1, and t2 = Test Material 2

ANOVA

Source	SSE	DF	MSE
Fit	820.44	3	273.48
Error	34.64	35	0.99
Total	855.07	38	22.50

Statistic	Estimate
F	276.325
P	<0.001
Adjusted R^2	0.9560

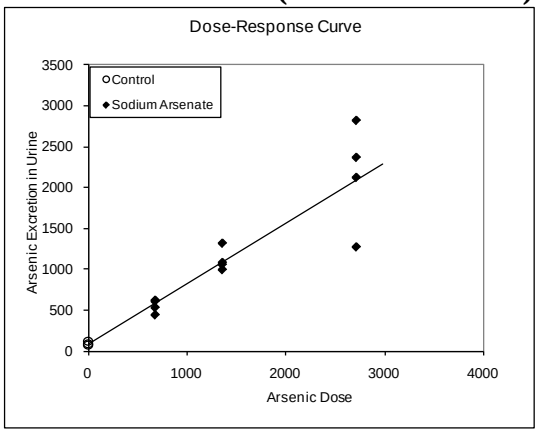
RBA and Uncertainty

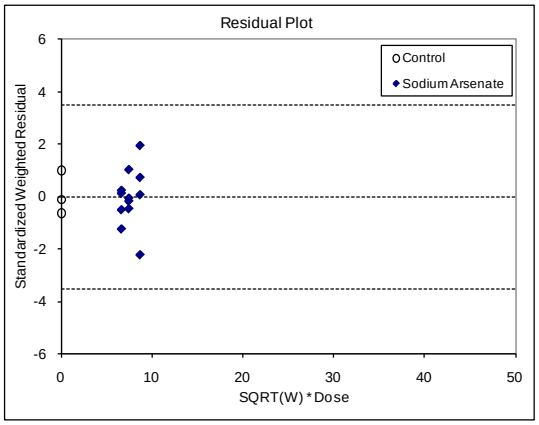
	Test Material 1	Test Material 2
RBA	0.49	0.48
Lower bound [b]	0.43	0.42
Upper bound [b]	0.56	0.55
Standard Error	0.049	0.037

[b] 90% confidence interval as calculated using Fieller's theorem

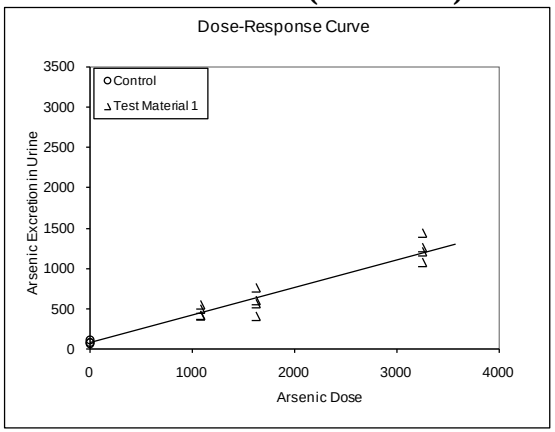
Figure 4-4. ASARCO and Hawaii Urinary Excretion of Arsenic: Days 12/13

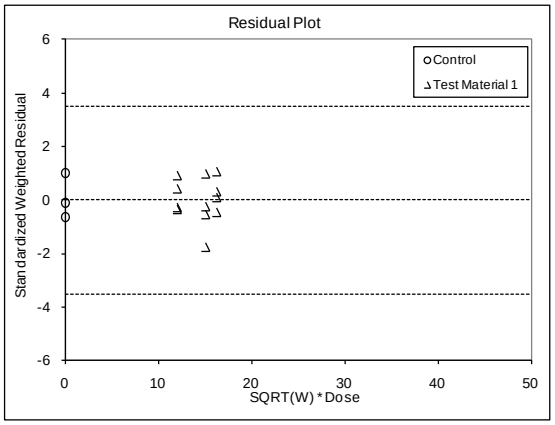
Reference Material (Sodium Arsenate)



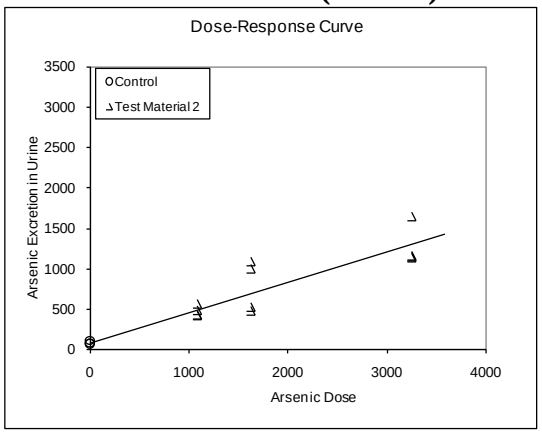


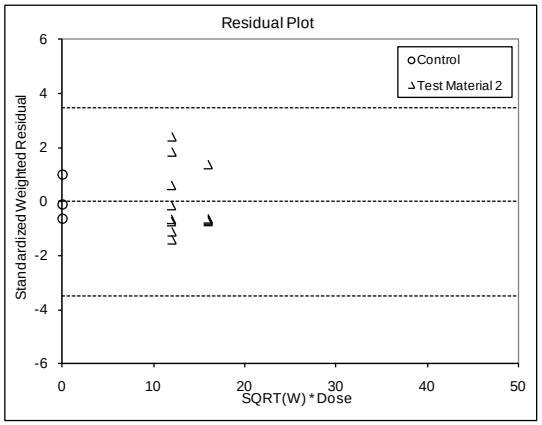
Test Material 1 (ASARCO)





Test Material 2 (Hawaii)





Summary of Fitting [a]

Parameter	Estimate	Standard Error
a	89.7	16.3
b_r	0.74	0.05
b_{t1}	0.34	0.03
b_{t2}	0.38	0.03
Covariance (b_r,b_{t1})	0.0882	–
Covariance (b_r,b_{t2})	0.0819	–
Degrees of Freedom	36	–

[a] $y = a + b_r*x_r + b_{t1}*x_{t1} + b_{t2}*x_{t2}$
where r = Reference Material, t1 = Test Material 1, and t2 = Test Material 2

ANOVA

Source	SSE	DF	MSE
Fit	757.73	3	252.58
Error	54.28	35	1.55
Total	812.02	38	21.37

Statistic	Estimate
F	162.849
P	<0.001
Adjusted R^2	0.9274

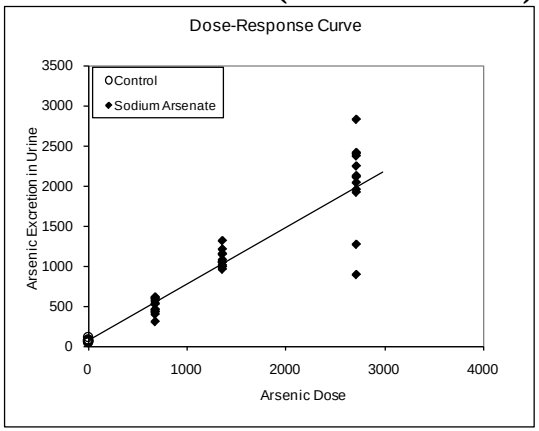
RBA and Uncertainty

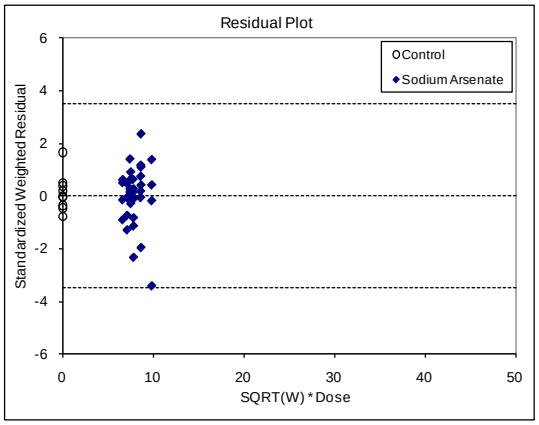
	Test Material 1	Test Material 2
RBA	0.49	0.48
Lower bound [b]	0.43	0.42
Upper bound [b]	0.56	0.55
Standard Error	0.049	0.037

[b] 90% confidence interval as calculated using Fieller's theorem

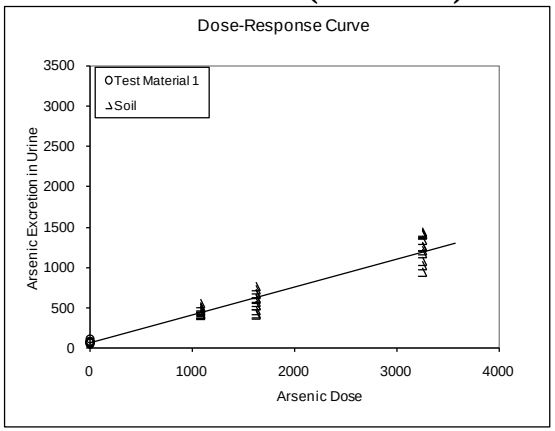
Figure 4-5. ASARCO and Hawaii Urinary Excretion of Arsenic: All Days

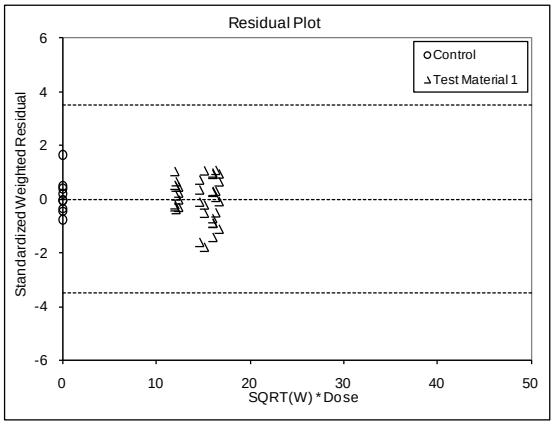
Reference Material (Sodium Arsenate)



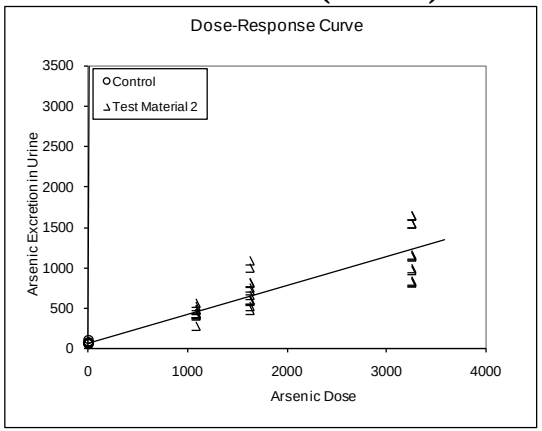


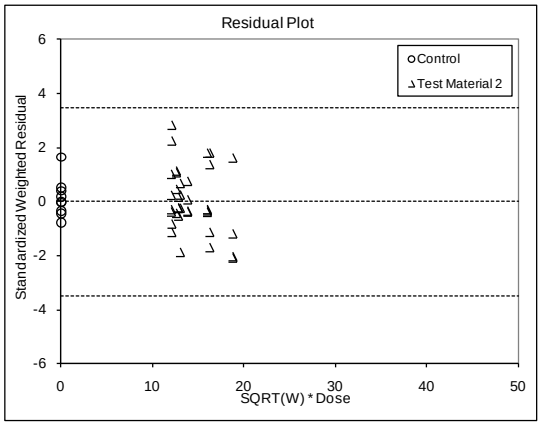
Test Material 1 (ASARCO)





Test Material 2 (Hawaii)





Summary of Fitting [a]

Parameter	Estimate	Standard Error
a	72.7	7.5
b_r	0.70	0.02
b_{t1}	0.34	0.01
b_{t2}	0.36	0.01
Covariance (b_r,b_{t1})	0.0706	–
Covariance (b_r,b_{t2})	0.0680	–
Degrees of Freedom	114	–

[a] $y = a + b_r*x_r + b_{t1}*x_{t1} + b_{t2}*x_{t2}$
where r = Reference Material, t1 = Test Material 1, and t2 = Test Material 2

ANOVA

Source	SSE	DF	MSE
Fit	2423.62	3	807.87
Error	154.85	113	1.37
Total	2578.47	116	22.23

Statistic	Estimate
F	589.531
P	< 0.001
Adjusted R^2	0.9384

RBA and Uncertainty

	Test Material 1	Test Material 2
RBA	0.49	0.51
Lower bound [b]	0.45	0.46
Upper bound [b]	0.53	0.55
Standard Error	0.049	0.025

[b] 90% confidence interval as calculated using Fieller's theorem

4.6 Calculated RBA Values

Estimated RBA values (mean and 90% confidence interval) are shown in Table 4-3. As shown, the best fit point estimate RBA of arsenic in an ASARCO and Hawaii soil sample observed was is 49 and 51%, respectively.

Table 4-3. Estimated Arsenic Relative Bioavailability (RBA) for Asarco and Hawaii Soils

Urine Collection Period (days)	Estimated RBA (90% Confidence Interval)	
	TM1 (ASARCO)	TM2 (Hawaii)
Days 6/7	0.52 (0.44–0.61)	0.52 (0.44–0.61)
Days 9/10	0.49 (0.43–0.56)	0.48 (0.42–0.55)
Days 12/13	0.46 (0.39–0.54)	0.51 (0.43–0.60)
All Days	**0.49 (0.45–0.53)**	**0.51 (0.46–0.55)**

4.7 Uncertainty

The bioavailability estimates above are subject to uncertainty that arises from several different sources. One source of uncertainty is the inherent biological variability between different animals in a dose group, which in turn causes variability in the amount of arsenic absorbed by the exposed animals. The between-animal variability results in statistical uncertainty in the best-fit dose-response curves and, hence, uncertainty in the calculated values of RBA. Such statistical uncertainty is accounted for by the statistical models used above and is characterized by the uncertainty range around the RBA estimates.

However, there is also uncertainty in the extrapolation of RBA values measured in juvenile swine to young children or adults, and this uncertainty is not included in the statistical confidence bounds above. Even though the immature swine is believed to be a useful and meaningful animal model for gastrointestinal absorption in humans, it is possible that there are differences in physiological parameters that may influence RBA; therefore, RBA values in swine may not be identical to values in children. In addition, RBA may depend on the amount and type of food in the stomach, since the presence of food can influence stomach pH, holding time, and possibly other factors that may influence solubilization of arsenic. RBA values measured in this study are based on animals that have little or no food in their stomach at the time of exposure and, hence, are likely to yield high-end values of RBA. Thus, these RBA values may be somewhat conservative for humans who ingest the site soils along with food. The magnitude of this bias is not known.

5.0 REFERENCES

Canavos, C. G. 1984. Applied Probability and Statistical Methods. Little, Brown and Co., Boston.

Casteel, S. W., R. P. Cowart, C. P. Weis, G. M. Henningsen, E. Hoffman, W. J. Brattin, M. F. Starost, J. T. Payne, S. L. Stockham, S. V. Becker, and J. R. Turk. 1996. A swine model for determining the bioavailability of lead from contaminated media. In: Advances in Swine in Biomedical Research. Volume 2, Tumbleson and Schook (editors). Plenum Press, New York. pp. 637–646.

Draper, N. R. and H. Smith. 1998. Applied Regression Analysis. 3rd Edition. John Wiley & Sons, New York, NY.

Finney, D. J. 1978. Statistical Method in Biological Assay. 3rd Edition. Charles Griffin and Co., London.

Gibaldi, M. and Perrier, D. 1982. Pharmacokinetics. 2nd edition. Marcel Dekker, Inc, New York, NY, pp 294–297.

Goodman, A. G., Rall, T. W., Nies, A. S., and Taylor, P. 1990. The Pharmacological Basis of Therapeutics. 8th edition. Pergamon Press, Inc. Elmsford, NY, pp. 5–21.

Klaassen, C. D., Amdur, M. O., and Doull, J. 1996. Cassarett and Doull's Toxicology: The Basic Science of Poisons. McGraw-Hill, Inc. New York, NY, pp. 190.

Miller, B. W. and Scheckel, K. G. 2012. Technical Review Workgroup for Metals and Asbestos: Bioavailability Committee. Mineralogical Report. XAS Data and Linear Combination Fitting Results. Available at: http://epa.gov/superfund/bioavailability/guidance.htm

NIST. 2003. Certificate of Analysis, Standard Reference Material® 2710 – Montana Soil, Highly Elevated Trace Element Concentrations. National Institute of Standards & Technology, Gaithersburg, MD. Certificate Issue Date: July 18, 2003.

NRC. 1988. Nutrient Requirements of Swine. A Report of the Committee on Animal Nutrition. National Research Council. National Academy Press, Washington, DC.

USEPA. 2007. Estimation of Relative Bioavailability of Lead in Soil and Soil-Like Materials by *In Vivo* and *In Vitro* Methods. U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response, Washington DC. OSWER 9285.7-77.

Weis, C. P. and LaVelle, J. M. 1991. Characteristics to consider when choosing an animal model for the study of lead bioavailability. In: The proceedings of the international symposium on the bioavailability and dietary uptake of lead. *Science and Technology Letters* 3:113–119.

Appendix A: Group Assignments

Table A-1. Group Assignments For The ASARCO-Hawaii Arsenic Study

Swine Number	Group	Treatment	Target Arsenic Dose (µg/kg-day)
714	1	NaAs	25
726			
741			
743			
702	2	NaAs	50
706			
710			
738			
704	3	NaAs	100
721			
730			
740			
705	4	TM1	40
728			
734			
735			
708	5	TM1	60
715			
717			
720			
713	6	TM1	120
718			
731			
733			
716	7	TM2	40
719			
737			
739			
711	8	TM2	60
723			
736			
742			
701	9	TM2	120
707			
709			
724			
703	10	Control	0
727			
729			

Appendix B: Body Weights

Table B-1. Body Weights

Group	Swine Number	Weight (kg)													
		Day -5	Group BW	Day -1	Group BW	Day 2	Group BW	Day 5	Group BW	Day 8	Group BW	Day 11	Group BW	Day 14	Group BW
		3/3/10	Mean ± SD	3/7/10	Mean ± SD	3/10/10	Mean ± SD	3/13/10	Mean ± SD	3/16/10	Mean ± SD	3/19/10	Mean ± SD	3/22/10	Mean ± SD
1 NaAs / 25	714	9.1		9.3		9.8		10.3		11		11.6		12.1	
	726	8.2		8.6		9.5		9.8		10.4		11.6		11.8	
	741	8		8.7		9.1		9.5		10.2		11		11.6	
	743	10	8.8 ± 0.9	10.5	9.3 ± 0.9	11.4	10.0 ± 1.0	12.1	10.4 ± 1.2	12.8	11.1 ± 1.2	13.6	12.0 ± 1.1	14	12.4 ± 1.1
2 NaAs / 50	702	10.4		10.9		11.7		12.1		12.6		13.6		13.9	
	706	10.5		10.9		11.6		12		12.7		13.7		14	
	710	9.6		10.3		11.1		11.6		12.4		13.5		14	
	738	10.5	10.3 ± 0.4	10.7	10.7 ± 0.3	11.5	11.5 ± 0.3	12.1	12.0 ± 0.2	12.8	12.6 ± 0.2	14.1	13.7 ± 0.3	14.6	14.1 ± 0.3
3 NaAs / 100	704	10		10.2		10.7		11.2		11.9		13.1		13.3	
	721	9.6		10.3		10.8		11.7		12.4		13.2		13.7	
	730	8.7		9		10		10.3		11.1		12.2		12.2	
	740	10.4	9.7 ± 0.7	10.9	10.1 ± 0.8	11.7	10.8 ± 0.7	12.3	11.4 ± 0.8	13	12.1 ± 0.8	14.2	13.2 ± 0.8	14.5	13.4 ± 1.0
4 TM1 / 40	705	9		9.8		10.9		11.5		12.1		13.1		13.6	
	728	10.1		10.4		10.6		11.5		12.2		13.4		13.8	
	734	9.2		9.5		10.1		10.7		11.6		12.9		13.4	
	735	8	9.1 ± 0.9	8	9.4 ± 1.0	8.2	10.0 ± 1.2	8.9	10.7 ± 1.2	9.8	11.4 ± 1.1	10.8	12.6 ± 1.2	11.2	13.0 ± 1.2
5 TM1 / 60	708	8.7		9.3		9.8		10.5		11		11.9		12.4	
	715	9.5		10.3		11.2		11.7		12.7		13.5		13.8	
	717	9.8		10.1		11		11.6		12.5		13.4		14	
	720	9.2	9.3 ± 0.5	9.6	9.8 ± 0.5	10.4	10.6 ± 0.6	10.8	11.2 ± 0.6	11.9	12.0 ± 0.8	13	13.0 ± 0.7	13.4	13.4 ± 0.7
6 TM1 / 120	713	9.1		9.5		10.2		11.2		11.9		12.9		13.2	
	718	10		10.4		11.5		12.3		13.2		14		14.2	
	731	8		8.6		9.4		9.8		10		11.4		12.2	
	733	9.1	9.1 ± 0.8	9.6	9.5 ± 0.7	10.2	10.3 ± 0.9	11.1	11.1 ± 1.0	11.8	11.7 ± 1.3	13.1	12.9 ± 1.1	13.4	13.3 ± 0.8
7 TM2 / 40	716	9.1		9.6		10.2		10.7		11.2		12.3		12.8	
	719	10.6		11.1		11.9		12.6		13		14.4		14.6	
	737	8.1		8.9		9.4		10.1		10.7		11.6		11.8	
	739	8.5	9.1 ± 1.1	9	9.7 ± 1.0	9.8	10.3 ± 1.1	10.2	10.9 ± 1.2	10.9	11.5 ± 1.1	11.1	12.4 ± 1.5	11.8	12.8 ± 1.3
8 TM2 / 60	711	10.3		10.9		11.4		11.9		12.6		14.2		14	
	723	8.4		9		9.6		10		10.6		11.2		11.4	
	736	9.6		9.7		10.1		10.8		10.9		12		12	
	742	8.1	9.1 ± 1.0	8.6	9.6 ± 1.0	9.2	10.1 ± 1.0	9.8	10.6 ± 1.0	9.5	10.9 ± 1.3	11	12.1 ± 1.5	11.4	12.2 ± 1.2
9 TM2 / 120	701	10.8		11.2		12		12.6		13.2		14.1		14.4	
	707	8.5		9.4		10		10.7		11.4		12.6		13.1	
	709	9.2		9.7		10		10.7		10.7		11.9		12.3	
	724	9.8	9.6 ± 1.0	10	10.1 ± 0.8	10.7	10.7 ± 0.9	10.9	11.2 ± 0.9	11.5	11.7 ± 1.1	12	12.7 ± 1.0	13	13.2 ± 0.9
10 Control / 0	703	8.7		9.3		10.1		10.4		11.3		12.8		12.9	
	727	9.3		9.7		10.3		11.2		11.6		12.4		12.8	
	729	10.3	9.4 ± 0.8	10.5	9.8 ± 0.6	10.5	10.3 ± 0.2	10.6	10.7 ± 0.4	11.8	11.6 ± 0.3	12.5	12.6 ± 0.2	13.5	13.1 ± 0.4

BW = body weight

Appendix C: Urine Volumes and Urinary Arsenic Analytical Results for Study Samples

Table C-1. Urinary Arsenic Analytical Results and Urine Volumes for Study Samples

Group	Material	Collection Period (days)	Sample ID	Swine Number	Urinary As (µg/L)	Urine Volume (mL)
1	NaAs	6/7	ASHI-714-U1	714	440	1060
			ASHI-726-U1	726	242	1800
			ASHI-741-U1	741	256	1240
			ASHI-743-U1	743	95	6060
		9/10	ASHI-714-U2	714	510	800
			ASHI-726-U2	726	226	2660
			ASHI-741-U2	741	150	3620
			ASHI-743-U2	743	130	3600
		12/13	ASHI-714-U3	714	760	700
			ASHI-726-U3	726	180	3380
			ASHI-741-U3	741	360	1230
			ASHI-743-U3	743	73	8520
2	NaAs	6/7	ASHI-702-U1	702	420	2420
			ASHI-706-U1	706	640	1560
			ASHI-710-U1	710	650	1620
			ASHI-738-U1	738	1010	1140
		9/10	ASHI-702-U2	702	440	2760
			ASHI-706-U2	706	660	1600
			ASHI-710-U2	710	460	2100
			ASHI-738-U2	738	950	1220
		12/13	ASHI-702-U3	702	420	2580
			ASHI-706-U3	706	600	1660
			ASHI-710-U3	710	249	5300
			ASHI-738-U3	738	790	1340
3	NaAs	6/7	ASHI-704-U1	704	4000	480
			ASHI-721-U1	721	950	2220
			ASHI-730-U1	730	900	2680
			ASHI-740-U1	740	500	1800
		9/10	ASHI-704-U2	704	2440	920
			ASHI-721-U2	721	540	4440
			ASHI-730-U2	730	660	2960
			ASHI-740-U2	740	1500	1360
		12/13	ASHI-704-U3	704	2890	820
			ASHI-721-U3	721	490	5760
			ASHI-730-U3	730	310	4110
			ASHI-740-U3	740	320	6640
4	TM1	6/7	ASHI-705-U1	705	93	5060
			ASHI-728-U1	728	530	840
			ASHI-734-U1	734	215	1940
			ASHI-735-U1	735	140	3520
		9/10	ASHI-705-U2	705	37	12440
			ASHI-728-U2	728	330	1560
			ASHI-734-U2	734	217	1880
			ASHI-735-U2	735	190	2600
		12/13	ASHI-705-U3	705	42	9960
			ASHI-728-U3	728	440	1140
			ASHI-734-U3	734	263	1620
			ASHI-735-U3	735	207	2680

Table C-1. Urinary Arsenic Analytical Results and Urine Volumes for Study Samples

Group	Material	Collection Period (days)	Sample ID	Swine Number	Urinary As (µg/L)	Urine Volume (mL)
5	TM1	6/7	ASHI-708-U1	708	221	2400
			ASHI-715-U1	715	75	6240
			ASHI-717-U1	717	760	880
			ASHI-720-U1	720	130	4070
		9/10	ASHI-708-U2	708	202	3360
			ASHI-715-U2	715	63	6800
			ASHI-717-U2	717	550	1320
			ASHI-720-U2	720	160	3880
		12/13	ASHI-708-U3	708	205	2780
			ASHI-715-U3	715	77	5360
			ASHI-717-U3	717	330	2320
			ASHI-720-U3	720	160	3800
6	TM1	6/7	ASHI-713-U1	713	400	3520
			ASHI-718-U1	718	630	1500
			ASHI-731-U1	731	390	3440
			ASHI-733-U1	733	700	1680
		9/10	ASHI-713-U2	713	290	3540
			ASHI-718-U2	718	380	3720
			ASHI-731-U2	731	330	4320
			ASHI-733-U2	733	590	2130
		12/13	ASHI-713-U3	713	270	4000
			ASHI-718-U3	718	273	4440
			ASHI-731-U3	731	370	3900
			ASHI-733-U3	733	540	2340
7	TM2	6/7	ASHI-716-U1	716	82	5300
			ASHI-719-U1	719	440	1080
			ASHI-737-U1	737	48	8480
			ASHI-739-U1	739	72	6070
		9/10	ASHI-716-U2	716	99	4400
			ASHI-719-U2	719	310	900
			ASHI-737-U2	737	63	7660
			ASHI-739-U2	739	140	3740
		12/13	ASHI-716-U3	716	90	4760
			ASHI-719-U3	719	214	1960
			ASHI-737-U3	737	79	6120
			ASHI-739-U3	739	130	4340
8	TM2	6/7	ASHI-711-U1	711	92	8820
			ASHI-723-U1	723	1400	420
			ASHI-736-U1	736	600	1200
			ASHI-742-U1	742	120	6840
		9/10	ASHI-711-U2	711	140	4320
			ASHI-723-U2	723	1300	580
			ASHI-736-U2	736	300	2000
			ASHI-742-U2	742	75	8820
		12/13	ASHI-711-U3	711	244	4100
			ASHI-723-U3	723	680	700
			ASHI-736-U3	736	540	2020
			ASHI-742-U3	742	74	7080

Table C-1. Urinary Arsenic Analytical Results and Urine Volumes for Study Samples

Group	Material	Collection Period (days)	Sample ID	Swine Number	Urinary As (µg/L)	Urine Volume (mL)
9	TM2	6/7	ASHI-701-U1	701	160	9700
			ASHI-707-U1	707	243	3400
			ASHI-709-U1	709	1020	800
			ASHI-724-U1	724	710	1400
		9/10	ASHI-701-U2	701	350	4700
			ASHI-707-U2	707	330	2940
			ASHI-709-U2	709	910	1700
			ASHI-724-U2	724	200	4200
		12/13	ASHI-701-U3	701	150	7680
			ASHI-707-U3	707	380	3060
			ASHI-709-U3	709	960	1720
			ASHI-724-U3	724	170	6700
10	Control	6/7	ASHI-703-U1	703	120	600
			ASHI-727-U1	727	34	1680
			ASHI-729-U1	729	56	1140
		9/10	ASHI-703-U2	703	65	1260
			ASHI-727-U2	727	23	3360
			ASHI-729-U2	729	55	1180
		12/13	ASHI-703-U3	703	37	2340
			ASHI-727-U3	727	10	11760
			ASHI-729-U3	729	53	1360

Appendix D: Analytical Results for Quality Control Samples

Table D-1. Blind Duplicate Samples

Blind Duplicate Sample ID	Sample Type	Swine Number	Collection Days	Original Sample Concentration	Duplicate Sample Concentration	Sample Units	RPD
ASHI-196	Urine	702	U-3	420	420	µg/L	0%
ASHI-201	Urine	709	U-3	960	940	µg/L	2%
ASHI-168	Urine	726	U-2	226	225	µg/L	0%
ASHI-129	Urine	727	U-1	34	3.7	µg/L	161%
ASHI-237	Urine	731	U-3	370	360	µg/L	3%
ASHI-109	Urine	733	U-1	700	710	µg/L	1%
ASHI-141	Urine	735	U-1	140	140	µg/L	0%
ASHI-181	Urine	736	U-2	300	310	µg/L	3%
ASHI-160	Urine	739	U-2	140	140	µg/L	0%

RPD = relative percent difference

Table D-2. Laboratory Spikes

Spike Sample ID	Sample Type	Original Sample Concentration (µg/L)	Added Spike Concentration (µg/L)	Measured Sample Concentration (µg/L)	Recovered Spike (µg/L)	Recovery
ASHI-110	Urine	48	200	250	202	101%
ASHI-120	Urine	82	200	280	198	99%
ASHI-130	Urine	92	200	300	208	104%
ASHI-140	Urine	4000	200	4220	220	110%
ASHI-150	Urine	310	200	510	200	100%
ASHI-160	Urine	140	200	350	210	105%
ASHI-170	Urine	202	200	390	188	94%
ASHI-180	Urine	440	200	660	220	110%
ASHI-190	Urine	950	200	974	24	12%
ASHI-200	Urine	150	200	360	210	105%
ASHI-210	Urine	273	200	478	205	103%
ASHI-220	Urine	74	200	280	206	103%
ASHI-230	Urine	42	200	240	198	99%
ASHI-240	Urine	205	200	400	195	98%
ASHI-276	Water	1	100	98	97	97%

Table D-3. Laboratory Duplicates

Duplicate Sample ID	Sample Type	Original Sample Concentration (ppb)	Duplicate Concentration (ppb)	RPD	Absolute Difference
ASHI-105	Urine	72	72	0%	0
ASHI-115	Urine	1400	1400	0%	0
ASHI-125	Urine	760	740	3%	20
ASHI-135	Urine	56	54	4%	2
ASHI-145	Urine	420	430	2%	10
ASHI-155	Urine	65	61	6%	4
ASHI-165	Urine	217	220	1%	3
ASHI-175	Urine	226	225	0%	1
ASHI-185	Urine	63	62	2%	1
ASHI-195	Urine	130	130	0%	0
ASHI-205	Urine	170	170	0%	0
ASHI-215	Urine	73	73	0%	0
ASHI-225	Urine	310	370	18%	60
ASHI-235	Urine	160	160	0%	0
ASHI-273	Water	<1	<1	0%	0
ASHI-277	Feed	0.2	0.1	67%	0.1

RPD = relative percent difference

Table D-4. Laboratory Quality Control Standards

Sample ID	Associated Sample Type	LET Number	Measured Concentration	Units	Reference Material ID	Certified Value (Mean ± SD)	Recovery
QC-1	Urine	L10030056	<5	ng/mL	NIST 2670a-L	3	83%
QC-2	Urine	L10030080	220	ng/mL	NIST 2670a-H	220 ± 10	100%
QC-3	Urine	L10030104	240	ng/mL	NIST 2670a-H	220 ± 10	109%
QC-4	Urine	L10030128	220	ng/mL	NIST 2670a-H	220 ± 10	100%
QC-5	Urine	L10030152	230	ng/mL	NIST 2670a-H	220 ± 10	105%
QC-6	Urine	L10030176	230	ng/mL	NIST 2670a-H	220 ± 10	105%
QC-7	Urine	L10030200	6	ng/mL	NIST 2670a-L	3	200%
QC-8	Water	L10030210	58	ng/mL	NIST 1643e	58.98 ± 0.7	98%
QC-9	Feed	L10030215	7.1	mcg/g	NIST 1566b	7.65 ± 0.65	93%

TABLE D-5. ARSENIC PERFORMANCE EVALUATION SAMPLES

Sample ID	PE ID	PE Standard	PE Concentration (µg/L)	Sample Concentration (µg/L)	Adjusted Concentration (µg/L)	RPD
ASHI-177	as3.100	Sodium arsenite	100	120	70	36%
ASHI-221	as3.20	Sodium arsenite	20	40	0	200%
ASHI-139	as5.100	Sodium arsenate	100	150	100	0%
ASHI-151	as5.20	Sodium arsenate	20	51	1	187%
ASHI-204	as5.400	Sodium arsenate	400	440	390	3%
ASHI-232	ctrl	Control urine	0	38	0	0%
ASHI-136	ctrl	Control urine	0	120	70	0%
ASHI-173	dma100	Disodium methylarsenate	100	150	100	0%
ASHI-122	dma20	Disodium methylarsenate	20	58	8	89%
ASHI-199	dma400	Disodium methylarsenate	400	460	410	2%
ASHI-234	mma100	Dimethyl arsenic acid	100	140	90	11%
ASHI-114	mma20	Dimethyl arsenic acid	20	79	29	36%
ASHI-163	mma400	Dimethyl arsenic acid	400	420	370	8%

PE = performance evaluation. Sample concentration adjusted by subtracting mean of background arsenic (~50 µg/L) from sample concentration.
RPD = relative percent difference

TABLE D-6. BLANKS

Sample ID	Associated Sample Type	Measured Concentration	Detection Limit	Units
Blank-1	Urine	<1	1	µg/L
Blank-2	Urine	<1	1	µg/L
Blank-3	Urine	<1	1	µg/L
Blank-4	Urine	<1	1	µg/L
Blank-5	Urine	<1	1	µg/L
Blank-6	Urine	<1	1	µg/L
Blank-7	Urine	<1	1	µg/L
Blank-8	Water	<1	1	µg/L
Blank-9	Feed	<0.1	0.1	µg/g

Figure D-1. Urinary Arsenic Blind Duplicates

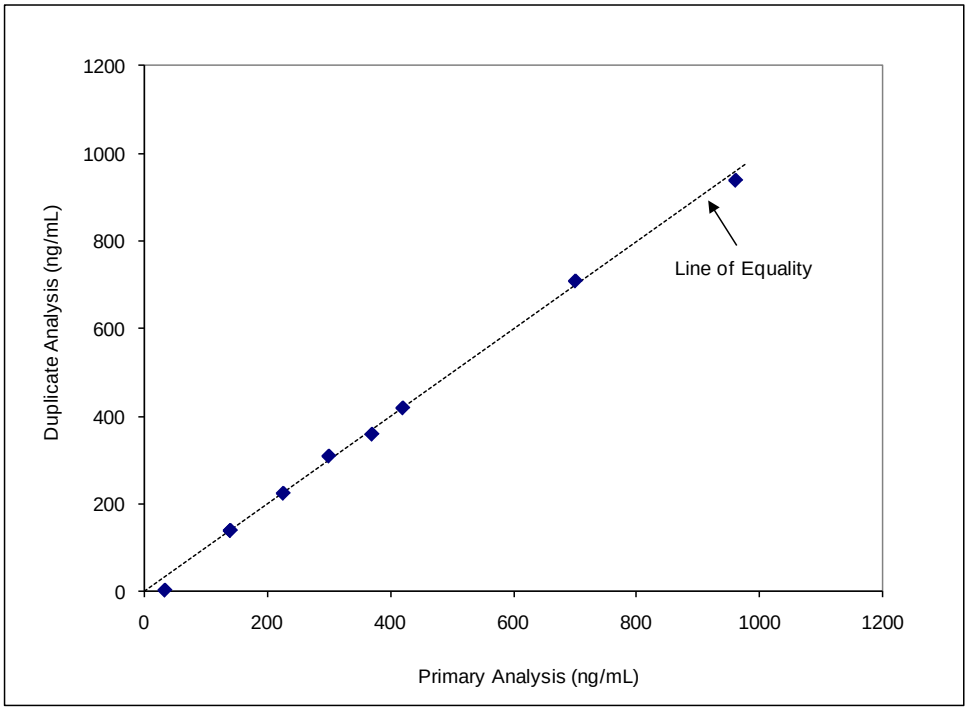
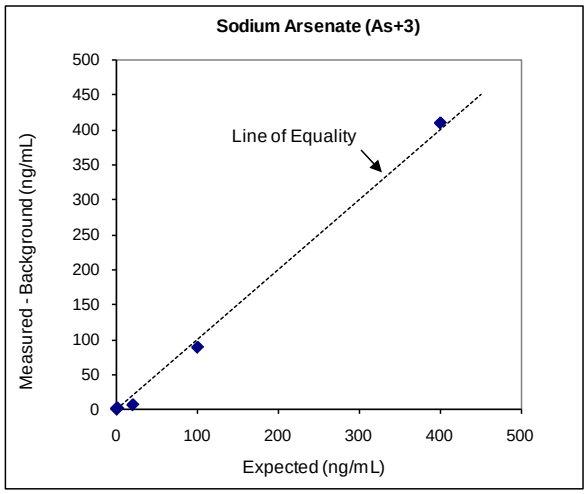
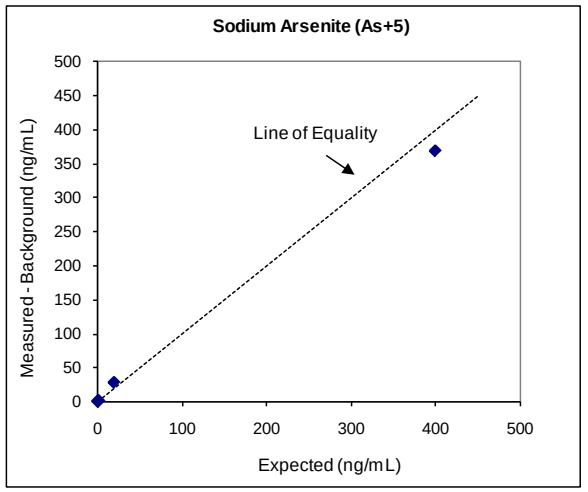
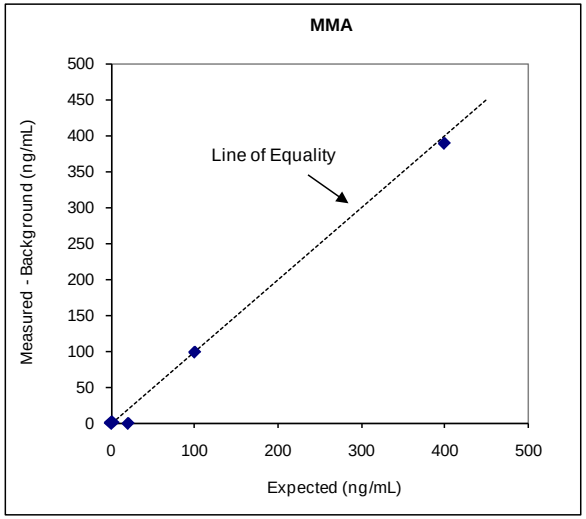
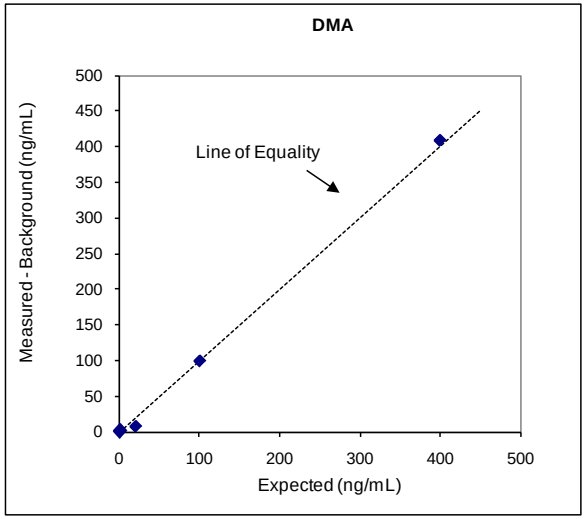


Figure D-2. Performance Evaluation Samples









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RELATIVE BIOAVAILABILITY OF ARSENIC IN BARBER ORCHARD SOILS

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EXECUTIVE SUMMARY

A study using juvenile swine as test animals was performed to measure the gastrointestinal absorption of arsenic from four Barber Orchard soil samples. The soil samples are identified as MS-1, MS-4, MS-5 and MS-8. The soil samples were collected from the Barber Orchard site located near Waynesville, Haywood County, NC. The property was used as a commercial apple orchard from 1903 until the mid-1980s. In 1999, elevated concentrations of arsenic, lead and organic pesticides were found in the soil.

The relative oral bioavailability of arsenic was assessed by comparing the absorption of arsenic from the Barber Orchard soils ("test materials") to that of sodium arsenate. Groups of four swine were given oral doses of sodium arsenate or the test material twice a day for 14 days. Groups of two or three non-treated swine served as a control.

The amount of arsenic absorbed by each animal was evaluated by measuring the amount of arsenic excreted in the urine (collected over 48-hour periods beginning on days 6, 9, and 12). The urinary excretion fraction (UEF) is the ratio of the amount excreted per 48 hours divided by the dose given per 48 hours. UEF was calculated for both test materials and sodium arsenate using simultaneous weighted linear regression. The relative bioavailability (RBA) of arsenic in each test material compared to sodium arsenate was calculated as follows:

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

Estimated RBA values (mean and 90% confidence interval) are shown below:

Time Interval	MS-1 Estimated RBA	MS-4 Estimated RBA	MS-5 Estimated RBA	MS-8 Estimated RBA
Days 6/7	0.38 (0.24 - 0.58)	0.43 (0.39 - 0.48)	0.62 (0.46 - 0.85)	0.53 (0.48 - 0.59)
Days 9/10	0.31 (0.20 - 0.45)	0.40 (0.36 - 0.45)	0.39 (0.29 - 0.53)	0.53 (0.47 - 0.59)
Days 12/13	0.27 (0.18 - 0.37)	0.39 (0.33 - 0.46)	0.44 (0.35 - 0.56)	0.53 (0.45 - 0.62)
All Days	0.31 (0.25 - 0.38)	0.41 (0.38 - 0.44)	0.49 (0.42 - 0.57)	0.53 (0.49 - 0.57)

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ACRONYMS AND ABBREVIATIONS

ABA	Absolute bioavailability
AF_o	Oral absorption fraction
As+3	Trivalent inorganic arsenic
As+5	Pentavalent inorganic arsenic
DMA	Dimethyl arsenic
D	Ingested dose
g	Gram
GLP	Good Laboratory Practices
kg	Kilogram
K_u	Fraction of absorbed arsenic which is excreted in urine
mL	Milliliter
MMA	Monomethyl arsenic
N	Number of data points
NaAs	Sodium arsenate
NIST	National Institute of Standards and Technology
NRCC	National Research Council of Canada
QC	Quality control
RBA	Relative bioavailability
ref	Reference material
RfD	Reference dose
SD	Standard deviation
SF	Slope factor
SRM	Standard reference material
TM	Test material
UEF	Urinary excretion fraction
USEPA	United States Environmental Protection Agency
µg	Microgram
µm	Micrometer
°C	Degrees Celsius

1.0 INTRODUCTION

1.1 Overview of Bioavailability

Reliable analysis of the potential hazard to humans from ingestion of a chemical depends upon accurate information on a number of key parameters, including the concentration of the chemical in environmental media (e.g., soil, dust, water, food, air, paint), intake rates of each medium, and the rate and extent of absorption ("bioavailability") of the chemical by the body from each ingested medium. The amount of a chemical that actually enters the body from an ingested medium depends on the physical-chemical properties of the chemical and of the medium. For example, some metals in soil may exist, at least in part, as poorly water-soluble minerals, and may also exist inside particles of inert matrix such as rock or slag of variable size, shape, and association. These chemical and physical properties may influence (usually decrease) the absorption (bioavailability) of the metals when ingested. Thus, equal ingested doses of different forms of a chemical in different media may not be of equal health concern.

Bioavailability of a chemical in a particular medium may be expressed either in absolute terms (absolute bioavailability) or in relative terms (relative bioavailability):

Absolute bioavailability (ABA) is the ratio of the amount of the chemical absorbed to the amount ingested:

$$ABA = \frac{Absorbed\ Dose}{Ingested\ Dose}$$

This ratio is also referred to as the oral absorption fraction (AF_o).

Relative bioavailability (RBA) is the ratio of the AF_o of the chemical present in some test material (*test*) to the AF_o of the chemical in some appropriate reference material (e.g., either the chemical dissolved in water or a solid form that is expected to fully dissolve in the stomach) (*ref*):

$$RBA(test\ vs\ ref) = \frac{AF_o(test)}{AF_o(ref)}$$

For example, if 100 micrograms (µg) of a chemical (e.g., arsenic) dissolved in drinking water were ingested and a total of 50 µg were absorbed into the body, the AF_o would be 50/100, or 0.50 (50%). Likewise, if 100 µg of a chemical contained in soil were ingested and 30 µg were absorbed into the body, the AF_o for this chemical in soil would be 30/100, or 0.30 (30%). If the chemical dissolved in water were used as the frame of reference for describing the relative amount of the same chemical absorbed from soil, the RBA would be 0.30/0.50, or 0.60 (60%).

For additional discussion about the concept and application of bioavailability, see Gibaldi and Perrier (1982), Goodman et al. (1990), and/or Klaassen et al. (1996).

1.2 Using RBA Data to Improve Risk Calculations

When reliable data are available on the relative bioavailability (RBA) of a chemical in a site medium (e.g., soil), the information can be used to improve the accuracy of exposure and risk calculations at that site. RBA data can be used to adjust default oral toxicity values (reference dose and slope factor) to account for differences in absorption between the chemical ingested in water and the chemical ingested in site media, assuming the toxicity factors are based on a readily soluble form of the chemical. For non-cancer effects, the default reference dose ($RfD_{default}$) can be adjusted ($RfD_{adjusted}$) as follows:

$$RfD_{adjusted} = \frac{RfD_{default}}{RBA}$$

For potential carcinogenic effects, the default slope factor ($SF_{default}$) can be adjusted ($SF_{adjusted}$) as follows:

$$SF_{adjusted} = SF_{default} \cdot RBA$$

Alternatively, it is also acceptable to adjust the dose (rather than the toxicity factors) as follows:

$$Dose_{adjusted} = Dose_{default} \cdot RBA$$

This dose adjustment is mathematically equivalent to adjusting the toxicity factors as described above.

1.3 Purpose of this Study

The objective of this study was to use juvenile swine as a test system in order to determine the RBA of arsenic in Barber Orchard soil samples compared to a soluble form of arsenic (sodium arsenate).

2.0 STUDY DESIGN

Test materials and a reference material (sodium arsenate, NaAs) were administered to groups of juvenile swine at two or three different dose levels for 14 days. Due to space constraints of the animal laboratory, the test materials were evaluated in three separate studies: (1) Study 1 (MS-1 test material); (2) Study 2 (MS-5 test material); and (3) Study 3 (MS-4 and MS-8 test materials).

Each study evaluated one or two test materials and a reference material, and included a non-treated group of two or three animals to serve as a control for determining background arsenic levels. The design for each of the studies is presented in Table 2-1. All doses were administered orally.

The study was performed as nearly as possible within the spirit and guidelines of Good Laboratory Practices (GLP: 40 CFR 792).

2.1 Test Materials

2.1.1 Sample Description

The test materials used in these investigations included Barber Orchard soil samples MS-1, MS-4, MS-5 and MS-8. The samples were collected from the Barber Orchard site located near Waynesville, Haywood County, NC. The property was used as a commercial apple orchard from 1903 until the mid-1980s. In the late 1980s, some of the land was parceled off and sold for residential properties, church properties, and commercial or light industrial property. The majority of the remaining acreage is slated for residential development. In 1999, elevated concentrations of arsenic, lead and organic pesticides were found in the soil.

2.1.2 Sample Preparation and Analysis

USEPA Region 4 collected the soil from the Barber Orchard site. The soil was placed in a large stainless steel mixing bowl and then homogenized. Homogenized soil was then shipped to USEPA's Office of Research and Development, National Exposure Research Laboratory (NERL) for processing. Soil was spread out in drying trays, placed in an air-drying oven and dried for about 4 days at <40 ºC. The soil was then added to a vibrating 2 mm stainless steel sieve screen to remove plant material, rocks and large chunks of aggregated soil. Material remaining on the screen was deaggregated and rescreened.

Bulk soil samples (unsieved) were measured at USEPA's laboratory in Athens, GA by inductively coupled plasma mass spectrometry (ICP-MS) following method EPA 200.8. Subsamples were then sieved to <250 µm by the Florida Department of Environmental Protection (FDEP) laboratory in Tallahassee, FL, prepared following method EPA 3050B, and analyzed following method EPA 6020. Total arsenic concentration in the unsieved and <250-µm sieved test materials are:

Sample Type	MS-1	MS-4	MS-5	MS-8
Bulk soil[1]	280 ppm	300 ppm	370 ppm	310 ppm
Sieved soil[2]	290 ppm	388 ppm	382 ppm	364 ppm

[1] Measured before sieving by EPA lab in Athens, GA. EPA method 200.8 was used for sample analysis.
[2] Measured on sieved (<250 μm) fractions by the FDEP lab. EPA method 3050B was used for sample preparation, and EPA Method 6020 (similar to EPA method 200.8) was used for sample analysis.

The sieved soil concentrations were used to calculate doses in these swine RBA studies.

X-ray absorption spectroscopy was conducted on the test materials to characterize the arsenic mineralogy (Miller and Scheckel, 2012).

2.2 Experimental Animals

Juvenile swine were selected for use because they are considered to be a good physiological model for gastrointestinal absorption in children (Weis and LaVelle, 1991; Casteel et al., 1996). The animals were intact males of the Pig Improvement Corporation genetically defined Line 26, and were purchased from Chinn Farms, Clarence, Missouri.

The number of animals purchased for each study was several more than required by the protocol. These animals were purchased at an age of about 5-6 weeks (weaning occurs at age 3 weeks) and housed in individual stainless steel cages. The animals were then held under quarantine for one week to observe their health before beginning exposure to dosing materials. Each animal was examined by a certified veterinary clinician (swine specialist) and any animals that appeared to be in poor health during this quarantine period were excluded from the study. To minimize weight variations among animals and groups, extra animals most different in body weight (either heavier or lighter) five days prior to exposure (day -5) were also excluded from the study. The remaining animals were assigned to dose groups at random (group assignments are presented in Appendix A).

When exposure began (day zero), the animals were about 6-7 weeks old. The animals were weighed at the beginning of the study and every three days during the course of the study. In each study, the rate of weight gain was comparable in all dosing groups. Body weight data are presented in Appendix B.

All animals were examined daily by an attending veterinarian while on study and were subjected to detailed examination at necropsy by a certified veterinary pathologist in order to assess overall animal health.

2.3 Diet

Animals were weaned onto standard pig chow (made at the University of Missouri Animal Science Feed Mill). The feed was nutritionally complete. The ingredients of the feed are presented in Table 2-2. Arsenic concentration in randomly selected feed samples measured <0.3 μg/g (Table 2-3).

Prior to the start of dosing and throughout the dosing period, each day every animal was given an amount of feed equal to 4.0% of the mean body weight of all animals on study. Feed amounts were adjusted every three days, when animals were weighed. Feed was administered in two equal portions, at 11:00 AM and 5:00 PM daily.

Drinking water was provided *ad libitum* via self-activated watering nozzles within each cage. Arsenic concentration of water samples from randomly selected drinking water nozzles were <1 µg/L (Table 2-3).

2.4 Dosing

Animals were exposed to dosing materials (sodium arsenate or sieved test material) for 14 days, with the dose for each day being administered in two equal portions beginning at 9:00 AM and 3:00 PM (two hours before feeding). Pigs were dosed two hours before feeding to ensure that they were in a semi-fasted state because the presence of food in the stomach is known to reduce arsenic absorption. To facilitate dose administration, dosing materials were placed in a small depression in a ball of dough consisting of moistened feed (typically about 5g) and the dough was pinched shut. This was then placed in the feeder at dosing time.

Target arsenic doses (expressed as µg of arsenic per kg of body weight per day) for animals in each group were determined in the study design (Table 2-1). The daily mass of arsenic administered (either as sodium arsenate or as sieved test material) to animals in each group was calculated by multiplying the target dose (µg/kg-day) for that group by the anticipated average weight of the animals (kg) over the course of the study:

$$Mass\ (\mu g / day) = Dose\ (\mu g / kg - day) \cdot Average\ Body\ Weight\ (kg)$$

The average body weight expected during the course of the study was estimated by measuring the average body weight of all animals one day before the study began, and then assuming an average weight gain of 0.5 kg/day during the study. After completion of the study, the true mean body weight was calculated using the actual body weights (measured every three days during the study), and the resulting true mean body weight was used to calculate the actual doses achieved. These calculations included adjustments for any partial or missed doses (see Appendix C). Actual doses for each group are shown in Table 2-1.

2.5 Collection and Preservation of Urine Samples

Samples of urine were collected from each animal for 48-hour periods on days 6 to 7 (U-1), 9 to 10 (U-2), and 12 to 13 (U-3) of the study. Collection began at 9:00 AM and ended 48 hours later. The urine was collected in a plastic bucket placed beneath each cage, which was emptied into a plastic storage bottle. Aluminum screens were placed under the cages to minimize contamination with feces, spilled food, or other debris. Due to the length of the collection period, collection containers were emptied periodically (typically twice daily) into a separate plastic bottles to ensure that there was no loss of sample due to overflow.

At the end of each collection period, the total urine volume for each animal was measured (Appendix D) and three 60-mL portions were removed and acidified with 0.6 mL concentrated

nitric acid. All samples were refrigerated. Two of the aliquots were archived and one aliquot was sent for arsenic analysis (refrigeration was maintained until arsenic analysis).

2.6 Arsenic Analysis

Urine samples were assigned random chain-of-custody tag numbers and submitted to the analytical laboratory for analysis in a blind fashion. The samples were analyzed for arsenic by L. E. T., Inc., (Columbia, Missouri). In brief, 25-mL samples of urine were digested by refluxing and then heating to dryness in the presence of magnesium nitrate and concentrated nitric acid. Following magnesium nitrate digestion, samples were transferred to a muffle furnace and ashed at 500°C. The digested and ashed residue was dissolved in hydrochloric acid and analyzed by the hydride generation technique using a PerkinElmer 3100 atomic absorption spectrometer. Preliminary tests of this method established that each of the different forms of arsenic that may occur in urine, including trivalent inorganic arsenic (As+3), pentavalent inorganic arsenic (As+5), monomethyl arsenic (MMA), and dimethyl arsenic (DMA) are all recovered with high efficiency.

Analytical results for the urine samples are presented in Appendix E.

2.7 Quality Control

A number of quality control (QC) steps were taken during this project to evaluate the accuracy of the analytical procedures. The results for QC samples are presented in Appendix F, and are summarized below.

Blind Duplicates (Sample Preparation Replicates)

A random selection of about 20% of all urine samples generated during each study were prepared for laboratory analysis in duplicate (i.e., two separate subsamples of urine were digested) and submitted to the laboratory in a blind fashion. There was generally good agreement between results for the duplicate pairs (Figure F-1 and Table F-1).

Spike Recovery

During arsenic analysis for each study, every tenth sample was spiked with known amounts of arsenic (sodium arsenate) and the recovery of the added arsenic was measured. Mean arsenic concentrations recovered from spiked samples were within 10% of actual arsenic concentrations (Table F-2).

Laboratory Duplicates

During arsenic analysis, every tenth sample was analyzed in duplicate (Table F-3). For urine samples, duplicate results agreed within ±1 times the detection limit or less than 10% relative percent difference (RPD). Most duplicate water samples were below the detection limit. Duplicate analysis for feed samples showed deviations between 35% (Study 1 and Study 2) and 67% (Study 3).

Laboratory Control Standards

Laboratory control standards (samples of reference materials for which a certified concentration of specific analytes has been established) were tested periodically during sample analysis. Recovery of arsenic from these standards was generally good and within the acceptable range (Table F-4).

Blanks

Blank samples run along with each batch of samples ($N \geq 6$) never yielded a measurable level of arsenic (Table F-5). The detection limit was 1 µg/L.

Summary of QC Results

Based on the results of all of the QC samples and steps described above, it is concluded that the analytical results are of sufficient quality for derivation of reliable estimates of arsenic absorption from the test materials.

3.0 DATA ANALYSIS

3.1 Overview

Figure 3-1 shows a conceptual model for the toxicokinetic fate of ingested arsenic. Key points of this model are as follows:

- In most animals (including humans), absorbed arsenic is excreted mainly in the urine over the course of several days. Thus, the UEF, defined as the amount excreted in the urine divided by the amount given, is usually a reasonable approximation of the AF_o or ABA. However, this ratio will underestimate total absorption, because some absorbed arsenic is excreted in the feces via the bile, and some absorbed arsenic enters tissue compartments (e.g., skin, hair) from which it is cleared very slowly or not at all. Thus, the urinary excretion fraction should not be equated with the absolute absorption fraction.

- The RBA of two orally administered materials (i.e., a test material and reference material) can be calculated from the ratio of the urinary excretion fraction of the two materials. This calculation is independent of the extent of tissue binding and of biliary excretion:

$$RBA(test\ vs\ ref) = \frac{AF_o(test)}{AF_o(ref)} = \frac{D \cdot AF_o(test) \cdot K_u}{D \cdot AF_o(ref) \cdot K_u} = \frac{UEF(test)}{UEF(ref)}$$

  where:

  D = Ingested dose (μg)

  K_u = Fraction of absorbed arsenic that is excreted in the urine

Based on the conceptual model above, the basic method used to estimate the RBA of arsenic in a particular test material compared to arsenic in a reference material (sodium arsenate) is as follows:

1. Plot the amount of arsenic excreted in the urine (μg per 48 hours) as a function of the administered amount of arsenic (μg per 48 hours), both for reference material and for test material.

2. Find the best fit linear regression line through the each data set. The slope of each line (μg per 48 hours excreted per μg per 48 hours ingested) is the best estimate of the urinary excretion fraction (UEF) for each material.

3. Calculate RBA for each test material as the ratio of the UEF for test material compared to UEF for reference material:

$$RBA(test\ vs\ ref) = \frac{UEF(test)}{UEF(ref)}$$

A detailed description of the curve-fitting methods and rationale and the methods used to quantify uncertainty in the arsenic RBA estimates for a test material are summarized below. All model fitting was performed in Microsoft Excel® using matrix functions.

3.2 Dose-Response Model

Simultaneous Regression

The techniques used to derive linear regression fits to the dose-response data are based on the methods recommended by Finney (1978). As noted by Finney (1978), when the data to be analyzed consist of two dose-response curves (the reference material and the test material), it is obvious that both curves must have the same intercept, since there is no difference between the curves when the dose is zero. This requirement is achieved by combining the two dose response equations into one and solving for the parameters simultaneously, as follows:

Separate Models:

$$\mu_r(i) = a + b_r \cdot x_r(i)$$

$$\mu_t(i) = a + b_t \cdot x_t(i)$$

Combined Model

$$\mu(i) = a + b_r \cdot x_r(i) + b_t \cdot x_t(i)$$

where $\mu(i)$ indicates the expected mean response of animals exposed at dose $x(i)$, and the subscripts r and t refer to reference and test material, respectively. The coefficients of this combined model are derived using multivariate regression, with the understanding that the combined data set is restricted to cases in which one (or both) of x_r and x_t are zero (Finney, 1978).

When a study consists of a reference group and two test materials (e.g., see Study 3), the same approach is used, except that all three curves are fit simultaneously:

$$\mu(i) = a + b_r \cdot x_r(i) + b_{t1} \cdot x_{t1}(i) + b_{t2} \cdot x_{t2}(i)$$

Weighted Regression

Regression analysis based on ordinary least squares assumes that the variance of the responses is independent of the dose and/or the response (Draper and Smith, 1998). It has previously been shown that this assumption is generally not satisfied in swine-based RBA studies, where there is a tendency toward increasing variance in response as a function of increasing dose (heteroscedasticity) (USEPA, 2005). One method for dealing with heteroscedasticity is through

the use of weighted least squares regression (Draper and Smith, 1998). In this approach, each observation in a group of animals is assigned a weight that is inversely proportional to the variance of the response in that group:

$$w_i = \frac{1}{\sigma_i^2}$$

where:

w_i = weight assigned to all data points in dose group *i*

σ_i^2 = variance of responses in animals in dose group *i*

When the distributions of responses at each dose level are normal, weighted regression is equivalent to the maximum likelihood method.

There are several alternative strategies for assigning weights. The method used in this study estimates the value of σ_i^2 using an "external" variance model based on an analysis of the relationship between variance and mean response using data consolidated across many different swine-based arsenic RBA studies. The data used to derive the variance model are shown in Figure 3-2. As seen, log-variance increases as an approximately linear function of log-mean response:

$$\ln(s_i^2) = k1 + k2 \cdot \ln(\bar{y}_i)$$

where:

s_i^2 = observed variance of responses of animals in dose group *i*
$\bar{y}_i$ = mean observed response of animals in dose group *i*

Based on these data, values of *k*1 and *k*2 were derived using ordinary least squares minimization. The resulting values were -1.10 for *k*1 and 1.64 for *k*2.

Goodness of Fit

The goodness-of-fit of each dose-response model was assessed using the F test statistic and the adjusted coefficient of multiple determination (Adj R^2) as described by Draper and Smith (1998). A fit is considered acceptable if the p-value is less than 0.05.

Assessment of Outliers

In biological assays, it is not uncommon to note the occurrence of individual measured responses that appear atypical compared to the responses from other animals in the same dose group. In this study, responses that yielded standardized weighted residuals greater than 3.5 or less than -3.5 were considered to be potential outliers (Canavos, 1984). When such data points were encountered in a data set, the RBA values were calculated both with and without the potential outlier(s) excluded, and the result with the outlier(s) excluded was used as the preferred estimate.

3.3 Calculation of RBA Estimates

The arsenic RBA values were calculated as the ratio of the slope term for the test material data set (b_t) and the reference material data set (b_r):

$$RBA = \frac{b_t}{b_r}$$

The uncertainly range about the RBA ratio was calculated using Fieller's Theorem as described by Finney (1978).

4.0 RESULTS

4.1 Clinical Signs

The doses of arsenic administered in this study are below a level that is expected to cause toxicological responses in swine. No clinical signs of arsenic-induced toxicity were noted in any of the animals used in the studies.

4.2 Background Arsenic Excretion

Measured values for urinary arsenic excretion (mean and standard deviation) for control animals from days 6 to 13 are shown below:

Study	Arsenic mass in urine (μg/48 hours)	Number of samples
Study 1 (MS-1)	66.0 ± 14.5	8
Study 2 (MS-5)	73.3 ± 35.8	6
Study 3 (MS-4 & MS-8)	85.0 ± 35.5	9

These values are representative of endogenous background levels in food and water and support the view that the animals were not exposed to any significant exogenous sources of arsenic throughout the study.

4.3 Urinary Arsenic Variance

As discussed in Section 3.2, the urinary arsenic dose-response data are analyzed using weighted least squares regression and the weights are assigned using an "external" variance model. To ensure that the variance model was valid, the variance values from each of dose groups in each of the Barber Orchard studies were superimposed on the historic data set (Figures 4-1 through 4-3). As seen, the variances of the urinary arsenic data from these studies are consistent with the data used to generate the variance model.

4.4 Dose-Response Modeling

Outlier Identification

For each study, the dose-response data for arsenic in urine were initially modeled using all data, and outliers were identified as discussed above. These results are shown in Appendix G.

STUDY 1

Initial modeling indicated that data collected from days 9/10 and 12/13 from pig 374 were outliers (standardized weighted residuals > 3.5) (see Appendix G, Figures G-1 through G-4). The residual calculated from urine data collected from the same pig on days 6/7 was 3.2, just below the criterion for outlier identification. Further review of the data for this pig showed that the urine output on all days was at least 10-times that of the other pigs. Based on the high urine output and the poor agreement of the data from this animal with other animals in the same dose group, data for pig 374 were excluded from the final evaluation for arsenic RBA. Figures 4-4 (days 6/7), 4-5 (days 9/10), 4-6 (days 12/13) and 4-7 (all days combined) show the revised fittings with the outlier excluded from the analysis.

STUDY 2

Initial modeling indicated that data collected on days 9/10 from pig 464 were outliers based on the standardized weighted residuals greater than 3.5 (see Appendix G, Figures G-5 through G-9). Based on this analysis, data for pig 464 on days 9/10 were excluded from the final evaluation for arsenic RBA. Final regression fittings are shown in Figures 4-8 through 4-11.

STUDY 3

Initial modeling using all the data did not indicate the presence of any outliers. Therefore, all data were included in the final analysis. Final regression fittings are shown in Figures 4-12 through 4-15.

Best Fit Results After Outlier Exclusion

After exclusion of outliers, all of the dose-response curves were approximately linear, with the slope of the best-fit straight line being equal to the best estimate of the UEF. Table 4-1 summarizes the resulting slopes (UEF estimates) for the final fittings of each test material and corresponding reference material.

4.5 Calculated RBA Values

Estimated RBA values (mean and 90% confidence interval) are shown below:

Time Interval	MS-1 Estimated RBA	MS-4 Estimated RBA	MS-5 Estimated RBA	MS-8 Estimated RBA
Days 6/7	0.38 (0.24 - 0.58)	0.43 (0.39 - 0.48)	0.62 (0.46 - 0.85)	0.53 (0.48 - 0.59)
Days 9/10	0.31 (0.20 - 0.45)	0.40 (0.36 - 0.45)	0.39 (0.29 - 0.53)	0.53 (0.47 - 0.59)
Days 12/13	0.27 (0.18 - 0.37)	0.39 (0.33 - 0.46)	0.44 (0.35 - 0.56)	0.53 (0.45 - 0.62)
All Days	0.31 (0.25 - 0.38)	0.41 (0.38 - 0.44)	0.49 (0.42 - 0.57)	0.53 (0.49 - 0.57)

4.6 Uncertainty

The bioavailability estimates above are subject to uncertainty that arises from several different sources. One source of uncertainty is the inherent biological variability between different animals in a dose group, which in turn causes variability in the amount of arsenic absorbed by the exposed animals. The between-animal variability results in statistical uncertainty in the best-fit dose-response curves and, hence, uncertainty in the calculated values of RBA. Such statistical uncertainty is accounted for by the statistical models used above and is characterized by the uncertainty range around the RBA estimates.

However, there is also uncertainty in the extrapolation of RBA values measured in juvenile swine to young children or adults, and this uncertainty is not included in the statistical confidence bounds above. Even though the immature swine is believed to be a useful and meaningful animal model for gastrointestinal absorption in humans, it is possible that there are differences in physiological parameters that may influence RBA; therefore, RBA values in swine may not be identical to values in children. In addition, RBA may depend on the amount and type of food in the stomach, since the presence of food can influence stomach pH, holding time, and possibly other factors that may influence solubilization of arsenic. In this regard, it is important to recall that RBA values measured in this study are based on animals that have little or no food in their stomach at the time of exposure and, hence, are likely to yield high-end values of RBA. Thus, these RBA values may be somewhat conservative for humans who ingest the site soils along with food. The magnitude of this bias is not known.

5.0 REFERENCES

Canavos, C. G. 1984. Applied Probability and Statistical Methods. Little, Brown and Co., Boston.

Casteel, S. W., R. P. Cowart, C. P. Weis, G. M. Henningsen, E. Hoffman, W. J. Brattin, M. F. Starost, J. T. Payne, S. L. Stockham, S. V. Becker, and J. R. Turk. 1996. A swine model for determining the bioavailability of lead from contaminated media. In: Advances in Swine in Biomedical Research. Tumbleson and Schook, eds. Vol 2, Plenum Press, New York. Pp. 637-46.

Draper, N. R., and H. Smith. 1998. Applied Regression Analysis (3rd Edition). John Wiley & Sons, New York.

Finney, D. J. 1978. Statistical Method in Biological Assay (3rd Edition). Charles Griffin and Co., London.

Gibaldi, M., and Perrier, D. 1982. Pharmacokinetics (2nd edition), pp 294-297. Marcel Dekker, Inc, NY, NY.

Goodman, A.G., Rall, T.W., Nies, A.S., and Taylor, P. 1990. The Pharmacological Basis of Therapeutics (8th ed.), pp. 5-21. Pergamon Press, Inc. Elmsford, NY.

Klaassen, C.D., Amdur, M.O., and Doull, J. (eds). 1996. Cassarett and Doull's Toxicology: The Basic Science of Poisons, pp. 190. McGraw-Hill, Inc. NY, NY.

Miller, B.W. and Scheckel, K.G. 2012. Technical Review Workgroup for Metals and Asbestos: Bioavailability Committee. Mineralogical Report. XAS Data and Linear Combination Fitting Results. Available at: http://epa.gov/superfund/bioavailability/guidance.htm.

NIST. 2003. Certificate of Analysis, Standard Reference Material® 2710 – Montana Soil, Highly Elevated Trace Element Concentrations. National Institute of Standards & Technology, Gaithersburg, MD. Certificate Issue Date: July 18, 2003.

NRC. 1988. Nutrient requirements of swine. A report of the Committee on Animal Nutrition. National Research Council. National Academy Press, Washington, DC.

USEPA. 2007. Estimation of Relative Bioavailability of Lead in Soil and Soil-Like Materials by *In Vivo* and *In Vitro* Methods OSWER9285.7-77. Office of Solid Waste and Emergency Response, Washington DC, USA.

Weis, C.P., and LaVelle, J.M. 1991. Characteristics to consider when choosing an animal model for the study of lead bioavailability. In: The proceedings of the international symposium on the bioavailability and dietary uptake of lead. Science and Technology Letters 3:113-119.

TABLES AND FIGURES

TABLE 2-1 STUDY DESIGNS AND DOSING INFORMATION

STUDY 1

Group	Group Name Abbreviation	Dose Material Administered	As Conc of the material (ug/g or ug/uL)	No. Pigs in Group	Arsenic Dose (µg/kg BW-day)	
					Target	Actual [a]
1	Control	(none)	0	3	0	0.0
2	NaAs	Sodium Arsenate	2	4	25	32.0
3	NaAs	Sodium Arsenate	10	4	50	55.7
4	NaAs	Sodium Arsenate	10	4	100	125.2
5	TM1	Barber Orchard Soil MS-1	290	4	60	72.9
6	TM1	Barber Orchard Soil MS-1	290	4	120	145.7

STUDY 2

Group	Group Name Abbreviation	Dose Material Administered	As Conc of the material (ug/g or ug/uL)	No. Pigs in Group	Arsenic Dose (µg/kg BW-day)	
					Target	Actual [a]
1	NaAs	Sodium Arsenate	2	4	25	29.7
2	NaAs	Sodium Arsenate	10	4	50	57.3
3	TM1	Barber orchard Soil MS-5	382	4	40	46.0
4	TM1	Barber orchard Soil MS-5	382	4	60	71.0
5	TM1	Barber orchard Soil MS-5	382	4	120	138.9
6	Control	(none)	0	2	0	0.0

TABLE 2-1 STUDY DESIGNS AND DOSING INFORMATION

STUDY 3

Group	Group Name Abbreviation	Dose Material Administered	As Conc of the material (ug/g or ug/uL)	No. Pigs in Group	Arsenic Dose (µg/kg BW-day)	
					Target	Actual [a]
1	NaAs	Sodium Arsenate	10.00	4	25	25.4
2	NaAs	Sodium Arsenate	10.00	4	50	53.6
3	NaAs	Sodium Arsenate	10.00	4	100	104.6
4	TM1	Barber Orchard Soil MS-4	300	4	40	52.6
5	TM1	Barber Orchard Soil MS-4	300	4	60	77.3
6	TM1	Barber Orchard Soil MS-4	300	4	120	144.4
7	TM2	Barber Orchard Soil MS-8	310	4	40	44.6
8	TM2	Barber Orchard Soil MS-8	310	4	60	72.0
9	TM2	Barber Orchard Soil MS-8	310	4	120	155.0
10	Control	(none)	0	3	0	0.0

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

Doses were administered in two equal portions given at 9:00 AM and 3:00 PM each day. Doses were held constant based on the expected mean weight during the exposure interval (14 days).

TABLE 2-2 TYPICAL FEED COMPOSITION

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

INGREDIENTS

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

NUTRITIONAL PROFILE[2]

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

FOOTNOTES

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

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

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

TABLE 2-3 ARSENIC CONCENTRATIONS IN FEED AND WATER SAMPLES

STUDY 1

Sample ID	Sample Type	Arsenic Concentration	Units
EP3-2-407	Feed	0.22	µg/g
EP3-2-408	Feed	<0.05	µg/g
EP3-2-409	Feed	<0.05	µg/g
EP3-2-410	Water	1	µg/L
EP3-2-411	Water	0.5	µg/L
EP3-2-412	Water	<0.6	µg/L
EP3-2-413	Water	1	µg/L
EP3-2-414	Water	<0.5	µg/L

STUDY 2

Sample ID	Sample Type	Arsenic Concentration	Units
MS-5-223	Feed	0.07	µg/g
MS-5-228	Water	<1	µg/L
MS-5-227	Water	<1	µg/L
MS-5-226	Water	<1	µg/L
MS-5-225	Water	<1	µg/L
MS-5-224	Water	<1	µg/L

STUDY 3

Sample ID	Sample Type	Arsenic Concentration	Units
BOrch-MS4&8-311	Feed	0.1	µg/g
BOrch-MS4&8-312	Water	<1	µg/L
BOrch-MS4&8-313	Water	<1	µg/L
BOrch-MS4&8-314	Water	<1	µg/L
BOrch-MS4&8-315	Water	<1	µg/L
BOrch-MS4&8-316	Water	<1	µg/L
BOrch-MS4&8-317	Water	<1	µg/L

TABLE 4-1 UEF ESTIMATES FOR EACH TEST MATERIAL AND CORRESPONDING REFERENCE MATERIAL

STUDY 1: MS-1 Data

Time Interval	Outliers Excluded	Slopes (UEF Estimates)	
		b_r	b_{t1}
Days 6/7	1	0.85	0.24
Days 9/10	1	0.79	0.25
Days 12/13	1	0.83	0.26
All Days	3	0.83	0.25

STUDY 2: MS-5 Data

Time Interval	Outliers Excluded	Slopes (UEF Estimates)	
		b_r	b_{t1}
Days 6/7	0	0.62	0.38
Days 9/10	1	0.80	0.31
Days 12/13	0	0.79	0.35
All Days	1	0.73	0.35

STUDY 3: MS-4 and MS-8 Data

Time Interval	Outliers Excluded	Slopes (UEF Estimates)		
		b_r	b_{t1}	b_{t2}
Days 6/7	0	0.67	0.29	0.36
Days 9/10	0	0.72	0.29	0.38
Days 12/13	0	0.70	0.28	0.37
All Days	0	0.70	0.28	0.37

Notes:

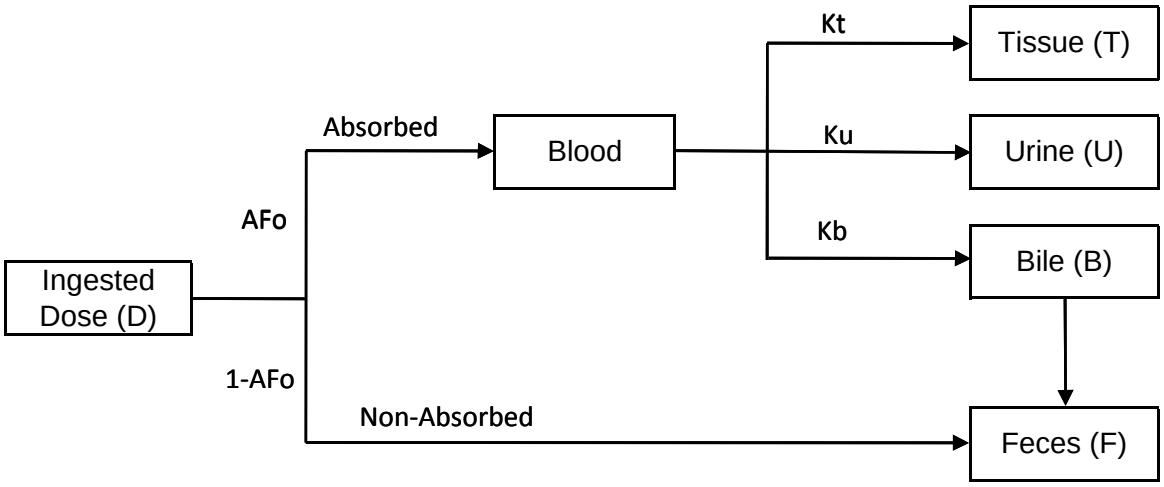
Slopes represent final fittings (outliers excluded).

b_r = slope term for the reference material data set

b_{t1} = slope term for the test material 1 data set

b_{t2} = slope term for the test material 2 data set

FIGURE 3-1. CONCEPTUAL MODEL FOR ARSENIC TOXICOKINETICS



where:

D = Ingested dose
AFo = Oral absorption fraction
Kt = Fraction of absorbed arsenic that is retained in tissues
Ku = Fraction of absorbed arsenic that is excreted in urine
Kb = Fraction of absorbed arsenic that is excreted in bile

Basic Equations

Amount absorbed	= D x AFo
Amount excreted in urine	= Amount absorbed x Ku = D x AFo x Ku
Urinary excretion fraction (UEF)	= Amount excreted / Amount ingested = D x AFo x Ku / D = AFo x Ku
Relative bioavailability (RBA)	= AFo(test) / AFo(reference) = AFo(test) x Ku / (AFo(reference) x Ku) = UEF(test) / UEF(reference)

FIGURE 3-2 URINARY ARSENIC VARIANCE MODEL

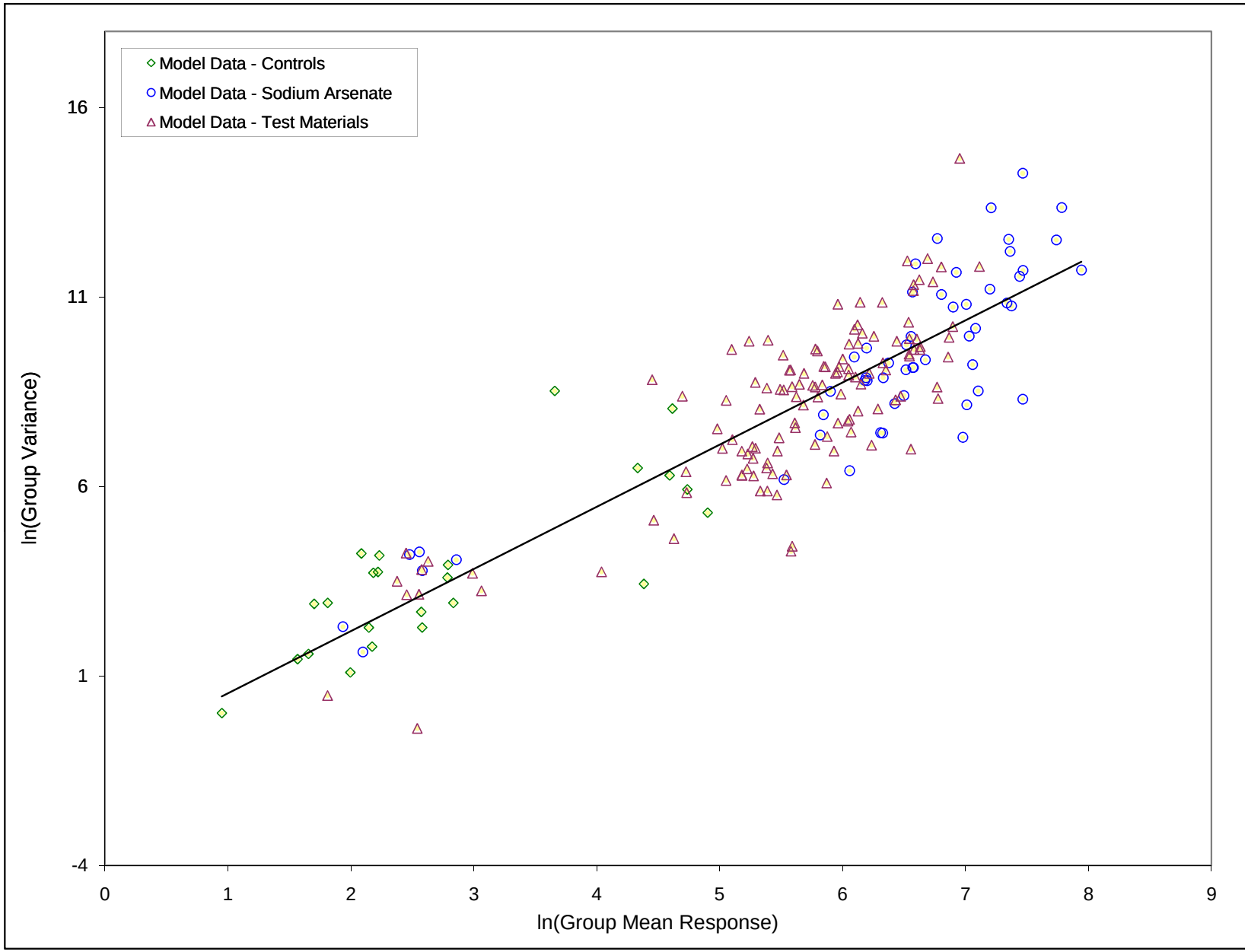
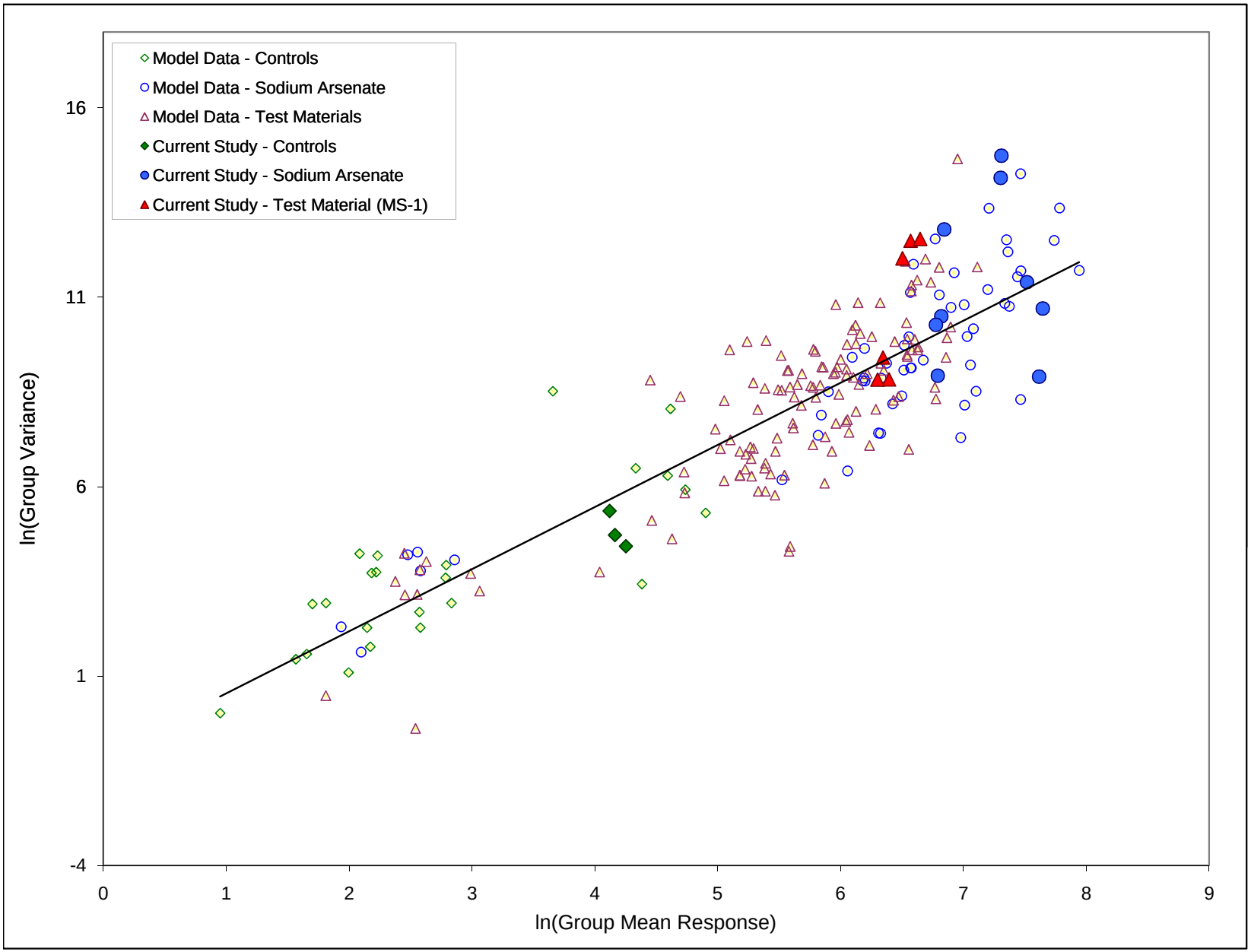


Figure 3-2 Barber Orchard.xls (Fig 4-1B)

FIGURE 4-1 STUDY 1 DATA COMPARED TO URINARY ARSENIC VARIANCE MODEL



\\DENVER2\Public\Swine Bioavailability\EPA3-2-ORCH Barber Orchard\Barber Orchard Report\Originals\Figure 4-1 MS-1 Barb Orch.xlsFig 4-1

FIGURE 4-2 STUDY 2 DATA COMPARED TO URINARY ARSENIC VARIANCE MODEL

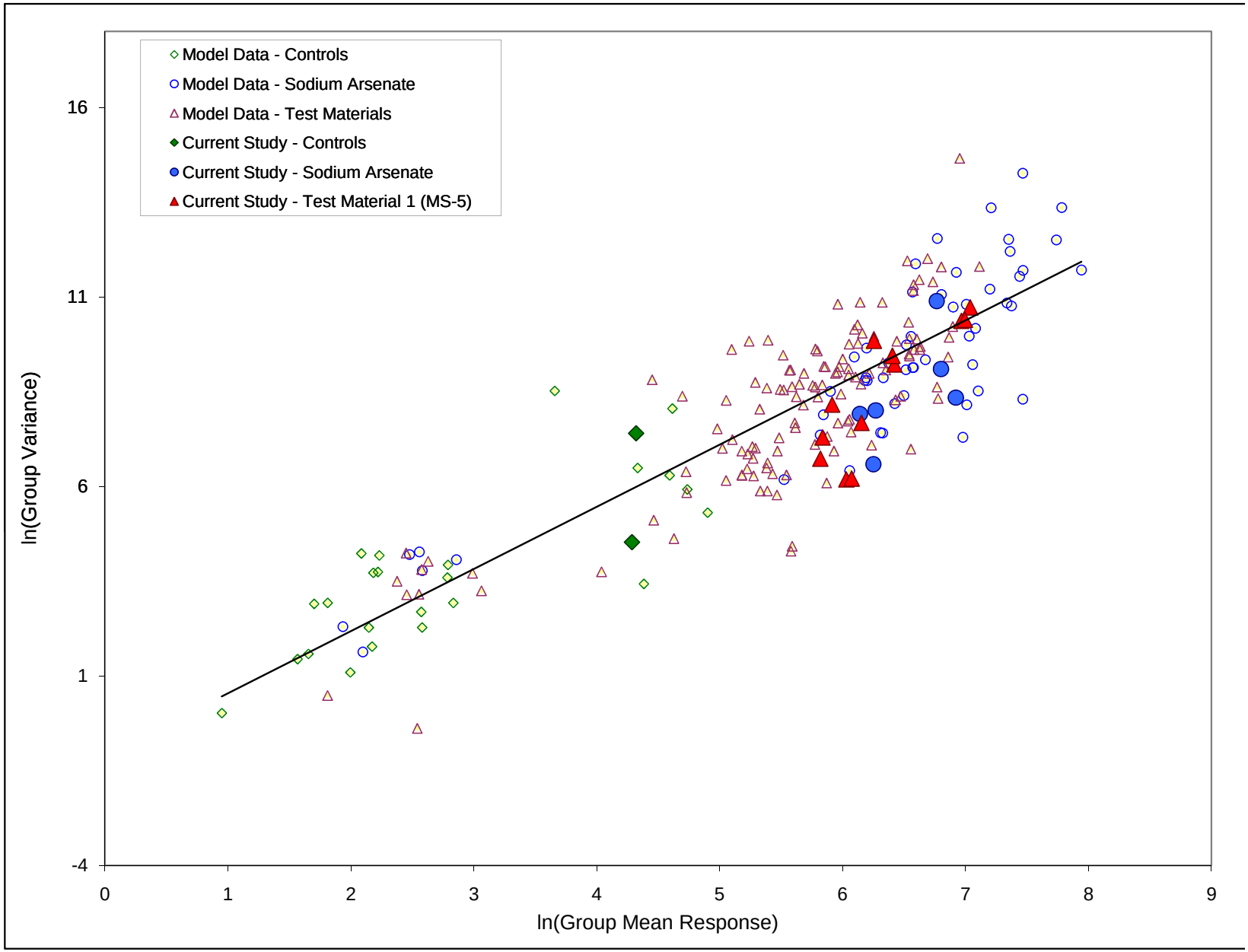


Figure 4-2 MS-5 Barb Orch.xls (Fig 4-1B)

FIGURE 4-3 STUDY 3 DATA COMPARED TO URINARY ARSENIC VARIANCE MODEL

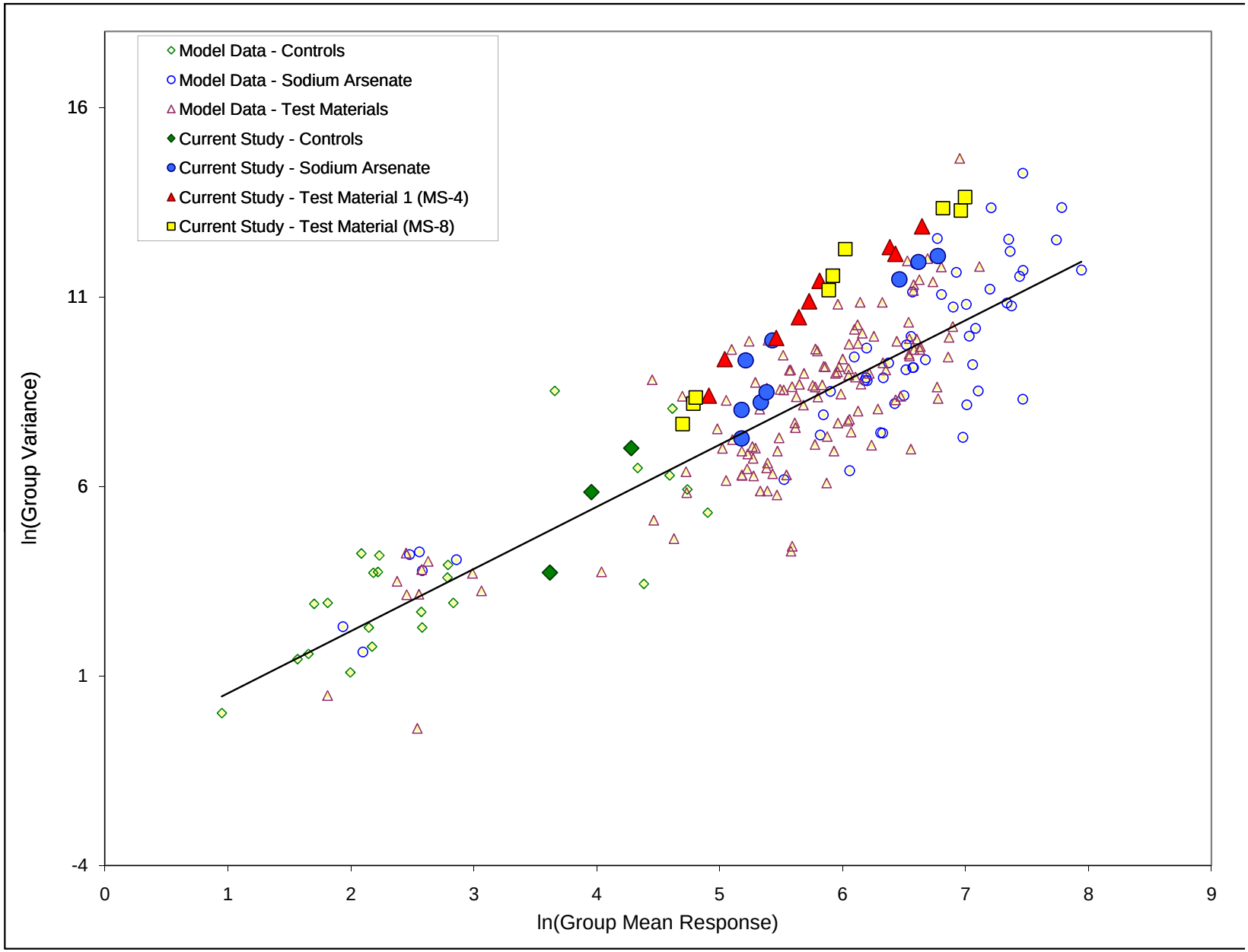


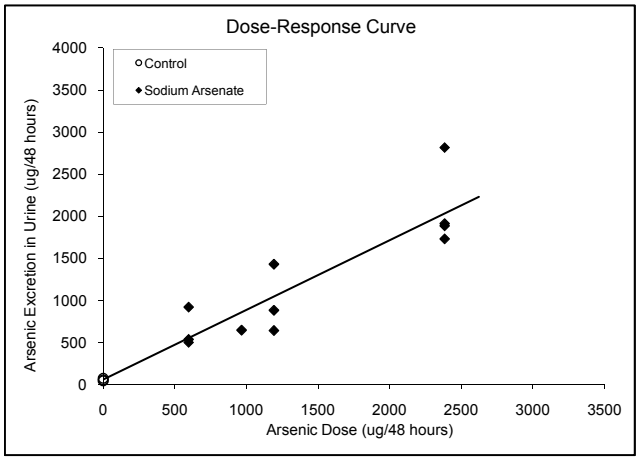
Figure 4-3 MS4+MS8 Barb Orch.xls (Fig 4-1B)

APPENDIX A

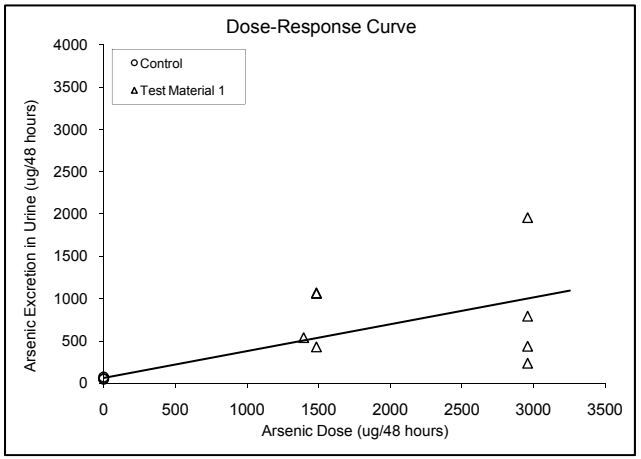
GROUP ASSIGNMENTS

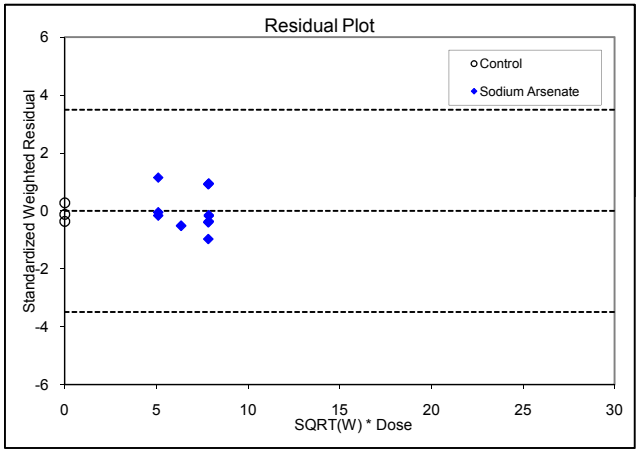
FIGURE 4-4 STUDY 1 URINARY EXCRETION OF ARSENIC: Days 6/7 (Outlier Excluded)

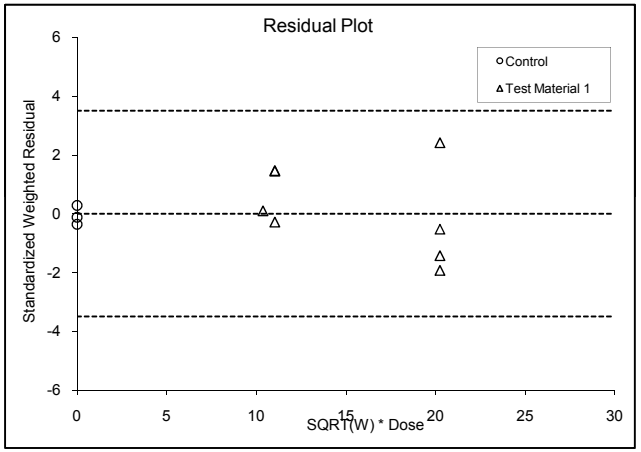
Reference Material (Sodium Arsenate)



Test Material 1 (MS-1)







Summary of Fitting[b]

Parameter	Estimate	Standard Error
a	61.2	26.5
b_r	0.83	0.12
b_{t1}	0.32	0.06
Covariance (b_r,b_{t1})	0.0292	--
Degrees of Freedom	20	--

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

where r = Reference Material, t1 = Test Material 1

ANOVA

Source	MSE
Fit	277.66
Error	8.17
Total	33.84

Statistic	Estimate
F	33.966
p	< 0.001
Adjusted R^2	0.7584

RBA and Uncertainty

	Test Material 1
RBA	0.38
Lower bound[c]	0.24
Upper bound[c]	0.58
Standard Error[c]	0.094**

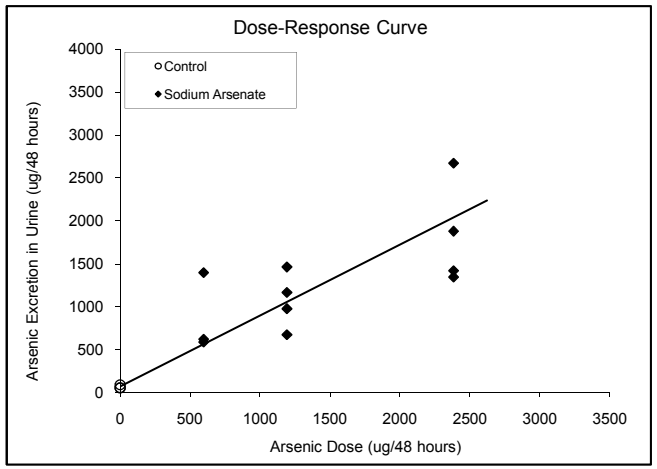
[c] 90% confidence interval calculated using Fieller's theorem

** g ≥ 0.05 (Feiller's SE is uncertain)

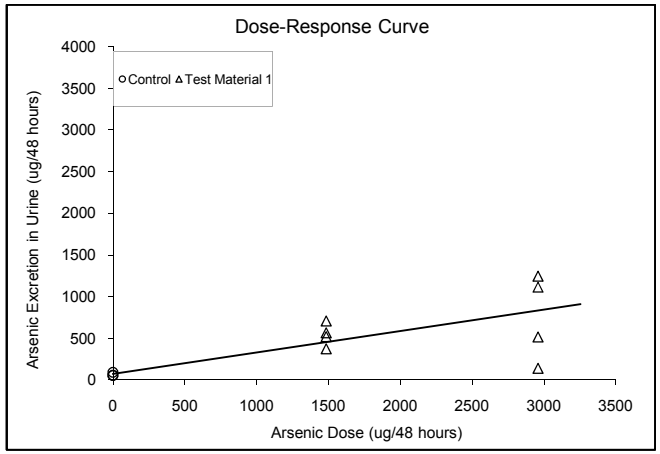
2_MS-1 As RBA Calcs all data 8-19-09 sieved no outlier v2.xls (Graph_1)

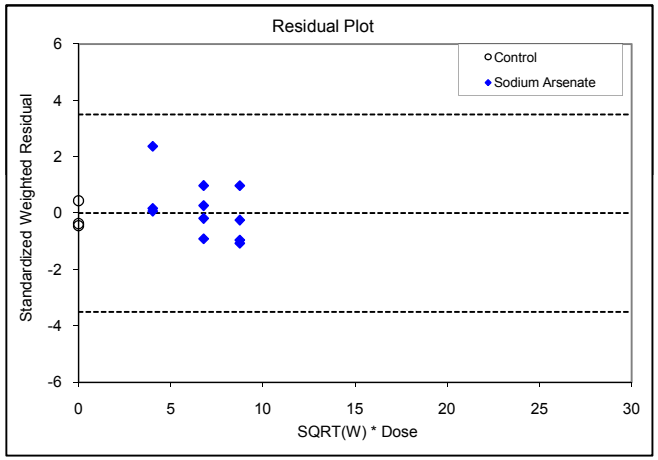
FIGURE 4-5 STUDY 1 URINARY EXCRETION OF ARSENIC: Days 9/10 (Outlier Excluded)

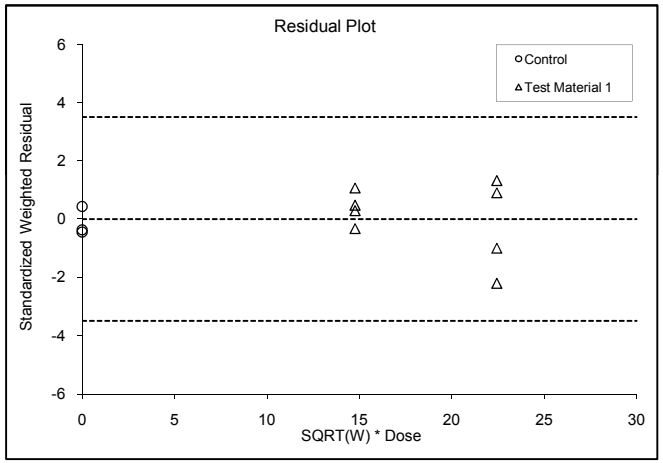
Reference Material (Sodium Arsenate)



Test Material 1 (MS-1)







Summary of Fitting[a]

Parameter	Estimate	SE
a	71.0	25.6
b_r	0.83	0.11
b_{t1}	0.26	0.05
Covariance (b_r,b_{t1})	0.0371	--
Degrees of Freedom	20	--

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

where r = Reference Material, t1 = Test Material 1

ANOVA

Source	MSE
Fit	260.44
Error	6.29
Total	30.49

Statistic	Estimate
F	41.420
p	< 0.001
Adjusted R^2	0.7938

RBA and Uncertainty

	Test Material 1
RBA	0.31
Lower bound[c]	0.20
Upper bound[c]	0.45
Standard Error[c]	0.070**

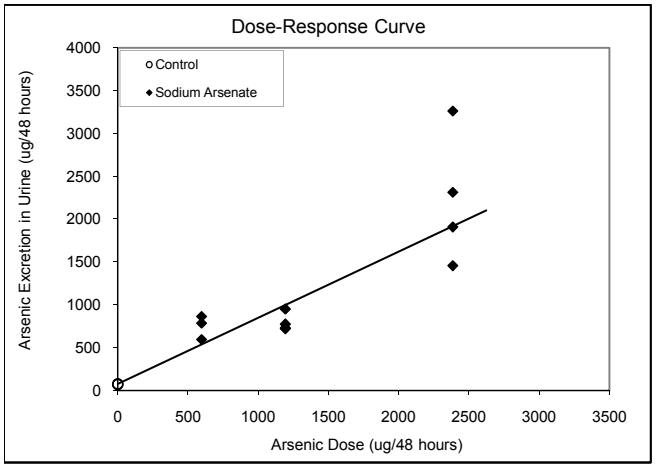
[c] 90% confidence interval calculated using Fieller's theorem

** g ≥ 0.05 (Feiller's SE is uncertain)

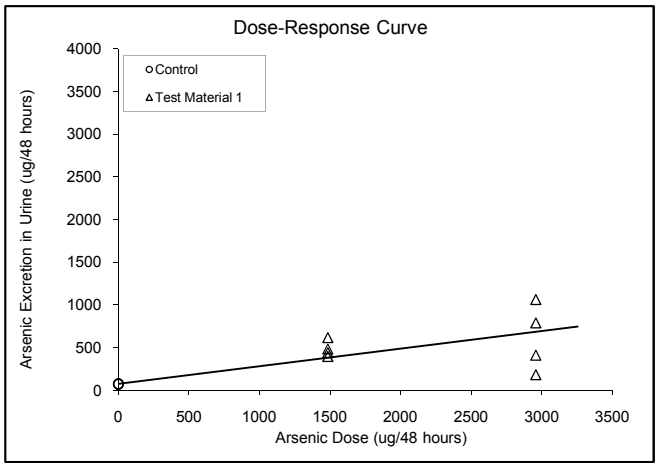
2_MS-1 As RBA Calcs all data 8-19-09 sieved no outlier v2.xls (Graph_2)

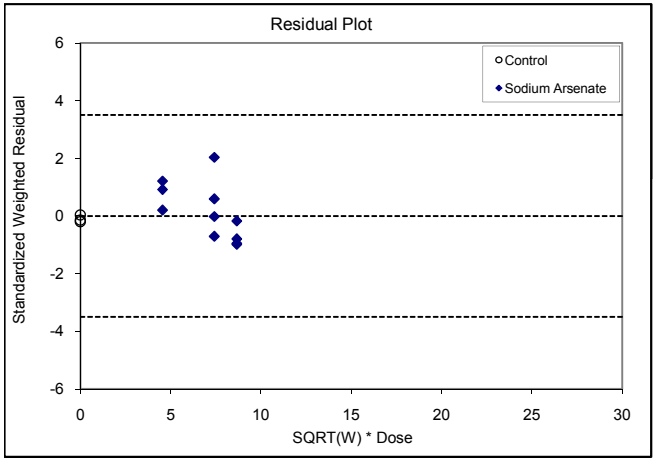
FIGURE 4-6 STUDY 1 URINARY EXCRETION OF ARSENIC: Days 12/13 (Outlier Excluded)

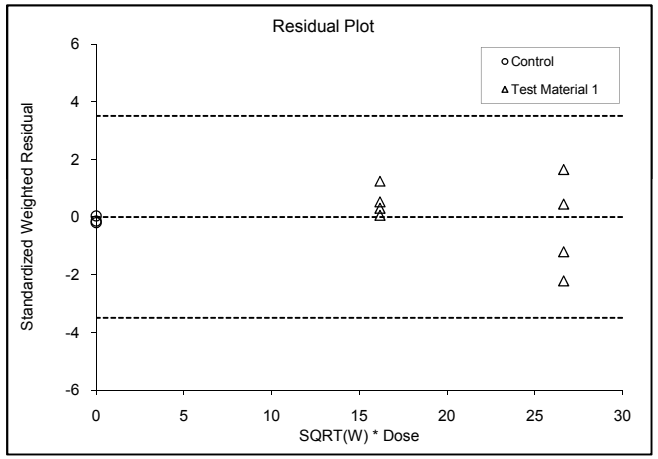
Reference Material (Sodium Arsenate)



Test Material 1 (MS-1)







Summary of Fitting[a]

Parameter	Estimate	SE
a	77.9	24.3
b_r	0.77	0.09
b_{t1}	0.21	0.04
Covariance (b_r,b_{t1})	0.0587	--
Degrees of Freedom	20	--

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

where r = Reference Material, t1 = Test Material 1

ANOVA

Source	MSE
Fit	229.41
Error	4.69
Total	26.09

Statistic	Estimate
F	48.928
p	< 0.001
Adjusted R^2	0.8203

RBA and Uncertainty

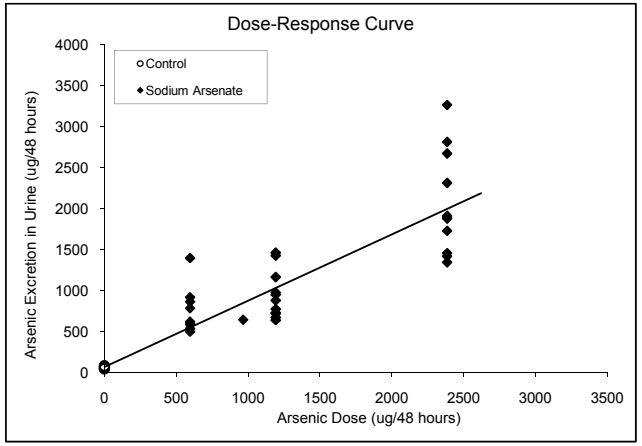
	Test Material 1
RBA	0.27
Lower bound[c]	0.18
Upper bound[c]	0.37
Standard Error[c]	0.055

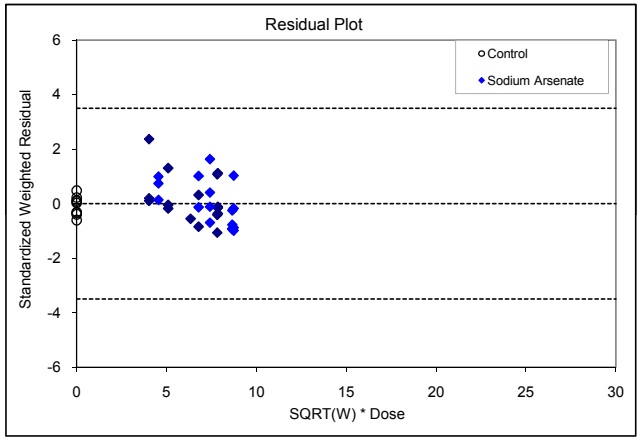
[c] 90% confidence interval calculated using Fieller's theorem

2_MS-1 As RBA Calcs all data 8-19-09 sieved no outlier v2.xls (Graph_3)

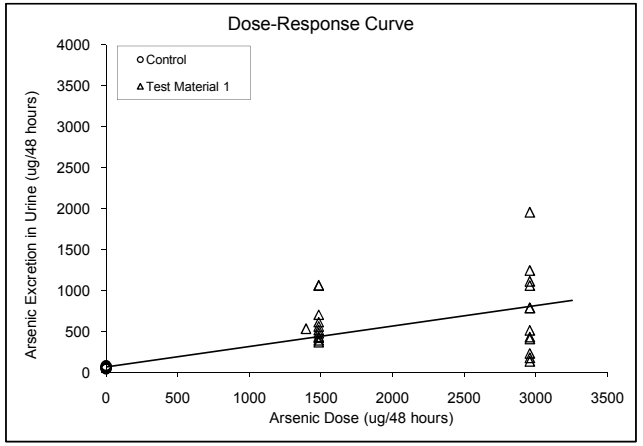
FIGURE 4-7 STUDY 1 URINARY EXCRETION OF ARSENIC: All Days (Outliers Excluded)

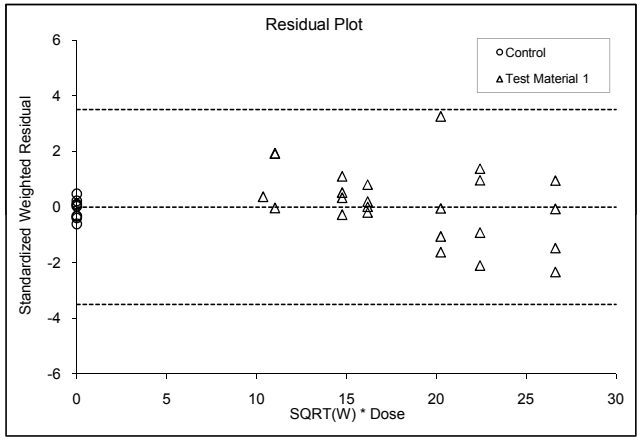
Reference Material (Sodium Arsenate)





Test Material 1 (MS-1)





Summary of Fitting[b]

Parameter	Estimate	SE
a	68.9	14.4
b_r	0.81	0.06
b_{t1}	0.25	0.03
Covariance (b_r,b_{t1})	0.0404	--
Degrees of Freedom	64	--

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

where r = Reference Material, t1 = Test Material 1

ANOVA

Source	MSE
Fit	763.36
Error	6.05
Total	29.35

Statistic	Estimate
F	126.105
p	< 0.001
Adjusted R^2	0.7938

RBA and Uncertainty

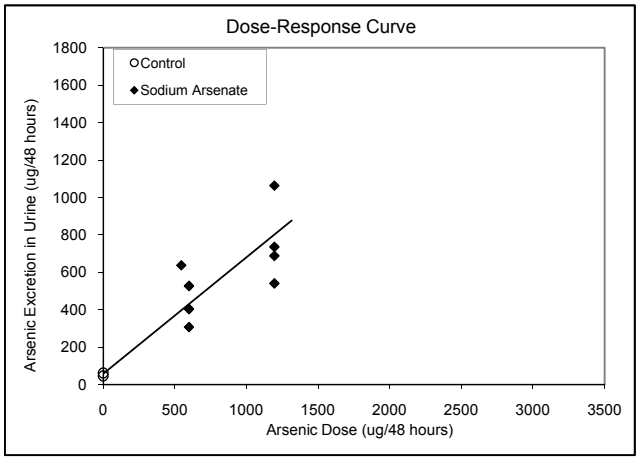
	Test Material 1
RBA	0.31
Lower bound[c]	0.25
Upper bound[c]	0.38
Standard Error[c]	0.040

[c] 90% confidence interval calculated using Fieller's theorem

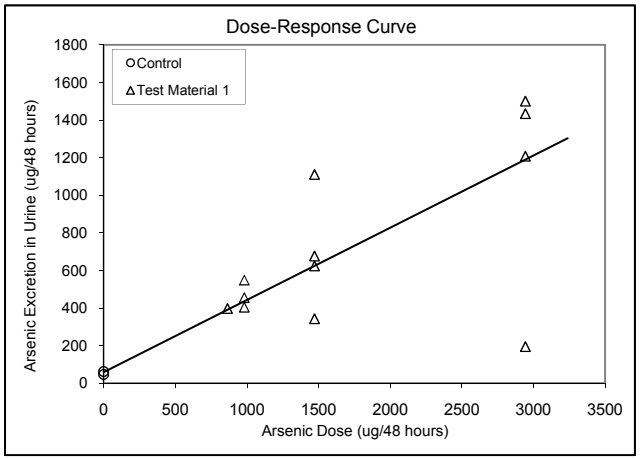
2_MS-1 As RBA Calcs all data 8-19-09 sieved no outlier v2.xls (Graph_All)

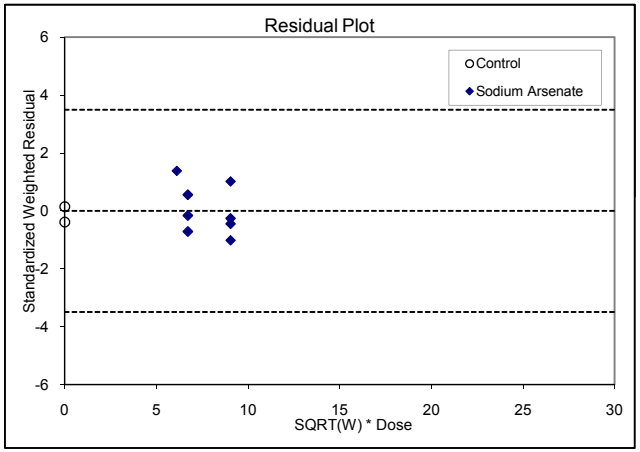
FIGURE 4-8 STUDY 2 URINARY EXCRETION OF ARSENIC: Days 6/7 (Outlier Excluded)

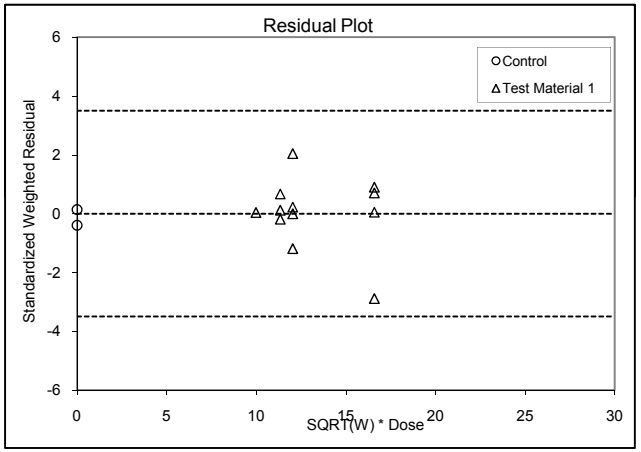
Reference Material (Sodium Arsenate)



Test Material 1 (MS-5)







Summary of Fitting[b]

Parameter	Estimate	Standard Error
a	58.3	21.8
b_r	0.62	0.09
b_{t1}	0.38	0.05
Covariance (b_r,b_{t1})	0.0733	--
Degrees of Freedom	20	--

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

where r = Reference Material, t1 = Test Material 1

ANOVA

Source	MSE
Fit	221.77
Error	4.18
Total	24.91

Statistic	Estimate
F	53.014
p	< 0.001
Adjusted R^2	0.8320

RBA and Uncertainty

	Test Material 1
RBA	0.62
Lower bound[c]	0.45
Upper bound[c]	0.87
Standard Error[c]	0.115**

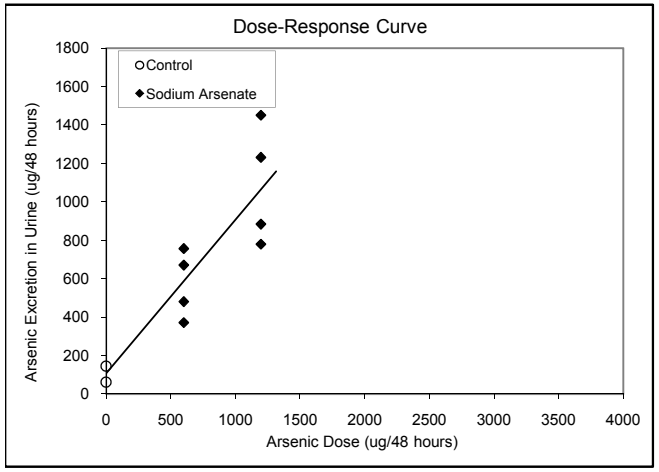
[c] 90% confidence interval calculated using Fieller's theorem

** g ≥ 0.05 (Feiller's SE is uncertain)

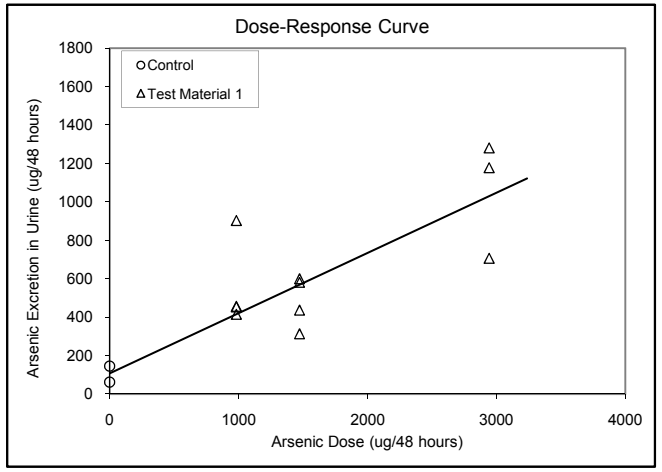
2_MS-5 As RBA Calcs all data 8-19-09 no outlier.xls (Graph_1)

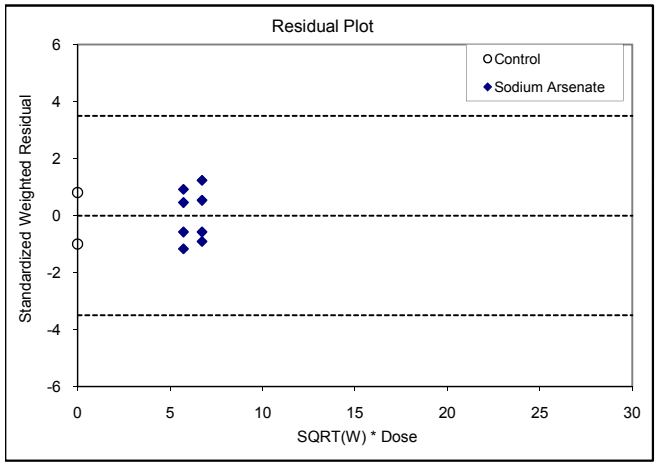
FIGURE 4-9 STUDY 2 URINARY EXCRETION OF ARSENIC: Days 9/10 (Outlier Excluded)

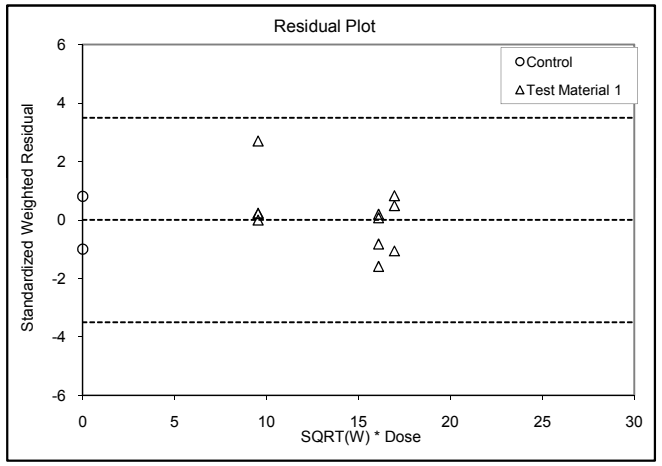
Reference Material (Sodium Arsenate)



Test Material 1 (MS-5)







Summary of Fitting[a]

Parameter	Estimate	SE
a	108.0	33.0
b_r	0.80	0.11
b_{t1}	0.31	0.04
Covariance (b_r,b_{t1})	0.1592	--
Degrees of Freedom	19	--

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

where r = Reference Material, t1 = Test Material 1

ANOVA

Source	MSE
Fit	150.99
Error	3.44
Total	18.19

Statistic	Estimate
F	43.920
p	< 0.001
Adjusted R^2	0.8110

RBA and Uncertainty

	Test Material 1
RBA	0.39
Lower bound[c]	0.28
Upper bound[c]	0.54
Lower bound[c]	0.071**

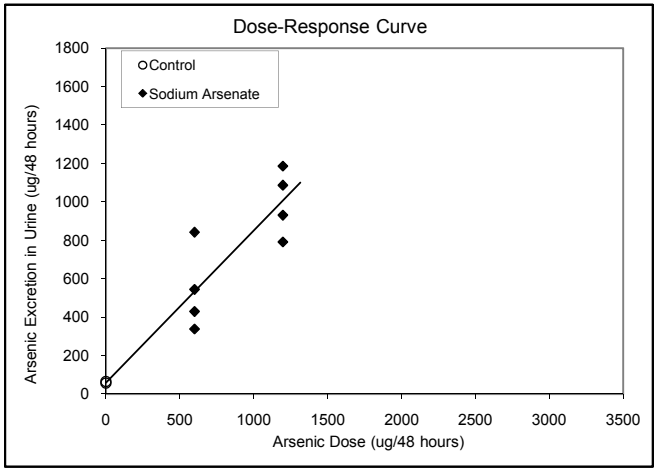
[c] 90% confidence interval calculated using Fieller's theorem

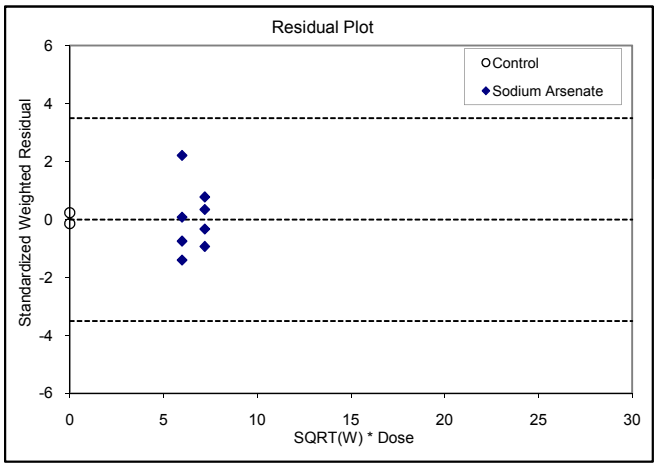
** g ≥ 0.05 (Feiller's SE is uncertain)

2_MS-5 As RBA Calcs all data 8-19-09 no outlier.xls (Graph_2)

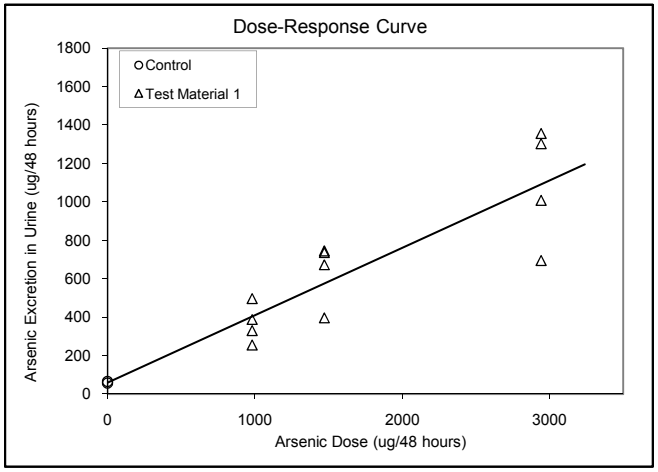
FIGURE 4-10 STUDY 2 URINARY EXCRETION OF ARSENIC: Days 12/13 (Outlier Excluded)

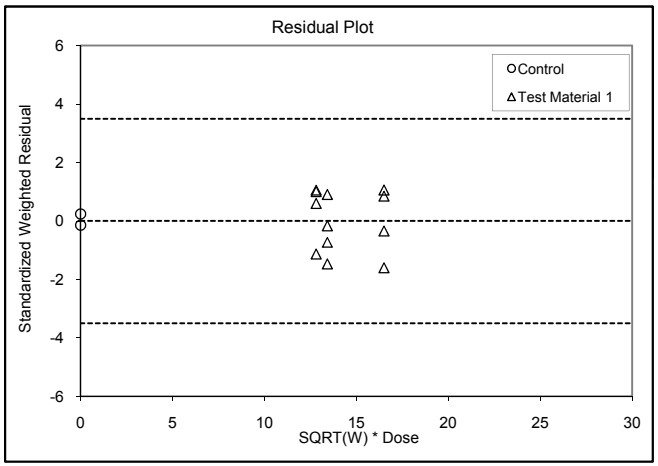
Reference Material (Sodium Arsenate)





Test Material 1 (MS-5)





Summary of Fitting[a]

Parameter	Estimate	SE
a	60.3	17.1
b_r	0.79	0.08
b_{t1}	0.35	0.03
Covariance (b_r,b_{t1})	0.0858	--
Degrees of Freedom	20	--

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

where r = Reference Material, t1 = Test Material 1

ANOVA

Source	MSE
Fit	217.93
Error	2.14
Total	22.69

Statistic	Estimate
F	101.741
p	< 0.001
Adjusted R^2	0.9056

RBA and Uncertainty

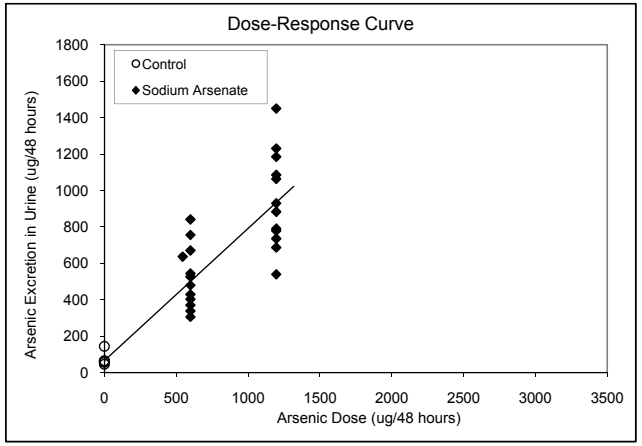
	Test Material 1
RBA	0.44
Lower bound[c]	0.35
Upper bound[c]	0.56
Lower bound[c]	0.058

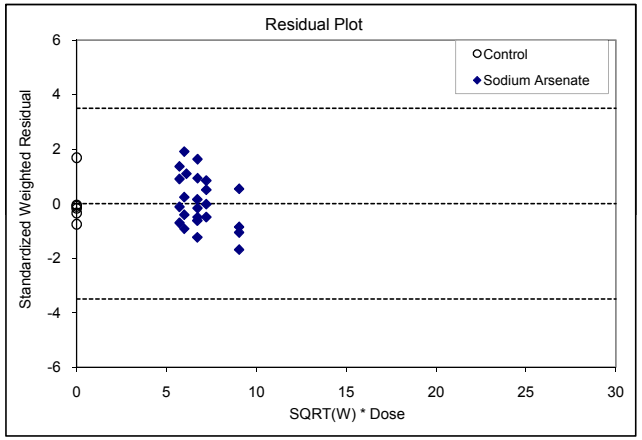
[c] 90% confidence interval calculated using Fieller's theorem

2_MS-5 As RBA Calcs all data 8-19-09 no outlier.xls (Graph_3)

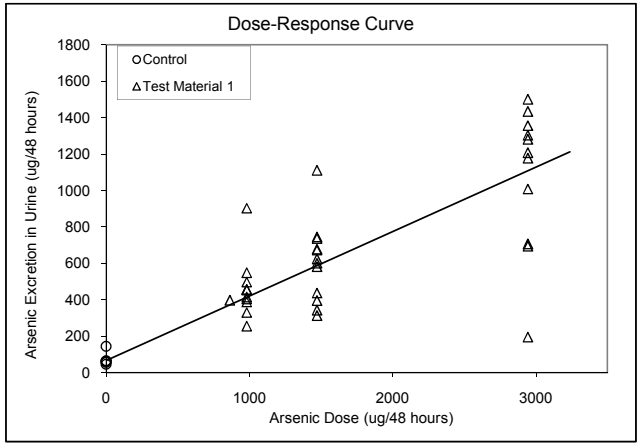
FIGURE 4-11 STUDY 2 URINARY EXCRETION OF ARSENIC: All Days (Outlier Excluded)

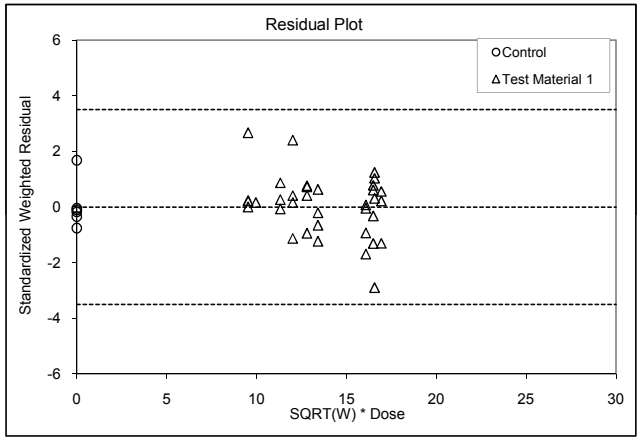
Reference Material (Sodium Arsenate)





Test Material 1 (MS-5)





Summary of Fitting[b]

Parameter	Estimate	SE
a	67.3	13.0
b_r	0.73	0.06
b_{t1}	0.35	0.02
Covariance (b_r,b_{t1})	0.0939	--
Degrees of Freedom	63	--

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

where r = Reference Material, t1 = Test Material 1

ANOVA

Source	MSE
Fit	607.89
Error	3.27
Total	22.16

Statistic	Estimate
F	185.930
p	< 0.001
Adjusted R^2	0.8525

RBA and Uncertainty

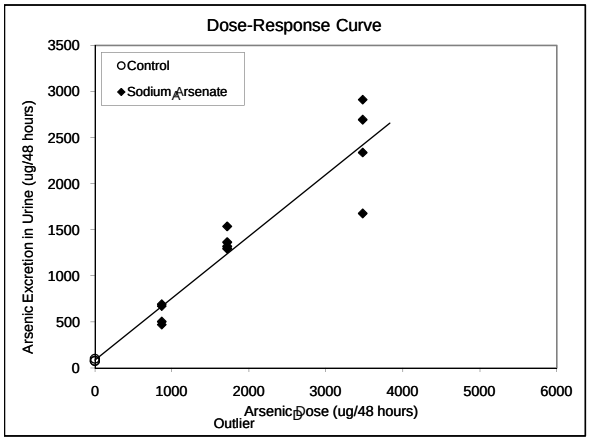
	Test Material 1
RBA	0.49
Lower bound[c]	0.42
Upper bound[c]	0.57
Standard Error[c]	0.047

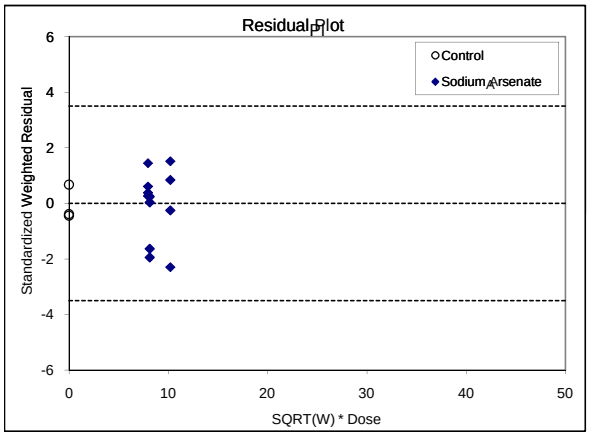
[c] 90% confidence interval calculated using Fieller's theorem

2_MS-5 As RBA Calcs all data 8-19-09 no outlier.xls (Graph_All)

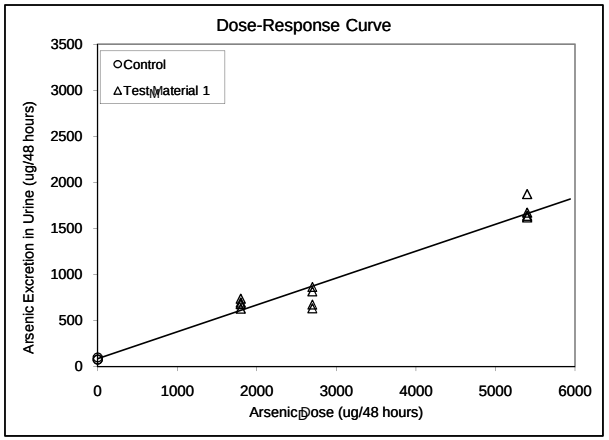
FIGURE 4-12 STUDY 3 URINARY EXCRETION OF ARSENIC: Days 6/7

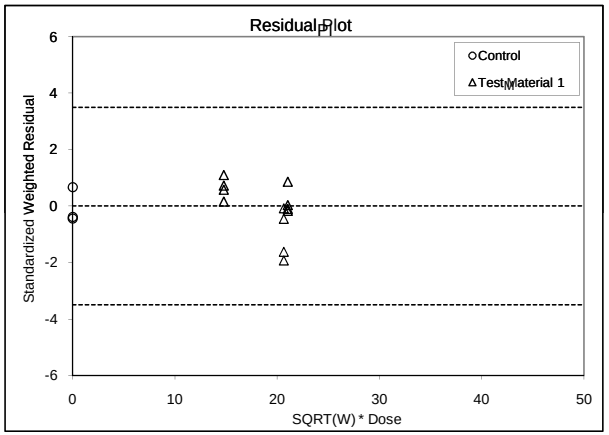
Reference Material (Sodium Arsenate)



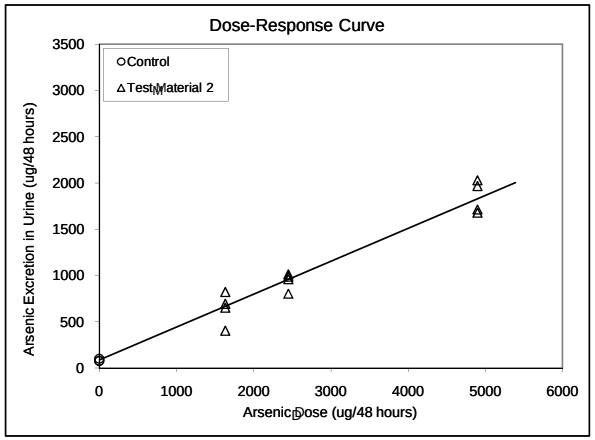


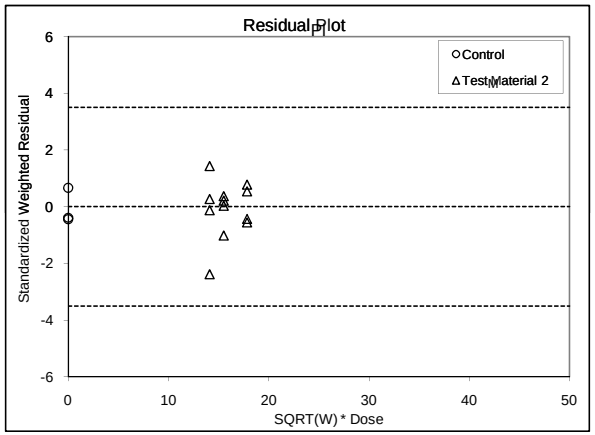
Test Material 1 (MS-4)





Test Material 2 (MS-8)





Summary of Fitting[a]

Parameter	Estimate	Standard Error
a	85.1	30.0
b_r	0.67	0.03
b_{t1}	0.29	0.02
b_{t2}	0.36	0.02
Covariance (b_r,b_{t1})	0.2916	--
Covariance (b_r,b_{t2})	0.2356	--
Degrees of Freedom	36	--

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

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

ANOVA

Source	SSE	DF	MSE
Fit	987.75	3	329.25
Error	34.45	35	0.98
Total	1022.19	38	26.90

Statistic	Estimate
F	334.537
p	< 0.001
Adjusted R^2	0.9634

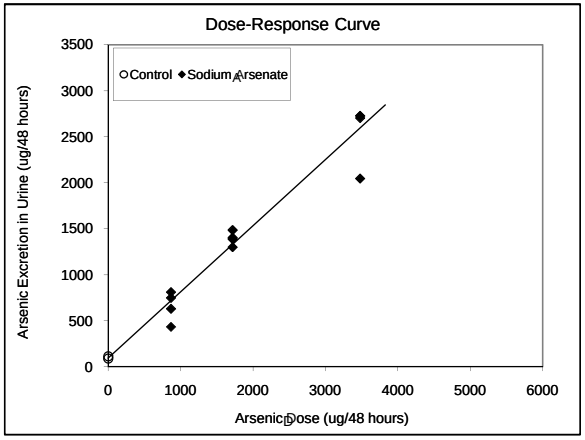
RBA and Uncertainty

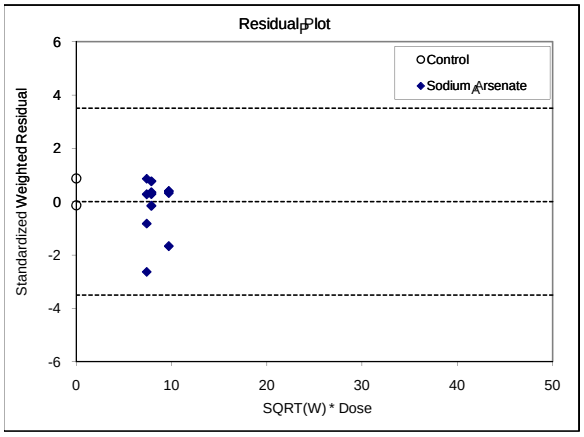
	Test Material 1	Test Material 2
RBA	0.43	0.53
Lower bound[c]	0.39	0.48
Upper bound[c]	0.48	0.59
Standard Error[c]	0.026	0.033

[c] 90% confidence interval as calculated using Fieller's theorem

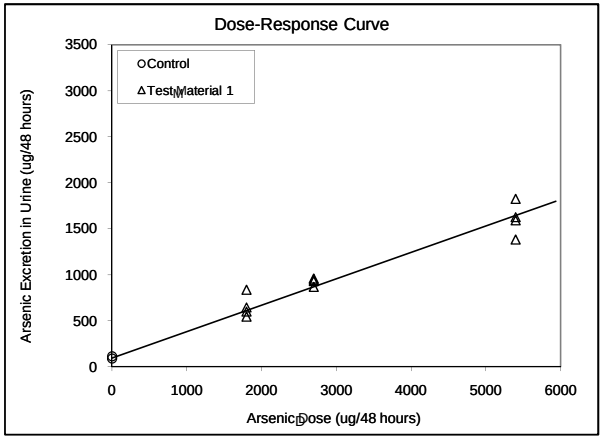
FIGURE 4-13 STUDY 3 URINARY EXCRETION OF ARSENIC: Days 9/10

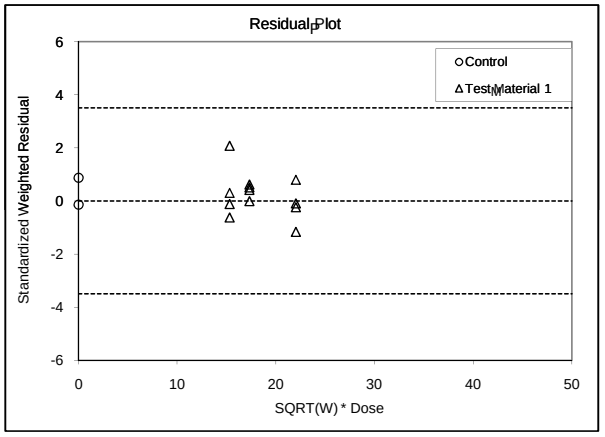
Reference Material (Sodium Arsenate)



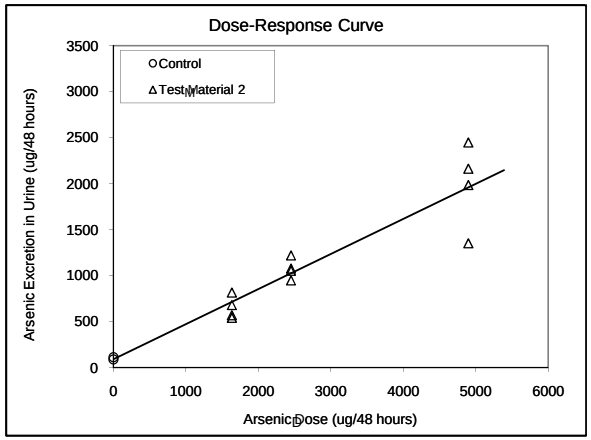


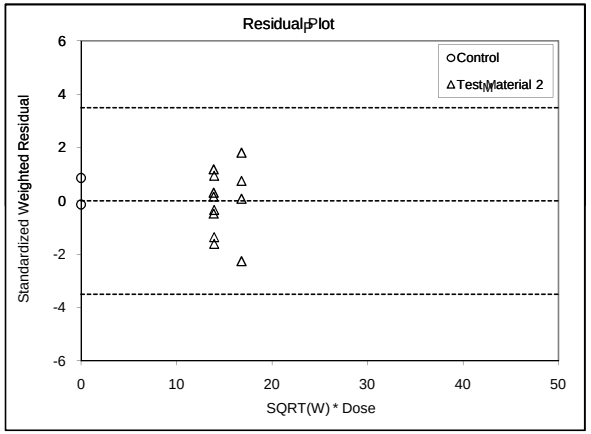
Test Material 1 (MS-4)





Test Material 2 (MS-8)





Summary of Fitting[a]

Parameter	Estimate	SE
a	93.5	43.5
b_r	0.72	0.04
b_{t1}	0.29	0.02
b_{t2}	0.38	0.02
Covariance (b_r,b_{t1})	0.3871	--
Covariance (b_r,b_{t2})	0.3359	--
Degrees of Freedom	35	--

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

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

ANOVA

Source	SSE	DF	MSE
Fit	874.36	3	291.45
Error	31.48	34	0.93
Total	905.84	37	24.48

Statistic	Estimate
F	314.800
p	< 0.001
Adjusted R^2	0.9622

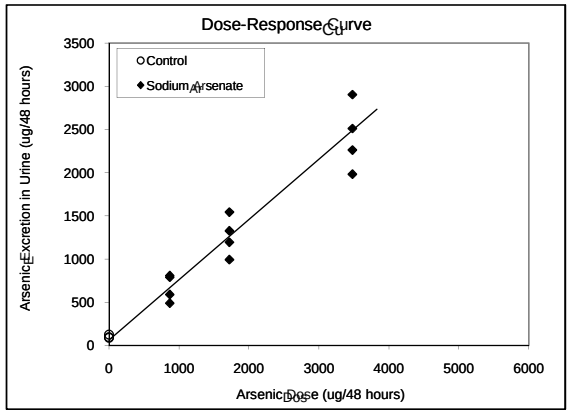
RBA and Uncertainty

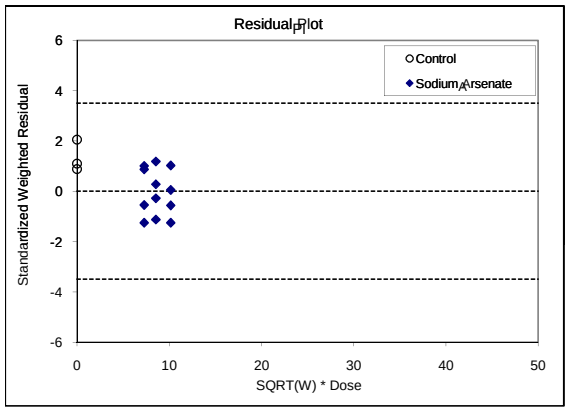
	Test Material 1	Test Material 2
RBA	0.40	0.53
Lower bound[c]	0.36	0.47
Upper bound[c]	0.45	0.59
Standard Error[c]	0.027	0.034

[c] 90% confidence interval as calculated using Fieller's theorem

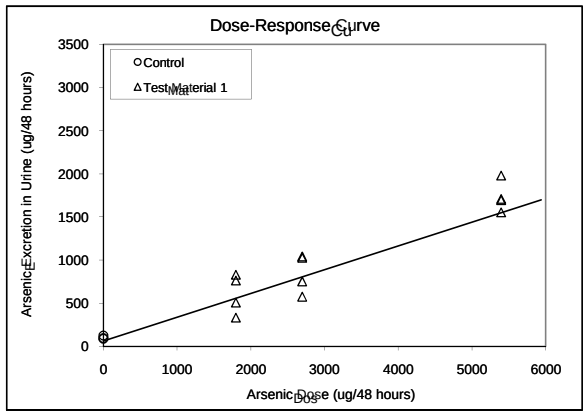
FIGURE 4-14 STUDY 3 URINARY EXCRETION OF ARSENIC: Days 12/13

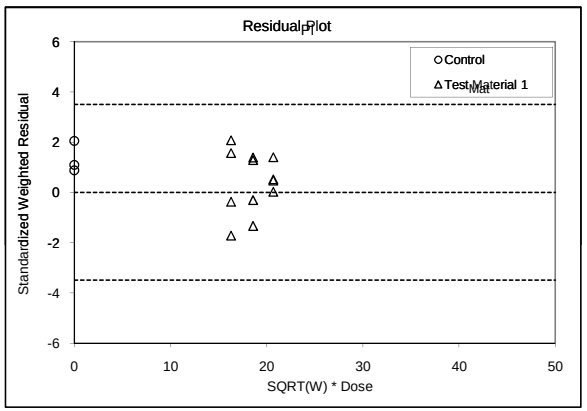
Reference Material (Sodium Arsenate)



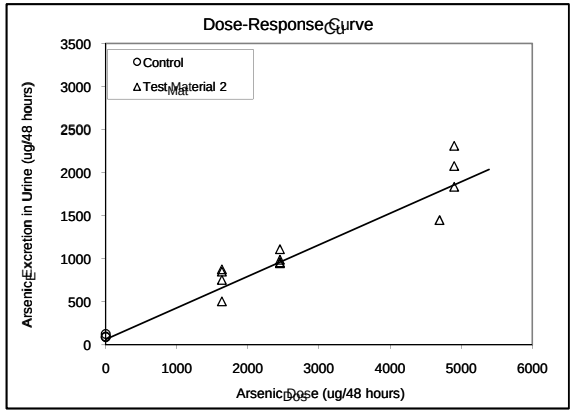


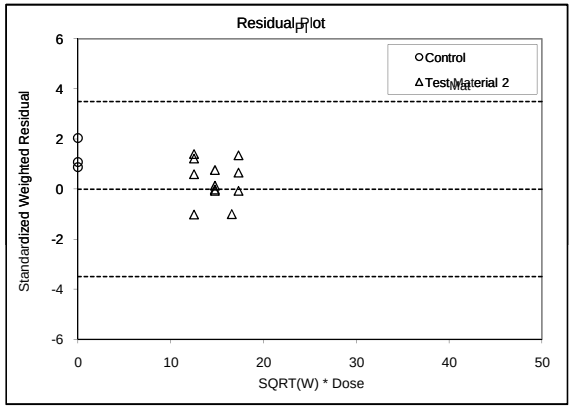
Test Material 1 (MS-4)





Test Material 2 (MS-8)





Summary of Fitting[a]

Parameter	Estimate	SE
a	62.0	39.6
b_r	0.70	0.05
b_{t1}	0.28	0.02
b_{t2}	0.37	0.03
Covariance (b_r,b_{t1})	0.2097	--
Covariance (b_r,b_{t2})	0.1477	--
Degrees of Freedom	36	--

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

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

ANOVA

Source	SSE	DF	MSE
Fit	918.67	3	306.22
Error	58.88	35	1.68
Total	977.55	38	25.73

Statistic	Estimate
F	182.013
p	< 0.001
Adjusted R^2	0.9346

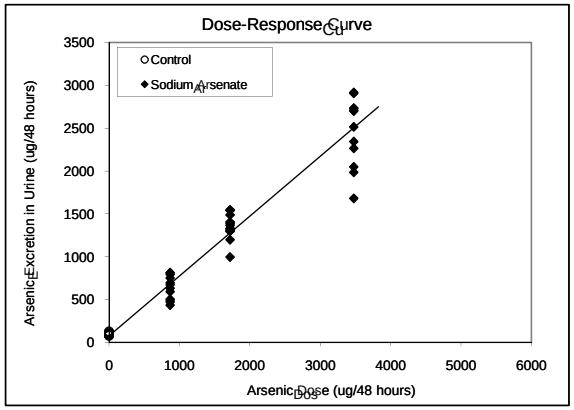
RBA and Uncertainty

	Test Material 1	Test Material 2
RBA	0.39	0.53
Lower bound[c]	0.33	0.45
Upper bound[c]	0.46	0.62
Standard Error[c]	0.038	0.049

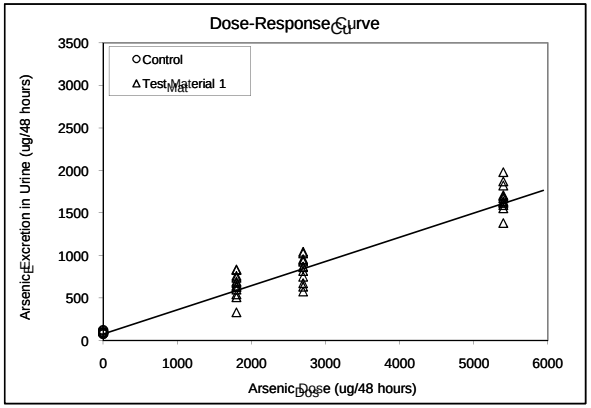
[c] 90% confidence interval as calculated using Fieller's theorem

FIGURE 4-15 STUDY 3 URINARY EXCRETION OF ARSENIC: All Days

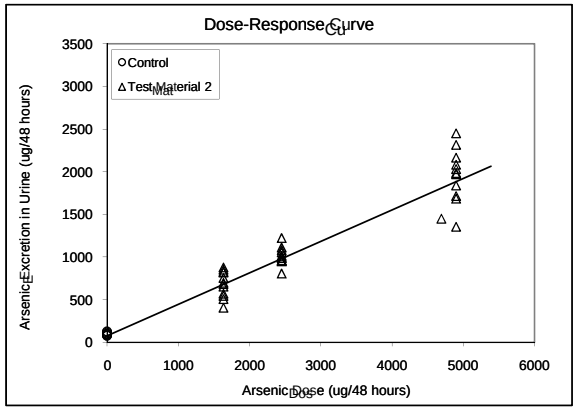
Reference Material (Sodium Arsenate)

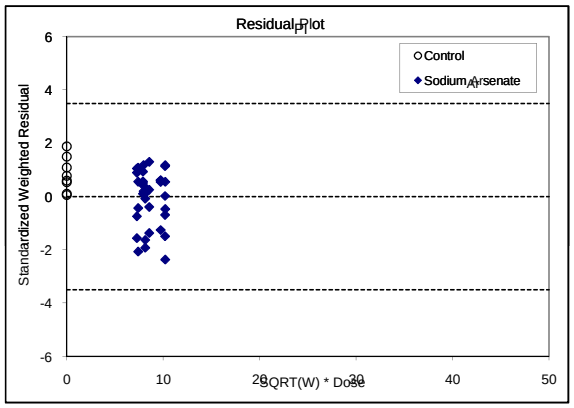


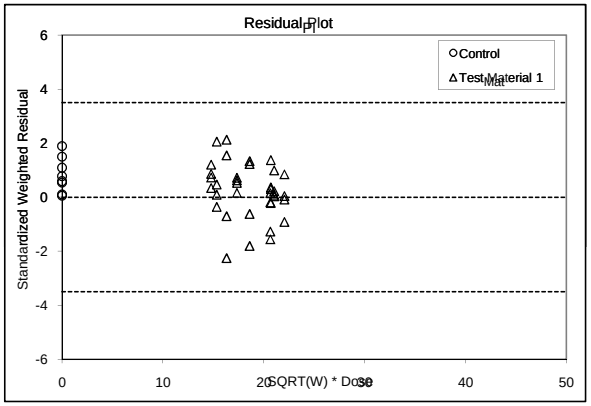
Test Material 1 (MS-4)

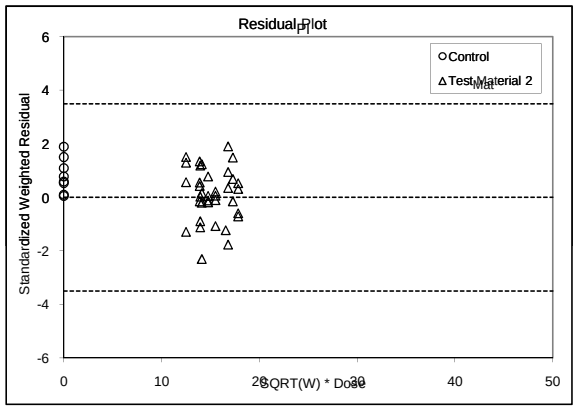


Test Material 2 (MS-8)









Summary of Fitting[a]

Parameter	Estimate	SE
a	74.6	21.3
b_r	0.70	0.02
b_{t1}	0.28	0.01
b_{t2}	0.37	0.01
Covariance (b_r,b_{t1})	0.2729	--
Covariance (b_r,b_{t2})	0.2135	--
Degrees of Freedom	113	--

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

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

ANOVA

Source	SSE	DF	MSE
Fit	2815.63	3	938.54
Error	122.72	112	1.10
Total	2938.34	115	25.55

Statistic	Estimate
F	856.575
p	< 0.001
Adjusted R^2	0.9571

RBA and Uncertainty

	Test Material 1	Test Material 2
RBA	0.41	0.53
Lower bound[c]	0.38	0.49
Upper bound[c]	0.44	0.57
Standard Error[c]	0.018	0.023

[c] 90% confidence interval as calculated using Fieller's theorem

APPENDIX A GROUP ASSIGNMENTS

STUDY 1 (MS-1 MATERIAL)

Pig Number	Group	Dosing Material	Target Arsenic Dose (μg/kg-day)
353 359 373	1	Control	0
368 374 367 370	2	NaAs	25
351 356 361 372	3	NaAs	50
358 365 366 371	4	NaAs	100
360 363 369 375	5	TM1	60
352 364 354 362	6	TM1	120

STUDY 2 (MS-5 MATERIAL)

Pig Number	Group	Dosing Material	Target Arsenic Dose (μg/kg-day)
463 465 474 475	1	NaAs	25
448 451 454 483	2	NaAs	50
450 466 468 481	3	TM1	40
445 452 470 482	4	TM1	60
250 449 455 464	5	TM1	120
249 446 453 467	6	TM2	40
469 473	7	Control	0

Notes:
MS-1 material was used for the TM2 group but RBA was not evaluated for TM2.

STUDY 3 (MS-4 and MS-8 MATERIALS)

Pig Number	Group	Dosing Material	Target Arsenic Dose (μg/kg-day)
503 511 529 543	1	NaAs	25
502 505 506 527	2	NaAs	50
501 516 521 531	3	NaAs	100
530 535 538 541	4	TM1	40
513 525 526 537	5	TM1	60
507 514 515 533	6	TM1	120
504 508 519 534	7	TM2	40
509 532 536 540	8	TM2	60
510 517 518 520	9	TM2	120
512 522 539	10	Control	0

APPENDIX B

BODY WEIGHTS

APPENDIX B BODY WEIGHTS

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

STUDY 1

Group	Pig #	Day -1	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Days 0-14
		BW (kg)	BW (kg)	BW (kg)	BW (kg)	BW (kg)	BW (kg)	BW (kg)	BW (kg)	BW (kg)	BW (kg)	BW (kg)	BW (kg)	BW (kg)	BW (kg)	BW (kg)	BW (kg)	Mean Daily BW Gain
1	503	14.8	15.1	15.3	15.6	15.9	16.2	16.5	16.8	17.2	17.5	17.9	18.3	18.7	19.0	19.2	19.5	0.32
1	511	11.7	11.9	12.2	12.4	12.8	13.1	13.5	13.8	14.1	14.4	14.7	15.0	15.3	15.8	16.2	16.7	0.34
1	529	13.4	13.7	14.1	14.4	14.7	15.1	15.4	15.7	16.0	16.3	16.7	17.1	17.5	17.9	18.3	18.7	0.35
1	543	15.1	15.4	15.7	16.0	16.4	16.7	17.1	17.3	17.5	17.7	18.2	18.8	19.3	19.7	20.1	20.5	0.36
2	502	12.8	13.0	13.2	13.4	13.7	14.0	14.3	14.7	15.0	15.4	15.8	16.2	16.6	16.9	17.3	17.6	0.33
2	505	12.0	12.3	12.6	12.9	13.2	13.6	13.9	14.4	14.8	15.3	15.7	16.1	16.5	16.9	17.2	17.6	0.38
2	506	12.5	12.8	13.1	13.4	13.7	13.9	14.2	14.5	14.9	15.2	15.6	16.0	16.4	16.7	17.1	17.4	0.33
2	527	13.4	13.7	14.1	14.4	14.6	14.8	15.0	15.4	15.7	16.1	16.3	16.5	16.7	17.1	17.6	18.0	0.30
3	501	11.7	12.0	12.3	12.6	12.9	13.3	13.6	13.9	14.2	14.5	14.9	15.2	15.6	15.9	16.3	16.6	0.33
3	516	15.2	15.5	15.9	16.2	16.5	16.7	17.0	17.3	17.7	18.0	18.4	18.8	19.2	19.5	19.9	20.2	0.33
3	521	12.0	12.4	12.7	13.1	13.4	13.6	13.9	14.3	14.8	15.2	15.5	15.8	16.1	16.4	16.8	17.1	0.34
3	531	13.9	14.2	14.5	14.8	15.2	15.5	15.9	16.2	16.6	16.9	17.3	17.8	18.2	18.6	18.9	19.3	0.36
4	530	14.2	14.5	14.9	15.2	15.6	16.0	16.4	16.8	17.2	17.6	17.9	18.2	18.5	18.9	19.3	19.7	0.37
4	535	13.0	13.3	13.6	13.9	14.0	14.1	14.2	14.6	15.1	15.5	15.8	16.1	16.4	16.7	17.1	17.4	0.29
4	538	14.3	14.6	15.0	15.3	15.7	16.1	16.5	16.8	17.1	17.4	17.8	18.2	18.6	19.1	19.5	20.0	0.38
4	541	13.1	13.4	13.6	13.9	14.1	14.4	14.6	15.0	15.3	15.7	16.0	16.4	16.7	17.1	17.4	17.8	0.32
5	513	14.3	14.6	14.8	15.1	15.5	15.8	16.2	16.5	16.8	17.1	17.5	17.9	18.3	18.6	18.9	19.2	0.33
5	525	13.6	13.8	14.1	14.3	14.6	14.9	15.2	15.6	16.0	16.4	16.8	17.3	17.7	18.2	18.7	19.2	0.38
5	526	15.9	16.1	16.4	16.6	16.9	17.2	17.5	17.9	18.4	18.8	19.2	19.6	20.0	20.4	20.9	21.3	0.37
5	537	12.1	12.4	12.8	13.1	13.5	13.9	14.3	14.6	14.9	15.2	15.6	16.0	16.4	16.7	17.0	17.3	0.35
6	507	14.4	14.8	15.1	15.5	15.6	15.8	15.9	16.3	16.8	17.2	17.6	17.9	18.3	18.7	19.0	19.4	0.33
6	514	15.3	15.7	16.2	16.6	16.9	17.3	17.6	18.0	18.3	18.7	19.1	19.6	20.0	20.3	20.6	20.9	0.37
6	515	14.8	15.2	15.6	16.0	16.4	16.7	17.1	17.4	17.8	18.1	18.6	19.0	19.5	19.9	20.3	20.7	0.39
6	533	14.6	15.0	15.4	15.8	16.0	16.2	16.4	16.8	17.3	17.7	18.3	18.8	19.4	19.7	20.0	20.3	0.38
7	504	16.2	16.7	17.1	17.6	17.8	18.0	18.2	18.5	18.8	19.1	19.5	20.0	20.4	20.7	21.1	21.4	0.34
7	508	15.0	15.4	15.7	16.1	16.3	16.6	16.8	17.0	17.3	17.5	17.9	18.4	18.8	19.1	19.4	19.7	0.31

STUDY 2

Group	Pig #	Day -1	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Days 0-14
		BW (kg)	BW (kg)	BW (kg)	BW (kg)	BW (kg)	BW (kg)	BW (kg)	BW (kg)	BW (kg)	BW (kg)	BW (kg)	BW (kg)	BW (kg)	BW (kg)	BW (kg)	BW (kg)	Mean Daily BW Gain
1	463	9.0	9.2	9.4	9.6	9.7	9.9	10.0	10.3	10.6	10.9	11.1	11.3	11.5	11.7	12.0	12.2	0.21
1	465	7.2	7.4	7.6	7.8	8.0	8.3	8.5	8.7	9.0	9.2	9.4	9.6	9.8	10.2	10.5	10.9	0.25
1	474	7.6	7.9	8.1	8.4	8.6	8.7	8.9	9.1	9.3	9.5	9.8	10.0	10.3	10.7	11.0	11.4	0.25
1	475	7.5	7.6	7.6	7.7	7.9	8.2	8.4	8.7	8.9	9.2	9.4	9.7	9.9	10.2	10.5	10.8	0.23
2	448	8.2	8.4	8.6	8.8	9.0	9.3	9.5	9.7	10.0	10.2	10.5	10.7	11.0	11.2	11.5	11.7	0.24
2	451	8.7	8.8	8.9	9.0	9.2	9.4	9.6	9.8	10.1	10.3	10.5	10.8	11.0	11.3	11.5	11.8	0.21
2	454	9.1	9.3	9.4	9.6	9.7	9.9	10.0	10.3	10.5	10.8	11.1	11.3	11.6	11.9	12.1	12.4	0.22
2	483	7.2	7.4	7.6	7.8	8.1	8.3	8.6	8.8	9.0	9.2	9.4	9.7	9.9	10.2	10.5	10.8	0.24
3	450	7.7	7.9	8.0	8.2	8.4	8.7	8.9	9.1	9.4	9.6	9.9	10.1	10.4	10.6	10.9	11.1	0.23
3	466	7.9	8.1	8.2	8.4	8.6	8.8	9.0	9.2	9.5	9.7	10.0	10.2	10.5	10.8	11.1	11.4	0.24
3	468	9.1	9.1	9.2	9.2	9.4	9.6	9.8	10.0	10.3	10.5	10.8	11.1	11.4	11.7	12.0	12.3	0.23
3	481	8.8	8.9	9.0	9.1	9.4	9.6	9.9	10.1	10.3	10.5	10.8	11.1	11.4	11.6	11.9	12.1	0.23
4	445	7.9	8.1	8.3	8.5	8.7	9.0	9.2	9.4	9.7	9.9	10.2	10.4	10.7	11.0	11.2	11.5	0.24
4	452	7.7	7.9	8.1	8.3	8.5	8.8	9.0	9.2	9.4	9.6	9.9	10.1	10.4	10.7	11.0	11.3	0.24
4	470	8.5	8.8	9.0	9.3	9.6	9.9	10.2	10.4	10.7	10.9	11.1	11.3	11.5	11.8	12.0	12.3	0.25
4	482	7.9	8.1	8.3	8.5	8.7	8.8	9.0	9.3	9.5	9.8	10.0	10.2	10.4	10.7	11.0	11.3	0.23
5	250	7.2	7.3	7.4	7.5	7.8	8.2	8.5	8.7	8.8	9.0	9.3	9.5	9.8	10.1	10.4	10.7	0.24
5	449	8.6	8.8	8.9	9.1	9.3	9.6	9.8	10.0	10.2	10.4	10.7	11.1	11.4	11.7	12.1	12.4	0.26
5	455	9.2	9.4	9.6	9.8	10.0	10.3	10.5	10.7	10.9	11.1	11.3	11.6	11.8	12.1	12.4	12.7	0.24
5	464	8.8	8.9	9.1	9.2	9.3	9.5	9.6	9.9	10.1	10.4	10.6	10.9	11.1	11.4	11.7	12.0	0.22
6	249	8.7	8.7	8.7	8.7	8.9	9.0	9.2	9.6	9.9	10.3	10.5	10.8	11.0	11.3	11.7	12.0	0.24
6	446	8.0	8.1	8.1	8.2	8.4	8.6	8.8	9.1	9.3	9.6	9.9	10.1	10.4	10.7	11.0	11.3	0.23
6	453	8.5	8.6	8.7	8.8	8.9	9.1	9.2	9.3	9.4	9.5	9.8	10.0	10.3	10.6	10.8	11.1	0.18
6	467	8.0	8.1	8.1	8.2	8.5	8.7	9.0	9.2	9.3	9.5	9.8	10.1	10.4	10.6	10.9	11.1	0.22
6	469	8.8	9.0	9.2	9.4	9.6	9.7	9.9	10.2	10.4	10.7	11.0	11.2	11.5	11.8	12.0	12.3	0.24
6	473	8.1	8.0	8.0	7.9	8.1	8.2	8.4	8.6	8.8	9.0	9.1	9.3	9.4	9.5	9.7	9.8	0.13

STUDY 3

Group	Pig #	Day -1	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Days 0-14
		BW (kg)	BW (kg)	BW (kg)	BW (kg)	BW (kg)	BW (kg)	BW (kg)	BW (kg)	BW (kg)	BW (kg)	BW (kg)	BW (kg)	BW (kg)	BW (kg)	BW (kg)	BW (kg)	Mean Daily BW Gain
1	503	14.8	15.1	15.3	15.6	15.9	16.2	16.5	16.8	17.2	17.5	17.9	18.3	18.7	19.0	19.2	19.5	0.32
1	511	11.7	11.9	12.2	12.4	12.8	13.1	13.5	13.8	14.1	14.4	14.7	15.0	15.3	15.8	16.2	16.7	0.34
1	529	13.4	13.7	14.1	14.4	14.7	15.1	15.4	15.7	16.0	16.3	16.7	17.1	17.5	17.9	18.3	18.7	0.35
1	543	15.1	15.4	15.7	16.0	16.4	16.7	17.1	17.3	17.5	17.7	18.2	18.8	19.3	19.7	20.1	20.5	0.36
2	502	12.8	13.0	13.2	13.4	13.7	14.0	14.3	14.7	15.0	15.4	15.8	16.2	16.6	16.9	17.3	17.6	0.33
2	505	12.0	12.3	12.6	12.9	13.2	13.6	13.9	14.4	14.8	15.3	15.7	16.1	16.5	16.9	17.2	17.6	0.38
2	506	12.5	12.8	13.1	13.4	13.7	13.9	14.2	14.5	14.9	15.2	15.6	16.0	16.4	16.7	17.1	17.4	0.33
2	527	13.4	13.7	14.1	14.4	14.6	14.8	15.0	15.4	15.7	16.1	16.3	16.5	16.7	17.1	17.6	18.0	0.30
3	501	11.7	12.0	12.3	12.6	12.9	13.3	13.6	13.9	14.2	14.5	14.9	15.2	15.6	15.9	16.3	16.6	0.33
3	516	15.2	15.5	15.9	16.2	16.5	16.7	17.0	17.3	17.7	18.0	18.4	18.8	19.2	19.5	19.9	20.2	0.33
3	521	12.0	12.4	12.7	13.1	13.4	13.6	13.9	14.3	14.8	15.2	15.5	15.8	16.1	16.4	16.8	17.1	0.34
3	531	13.9	14.2	14.5	14.8	15.2	15.5	15.9	16.2	16.6	16.9	17.3	17.8	18.2	18.6	18.9	19.3	0.36
4	530	14.2	14.5	14.9	15.2	15.6	16.0	16.4	16.8	17.2	17.6	17.9	18.2	18.5	18.9	19.3	19.7	0.37
4	535	13.0	13.3	13.6	13.9	14.0	14.1	14.2	14.6	15.1	15.5	15.8	16.1	16.4	16.7	17.1	17.4	0.29
4	538	14.3	14.6	15.0	15.3	15.7	16.1	16.5	16.8	17.1	17.4	17.8	18.2	18.6	19.1	19.5	20.0	0.38
4	541	13.1	13.4	13.6	13.9	14.1	14.4	14.6	15.0	15.3	15.7	16.0	16.4	16.7	17.1	17.4	17.8	0.32
5	513	14.3	14.6	14.8	15.1	15.5	15.8	16.2	16.5	16.8	17.1	17.5	17.9	18.3	18.6	18.9	19.2	0.33
5	525	13.6	13.8	14.1	14.3	14.6	14.9	15.2	15.6	16.0	16.4	16.8	17.3	17.7	18.2	18.7	19.2	0.38
5	526	15.9	16.1	16.4	16.6	16.9	17.2	17.5	17.9	18.4	18.8	19.2	19.6	20.0	20.4	20.9	21.3	0.37
5	537	12.1	12.4	12.8	13.1	13.5	13.9	14.3	14.6	14.9	15.2	15.6	16.0	16.4	16.7	17.0	17.3	0.35
6	507	14.4	14.8	15.1	15.5	15.6	15.8	15.9	16.3	16.8	17.2	17.6	17.9	18.3	18.7	19.0	19.4	0.33
6	514	15.3	15.7	16.2	16.6	16.9	17.3	17.6	18.0	18.3	18.7	19.1	19.6	20.0	20.3	20.6	20.9	0.37
6	515	14.8	15.2	15.6	16.0	16.4	16.7	17.1	17.4	17.8	18.1	18.6	19.0	19.5	19.9	20.3	20.7	0.39
6	533	14.6	15.0	15.4	15.8	16.0	16.2	16.4	16.8	17.3	17.7	18.3	18.8	19.4	19.7	20.0	20.3	0.38
7	504	16.2	16.7	17.1	17.6	17.8	18.0	18.2	18.5	18.8	19.1	19.5	20.0	20.4	20.7	21.1	21.4	0.34
7	508	15.0	15.4	15.7	16.1	16.3	16.6	16.8	17.0	17.3	17.5	17.9	18.4	18.8	19.1	19.4	19.7	0.31
7	519	12	12.77	13.13	13.5	13.8	14.1	14.4	14.7	15	15.3	15.8	16.3	16.8	17.17	17.53	17.9	0.37
7	534	15	15.367	15.733	16.1	16.467	16.833	17.2	17.567	17.933	18.3	18.667	19.033	19.4	19.7	20	20.3	0.35
8	509	13	13.367	13.633	13.9	14.1	14.3	14.5	14.833	15.167	15.5	15.9	16.3	16.7	17	17.3	17.6	0.30
8	532	14	14.5	14.8	15.1	15.5	15.9	16.3	16.767	17.233	17.7	17.867	18.033	18.2	18.533	18.867	19.2	0.34
8	536	13	13.633	13.967	14.3	14.667	15.033	15.4	15.5	15.6	15.7	16.5	17.3	18.1	18.333	18.567	18.8	0.37
8	540	13	13.667	13.933	14.2	14.467	14.733	15	15.567	16.133	16.7	17.033	17.367	17.7	18	18.3	18.6	0.35
9	510	11	11.6	12.1	12.6	13.133	13.667	14.2	14.433	14.667	14.9	15.4	15.9	16.4	16.8	17.2	17.6	0.43
9	517	12	11.7	11.6	11.5	11.933	12.367	12.8	13.167	13.533	13.9	14.067	14.233	14.4	14.767	15.133	15.5	0.27
9	518	13	13.367	13.733	14.1	14.5	14.9	15.3	15.6	15.9	16.2	16.633	17.067	17.5	17.767	18.033	18.3	0.35
9	520	14	14.067	14.333	14.6	14.933	15.267	15.6	15.867	16.133	16.4	16.733	17.067	17.4	17.633	17.867	18.1	0.29
10	512	13	13.333	13.767	14.2	14.533	14.867	15.2	15.4	15.6	15.8	16.333	16.867	17.4	17.5	17.6	17.7	0.31
10	522	14	14.333	14.567	14.8	15.167	15.533	15.9	16.133	16.367	16.6	17	17.4	17.8	18.1	18.4	18.7	0.31
10	539	15	14.767	15.033	15.3	15.667	16.033	16.4	16.8	17.2	17.6	17.933	18.267	18.6	18.9	19.2	19.5	0.34

APPENDIX C

MISSED AND LATE DOSE CONSUMPTION

APPENDIX C MISSED AND LATE DOSE CONSUMPTION

STUDY 1

Day	Pig No.	Note
0	360	Day 0 - Pig 360 at only 1/2 of his dose in the AM and the PM. Dose adjusted to 50%.
0	368	Day 0 - Pig 368 slow to eat AM dose but did finish it by PM dose time.
1	360	Day 1 - Pig 360 slow to eat AM dose but did eat it all by 3 PM.
2	372	Day 2 - Pig 372 did not eat his dose in the AM or PM.
2	360	Day 2 - Pig 360 slow to eat AM dose but did eat it all by 3 PM.
3	372	Day 3 - Pig 372 at only 1/2 of his dose in the AM. AM dose adjusted to 50%.

STUDY 2

Day	Pig No.	Note
0	468	Day 0 - Pig 468 did not consume PM dose.
0	445	Day 0 - Pig 445 lost 10% of dose.
1	468	Day 1 - Pig 468 did not consume AM dose.
2	474	Day 2 - Pig 474 lose 5% of PM dose.
3	474	Day 3 - Pig 474 ate 70% of AM dose and 90% of PM dose.
4	474	Day 4 - Pig 474 ate 30% of AM dose.
4	453	Day 4 - Pig 453 lost 10% of PM dose
9	469	Day 9 - Pig 469 did not eat AM dose
10	469	Day 10 - Pig 469 did not eat AM dose
11	469	Day 11 - Pig 469 did not eat AM dose

STUDY 3

Day	Pig No.	Note
0 - 14	510	Throughout dosing period pig 510 was slow to consume doses.
11	518	Day 11 - Pig 518 dropped doughball on cage floor and lost 25% of dose.

APPENDIX D

URINE VOLUMES

APPENDIX D URINE VOLUMES

STUDY 1

Group	Pig Number	Urine Collection (mL)		
		U-1 Days 6-7	**U-2** Days 9-10	**U-3** Days 12-13
1	353	3240	9790	8290
	359	4710	4000	5400
	373	6120	5030	6123
2	368	3540	3420	4660
	374	20130	42300	37980
	367	7730	8520	7680
	370	4075	4140	5030
3	351	3890	3530	3660
	356	2920	2700	3860
	361	5890	6220	7520
	372	2870	1900	1940
4	358	2390	2000	3350
	365	12560	16320	10360
	366	3260	2370	2480
	371	2660	2137	2420
5	360	2560	5260	5660
	363	1780	2950	2102
	369	1980	3930	3140
	375	5960	9220	8840
6	352	6960	6480	4830
	364	7180	6560	12230
	354	2560	3380	4040
	362	9960	9840	11410

STUDY 2

Group	Pig ID	Urine Collection (mL)		
		U-1 Days 6-7	**U-2** Days 9-10	**U-3** Days 12-13
1	463	3740	5380	6780
	465	1720	1180	2370
	474	3200	3460	4250
	475	6940	9680	6640
2	448	1040	1020	1980
	451	2280	1500	1990
	454	3800	10280	13100
	483	11160	7560	5430
3	450	3000	2080	4220
	466	6220	10200	20520
	468	3780	3460	2760
	481	2280	1660	2400
4	445	4700	5260	4540
	452	2800	5850	8200
	470	440	520	520
	482	10590	8280	7600
5	250	7600	10010	9040
	449	2480	3020	3260
	455	6040	5430	5340
	464	5020	1220	560
6	249	1860	2250	2000
	446	5620	4640	3560
	453	500	760	1060
	467	1860	2170	1790
7	469	3680	3350	4680
	473	5700	4240	3880

STUDY 3

Group	Pig Number	Urine Collection (mL)		
		U-1 Days 6-7	**U-2** Days 9-10	**U-3** Days 12-13
1	503	3350	5240	5900
	511	3200	3120	4160
	529	3360	3400	4260
	543	3300	1880	2130
2	502	3160	5120	4680
	505	14340	9920	9960
	506	9610	7220	10570
	527	5460	5390	6990
3	501	4085	5680	5460
	516	4700	4260	4130
	521	2330	3740	4350
	531	1560	2080	2640
4	530	3700	5490	5440
	535	2460	2320	2070
	538	880	980	1080
	541	7020	4020	3810
5	513	7850	5460	6130
	525	4300	3420	6460
	526	3540	2300	3420
	537	11740	10720	12520
6	507	13640	9360	7430
	514	1740	1160	1650
	515	1620	1520	2320
	533	4680	4460	7400
7	504	4040	3800	4200
	508	3660	3400	5000
	519	16800	10360	11250
	534	4660	9040	10600
8	509	1130	1250	860
	532	5740	6100	6380
	536	4400	4310	5840
	540	3660	4580	4700
9	510	7310	5100	2460
	517	3500	4240	6300
	518	840	820	680
	520	2460	1410	2240
10	512	1070	not measured	2380
	522	1980	1920	2960
	539	2170	1900	1540

APPENDIX E

URINARY ARSENIC ANALYTICAL RESULTS FOR STUDY SAMPLES

APPENDIX E URINARY ARSENIC ANALYTICAL RESULTS FOR STUDY SAMPLES

STUDY 1

sample number	tag number	Pig#	Group	Material	Urine Collection Day(s)	Arsenic Dose (ug As/48 hrs)	Q	Urine As Concentration (ug/L)	Urine Volume (mL/48 hrs)	Total As Excreted (ug As/48 hrs)
EP3-2-353-U1	EP3-2-127	353	1	Control	6/7	0		14	3240	45.36
EP3-2-359-U1	EP3-2-123	359	1	Control	6/7	0		14	4000	56
EP3-2-373-U1	EP3-2-105	373	1	Control	6/7	0		12	6123	73.476
EP3-2-354-U1	EP3-2-108	354	6	TM1	6/7	2958		170	2560	435.2
EP3-2-362-U1	EP3-2-126	362	6	TM1	6/7	2958		24	9840	236.16
EP3-2-367-U1	EP3-2-118	367	2	NaAs	6/7	596		70	7680	537.6
EP3-2-370-U1	EP3-2-113	370	2	NaAs	6/7	596		123	4075	501.225
EP3-2-351-U1	EP3-2-130	351	3	NaAs	6/7	1192		250	3530	882.5
EP3-2-356-U1	EP3-2-124	356	3	NaAs	6/7	1192		370	3860	1428.2
EP3-2-361-U1	EP3-2-129	361	3	NaAs	6/7	1192		109	5890	642.01
EP3-2-372-U1	EP3-2-122	372	3	NaAs	6/7	965.52		340	1900	646
EP3-2-358-U1	EP3-2-107	358	4	NaAs	6/7	2384		840	3350	2814
EP3-2-365-U1	EP3-2-128	365	4	NaAs	6/7	2384		150	12560	1884
EP3-2-366-U1	EP3-2-109	366	4	NaAs	6/7	2384		730	2370	1730.1
EP3-2-371-U1	EP3-2-120	371	4	NaAs	6/7	2384		790	2420	1911.8
EP3-2-360-U1	EP3-2-131	360	5	TM1	6/7	1395.712		210	2560	537.6
EP3-2-363-U1	EP3-2-115	363	5	TM1	6/7	1484.8		360	2950	1062
EP3-2-369-U1	EP3-2-110	369	5	TM1	6/7	1484.8		340	3140	1067.6
EP3-2-375-U1	EP3-2-125	375	5	TM1	6/7	1484.8		72	5960	429.12
EP3-2-352-U1	EP3-2-114	352	6	TM1	6/7	2958		122	6480	790.56
EP3-2-364-U1	EP3-2-103	364	6	TM1	6/7	2958		160	12230	1956.8
EP3-2-368-U1	EP3-2-111	368	2	NaAs	6/7	596		260	3540	920.4
EP3-2-374-U1	EP3-2-101	374	2	NaAs	6/7	596		89	42300	3764.7
EP3-2-353-U2	EP3-2-133	353	1	Control	9/10	0		6.3	8290	52.227
EP3-2-359-U2	EP3-2-158	359	1	Control	9/10	0		19	4710	89.49
EP3-2-373-U2	EP3-2-150	373	1	Control	9/10	0		11	5030	55.33
EP3-2-354-U2	EP3-2-144	354	6	TM1	9/10	2958		128	4040	517.12
EP3-2-362-U2	EP3-2-142	362	6	TM1	9/10	2958		14	9960	139.44
EP3-2-367-U2	EP3-2-145	367	2	NaAs	9/10	596		73	8520	621.96
EP3-2-370-U2	EP3-2-137	370	2	NaAs	9/10	596		117	5030	588.51
EP3-2-351-U2	EP3-2-153	351	3	NaAs	9/10	1192		300	3890	1167
EP3-2-356-U2	EP3-2-161	356	3	NaAs	9/10	1192		250	2700	675
EP3-2-361-U2	EP3-2-140	361	3	NaAs	9/10	1192		130	7520	977.6
EP3-2-372-U2	EP3-2-139	372	3	NaAs	9/10	1192		510	2870	1463.7
EP3-2-358-U2	EP3-2-135	358	4	NaAs	9/10	2384		710	2000	1420
EP3-2-365-U2	EP3-2-157	365	4	NaAs	9/10	2384		130	10360	1346.8
EP3-2-366-U2	EP3-2-132	366	4	NaAs	9/10	2384		820	3260	2673.2
EP3-2-371-U2	EP3-2-156	371	4	NaAs	9/10	2384		880	2137	1880.56
EP3-2-360-U2	EP3-2-154	360	5	TM1	9/10	1484.8		100	5660	566
EP3-2-363-U2	EP3-2-138	363	5	TM1	9/10	1484.8		210	1780	373.8
EP3-2-369-U2	EP3-2-141	369	5	TM1	9/10	1484.8		180	3930	707.4
EP3-2-375-U2	EP3-2-147	375	5	TM1	9/10	1484.8		59	8840	521.56
EP3-2-352-U2	EP3-2-149	352	6	TM1	9/10	2958		160	6960	1113.6
EP3-2-364-U2	EP3-2-146	364	6	TM1	9/10	2958		190	6560	1246.4
EP3-2-368-U2	EP3-2-160	368	2	NaAs	9/10	596		300	4660	1398
EP3-2-374-U2	EP3-2-136	374	2	NaAs	9/10	596		91	20130	1831.83
EP3-2-353-U3	EP3-2-178	353	1	Control	12/13	0		7.4	9790	72.446
EP3-2-359-U3	EP3-2-182	359	1	Control	12/13	0		13	5400	70.2
EP3-2-373-U3	EP3-2-173	373	1	Control	12/13	0		13	6120	79.56
EP3-2-354-U3	EP3-2-185	354	6	TM1	12/13	2958		122	3380	412.36
EP3-2-362-U3	EP3-2-181	362	6	TM1	12/13	2958		16	11410	182.56
EP3-2-367-U3	EP3-2-174	367	2	NaAs	12/13	596		77	7730	595.21
EP3-2-370-U3	EP3-2-186	370	2	NaAs	12/13	596		209	4140	865.26
EP3-2-351-U3	EP3-2-189	351	3	NaAs	12/13	1192		260	3660	951.6
EP3-2-356-U3	EP3-2-184	356	3	NaAs	12/13	1192		250	2920	730
EP3-2-361-U3	EP3-2-168	361	3	NaAs	12/13	1192		116	6220	721.52
EP3-2-372-U3	EP3-2-169	372	3	NaAs	12/13	1192		400	1940	776
EP3-2-358-U3	EP3-2-188	358	4	NaAs	12/13	2384		610	2390	1457.9
EP3-2-365-U3	EP3-2-187	365	4	NaAs	12/13	2384		200	16320	3264
EP3-2-366-U3	EP3-2-183	366	4	NaAs	12/13	2384		770	2480	1909.6
EP3-2-371-U3	EP3-2-175	371	4	NaAs	12/13	2384		870	2660	2314.2
EP3-2-360-U3	EP3-2-167	360	5	TM1	12/13	1484.8		92	5260	483.92
EP3-2-363-U3	EP3-2-172	363	5	TM1	12/13	1484.8		210	2102	441.42
EP3-2-369-U3	EP3-2-180	369	5	TM1	12/13	1484.8		200	1980	396
EP3-2-375-U3	EP3-2-177	375	5	TM1	12/13	1484.8		67	9220	617.74
EP3-2-352-U3	EP3-2-176	352	6	TM1	12/13	2958		220	4830	1062.6
EP3-2-364-U3	EP3-2-166	364	6	TM1	12/13	2958		110	7180	789.8
EP3-2-368-U3	EP3-2-171	368	2	NaAs	12/13	596		230	3420	786.6
EP3-2-374-U3	EP3-2-170	374	2	NaAs	12/13	596		85	37980	3228.3

STUDY 2

sample number	tag number	Pig#	Group	Material	Urine Collection Day(s)	Arsenic Dose	Q	Urine As Concentration (ug/L)	Urine Volume (mL/48 hrs)	Total As Excreted (ug As/48 hrs)
MS-5-463-U1	MS-5-126	463	1	NaAs	6/7	599		108	3740	403.92
MS-5-465-U1	MS-5-120	465	1	NaAs	6/7	599		260	1180	306.8
MS-5-474-U1	MS-5-119	474	1	NaAs	6/7	545		150	4250	637.5
MS-5-475-U1	MS-5-130	475	1	NaAs	6/7	599		76	6940	527.44
MS-5-448-U1	MS-5-118	448	2	NaAs	6/7	1196		530	1020	540.6
MS-5-451-U1	MS-5-128	451	2	NaAs	6/7	1196		370	1990	736.3
MS-5-454-U1	MS-5-110	454	2	NaAs	6/7	1196		280	3800	1064
MS-5-483-U1	MS-5-121	483	2	NaAs	6/7	1196		91	7560	687.96
MS-5-450-U1	MS-5-103	450	3	TM1	6/7	981		130	4220	548.6
MS-5-466-U1	MS-5-102	466	3	TM1	6/7	981		65	6220	404.3
MS-5-468-U1	MS-5-112	468	3	TM1	6/7	864		115	3460	397.9
MS-5-481-U1	MS-5-108	481	3	TM1	6/7	981		190	2400	456
MS-5-445-U1	MS-5-114	445	4	TM1	6/7	1472		73	4700	343.1
MS-5-452-U1	MS-5-101	452	4	TM1	6/7	1472		190	5850	1111.5
MS-5-470-U1	MS-5-109	470	4	TM1	6/7	1472		1200	520	624
MS-5-482-U1	MS-5-122	482	4	TM1	6/7	1472		64	10590	677.76
MS-5-250-U1	MS-5-116	250	5	TM1	6/7	2944		150	10010	1501.5
MS-5-449-U1	MS-5-123	449	5	TM1	6/7	2944		440	3260	1434.4
MS-5-455-U1	MS-5-133	455	5	TM1	6/7	2944		200	6040	1208
MS-5-464-U1	MS-5-105	464	5	TM1	6/7	2944		160	1220	195.2
MS-5-249-U1	MS-5-124	249	6	TM2	6/7	968		200	2000	400
MS-5-446-U1	MS-5-131	446	6	TM2	6/7	968		62	5620	348.44
MS-5-453-U1	MS-5-106	453	6	TM2	6/7	968		620	760	471.2
MS-5-467-U1	MS-5-134	467	6	TM2	6/7	968		170	1790	304.3
MS-5-469-U1	MS-5-115	469	7	Control	6/7	0		17	3680	62.56
MS-5-473-U1	MS-5-127	473	7	Control	6/7	0		11	4240	46.64
MS-5-463-U2	MS-5-144	463	1	NaAs	9/10	599		99	6780	671.22
MS-5-465-U2	MS-5-167	465	1	NaAs	9/10	599		440	1720	756.8
MS-5-474-U2	MS-5-161	474	1	NaAs	9/10	599		139	3460	480.94
MS-5-475-U2	MS-5-136	475	1	NaAs	9/10	599		56	6640	371.84
MS-5-448-U2	MS-5-150	448	2	NaAs	9/10	1196		850	1040	884
MS-5-451-U2	MS-5-142	451	2	NaAs	9/10	1196		520	1500	780
MS-5-454-U2	MS-5-158	454	2	NaAs	9/10	1196		94	13100	1231.4
MS-5-483-U2	MS-5-163	483	2	NaAs	9/10	1196		130	11160	1450.8
MS-5-450-U2	MS-5-164	450	3	TM1	9/10	981		220	2080	457.6
MS-5-466-U2	MS-5-154	466	3	TM1	9/10	981		44	20520	902.88
MS-5-468-U2	MS-5-145	468	3	TM1	9/10	981		120	3780	453.6
MS-5-481-U2	MS-5-143	481	3	TM1	9/10	981		250	1660	415
MS-5-445-U2	MS-5-153	445	4	TM1	9/10	1472		128	4540	581.12
MS-5-452-U2	MS-5-165	452	4	TM1	9/10	1472		112	2800	313.6
MS-5-470-U2	MS-5-159	470	4	TM1	9/10	1472		840	520	436.8
MS-5-482-U2	MS-5-162	482	4	TM1	9/10	1472		79	7600	600.4
MS-5-250-U2	MS-5-155	250	5	TM1	9/10	2944		93	7600	706.8
MS-5-449-U2	MS-5-139	449	5	TM1	9/10	2944		390	3020	1177.8
MS-5-455-U2	MS-5-135	455	5	TM1	9/10	2944		240	5340	1281.6
MS-5-464-U2	MS-5-160	464	5	TM1	9/10	2944		780	5020	3915.6
MS-5-249-U2	MS-5-140	249	6	TM2	9/10	968		150	2250	337.5
MS-5-446-U2	MS-5-138	446	6	TM2	9/10	968		84	3560	299.04
MS-5-453-U2	MS-5-149	453	6	TM2	9/10	968		390	500	195
MS-5-467-U2	MS-5-141	467	6	TM2	9/10	968		160	2170	347.2
MS-5-469-U2	MS-5-146	469	7	Control	9/10	0		31	4680	145.08
MS-5-473-U2	MS-5-156	473	7	Control	9/10	0		11	5700	62.7
MS-5-463-U3	MS-5-172	463	1	NaAs	12/13	599		80	5380	430.4
MS-5-465-U3	MS-5-173	465	1	NaAs	12/13	599		230	2370	545.1
MS-5-474-U3	MS-5-201	474	1	NaAs	12/13	599		106	3200	339.2
MS-5-475-U3	MS-5-182	475	1	NaAs	12/13	599		87	9680	842.16
MS-5-448-U3	MS-5-169	448	2	NaAs	12/13	1196		470	1980	930.6
MS-5-451-U3	MS-5-184	451	2	NaAs	12/13	1196		520	2280	1185.6
MS-5-454-U3	MS-5-192	454	2	NaAs	12/13	1196		77	10280	791.56

STUDY 2

sample number	tag number	Pig#	Group	Material	Urine Collection Day(s)	Arsenic Dose	Q	Urine As Concentration (ug/L)	Urine Volume (mL/48 hrs)	Total As Excreted (ug As/48 hrs)
MS-5-483-U3	MS-5-189	483	2	NaAs	12/13	1196		200	5430	1086
MS-5-450-U3	MS-5-197	450	3	TM1	12/13	981		110	3000	330
MS-5-466-U3	MS-5-177	466	3	TM1	12/13	981		25	10200	255
MS-5-468-U3	MS-5-185	468	3	TM1	12/13	981		180	2760	496.8
MS-5-481-U3	MS-5-187	481	3	TM1	12/13	981		170	2280	387.6
MS-5-445-U3	MS-5-168	445	4	TM1	12/13	1472		140	5260	736.4
MS-5-452-U3	MS-5-190	452	4	TM1	12/13	1472		82	8200	672.4
MS-5-470-U3	MS-5-181	470	4	TM1	12/13	1472		900	440	396
MS-5-482-U3	MS-5-174	482	4	TM1	12/13	1472		90	8280	745.2
MS-5-250-U3	MS-5-198	250	5	TM1	12/13	2944		150	9040	1356
MS-5-449-U3	MS-5-176	449	5	TM1	12/13	2944		280	2480	694.4
MS-5-455-U3	MS-5-178	455	5	TM1	12/13	2944		240	5430	1303.2
MS-5-464-U3	MS-5-194	464	5	TM1	12/13	2944		1800	560	1008
MS-5-249-U3	MS-5-175	249	6	TM2	12/13	968		170	1860	316.2
MS-5-446-U3	MS-5-196	446	6	TM2	12/13	968		122	4640	566.08
MS-5-453-U3	MS-5-200	453	6	TM2	12/13	968		380	1060	402.8
MS-5-467-U3	MS-5-183	467	6	TM2	12/13	968		170	1860	316.2
MS-5-469-U3	MS-5-199	469	7	Control	12/13	0		17	3350	56.95
MS-5-473-U3	MS-5-170	473	7	Control	12/13	0		17	3880	65.96

STUDY 3

sample number	tag number	Pig#	Group	Material	Urine Collection Day(s)	Arsenic Dose	Q	Urine As Concentration (ug/L)	Urine Volume (mL/48 hrs)	Total As Excreted (ug As/48 hrs)
BOrch-MS4&8-503-U1	BOrch-MS4&8-123	503	1	NaAs	6/7	868		150	3350	504
BOrch-MS4&8-511-U1	BOrch-MS4&8-105	511	1	NaAs	6/7	868		210	3200	693
BOrch-MS4&8-529-U1	BOrch-MS4&8-122	529	1	NaAs	6/7	868		140	3360	442.4
BOrch-MS4&8-543-U1	BOrch-MS4&8-106	543	1	NaAs	6/7	868		210	3300	3011.4
BOrch-MS4&8-502-U1	BOrch-MS4&8-102	502	2	NaAs	6/7	1720		410	3160	3940.1
BOrch-MS4&8-505-U1	BOrch-MS4&8-119	505	2	NaAs	6/7	1720		92	14340	502.32
BOrch-MS4&8-506-U1	BOrch-MS4&8-110	506	2	NaAs	6/7	1720		160	9610	653.6
BOrch-MS4&8-527-U1	BOrch-MS4&8-103	527	2	NaAs	6/7	1720		250	5460	1175
BOrch-MS4&8-501-U1	BOrch-MS4&8-101	501	3	NaAs	6/7	3480		660	4085	1537.8
BOrch-MS4&8-516-U1	BOrch-MS4&8-120	516	3	NaAs	6/7	3480		620	4700	967.2
BOrch-MS4&8-521-U1	BOrch-MS4&8-121	521	3	NaAs	6/7	3480		720	2330	2664
BOrch-MS4&8-531-U1	BOrch-MS4&8-129	531	3	NaAs	6/7	3480		1500	1560	3690
BOrch-MS4&8-530-U1	BOrch-MS4&8-117	530	4	TM1	6/7	1799		170	3700	149.6
BOrch-MS4&8-535-U1	BOrch-MS4&8-118	535	4	TM1	6/7	1799		300	2460	2106
BOrch-MS4&8-538-U1	BOrch-MS4&8-124	538	4	TM1	6/7	1799		770	880	6044.5
BOrch-MS4&8-541-U1	BOrch-MS4&8-113	541	4	TM1	6/7	1799		99	7020	425.7
BOrch-MS4&8-513-U1	BOrch-MS4&8-128	513	5	TM1	6/7	2699		110	7850	389.4
BOrch-MS4&8-525-U1	BOrch-MS4&8-116	525	5	TM1	6/7	2699		190	4300	2230.6
BOrch-MS4&8-526-U1	BOrch-MS4&8-107	526	5	TM1	6/7	2699		190	3540	2591.6
BOrch-MS4&8-537-U1	BOrch-MS4&8-125	537	5	TM1	6/7	2699		54	11740	93.96
BOrch-MS4&8-507-U1	BOrch-MS4&8-126	507	6	TM1	6/7	5397		120	13640	194.4
BOrch-MS4&8-514-U1	BOrch-MS4&8-127	514	6	TM1	6/7	5397		960	1740	4492.8
BOrch-MS4&8-515-U1	BOrch-MS4&8-112	515	6	TM1	6/7	5397		1000	1620	4040
BOrch-MS4&8-533-U1	BOrch-MS4&8-218	533	6	TM1	6/7	5397		400	4680	1464
BOrch-MS4&8-504-U1	BOrch-MS4&8-221	504	7	TM2	6/7	1633		100	4040	1680
BOrch-MS4&8-508-U1	BOrch-MS4&8-217	508	7	TM2	6/7	1633		190	3660	885.4
BOrch-MS4&8-519-U1	BOrch-MS4&8-212	519	7	TM2	6/7	1633		49	16800	55.37
BOrch-MS4&8-534-U1	BOrch-MS4&8-220	534	7	TM2	6/7	1633		140	4660	803.6
BOrch-MS4&8-509-U1	BOrch-MS4&8-215	509	8	TM2	6/7	2450		850	1130	3740
BOrch-MS4&8-532-U1	BOrch-MS4&8-216	532	8	TM2	6/7	2450		140	5740	512.4
BOrch-MS4&8-536-U1	BOrch-MS4&8-219	536	8	TM2	6/7	2450		230	4400	1681.3
BOrch-MS4&8-540-U1	BOrch-MS4&8-225	540	8	TM2	6/7	2450		270	3660	945
BOrch-MS4&8-510-U1	BOrch-MS4&8-222	510	9	TM2	6/7	4900		230	7310	193.2
BOrch-MS4&8-517-U1	BOrch-MS4&8-224	517	9	TM2	6/7	4900		580	3500	1426.8
BOrch-MS4&8-518-U1	BOrch-MS4&8-213	518	9	TM2	6/7	4900		2040	840	2182.8
BOrch-MS4&8-520-U1	BOrch-MS4&8-211	520	9	TM2	6/7	4900		800	2460	1584
BOrch-MS4&8-512-U1	BOrch-MS4&8-226	512	10	Control	6/7	0		72	1070	156.24
BOrch-MS4&8-522-U1	BOrch-MS4&8-223	522	10	Control	6/7	0		50	1980	262
BOrch-MS4&8-539-U1	BOrch-MS4&8-214	539	10	Control	6/7	0		35	2170	109.2
BOrch-MS4&8-503-U2	BOrch-MS4&8-148	503	1	NaAs	9/10	868		120	5240	408
BOrch-MS4&8-511-U2	BOrch-MS4&8-146	511	1	NaAs	9/10	868		260	3120	488.8
BOrch-MS4&8-529-U2	BOrch-MS4&8-152	529	1	NaAs	9/10	868		220	3400	1126.4
BOrch-MS4&8-543-U2	BOrch-MS4&8-143	543	1	NaAs	9/10	868		230	1880	2281.6
BOrch-MS4&8-502-U2	BOrch-MS4&8-158	502	2	NaAs	9/10	1720		290	5120	2093.8
BOrch-MS4&8-505-U2	BOrch-MS4&8-135	505	2	NaAs	9/10	1720		140	9920	754.6
BOrch-MS4&8-506-U2	BOrch-MS4&8-144	506	2	NaAs	9/10	1720		180	7220	1022.4
BOrch-MS4&8-527-U2	BOrch-MS4&8-155	527	2	NaAs	9/10	1720		260	5390	1107.6
BOrch-MS4&8-501-U2	BOrch-MS4&8-134	501	3	NaAs	9/10	3480		480	5680	1795.2
BOrch-MS4&8-516-U2	BOrch-MS4&8-132	516	3	NaAs	9/10	3480		480	4260	998.4
BOrch-MS4&8-521-U2	BOrch-MS4&8-154	521	3	NaAs	9/10	3480		730	3740	4007.7
BOrch-MS4&8-531-U2	BOrch-MS4&8-136	531	3	NaAs	9/10	3480		1300	2080	3016
BOrch-MS4&8-530-U2	BOrch-MS4&8-150	530	4	TM1	9/10	1799		99	5490	97.02
BOrch-MS4&8-535-U2	BOrch-MS4&8-160	535	4	TM1	9/10	1799		360	2320	1447.2
BOrch-MS4&8-538-U2	BOrch-MS4&8-159	538	4	TM1	9/10	1799		610	980	3330.6
BOrch-MS4&8-541-U2	BOrch-MS4&8-133	541	4	TM1	9/10	1799		160	4020	547.2
BOrch-MS4&8-513-U2	BOrch-MS4&8-140	513	5	TM1	9/10	2699		170	5460	391
BOrch-MS4&8-525-U2	BOrch-MS4&8-141	525	5	TM1	9/10	2699		280	3420	3001.6
BOrch-MS4&8-526-U2	BOrch-MS4&8-139	526	5	TM1	9/10	2699		410	2300	3837.6
BOrch-MS4&8-537-U2	BOrch-MS4&8-161	537	5	TM1	9/10	2699		81	10720	93.96
BOrch-MS4&8-507-U2	BOrch-MS4&8-157	507	6	TM1	9/10	5397		170	9360	258.4
BOrch-MS4&8-514-U2	BOrch-MS4&8-142	514	6	TM1	9/10	5397		1400	1160	6244
BOrch-MS4&8-515-U2	BOrch-MS4&8-137	515	6	TM1	9/10	5397		1200	1520	4560
BOrch-MS4&8-533-U2	BOrch-MS4&8-236	533	6	TM1	9/10	5397		310	4460	1054
BOrch-MS4&8-504-U2	BOrch-MS4&8-234	504	7	TM2	9/10	1633		150	3800	1554
BOrch-MS4&8-508-U2	BOrch-MS4&8-235	508	7	TM2	9/10	1633		200	3400	1808
BOrch-MS4&8-519-U2	BOrch-MS4&8-240	519	7	TM2	9/10	1633		79	10360	98.75
BOrch-MS4&8-534-U2	BOrch-MS4&8-228	534	7	TM2	9/10	1633		60	9040	366
BOrch-MS4&8-509-U2	BOrch-MS4&8-231	509	8	TM2	9/10	2450		760	1250	3275.6
BOrch-MS4&8-532-U2	BOrch-MS4&8-238	532	8	TM2	9/10	2450		200	6100	916
BOrch-MS4&8-536-U2	BOrch-MS4&8-232	536	8	TM2	9/10	2450		250	4310	1275

STUDY 3

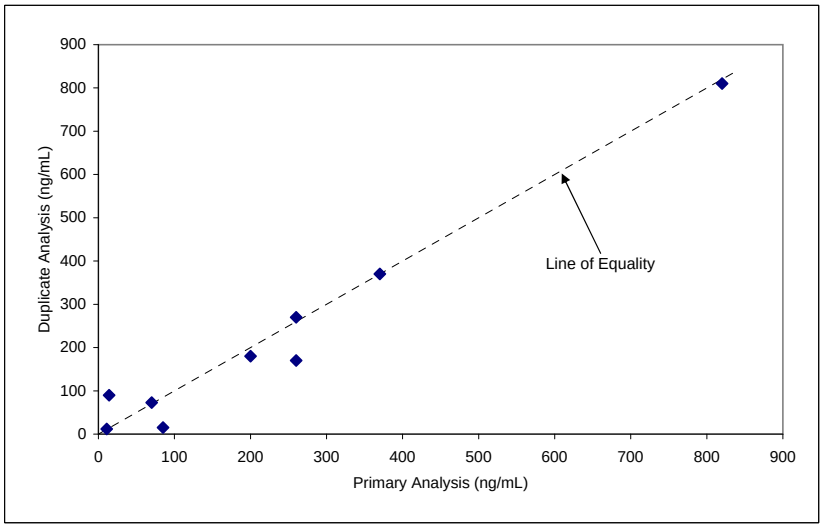
sample number	tag number	Pig#	Group	Material	Urine Collection Day(s)	Arsenic Dose	Q	Urine As Concentration (ug/L)	Urine Volume (mL/48 hrs)	Total As Excreted (ug As/48 hrs)
BOrch-MS4&8-540-U2	BOrch-MS4&8-241	540	8	TM2	9/10	2450		230	4580	975.2
BOrch-MS4&8-510-U2	BOrch-MS4&8-239	510	9	TM2	9/10	4900		480	5100	393.6
BOrch-MS4&8-517-U2	BOrch-MS4&8-233	517	9	TM2	9/10	4900		510	4240	719.1
BOrch-MS4&8-518-U2	BOrch-MS4&8-237	518	9	TM2	9/10	4900		2420	820	0
BOrch-MS4&8-520-U2	BOrch-MS4&8-230	520	9	TM2	9/10	4900		960	1410	1843.2
BOrch-MS4&8-512-U2	BOrch-MS4&8-229	512	10	Control	9/10	0		110		209
BOrch-MS4&8-522-U2	BOrch-MS4&8-227	522	10	Control	9/10	0		47	1920	277.3
BOrch-MS4&8-522-U2	BOrch-MS4&8-309	539	10	Control	9/10	0		60	1900	249.6
BOrch-MS4&8-503-U3	BOrch-MS4&8-183	503	1	NaAs	12/13	868		100	5900	426
BOrch-MS4&8-511-U3	BOrch-MS4&8-171	511	1	NaAs	12/13	868		190	4160	404.7
BOrch-MS4&8-529-U3	BOrch-MS4&8-177	529	1	NaAs	12/13	868		190	4260	889.2
BOrch-MS4&8-543-U3	BOrch-MS4&8-192	543	1	NaAs	12/13	868		230	2130	2290.8
BOrch-MS4&8-502-U3	BOrch-MS4&8-182	502	2	NaAs	12/13	1720		330	4680	3488.1
BOrch-MS4&8-505-U3	BOrch-MS4&8-185	505	2	NaAs	12/13	1720		120	9960	838.8
BOrch-MS4&8-506-U3	BOrch-MS4&8-180	506	2	NaAs	12/13	1720		94	10570	513.24
BOrch-MS4&8-527-U3	BOrch-MS4&8-166	527	2	NaAs	12/13	1720		190	6990	784.7
BOrch-MS4&8-501-U3	BOrch-MS4&8-189	501	3	NaAs	12/13	3480		460	5460	2001
BOrch-MS4&8-516-U3	BOrch-MS4&8-181	516	3	NaAs	12/13	3480		480	4130	1267.2
BOrch-MS4&8-521-U3	BOrch-MS4&8-178	521	3	NaAs	12/13	3480		520	4350	2828.8
BOrch-MS4&8-531-U3	BOrch-MS4&8-162	531	3	NaAs	12/13	3480		1100	2640	2277
BOrch-MS4&8-530-U3	BOrch-MS4&8-184	530	4	TM1	12/13	1799		61	5440	65.88
BOrch-MS4&8-535-U3	BOrch-MS4&8-172	535	4	TM1	12/13	1799		400	2070	1524
BOrch-MS4&8-538-U3	BOrch-MS4&8-191	538	4	TM1	12/13	1799		470	1080	2881.1
BOrch-MS4&8-541-U3	BOrch-MS4&8-173	541	4	TM1	12/13	1799		200	3810	1292
BOrch-MS4&8-513-U3	BOrch-MS4&8-167	513	5	TM1	12/13	2699		170	6130	581.4
BOrch-MS4&8-525-U3	BOrch-MS4&8-174	525	5	TM1	12/13	2699		89	6460	1114.28
BOrch-MS4&8-526-U3	BOrch-MS4&8-163	526	5	TM1	12/13	2699		300	3420	2229
BOrch-MS4&8-537-U3	BOrch-MS4&8-186	537	5	TM1	12/13	2699		60	12520	99
BOrch-MS4&8-507-U3	BOrch-MS4&8-170	507	6	TM1	12/13	5397		230	7430	533.6
BOrch-MS4&8-514-U3	BOrch-MS4&8-188	514	6	TM1	12/13	5397		1200	1650	8880
BOrch-MS4&8-515-U3	BOrch-MS4&8-165	515	6	TM1	12/13	5397		730	2320	3066
BOrch-MS4&8-533-U3	BOrch-MS4&8-244	533	6	TM1	12/13	5397		210	7400	1050
BOrch-MS4&8-504-U3	BOrch-MS4&8-246	504	7	TM2	12/13	1633		120	4200	1350
BOrch-MS4&8-508-U3	BOrch-MS4&8-256	508	7	TM2	12/13	1633		170	5000	1802
BOrch-MS4&8-519-U3	BOrch-MS4&8-245	519	7	TM2	12/13	1633		78	11250	67.08
BOrch-MS4&8-534-U3	BOrch-MS4&8-252	534	7	TM2	12/13	1633		71	10600	452.98
BOrch-MS4&8-509-U3	BOrch-MS4&8-249	509	8	TM2	12/13	2450		1100	860	6424
BOrch-MS4&8-532-U3	BOrch-MS4&8-250	532	8	TM2	12/13	2450		150	6380	705
BOrch-MS4&8-536-U3	BOrch-MS4&8-247	536	8	TM2	12/13	2450		190	5840	467.4
BOrch-MS4&8-540-U3	BOrch-MS4&8-248	540	8	TM2	12/13	2450		210	4700	1323
BOrch-MS4&8-510-U3	BOrch-MS4&8-255	510	9	TM2	12/13	4900		940	2460	639.2
BOrch-MS4&8-517-U3	BOrch-MS4&8-242	517	9	TM2	12/13	4900		330	6300	739.2
BOrch-MS4&8-518-U3	BOrch-MS4&8-253	518	9	TM2	12/13	4694		2130	680	5069.4
BOrch-MS4&8-520-U3	BOrch-MS4&8-243	520	9	TM2	12/13	4900		820	2240	2427.2
BOrch-MS4&8-512-U3	BOrch-MS4&8-254	512	10	Control	12/13	0		40	2380	61.6
BOrch-MS4&8-522-U3	BOrch-MS4&8-251	522	10	Control	12/13	0		30	2960	88.8
BOrch-MS4&8-522-U3	BOrch-MS4&8-310	539	10	Control	12/13	0		42	1540	64.68

APPENDIX F

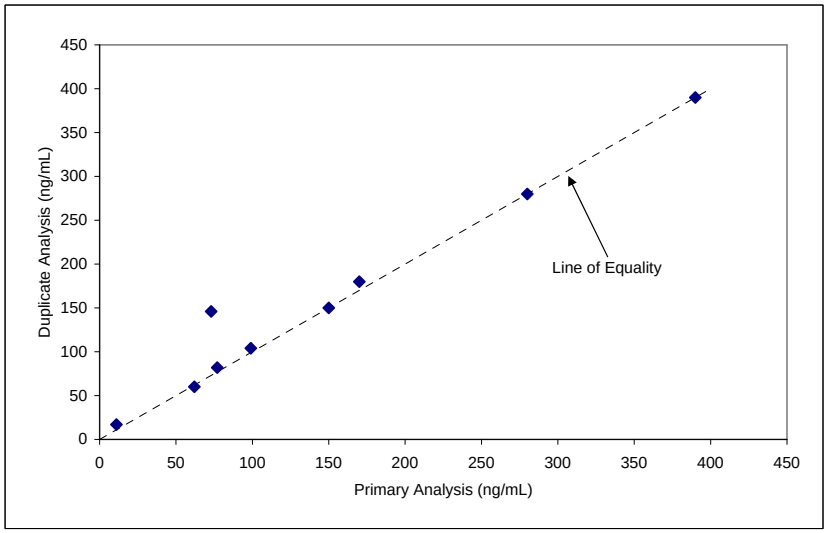
ARSENIC ANALYTICAL RESULTS FOR QUALITY CONTROL SAMPLES

FIGURE F-1 URINARY ARSENIC BLIND DUPLICATES

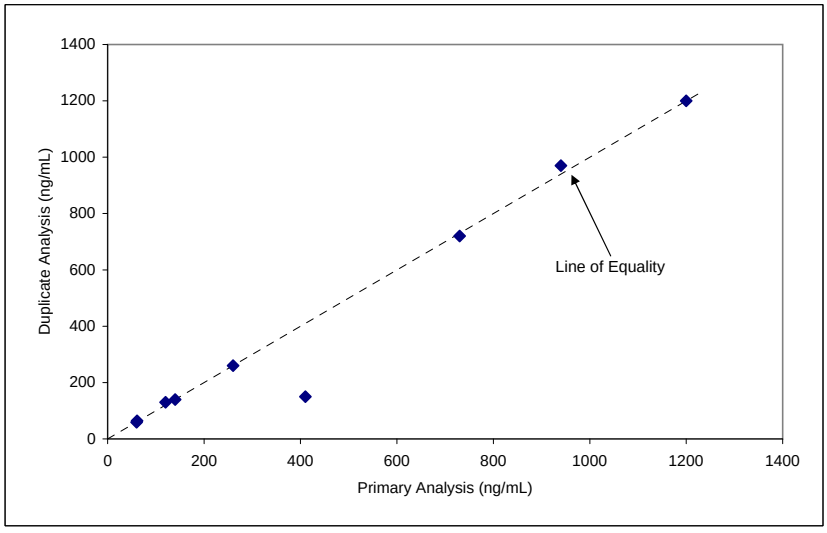
STUDY 1



STUDY 2



STUDY 3



APPENDIX F ARSENIC ANALYTICAL RESULTS FOR QUALITY CONTROL SAMPLES

TABLE F-1 BLIND DUPLICATE SAMPLES

STUDY 1

Blind Duplicate Sample ID	Sample type	Pig Number	Urine Collection Days	Duplicate concentration (ppb)	Sample Concentration (ppb)	RPD
EP3-2-119	Urine	367	U1	73	70	4%
EP3-2-121	Urine	356	U1	370	370	0%
EP3-2-112	Urine	368	U1	170	260	42%
EP3-2-148	Urine	373	U2	12	11	9%
EP3-2-151	Urine	362	U2	90	14	146%
EP3-2-159	Urine	366	U2	810	820	1%
EP3-2-179	Urine	351	U3	270	260	4%
EP3-2-164	Urine	369	U3	180	200	11%
EP3-2-162	Urine	374	U3	15	85	140%

STUDY 2

Blind Duplicate Sample ID	Sample type	Pig Number	Urine Collection Days	Duplicate concentration (ppb)	Sample Concentration (ppb)	RPD
MS-5-113	Urine	445	U1	146	73	67%
MS-5-125	Urine	446	U1	60	62	3%
MS-5-132	Urine	474	U1	150	280	60%
MS-5-166	Urine	453	U2	390	390	0%
MS-5-151	Urine	463	U2	104	77	30%
MS-5-152	Urine	473	U2	17	99	141%
MS-5-179	Urine	449	U3	280	11	185%
MS-5-191	Urine	454	U3	82	150	59%
MS-5-171	Urine	481	U3	180	170	6%

STUDY 3

Blind Duplicate Sample ID	Sample type	Pig Number	Urine Collection Days	Duplicate concentration (ppb)	Sample Concentration (ppb)	RPD
BOrch-MS4&8-104	Urine	502	U1	150	410	93%
BOrch-MS4&8-114	Urine	507	U1	130	120	8%
BOrch-MS4&8-109	Urine	532	U1	140	140	0%
BOrch-MS4&8-145	Urine	511	U2	260	260	0%
BOrch-MS4&8-147	Urine	521	U2	720	730	1%
BOrch-MS4&8-153	Urine	539	U2	59	60	2%
BOrch-MS4&8-168	Urine	510	U3	970	940	3%
BOrch-MS4&8-164	Urine	514	U3	1200	1200	0%
BOrch-MS4&8-190	Urine	530	U3	64	61	5%

TABLE F-2. LABORATORY SPIKES

STUDY 1

Sample ID	Sample Type	Nominal Arsenic (ppb)	Spiked Concentration (ppb)	Sample concentration (ppb)	Recovered Spike (ppb)	% Recovery
EP3-2-129	Urine	200	320	109	211	106%
EP3-2-114	Urine	200	340	122	218	109%
EP3-2-144	Urine	200	330	128	202	101%
EP3-2-132	Urine	200	1000	820	180	90%
EP3-2-148	Urine	200	210	12	198	99%
EP3-2-189	Urine	200	460	260	200	100%
EP3-2-180	Urine	200	390	200	190	95%
EP3-2-162	Urine	200	220	15	205	103%
EP3-2-409	Feed	9880	10000	<50	10000	101%
EP3-2-414	Water	100	100	<0.5	100	100%

STUDY 2

Sample ID	Sample Type	Nominal Arsenic (ppb)	Spiked Concentration (ppb)	Sample concentration (ppb)	Recovered Spike (ppb)	% Recovery
MS-5-113	Urine	200	340	146	194	97%
MS-5-124	Urine	200	406	200	206	103%
MS-5-135	Urine	200	453	240	213	107%
MS-5-146	Urine	200	220	31	189	95%
MS-5-159	Urine	200	1100	840	260	130%
MS-5-169	Urine	294	800	470	330	112%
MS-5-179	Urine	200	510	280	230	115%
MS-5-192	Urine	200	290	77	213	107%
MS-5-200	Urine	200	556	380	176	88%
MS-5-223	Feed	9940	9600	70	9530	96%
MS-5-225	Water	111	110	<1	110	99%

STUDY 3

Sample ID	Sample Type	Nominal Arsenic (ppb)	Spiked Concentration (ppb)	Sample concentration (ppb)	Recovered Spike (ppb)	% Recovery
BOrch-MS4&8-110	Urine	200	360	160	200	100%
BOrch-MS4&8-121	Urine	1000	1700	720	980	98%
BOrch-MS4&8-131	Urine	200	260	55	205	103%
BOrch-MS4&8-135	Urine	200	350	140	210	105%
BOrch-MS4&8-145	Urine	200	480	260	220	110%
BOrch-MS4&8-155	Urine	200	460	260	200	100%
BOrch-MS4&8-169	Urine	200	240	38	202	101%
BOrch-MS4&8-179	Urine	200	260	55	205	103%
BOrch-MS4&8-189	Urine	1000	1400	460	940	94%
BOrch-MS4&8-220	Urine	200	350	140	210	105%
BOrch-MS4&8-230	Urine	200	1130	960	170	85%
BOrch-MS4&8-240	Urine	200	280	79	201	101%
BOrch-MS4&8-248	Urine	200	426	210	216	108%
BOrch-MS4&8-311	Feed	9980	10000	0.1	9999.9	100%
BOrch-MS4&8-312	Water	100	97	<1	97	97%
BOrch-MS4&8-316	Water	100	99	<1	99	99%

TABLE F-3 LABORATORY DUPLICATES

STUDY 1

Sample ID	Sample Type	Duplicate Concentration	Sample Concentration	Units	RPD
EP3-2-120	Urine	790	790	ug/L	0%
EP3-2-121	Urine	380	370	ug/L	3%
EP3-2-126	Urine	23	24	ug/L	4%
EP3-2-147	Urine	58	59	ug/L	2%
EP3-2-161	Urine	260	250	ug/L	4%
EP3-2-170	Urine	85	85	ug/L	0%
EP3-2-173	Urine	13	13	ug/L	0%
EP3-2-187	Urine	220	200	ug/L	10%
EP3-2-407	Feed	150	220	ng/g	38%
EP3-2-410	Water	0.7	1	ug/L	35%

STUDY 2

Sample ID	Sample Type	Duplicate Concentration	Sample Concentration	Units	RPD
MS-5-106	Urine	600	620	ug/L	3%
MS-5-119	Urine	140	150	ug/L	7%
MS-5-130	Urine	76	76	ug/L	0%
MS-5-141	Urine	160	160	ug/L	0%
MS-5-153	Urine	129	128	ug/L	1%
MS-5-164	Urine	220	220	ug/L	0%
MS-5-174	Urine	89	90	ug/L	1%
MS-5-185	Urine	180	180	ug/L	0%
MS-5-197	Urine	117	110	ug/L	6%
MS-5-227	Water	<1	<1	ug/L	0%
MS-5-223	Feed	100	70	ng/g	35%

STUDY 3

Sample ID	Sample Type	Duplicate Concentration	Sample Concentration	Units	RPD
BOrch-MS4&8-105	Urine	210	210	ug/L	0%
BOrch-MS4&8-116	Urine	200	190	ug/L	5%
BOrch-MS4&8-126	Urine	120	120	ug/L	0%
BOrch-MS4&8-140	Urine	170	170	ug/L	0%
BOrch-MS4&8-150	Urine	99	99	ug/L	0%
BOrch-MS4&8-160	Urine	370	360	ug/L	3%
BOrch-MS4&8-164	Urine	1200	1200	ug/L	0%
BOrch-MS4&8-174	Urine	94	89	ug/L	5%
BOrch-MS4&8-184	Urine	60	61	ug/L	2%
BOrch-MS4&8-215	Urine	850	850	ug/L	0%
BOrch-MS4&8-225	Urine	270	270	ug/L	0%
BOrch-MS4&8-235	Urine	190	200	ug/L	5%
BOrch-MS4&8-243	Urine	840	820	ug/L	2%
BOrch-MS4&8-253	Urine	2230	2130	ug/L	5%
BOrch-MS4&8-311	Feed	0.2	0.1	ug/g	67%
BOrch-MS4&8-314	Water	<1	<1	ug/L	0%

TABLE F-4 LABORATORY QUALITY CONTROL STANDARDS

STUDY 1

Tag Number	Arsenic Concentration	DL	Units	SRMID	Certified Mean
QC-1	<3	3	ug/L	NIST 2670a-L	3
QC-2	230	5	ug/L	NIST 2670a-H	220 ± 10
QC-3	<3	3	ug/L	NIST 2670a-L	3
QC-4	220	5	ug/L	NIST 2670a-H	220 ± 10
QC-5	28	1	ug/L	NIST 1640	26.7 ± 0.41
QC-6	21000	500	ng/g	NRCC TORT-2	21,600 ± 1,800

STUDY 2

Tag Number	Arsenic Concentration	DL	Units	SRMID	Certified Mean
QC-1	4	2	ug/L	NIST 2670a-L	3
QC-2	220	4	ug/L	NIST 2670a-H	220 ± 10
QC-3	<3	3	ug/L	NIST 2670a-L	3
QC-4	230	4	ug/L	NIST 2670a-H	220 ± 10
QC-5	220	4	ug/L	NIST 2670a-H	220 ± 10
QC-6	55	1	ug/L	NIST 1643e	60.5
QC-7	7.1	200	ug/g	NIST 1566b	7.65 +/-0.65

STUDY 3

Tag Number	Arsenic Concentration	DL	Units	SRMID	Certified Mean
QC-1	6	2	ug/L	NIST 2670a-L	3
QC-2	210	9	ug/L	NIST 2670a-H	220 ± 10
QC-3	230	9	ug/L	NIST 2670a-H	220 ± 10
QC-4	230	9	ug/L	NIST 2670a-H	220 ± 10
QC-5	3	2	ug/L	NIST 2670a-L	3
QC-6	220	9	ug/L	NIST 2670a-H	220 ± 10
QC-7	220	9	ug/L	NIST 2670a-H	220 ± 10
QC-8	57	1	ug/L	NIST 1643e	60.5
QC-9	7.2	0.2	ug/g	NIST 1566b	7.65 +/-0.65

TABLE F-5 BLANKS

STUDY 1

Tag Number	Arsenic Concentration	DL	Units
Blank-1	<1	1	ug/L
Blank-2	<1	1	ug/L
Blank-3	<1	1	ug/L
Blank-4	<1	1	ug/L
Blank-5	<0.5	0.5	ug/L
Blank-6	<50	50	ng/g

STUDY 2

Tag Number	Arsenic Concentration	DL	Units
Blank-1	<1	1	ug/L
Blank-2	<1	1	ug/L
Blank-3	<1	1	ug/L
Blank-4	<1	1	ug/L
Blank-5	<1	1	ug/L
Blank-6	<1	1	ug/L
Blank-7	<50	50	ng/g

STUDY 3

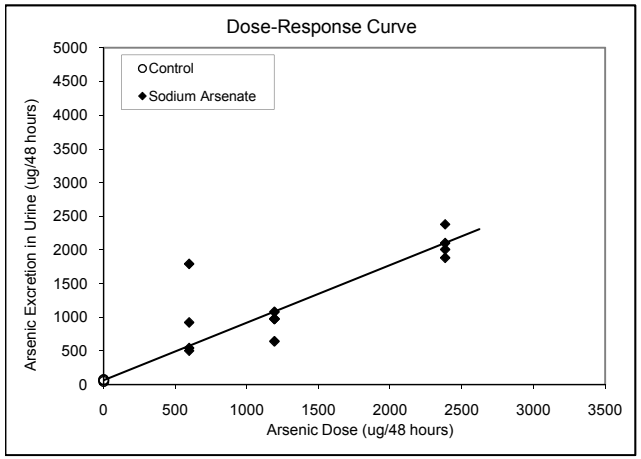
Tag Number	Arsenic Concentration	DL	Units
Blank-1	<1	1	ug/L
Blank-2	<1	1	ug/L
Blank-3	<1	1	ug/L
Blank-4	<1	1	ug/L
Blank-5	<1	1	ug/L
Blank-6	<1	1	ug/L
Blank-7	<1	1	ug/L
Blank-8	<1	1	ug/L
Blank-9	<0.1	0.1	ug/g

APPENDIX G

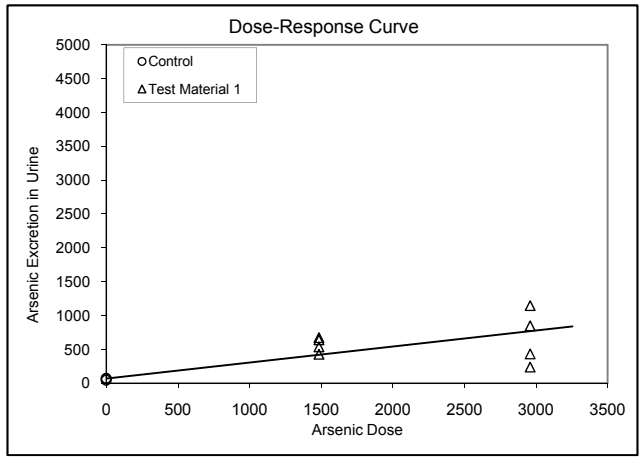
INITIAL ARSENIC DOSE-RESPONSE MODELING FOR STUDY 1 AND STUDY 2

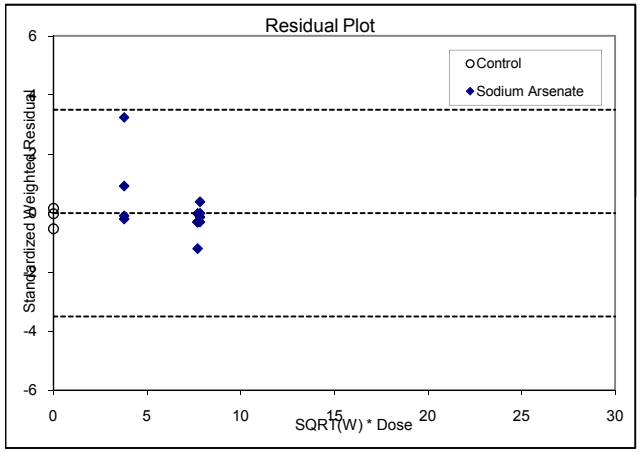
FIGURE G-1 STUDY 1 URINARY EXCRETION OF ARSENIC: Days 6/7 (All Data)

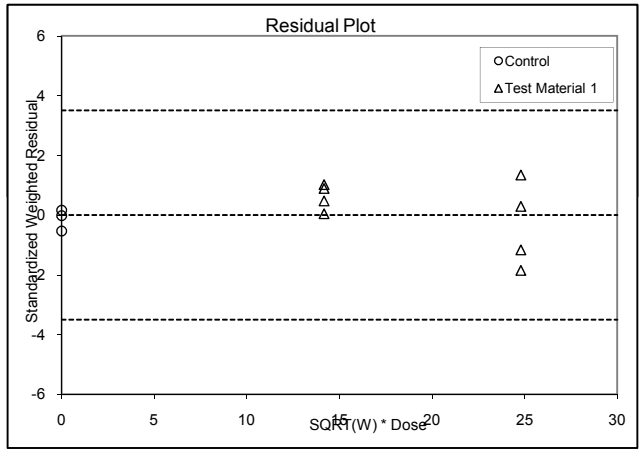
Reference Material (Sodium Arsenate)



Test Material 1 (MS-1)







[a] Note that the data from this figure were refitted with the outlier excluded (see Figure 4-6); this outlier was excluded from the final evaluation for arsenic R

Summary of Fitting[b]

Parameter	Estimate	Standard Error
a	67.0	24.4
b_r	0.85	0.11
b_{t1}	0.24	0.05
Covariance (b_r,b_{t1})	0.0371	--
Degrees of Freedom	21	--

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

where r = Reference Material, t1 = Test Material 1

ANOVA

Source	MSE
Fit	269.24
Error	6.33
Total	30.23

Statistic	Estimate
F	42.524
p	< 0.001
Adjusted R^2	0.7906

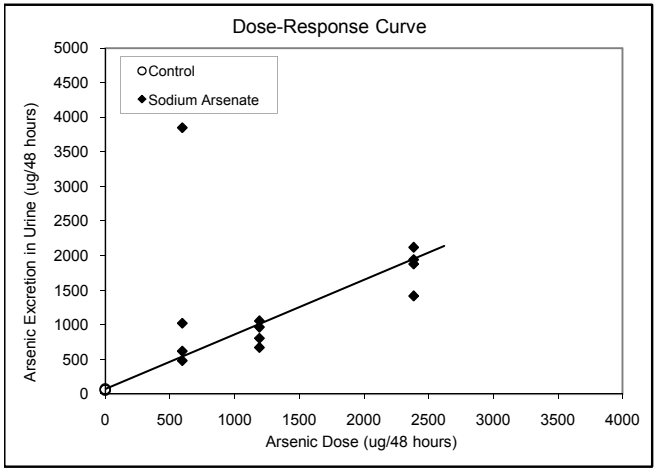
RBA and Uncertainty

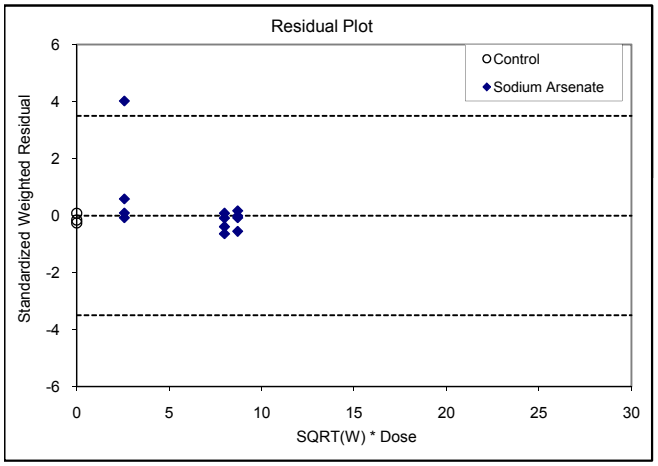
	Test Material 1
RBA	0.28
Lower bound[c]	0.18
Upper bound[c]	0.40
Standard Error[c]	0.063

[c] 90% confidence intervals calculated using Fieller's theorem

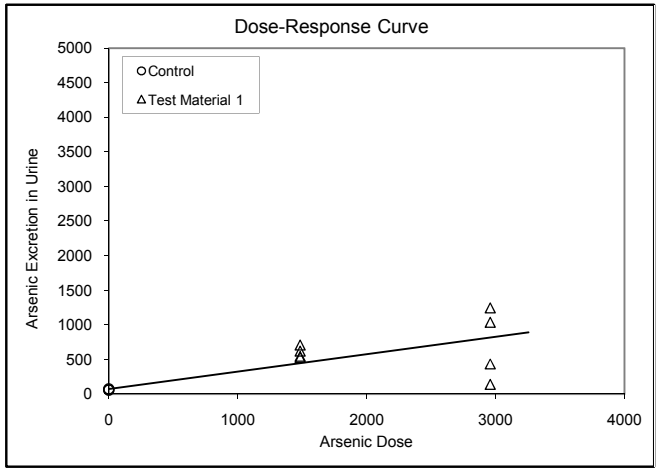
FIGURE G-2 STUDY 1 URINARY EXCRETION OF ARSENIC: Days 9/10 (All Data)

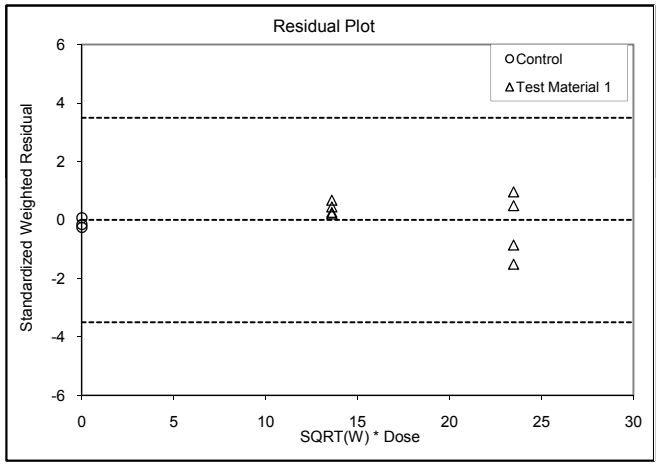
Reference Material (Sodium Arsenate)





Test Material 1 (MS-1)





Summary of Fitting[a]

Parameter	Estimate	SE
a	71.2	37.9
b_r	0.79	0.16
b_{t1}	0.25	0.07
Covariance (b_r,b_{t1})	0.0355	--
Degrees of Freedom	21	--

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

where r = Reference Material, t1 = Test Material 1

ANOVA

Source	MSE
Fit	257.39
Error	14.24
Total	36.34

Statistic	Estimate
F	18.078
p	< 0.001
Adjusted R^2	0.6082

RBA and Uncertainty

	Test Material 1
RBA	0.32
Lower bound[c]	0.16
Upper bound[c]	0.56
Standard Error[c]	0.109**

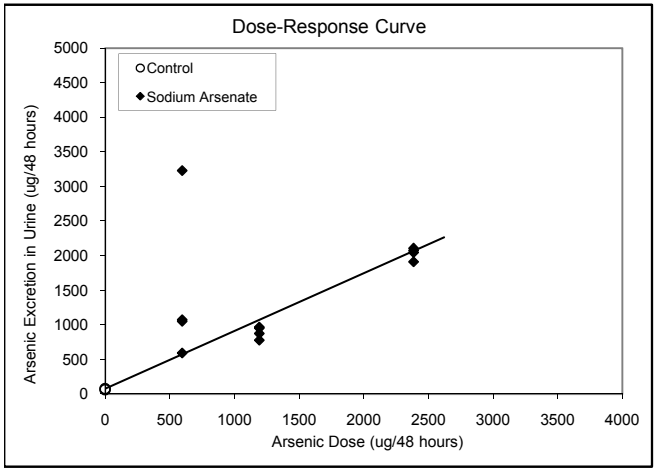
[c] 90% confidence intervals calculated using Fieller's theorem

** g ≥ 0.05 (Feiller's SE is uncertain)

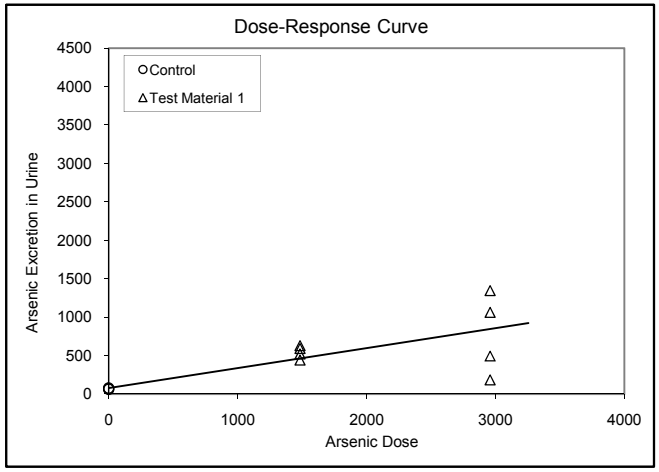
2_MS-1 As RBA Calcs 8-19-09 sieved.xls (Graph_2)

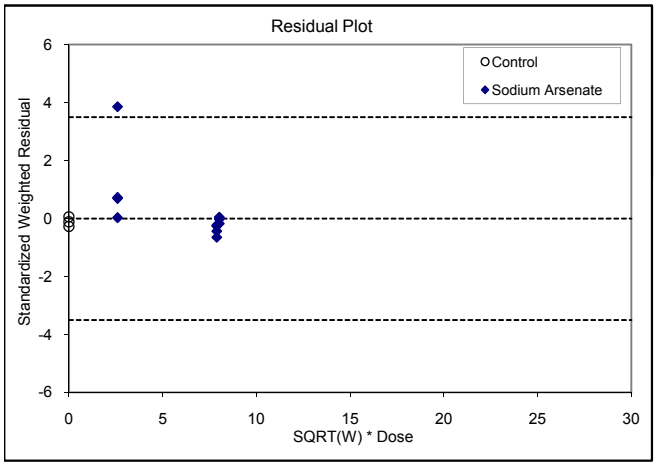
FIGURE G-3 STUDY 1 URINARY EXCRETION OF ARSENIC: Days 12/13 (All Data)

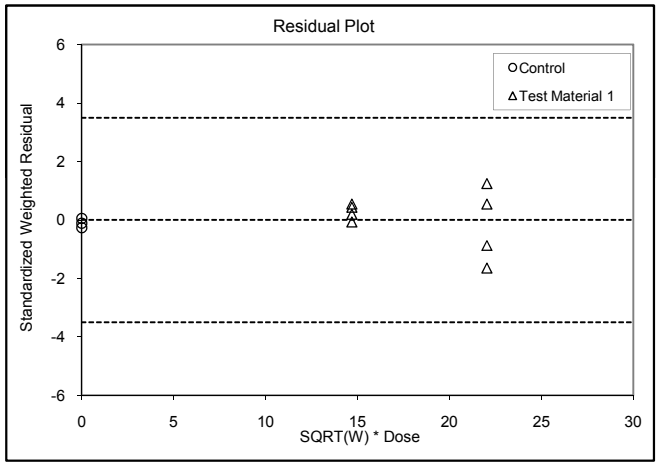
Reference Material (Sodium Arsenate)



Test Material 1 (MS-1)







Summary of Fitting[a]

Parameter	Estimate	SE
a	76.6	34.4
b_r	0.83	0.14
b_{t1}	0.26	0.06
Covariance (b_r,b_{t1})	0.0409	--
Degrees of Freedom	21	--

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

where r = Reference Material, t1 = Test Material 1

ANOVA

Source	MSE
Fit	259.41
Error	10.13
Total	32.79

Statistic	Estimate
F	25.602
p	< 0.001
Adjusted R^2	0.6910

RBA and Uncertainty

	Test Material 1
RBA	0.31
Lower bound[c]	0.18
Upper bound[c]	0.50
Standard Error[c]	0.089**

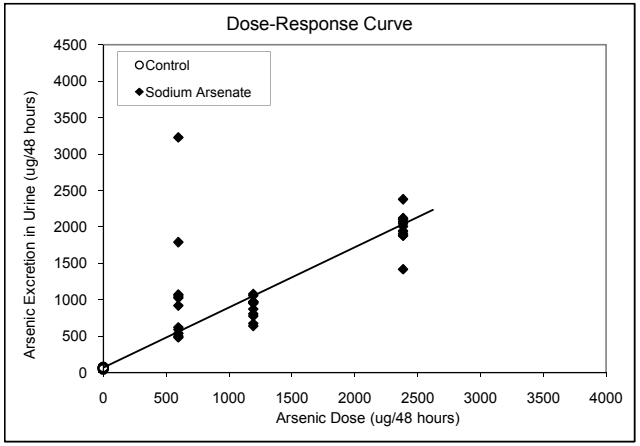
[c] 90% confidence intervals calculated using Fieller's theorem

** g ≥ 0.05 (Feiller's SE is uncertain)

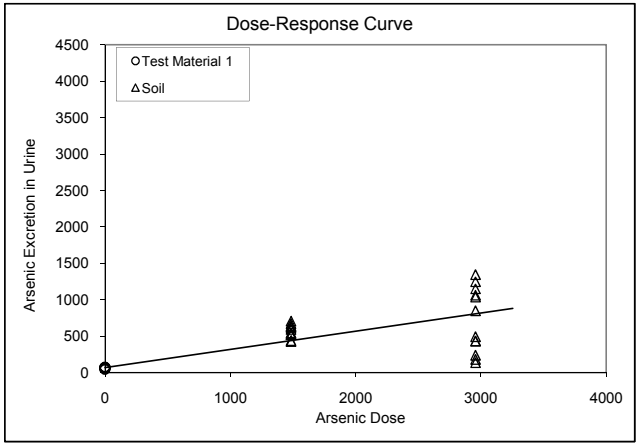
2_MS-1 As RBA Calcs 8-19-09 sieved.xls (Graph_3)

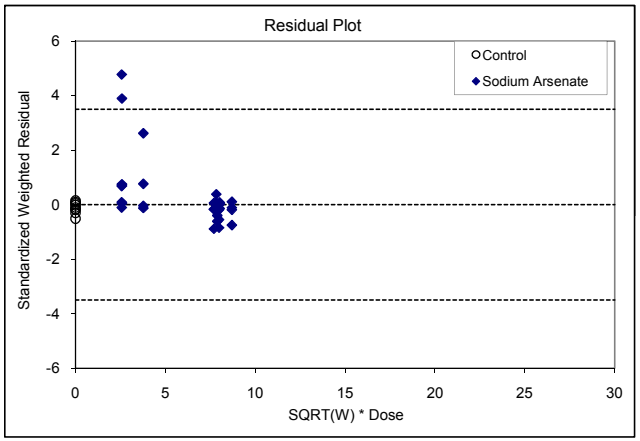
FIGURE G-4 STUDY 1 URINARY EXCRETION OF ARSENIC: All Days (All Data)

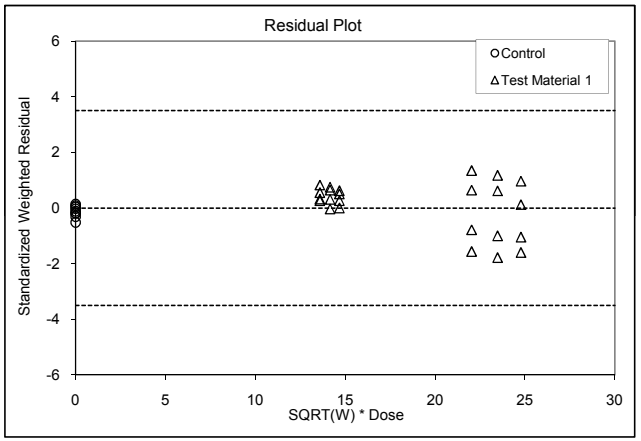
Reference Material (Sodium Arsenate)



Test Material 1 (MS-1)







[a] Note that the data from this figure were refitted with the outlier excluded (see Figure 4-7); this outlier was excluded from the final evaluation for arsenic RBA.

Summary of Fitting[b]

Parameter	Estimate	SE
a	71.3	17.9
b_r	0.83	0.08
b_{t1}	0.25	0.03
Covariance (b_r,b_{t1})	0.0376	--
Degrees of Freedom	67	--

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

where r = Reference Material, t1 = Test Material 1

ANOVA

Source	MSE
Fit	785.57
Error	9.35
Total	32.18

Statistic	Estimate
F	84.050
p	< 0.001
Adjusted R^2	0.7095

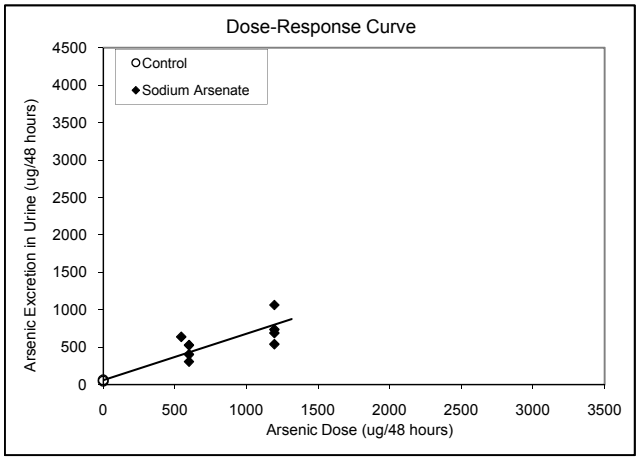
RBA and Uncertainty

	Test Material 1
RBA	0.30
Lower bound[c]	0.23
Upper bound[c]	0.39
Standard Error[c]	0.048

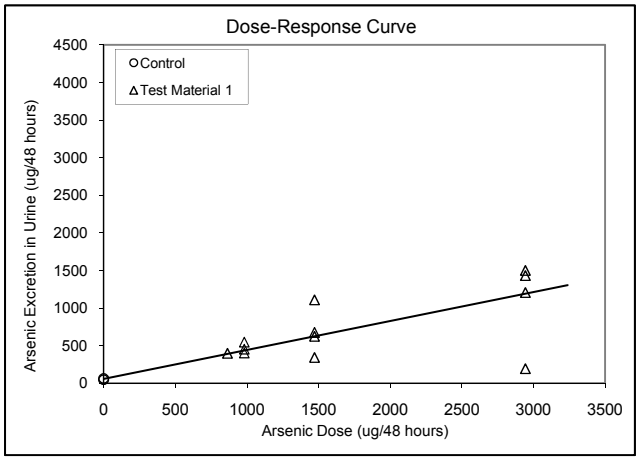
[c] 90% confidence intervals calculated using Fieller's theorem

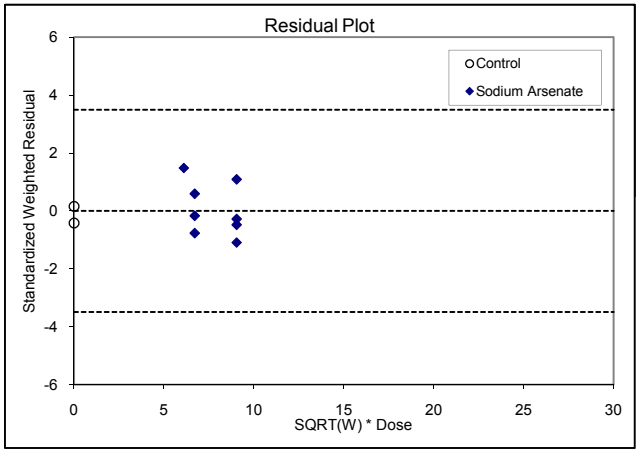
FIGURE G-5 STUDY 2 URINARY EXCRETION OF ARSENIC: Days 6/7 (All Data)

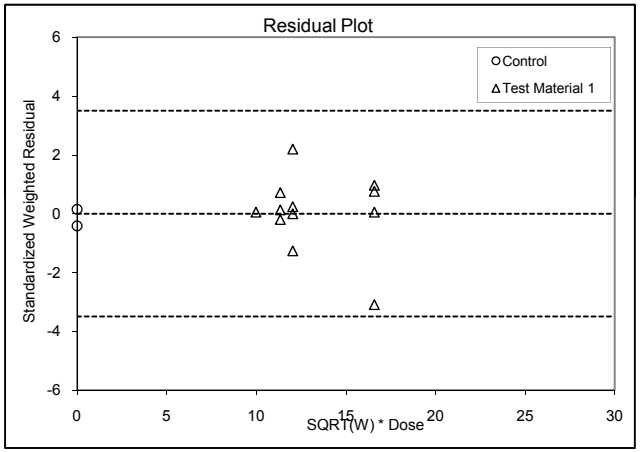
Reference Material (Sodium Arsenate)



Test Material 1 (MS-5)







Summary of Fitting[b]

Parameter	Estimate	Standard Error
a	58.3	20.6
b_r	0.62	0.09
b_{t1}	0.38	0.04
Covariance (b_r,b_{t1})	0.0733	--
Degrees of Freedom	23	--

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

where r = Reference Material, t1 = Test Material 1

ANOVA

Source	MSE
Fit	160.52
Error	3.74
Total	22.55

Statistic	Estimate
F	42.958
p	< 0.001
Adjusted R^2	0.8343

RBA and Uncertainty

	Test Material 1
RBA	0.62
Lower bound[c]	0.46
Upper bound[c]	0.85
Standard Error[c]	0.109**

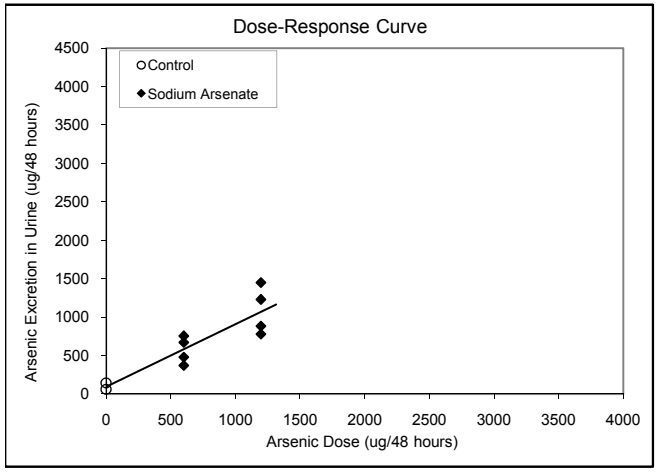
[c] 90% Confidence limit calculated using Fieller's theorem

** g ≥ 0.05 (Feiller's SE is uncertain)

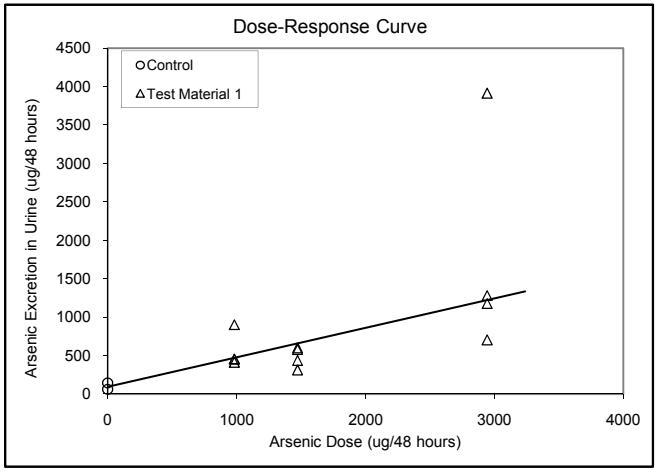
2_MS-5 As RBA Calcs all data 8-19-09.xls (Graph_1)

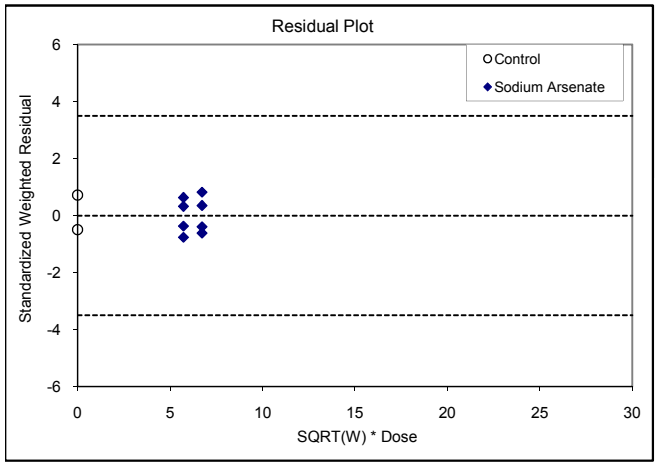
FIGURE G-6 STUDY 2 URINARY EXCRETION OF ARSENIC: Days 9/10 (All Data)

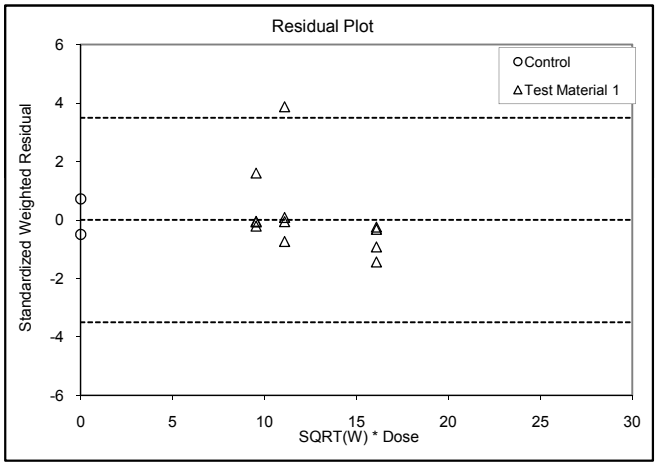
Reference Material (Sodium Arsenate)



Test Material 1 (MS-5)







Summary of Fitting[a]

Parameter	Estimate	SE
a	96.3	50.2
b_r	0.81	0.17
b_{t1}	0.38	0.07
Covariance (b_r,b_{t1})	0.1596	--
Degrees of Freedom	23	--

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

where r = Reference Material, t1 = Test Material 1

ANOVA

Source	MSE
Fit	115.10
Error	7.86
Total	20.73

Statistic	Estimate
F	14.646
p	< 0.001
Adjusted R^2	0.6209

RBA and Uncertainty

	Test Material 1
RBA	0.47
Lower bound[c]	0.30
Upper bound[c]	0.76
Standard Error[c]	0.122**

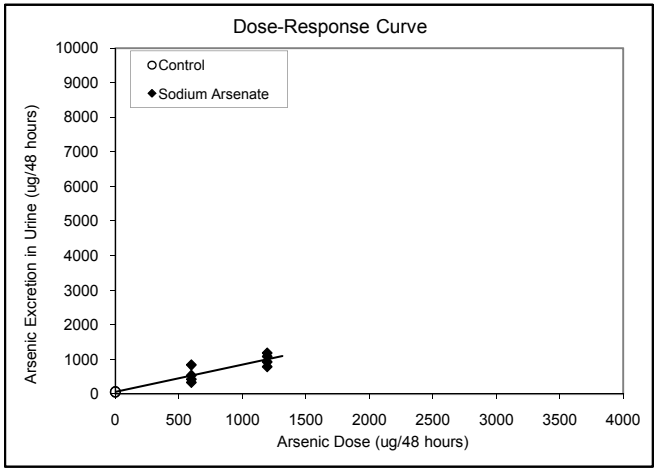
[c] 90% Confidence limit calculated using Fieller's theorem

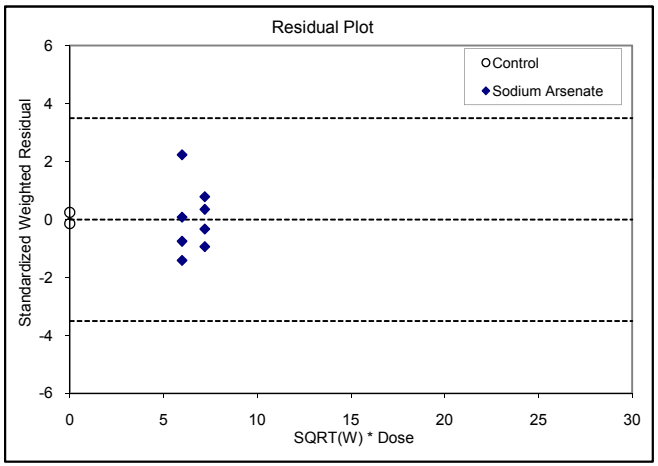
** g ≥ 0.05 (Feiller's SE is uncertain)

2_MS-5 As RBA Calcs all data 8-19-09.xls (Graph_2)

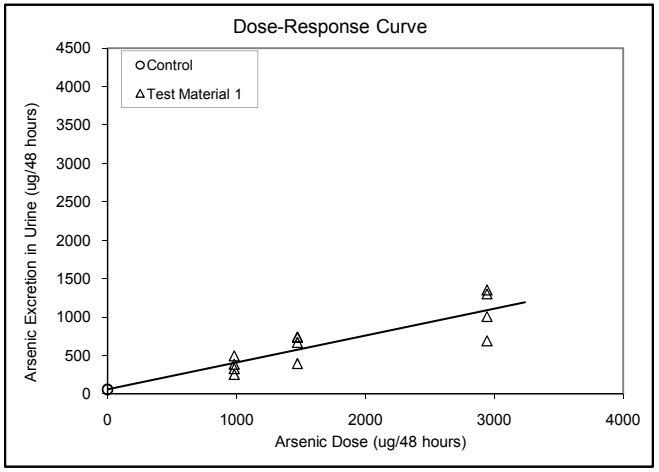
FIGURE G-7 STUDY 2 URINARY EXCRETION OF ARSENIC: Days 12/13 (All Data)

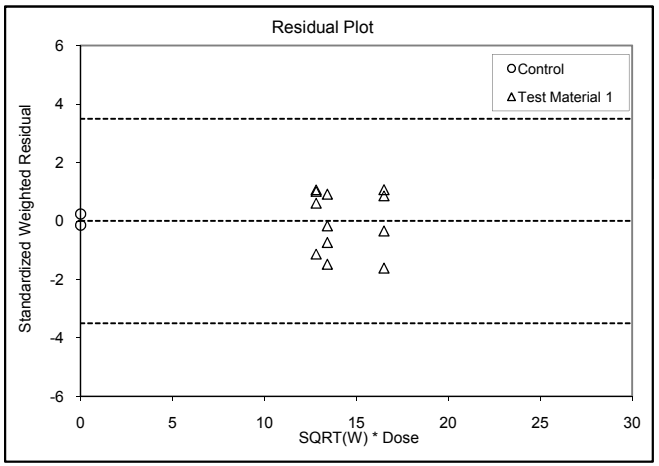
Reference Material (Sodium Arsenate)





Test Material 1 (MS-5)





Summary of Fitting[a]

Parameter	Estimate	SE
a	60.3	17.2
b_r	0.79	0.08
b_{t1}	0.35	0.03
Covariance (b_r,b_{t1})	0.0858	--
Degrees of Freedom	23	--

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

where r = Reference Material, t1 = Test Material 1

ANOVA

Source	MSE
Fit	156.97
Error	2.16
Total	20.74

Statistic	Estimate
F	72.750
p	< 0.001
Adjusted R^2	0.8959

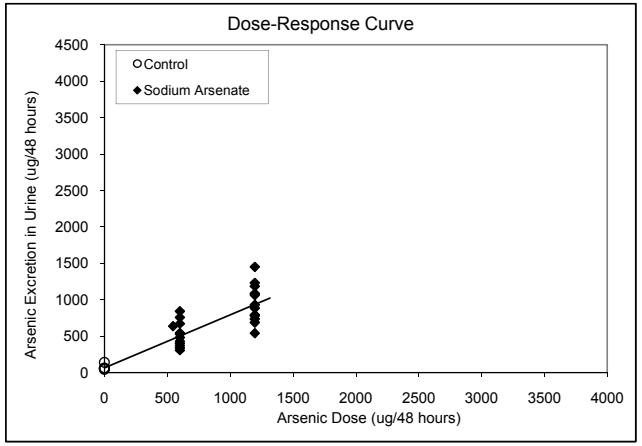
RBA and Uncertainty

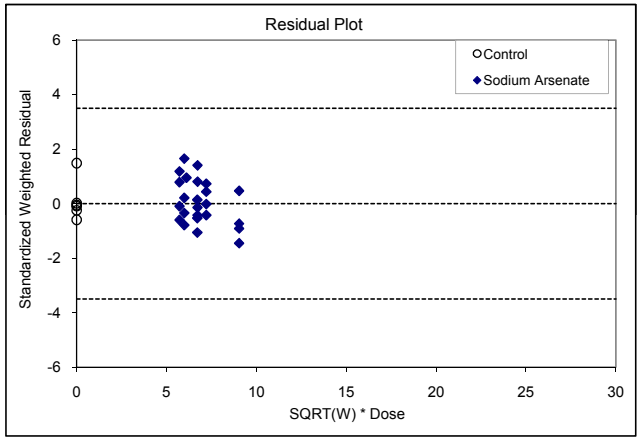
	Test Material 1
RBA	0.44
Lower bound[c]	0.35
Upper bound[c]	0.56
Standard Error[c]	0.058

[c] 90% Confidence limit calculated using Fieller's theorem

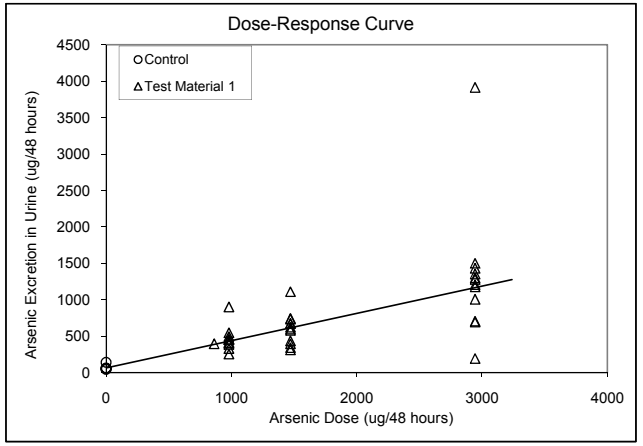
FIGURE G-8 STUDY 2 URINARY EXCRETION OF ARSENIC: All Days (All Data)

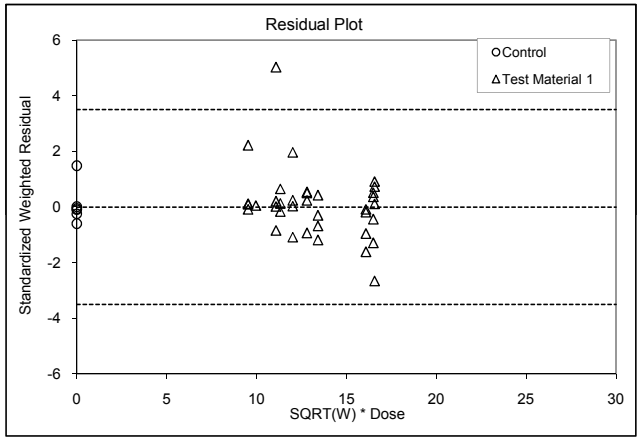
Reference Material (Sodium Arsenate)





Test Material 1 (MS-5)





Summary of Fitting[b]

Parameter	Estimate	SE
a	65.4	15.2
b_r	0.73	0.06
b_{t1}	0.37	0.03
Covariance (b_r,b_{t1})	0.0938	--
Degrees of Freedom	75	--

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

where r = Reference Material, t1 = Test Material 1

ANOVA

Source	MSE
Fit	442.90
Error	4.42
Total	21.51

Statistic	Estimate
F	100.115
p	< 0.001
Adjusted R^2	0.7943

RBA and Uncertainty

	Test Material 1
RBA	0.51
Lower bound[c]	0.43
Upper bound[c]	0.62
Standard Error[c]	0.056

[c] 90% Confidence limit calculated using Fieller's theorem

SRC TR-09-041

RELATIVE BIOAVAILABILITY OF ARSENIC IN TWO SOILS FROM THE IRON KING MINE

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Office of Superfund Remediation Technology Innovation

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EXECUTIVE SUMMARY

A study using juvenile swine as test animals was performed to measure the gastrointestinal absorption of arsenic from two soil samples collected from the Iron King mine – Humboldt Smelter Superfund Site. The mine operated from 1906 until the 1960's and was active in gold, silver, copper, lead, and zinc mining. The Humboldt Smelter performed custom smelting for many mines in the area and was active from 1870 to 1937. The soil samples (HSJ583 and IKJ583) were collected from the Chaparral Gulch near a residential area (HSJ583) and a tailings pile (IKJ583). The arsenic concentrations (mean ± standard deviation) of the soil samples are 200.4 ± 5.3 (HSJ583, TM1) and 3957.2 ± 332.7 (IKJ583, TM2) mg/kg.

The relative oral bioavailability of arsenic was assessed by comparing the absorption of arsenic from the Iron King soils ("test materials") to that of sodium arsenate. Groups of four swine were given oral doses of sodium arsenate or a test material twice a day for 14 days. Groups of three non-treated swine served as a control.

The amount of arsenic absorbed by each animal was evaluated by measuring the amount of arsenic excreted in the urine (collected over 48-hour periods beginning on days 5, 9, and 12). The urinary excretion fraction (UEF) is the ratio of the amount excreted per 48 hours divided by the dose given per 48 hours. UEF was calculated for each test material and the sodium arsenate using simultaneous weighted linear regression. The relative bioavailability (RBA) of arsenic in each test material compared to sodium arsenate was calculated as follows:

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

Estimated RBA values (mean and 90% confidence interval) are shown below:

Collection Interval	Estimated RBA (90% Confidence Interval)	
	Test Material 1 (HSJ583)	Test Material 2 (IKJ583)
Days 5/6	0.57 (0.50–0.65)	0.18 (0.16–0.21)
Days 9/10	0.70 (0.59–0.82)	0.21 (0.18–0.25)
Days 12/13	0.57 (0.51–0.63)	0.17 (0.16–0.19)
All Days	0.60 (0.56–0.65)	0.19 (0.17–0.20)

The best fit point estimate RBAs for the Iron King soil samples are 60% and 19% for TM1 and TM2, respectively.

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ACRONYMS AND ABBREVIATIONS

ABA	Absolute bioavailability
AF_o	Oral absorption fraction
As^{+3}	Trivalent inorganic arsenic
As^{+5}	Pentavalent inorganic arsenic
DMA	Dimethyl arsenic
D	Ingested dose
g	Gram
GLP	Good Laboratory Practices
ICP MS	Inductively coupled plasma mass spectrometry
kg	Kilogram
K_u	Fraction of absorbed arsenic which is excreted in urine
mL	Milliliter
MMA	Monomethyl arsenic
N	Number of data points
NIST	National Institute of Standards and Technology
ORD NERL	Office of Research and Development National Exposure Research Laboratory
PE	Performance Evaluation
QC	Quality control
RBA	Relative bioavailability
ref	Reference material
RfD	Reference dose
RPD	Relative percent difference
SF	Slope factor
SRM	Standard reference material
TM	Test material
UEF	Urinary excretion fraction
USEPA	United States Environmental Protection Agency
µg	Microgram
µm	Micrometer
°C	Degrees Celsius

1.0 INTRODUCTION

1.1 Overview of Bioavailability

Reliable analysis of the potential hazard to humans from ingestion of a chemical depends upon accurate information on a number of key parameters, including the concentration of the chemical in environmental media (e.g., soil, dust, water, food, air, paint), intake rates of each medium, and the rate and extent of absorption ("bioavailability") of the chemical by the body from each ingested medium. The amount of a chemical that actually enters the body from an ingested medium depends on the physical-chemical properties of the chemical and of the medium. For example, some metals in soil may exist, at least in part, as poorly water-soluble minerals, and may also exist inside particles of inert matrix such as rock or slag of variable size, shape, and association. These chemical and physical properties may influence (usually decrease) the absorption (bioavailability) of the metals when ingested. Thus, equal ingested doses of different forms of a chemical in different media may not be of equal health concern.

Bioavailability of a chemical in a particular medium may be expressed either in absolute terms (absolute bioavailability) or in relative terms (relative bioavailability):

> Absolute bioavailability (ABA) is the ratio of the amount of the chemical absorbed to the amount ingested:
>
> $$ABA = \frac{Absorbed\ Dose}{Ingested\ Dose}$$
>
> This ratio is also referred to as the oral absorption fraction (AF_o).
>
> Relative bioavailability (RBA) is the ratio of the AF_o of the chemical present in some test material (*test*) to the AF_o of the chemical in some appropriate reference material (e.g., either the chemical dissolved in water or a solid form that is expected to fully dissolve in the stomach) (*ref*):
>
> $$RBA(test\ vs\ ref) = \frac{AF_o(test)}{AF_o(ref)}$$

For example, if 100 micrograms (μg) of a chemical (e.g., arsenic) dissolved in drinking water were ingested and a total of 50 μg were absorbed into the body, the AF_o would be 50/100, or 0.50 (50%). Likewise, if 100 μg of a chemical contained in soil were ingested and 30 μg were absorbed into the body, the AF_o for this chemical in soil would be 30/100, or 0.30 (30%). If the chemical dissolved in water were used as the frame of reference for describing the relative amount of the same chemical absorbed from soil, the RBA would be 0.30/0.50, or 0.60 (60%).

For additional discussion about the concept and application of bioavailability, see Gibaldi and Perrier (1982), Goodman et al. (1990), and/or Klaassen et al. (1996).

1.2 Using RBA Data to Improve Risk Calculations

When reliable data are available on the RBA of a chemical in a site medium (e.g., soil), the information can be used to improve the accuracy of exposure and risk calculations at that site. RBA data can be used to adjust default oral toxicity values (reference dose and slope factor) to account for differences in absorption between the chemical ingested in water and the chemical ingested in site media, assuming the toxicity factors are based on a readily soluble form of the chemical. For non-cancer effects, the default reference dose ($RfD_{default}$) can be adjusted ($RfD_{adjusted}$) as follows:

$$RfD_{adjusted} = \frac{RfD_{default}}{RBA}$$

For potential carcinogenic effects, the default slope factor ($SF_{default}$) can be adjusted ($SF_{adjusted}$) as follows:

$$SF_{adjusted} = SF_{default} \cdot RBA$$

Alternatively, it is also acceptable to adjust the dose (rather than the toxicity factors) as follows:

$$Dose_{adjusted} = Dose_{default} \cdot RBA$$

This dose adjustment is mathematically equivalent to adjusting the toxicity factors as described above.

1.3 Purpose of this Study

The objective of this study was to use juvenile swine as a test system in order to determine the RBA of arsenic in two Iron King soil samples compared to a soluble form of arsenic (sodium arsenate).

2.0 STUDY DESIGN

The test materials and a reference material (sodium arsenate) were administered to groups of four juvenile swine at three different dose levels for 14 days. The study included a non-treated group of three animals to serve as a control for determining background arsenic levels. Study details are presented in Table 2-1. All doses were administered orally. The study was performed as nearly as possible within the spirit and guidelines of Good Laboratory Practices (GLP: 40 CFR 792).

2.1 Test Materials

2.1.1 Sample Description

The Iron King Mine – Humboldt Smelter Superfund Site is located near Humboldt Arizona. The site operated from 1906 to the 1960's and was active gold, silver, copper, lead, and zinc. The Humboldt Smelter performed custom smelting for many mines in the area and was active from

1870 to 1937. Arsenic and lead have been detected in site materials, including tailings deposits, at elevated concentrations. These materials are migrating off-site. Residential properties and the town of Humboldt are located immediately adjacent to the site and between the mine and smelter. Samples were collected from the Chaparral Gulch near a residential area (HSJ583) and a tailings pile (sample IKJ583). The arsenic concentrations (mean ± standard deviation) of the soil samples are 200.4 ± 5.3 (HSJ583, TM1) and 3957.2 ± 332.7 (IKJ583, TM2) mg/kg.

2.1.2 Sample Preparation and Analysis

USEPA Region 9 collected the soil from Iron King Mine – Humboldt Smelter Superfund Site. Soil was sieved to remove large chunks and rocks and shipped to the EPA Office of Research and Development National Exposure Research Laboratory (ORD NERL) where the soils were then sieved to <250 µm and homogenized using a vortex mixer. For arsenic analysis, sieved soil samples were digested following EPA Method 3051A (microwave digestion) and analyzed following EPA Method 6020 (inductively coupled plasma mass spectrometry [ICP MS]); four replicates of each sample were analyzed.

X-ray absorption spectroscopy was conducted on the test materials to characterize the arsenic mineralogy (Miller and Scheckel, 2012).

2.2 Experimental Animals

Juvenile swine were selected for use because they are considered to be a good physiological model for gastrointestinal absorption in children (Weis and LaVelle, 1991; Casteel et al., 1996). The animals were intact males of the Pig Improvement Corporation genetically defined Line 26, and were purchased from Chinn Farms, Clarence, Missouri.

The number of animals purchased for the study was several more than required by the protocol. These animals were purchased at an age of about 5–6 weeks (weaning occurs at age 3 weeks) and housed in individual stainless steel cages. The animals were then held under quarantine for one week to observe their health before beginning exposure to dosing materials. Each animal was examined by a certified veterinary clinician (swine specialist) and any animals that appeared to be in poor health during this quarantine period were excluded from the study. To minimize weight variations among animals and groups, extra animals most different in body weight (either heavier or lighter) five days prior to exposure (day 5) were also excluded from the study. The remaining animals were assigned to dose groups at random (group assignments are presented in Appendix A).

When exposure began (day 0), the animals were about 6–7 weeks old. The animals were weighed at the beginning of the study and every three days during the course of the study. In each study, the rate of weight gain was comparable in all dosing groups. Body weight data are presented in Appendix B.

All animals were examined daily by an attending veterinarian while on study and were subjected to detailed examination at necropsy by a certified veterinary pathologist in order to assess overall animal health.

2.3 Diet

Animals were weaned onto standard swine chow (made at the University of Missouri Animal Science Feed Mill). The feed was nutritionally complete (NRC 1988). The ingredients of the feed are presented in Appendix C. Arsenic concentration in a randomly selected feed sample measured <0.1 µg/g.

Prior to the start of dosing and throughout the dosing period, each day every animal was given an amount of feed equal to 4.0% of the mean body weight of all animals on study. Feed amounts were adjusted every three days, when animals were weighed. Feed was administered in two equal portions, at 11:00 AM and 5:00 PM daily.

Drinking water was provided *ad libitum* via self-activated watering nozzles within each cage. Arsenic concentration of five water samples from randomly selected drinking water nozzles were <0.6 µg/L.

2.4 Dosing

Animals were exposed to dosing materials (sodium arsenate or sieved test material) for 14 days, with the dose for each day being administered in two equal portions beginning at 9:00 AM and 3:00 PM (two hours before feeding). Swine were dosed two hours before feeding to ensure that they were in a semi-fasted state. To facilitate dose administration, dosing materials were placed in a small depression in a ball of dough consisting of moistened feed (typically about 5g) and the dough was pinched shut. This was then placed in the feeder at dosing time.

Target arsenic doses (expressed as µg of arsenic per kg of body weight per day) for animals in each group were determined prior to the study and are shown in the study design (see Table 2-1). Based on the target arsenic dose, a daily mass of arsenic administered (either as sodium arsenate or as sieved test material) to animals in each group is calculated by multiplying the target dose (µg/kg-day) for that group by the anticipated average weight of the animals (kg) over the course of the study:

$$Mass\ (\mu g / day) = Dose\ (\mu g / kg - day) \cdot Average\ Body\ Weight\ (kg)$$

The average body weight expected during the course of the study is estimated by measuring the average body weight of all animals one day before the study began, and then assuming an average weight gain of 0.5 kg/day during the study.

In planning for this study, the soil concentration for TM2 was reported incorrectly in the file used to calculate study doses. As a result, soil doses administered to swine in the TM2 groups were larger than needed, and actual doses were about 3-fold greater than the target dose (see Section 4.2 for further discussion).

After completion of the study, the true mean body weight of all swine combined was calculated using the actual body weights (measured every three days during the study), and the resulting true mean body weight was used to calculate the actual doses achieved. Any missed or late doses were recorded and the actual doses adjusted accordingly. Actual doses (µg arsenic per day) for each group are shown in Table 2-1.

2.5 Collection and Preservation of Urine Samples

Samples of urine were collected from each animal for 48-hour periods on days 5 to 6 (U-1), 9 to 10 (U-2), and 12 to 13 (U-3) of the study. Collection began at 8:00 AM and ended 48 hours later. The urine was collected in a plastic bucket placed beneath each cage, which was emptied into a plastic storage bottle. Aluminum screens were placed under the cages to minimize contamination with feces or spilled food. Due to the length of the collection period, collection containers were emptied periodically (typically twice daily) into a separate plastic bottles to ensure that there was no loss of sample due to overflow.

At the end of each collection period, the total urine volume for each animal was measured (Appendix D) and three 60-mL portions were removed and acidified with 0.6 mL concentrated nitric acid. All samples were refrigerated. Two of the aliquots were archived and one aliquot was sent for arsenic analysis (refrigeration was maintained until arsenic analysis).

2.6 Arsenic Analysis

Urine samples were assigned random chain-of-custody tag numbers and submitted to the analytical laboratory for analysis in a blind fashion. The samples were analyzed for arsenic by L. E. T., Inc. (Columbia, Missouri). In brief, 25-mL samples of urine were digested by refluxing and then heating to dryness in the presence of magnesium nitrate and concentrated nitric acid. Following magnesium nitrate digestion, samples were transferred to a muffle furnace and ashed at 500°C. The digested and ashed residue was dissolved in hydrochloric acid and analyzed by the hydride generation technique using a PerkinElmer 3100 atomic absorption spectrometer. Previous tests of this method established that each of the different forms of arsenic that may occur in urine, including trivalent inorganic arsenic (As^{+3}), pentavalent inorganic arsenic (As^{+5}), monomethyl arsenic (MMA), and dimethyl arsenic (DMA) are all recovered with high efficiency.

Analytical results for the urine samples are presented in Appendix D.

2.7 Quality Control

A number of quality control (QC) steps were taken during this project to evaluate the accuracy of the analytical procedures. The results for QC samples are presented in Appendix E and are summarized below.

Blind Duplicates (Sample Preparation Replicates)

A random selection of about 8% of all urine samples generated during the study were prepared for laboratory analysis in duplicate (i.e., two separate subsamples of urine were digested) and submitted to the laboratory in a blind fashion. Results are shown in Appendix E (see Table E-1 and Figure E-1). There was generally good agreement between results for the duplicate pairs.

Spike Recovery

During arsenic analysis, one feed sample and every tenth urine sample was spiked with known amounts of arsenic (sodium arsenate) and the recovery of the added arsenic was measured.

Results (see Table E-2) show that mean arsenic concentrations recovered from spiked samples were usually within 10% of actual arsenic concentrations.

Laboratory Duplicates

During arsenic analysis, every tenth sample was analyzed in duplicate. Duplicate results for urine samples (see Table E-3) typically agreed within 10% relative percent difference (RPD). The duplicate water and feed samples were below the detection limit.

Laboratory Control Standards

National Institute of Standards and Technology (NIST) Standard Reference Materials® (SRM), (2003) for which a certified concentration of specific analytes has been established, were tested periodically during sample analysis. Recovery of arsenic from these standards was generally good and within the acceptable range (see Table E-4).

Performance Evaluation Samples

A number of Performance Evaluation (PE) samples (urine samples of known arsenic concentration) were submitted to the laboratory in a blind fashion. The PE samples included varying concentrations (20, 100, or 400 µg/L) each of four different types of arsenic (As^{+3}, As^{+5}, MMA, and DMA). The results for the PE samples are shown in Table E-5 and Figure E-2. All sample results were close to the expected values, indicating that there was good recovery of the arsenic in all cases.

Blanks

Blank samples were run along with each batch of samples (n=8). Blanks never yielded a measurable level of arsenic (all results <1 µg/L). Results are shown in Table E-6.

Summary of QC Results

Based on the results of all of the QC samples and steps described above, it is concluded that the analytical results are of sufficient quality for derivation of reliable estimates of arsenic absorption from the test materials.

3.0 DATA ANALYSIS

3.1 Overview

Figure 3-1 shows a conceptual model for the toxicokinetic fate of ingested arsenic. Key points of this model are as follows:

- In most animals (including humans), absorbed arsenic is excreted mainly in the urine over the course of several days. Thus, the urinary excretion fraction (UEF), defined as the amount excreted in the urine divided by the amount given, is usually a reasonable approximation of the AF_o or ABA. However, this ratio will underestimate total absorption, because some absorbed arsenic is excreted in the feces via the bile, and some

absorbed arsenic enters tissue compartments (e.g., skin, hair) from which it is cleared very slowly or not at all. Thus, the UEF should not be equated with the absolute absorption fraction.

- The RBA of two orally administered materials (i.e., a test material and reference material) can be calculated from the ratio of the UEF of the two materials. This calculation is independent of the extent of tissue binding and of biliary excretion:

$$RBA(test\ vs\ ref) = \frac{AF_o(test)}{AF_o(ref)} = \frac{D \cdot AF_o(test) \cdot K_u}{D \cdot AF_o(ref) \cdot K_u} = \frac{UEF(test)}{UEF(ref)}$$

where:

D = ingested dose (μg)

K_u = fraction of absorbed arsenic that is excreted in the urine

Based on the conceptual model above, the basic method used to estimate the RBA of arsenic in a particular test material compared to arsenic in a reference material (sodium arsenate) is as follows:

1. Plot the amount of arsenic excreted in the urine (μg per 48 hours) as a function of the administered amount of arsenic (μg per 48 hours), both for reference material and for test material.

2. Find the best fit linear regression line through each data set. The slope of each line (μg per 48 hours excreted per μg per 48 hours ingested) is the best estimate of the UEF for each material.

3. Calculate RBA for each test material as the ratio of the UEF for test material compared to UEF for reference material:

$$RBA(test\ vs\ ref) = \frac{UEF(test)}{UEF(ref)}$$

A detailed description of the curve-fitting methods and rationale and the methods used to quantify uncertainty in the arsenic RBA estimates for a test material are summarized below. All model fitting was performed in Microsoft Excel® using matrix functions.

3.2 Dose-Response Model

Simultaneous Regression

The techniques used to derive linear regression fits to the dose-response data are based on the methods recommended by Finney (1978). As noted by Finney (1978), when the data to be analyzed consist of two dose-response curves (the reference material and the test material), it is obvious that both curves must have the same intercept, since there is no difference between the

curves when the dose is zero. This requirement is achieved by combining the two dose response equations into one and solving for the parameters simultaneously, as follows:

Separate models:

$$\mu_r(i) = a + b_r \cdot x_r(i)$$

$$\mu_t(i) = a + b_t \cdot x_t(i)$$

Combined model:

$$\mu(i) = a + b_r \cdot x_r(i) + b_t \cdot x_t(i)$$

where $\mu(i)$ indicates the expected mean response of animals exposed at dose $x(i)$, and the subscripts r and t refer to reference and test material, respectively. The coefficients of this combined model are derived using multivariate regression, with the understanding that the combined data set is restricted to cases in which one (or both) of x_r and x_t are zero (Finney, 1978). When a study consists of a reference group and two test materials, as is the case for this study, the same approach is used, except that all three curves are fit simultaneously:

$$\mu(i) = a + b_r \cdot x_r(i) + b_{t1} \cdot x_{t1}(i) + b_{t2} \cdot x_{t2}(i)$$

Weighted Regression

Regression analysis based on ordinary least squares assumes that the variance of the responses is independent of the dose and/or the response (Draper and Smith, 1998). It has previously been shown that this assumption is generally not satisfied in swine-based RBA studies, where there is a tendency toward increasing variance in response as a function of increasing dose (heteroscedasticity) (USEPA, 2007). One method for dealing with heteroscedasticity is through the use of weighted least squares regression (Draper and Smith, 1998). In this approach, each observation in a group of animals is assigned a weight that is inversely proportional to the variance of the response in that group:

$$w_i = \frac{1}{\sigma_i^2}$$

where:

w_i = weight assigned to all data points in dose group i

σ_i^2 = variance of responses in animals in dose group i

When the distributions of responses at each dose level are normal, weighted regression is equivalent to the maximum likelihood method.

There are several alternative strategies for assigning weights. The method used in this study estimates the value of σ_i^2 using an "external" variance model based on an analysis of the

relationship between variance and mean response using data consolidated across many different swine-based arsenic RBA studies. The data used to derive the variance model are shown in Figure 3-2. As seen, log-variance increases as an approximately linear function of log-mean response:

$$\ln(s_i^2) = k1 + k2 \cdot \ln(\bar{y}_i)$$

where:

s_i^2 = observed variance of responses of animals in dose group *i*

$\bar{y}_i$ = mean observed response of animals in dose group *i*

Based on these data, values of *k*1 and *k*2 were derived using ordinary least squares minimization. The resulting values were -1.10 for *k*1 and 1.64 for *k*2.

Goodness-of-Fit

The goodness-of-fit of each dose-response model was assessed using the F test statistic and the adjusted coefficient of multiple determination (Adj R^2) as described by Draper and Smith (1998). A fit is considered acceptable if the p-value is less than 0.05.

Assessment of Outliers

In biological assays, it is not uncommon to note the occurrence of individual measured responses that appear atypical compared to the responses from other animals in the same dose group. In this study, responses that yielded standardized weighted residuals greater than 3.5 or less than -3.5 were considered to be potential outliers (Canavos, 1984). Such a data point was encountered in the data set for this study. Therefore, RBA values were calculated both for all the data (outliers included) and without the outlier, and the result with the outlier excluded was used as the preferred estimate.

3.3 Calculation of RBA Estimates

The arsenic RBA values were calculated as the ratio of the slope term for the test material data set (b_t) and the reference material data set (b_r):

$$RBA = \frac{b_t}{b_r}$$

The uncertainly range about the RBA ratio was calculated using Fieller's Theorem as described by Finney (1978).

4.0 RESULTS

4.1 Clinical Signs

The doses of arsenic administered in this study are below a level that is expected to cause toxicological responses in swine. No clinical signs of arsenic-induced toxicity were noted in any of the animals used in the studies. Three swine received 1 cc Naxcel once per day on days 2, 3, and 4 (swines 606 and 609) or days 11, 12, and 13 (swine 636) during the study to treat a systemic bacterial infection (swine were found with fever ≥104°).

4.2 Dosing Deviations

Missed doses are summarized in Table 4-1. Most missed doses occurred on the first two days of dosing and were not specific to any particular group.

As noted in Section 2, the soil concentration for TM2 was reported incorrectly in the file used to calculate study doses (reported values were lower than actual). As a result, soil doses administered to swine in the TM2 groups were about 3-fold larger than targeted, and therefore the actual doses administered were greater than the target doses specified in the study design (see Table 2-1).

Although the administered arsenic doses for TM2 were higher than the target doses, this did not affect the study outcome because the dose-response pattern remained approximately linear. Since it is the ratio of administered arsenic to excreted arsenic between test and reference materials that is used to compute relative bioavailability, differences in administered doses between groups is accounted for in the calculations. Additionally, there were no observed signs of toxicity in any of the groups. Therefore, the higher doses administered in the TM2 group compared to target doses did not impact study performance or outcome.

4.3 Background Arsenic Excretion

Measured values for urinary arsenic excretion (mean and standard deviation) for control animals from days 5 to 13 are shown in Table 4-2. Mean urinary arsenic concentration (± standard deviation) was 49.8 ± 10.0 µg/L. The values shown are representative of levels in urine due to endogenous background levels in food and water and support the view that the animals were not exposed to any significant exogenous sources of arsenic throughout the study.

4.4 Urinary Arsenic Variance

As discussed in Section 3.2, the urinary arsenic dose-response data are analyzed using weighted least squares regression and the weights are assigned using an “external” variance model. To ensure that the variance model was valid, the variance values from each of the dose groups were superimposed on the historic data set (see Figure 4-1). As shown in Figure 4-1, the variances of the urinary arsenic data from this study are consistent with the data used to generate the variance model.

4.5 Dose-Response Modeling

The dose-response data for arsenic in urine were modeled using all of the data (no outliers were identified). Modeling results are shown in Figures 4-2 through 4-5.

All of the dose-response curves were approximately linear, with the slope of the best fit straight line being equal to the best estimate of the UEF. The resulting slopes (UEF estimates) for the final fittings of the test material and corresponding reference material are shown in Table 4-3.

4.6 Calculated RBA Values

Estimated RBA values (mean and 90% confidence interval) are shown in Table 4-4. The best fit point estimate RBA for the Iron King soil samples is 60% and 19% for TM1 and TM2, respectively.

4.7 Uncertainty

The bioavailability estimates above are subject to uncertainty that arises from several different sources. One source of uncertainty is the inherent biological variability between different animals in a dose group, which in turn causes variability in the amount of arsenic absorbed by the exposed animals. The between-animal variability results in statistical uncertainty in the best fit dose-response curves and, hence, uncertainty in the calculated values of RBA. Such statistical uncertainty is accounted for by the statistical models used above and is characterized by the uncertainty range around the RBA estimates.

However, there is also uncertainty in the extrapolation of RBA values measured in juvenile swine to young children or adults, and this uncertainty is not included in the statistical confidence bounds above. Even though the immature swine is believed to be a useful and meaningful animal model for gastrointestinal absorption in humans, it is possible that there are differences in physiological parameters that may influence RBA; therefore, RBA values in swine may not be identical to values in children. In addition, RBA may depend on the amount and type of food in the stomach, since the presence of food can influence stomach pH, holding time, and possibly other factors that may influence solubilization and absorption of arsenic. RBA values measured in this study are based on animals that have little or no food in their stomach at the time of exposure and, hence, are likely to yield high-end values of RBA. Thus, these RBA values may be somewhat conservative for humans who ingest the site soils along with food. The magnitude of this bias is not known.

5.0 REFERENCES

Canavos, C. G. 1984. Applied Probability and Statistical Methods. Little, Brown and Co., Boston, MA.

Casteel, S. W., R. P. Cowart, C. P. Weis, G. M. Henningsen, E. Hoffman, W. J. Brattin, M. F. Starost, J. T. Payne, S. L. Stockham, S. V. Becker, and J. R. Turk. 1996. A swine model for determining the bioavailability of lead from contaminated media. In: Advances in Swine in Biomedical Research. Tumbleson, M.E. and L.B. Schook (eds.), Volume 2, Plenum Press, New York, NY. pp. 637–46.

Draper, N. R. and H. Smith. 1998. Applied Regression Analysis. 3rd edition. John Wiley & Sons, New York, NY.

Finney, D. J. 1978. Statistical Method in Biological Assay. 3rd edition. Charles Griffin and Co., London, England.

Gibaldi, M. and D. Perrier. 1982. Pharmacokinetics. 2nd edition. Marcel Dekker, Inc., New York, NY. pp. 294–297.

Goodman, A. G., T. W. Rall, A. S. Nies, and P. Taylor. 1990. The Pharmacological Basis of Therapeutics. 8th edition. Pergamon Press, Inc., Elmsford, NY. pp. 5–21.

Klaassen, C. D., M. O. Amdur, and J. Doull (eds.). 1996. Cassarett and Doull's Toxicology: The Basic Science of Poisons. McGraw-Hill, Inc., New York, NY. p. 190.

Miller, B. W. and K. G. Scheckel. 2012. Technical Review Workgroup for Metals and Asbestos: Bioavailability Committee. Mineralogical Report. XAS Data and Linear Combination Fitting Results. Available at: http://epa.gov/superfund/bioavailability/guidance.htm

NIST. 2003. Certificate of Analysis, Standard Reference Material® 2710 – Montana Soil, Highly Elevated Trace Element Concentrations. National Institute of Standards & Technology, Gaithersburg, MD. Certificate Issue Date: July 18, 2003.

NRC. 1988. Nutrient Requirements of Swine. A Report of the Committee on Animal Nutrition. National Research Council. National Academy Press, Washington, DC.

USEPA. 2007. Estimation of Relative Bioavailability of Lead in Soil and Soil-Like Materials by *In Vivo* and *In Vitro* Methods. OSWER9285.7-77. U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response, Washington DC.

Weis, C.P. and J. M. LaVelle. 1991. Characteristics to consider when choosing an animal model for the study of lead bioavailability. In: The Proceedings of the International Symposium on the Bioavailability and Dietary Uptake of Lead. *Science Technology Letters* 3:113–119.

TABLES & FIGURES

TABLE 2-1. Study Design and Dosing Information

Group	Group Name Abbreviation	Dose Material Administered	As Concentration of the Material (µg/g or µg/µL)	Number of Swine in Group	Arsenic Dose		
					Target (µg/kg bw-day)	Actual [a] (µg/kg BW-day)	Actual [b] (µg-day)
1	NaAs	Sodium arsenate	2	4	25	25	307
2	NaAs	Sodium arsenate	10	4	50	50	614
3	NaAs	Sodium arsenate	10	4	100	100	1228
4	TM1	Iron King TM1 HSJ583	200	4	40	40	492
5	TM1	Iron King TM1 HSJ584	200	4	60	60	736
6	TM1	Iron King TM1 HSJ585	200	4	120	120	1476
7	TM2	Iron King TM2 IKJ583	3957	4	40	116	1425
8	TM2	Iron King TM2 IKJ584	3957	4	60	175	2137
9	TM2	Iron King TM2 IKJ585	3957	4	120	349	4274
10	Control	None (negative control)	–	3	0	0	0

[a] Calculated as the administered daily dose divided by the measured or extrapolated daily body weight, averaged over days 0–14 for each animal and each group.
[b] Calculated as the mass of soil or sodium arsenate solution administered times the concentration of the soil or sodium arsenate solution.

Doses were administered in two equal portions given at 9:00 AM and 3:00 PM each day. Doses were held constant based on the expected mean weight during the exposure interval (14 days).

TABLE 4-1. Missed Dose Consumption

Study Day	Swine Number	Note
0	601	Day 0 – Swine 601 did not eat AM or PM dose. Daily dose adjusted to 0%.
	605	Day 0 – Swine 605 did not eat AM or PM dose. Daily dose adjusted to 0%.
	606	Day 0 – Swine 606 did not eat AM dose. Daily dose adjusted to 50%.
	609	Day 0 – Swine 609 did not eat AM dose. Daily dose adjusted to 50%.
	615	Day 0 – Swine 615 did not eat AM dose. Daily dose adjusted to 50%.
	628	Day 0 – Swine 628 did not eat AM dose. Daily dose adjusted to 50%.
	635	Day 0 – Swine 635 did not eat AM or PM dose. Daily dose adjusted to 0%.
	643	Day 0 – Swine 643 did not eat AM dose. Daily dose adjusted to 50%.
1	601	Day 1 – Swine 601 did not eat AM or PM dose. Daily dose adjusted to 0%.
	605	Day 1 – Swine 605 did not eat AM or PM dose. Daily dose adjusted to 0%.
	606	Day 1 – Swine 606 did not eat PM dose. Daily dose adjusted to 50%.
	609	Day 1 – Swine 609 did not eat AM or PM dose. Daily dose adjusted to 0%.
	635	Day 1 – Swine 635 did not eat AM dose. Daily dose adjusted to 50%.
10	636	Day 10 – Swine 636 did not eat AM dose and only 50% of PM dose. Daily dose adjusted to 25%.

TABLE 4-2. Background Urinary Arsenic

Swine Number	Urine Collection Period (days)	As Dose (µg per collection period)	As Concentration in Urine (µg/L)	Urine Volume (µL)	Total As Excreted (µg/48 hours)
608	5/6	0	51	880	44.88
612	5/6	0	46	800	36.8
640	5/6	0	43	1110	47.73
608	9/10	0	45	1710	76.95
612	9/10	0	52	1400	72.8
640	9/10	0	57	1310	74.67
608	12/13	0	43	1810	77.83
612	12/13	0	72	900	64.8
640	12/13	0	39	1360	53.04

TABLE 4-3. Urine Excretion Fraction (UEF) Estimates

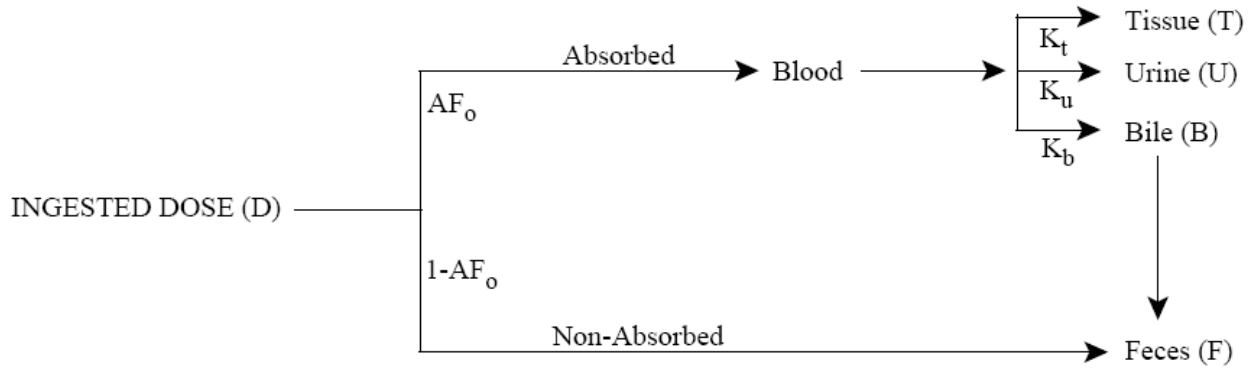
Urine Collection Period (days)	Outliers Excluded	Slopes (UEF Estimates)		
		b_r	b_{t1}	b_{t2}
Days 5/6	0	0.67	0.38	0.12
Days 9/10	0	0.64	0.45	0.14
Days 12/13	0	0.76	0.43	0.13
All Days	0	0.68	0.41	0.13

b_r = slope for reference material dose-response
b_{t1} = slope for test material 1 dose-response
b_{t2} = slope for test material 2 dose-response

TABLE 4-4. Estimated RBA for Iron King Soils

Urine Collection Period (days)	Estimated RBA (90% Confidence Interval)	
	Test Material 1 (HSJ583)	Test Material 2 (IKJ583)
Days 5/6	0.57 (0.50 - 0.65)	0.18 (0.16 - 0.21)
Days 9/10	0.70 (0.59 - 0.82)	0.21 (0.18 - 0.25)
Days 12/13	0.57 (0.51 - 0.63)	0.17 (0.16 - 0.19)
All Days	0.60 (0.56 -0.65)	0.19 (0.17 -0.20)

FIGURE 3-1. Conceptual Model for Arsenic Toxicokinetics



where:

D = ingested dose (µg)
AF_o = oral absorption fraction
K_t = fraction of absorbed arsenic which is retained in tissues
K_u = fraction of absorbed arsenic which is excreted in urine
K_b = fraction of absorbed arsenic which is excreted in the bile

Basic equations:

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

Amount Excreted (µg) $=$ Amount absorbed $\times K_u = D \times AF_o \times K_u$

Urinary Excretion Fraction (UEF) $=$ Amount excreted / Amount Ingested
$= (D \times AF_o \times K_u) / D$
$= AF_o \times K_u$

Relative Bioavailability (*x* vs. *y*) $= UEF(x) / UEF(y)$
$= (AF_o(x) \times K_u) / (AF_o(y) \times K_u)$
$= AF_o(x) / AF_o(y)$

FIGURE 3-2. Urinary Arsenic Variance Model

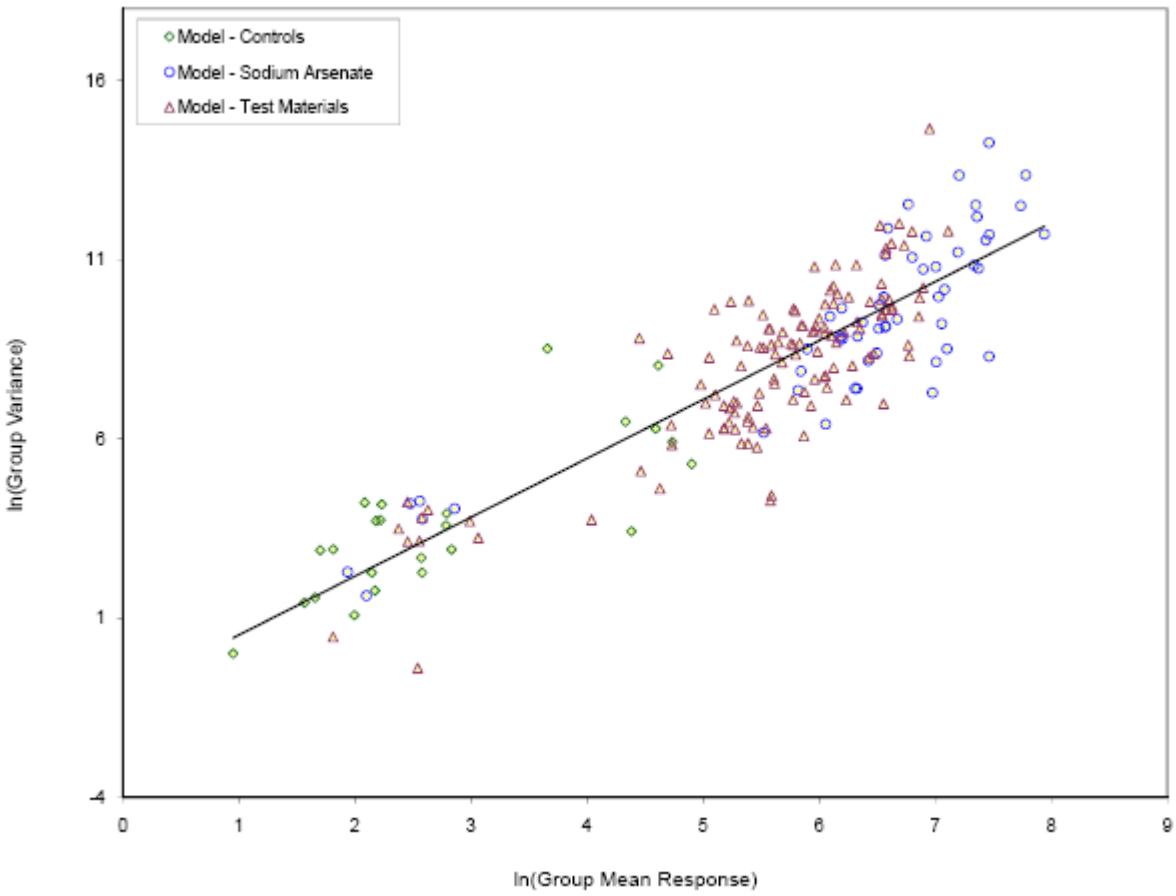


FIGURE 4-1. Iron King Data Compared to Urinary Arsenic Variance Model

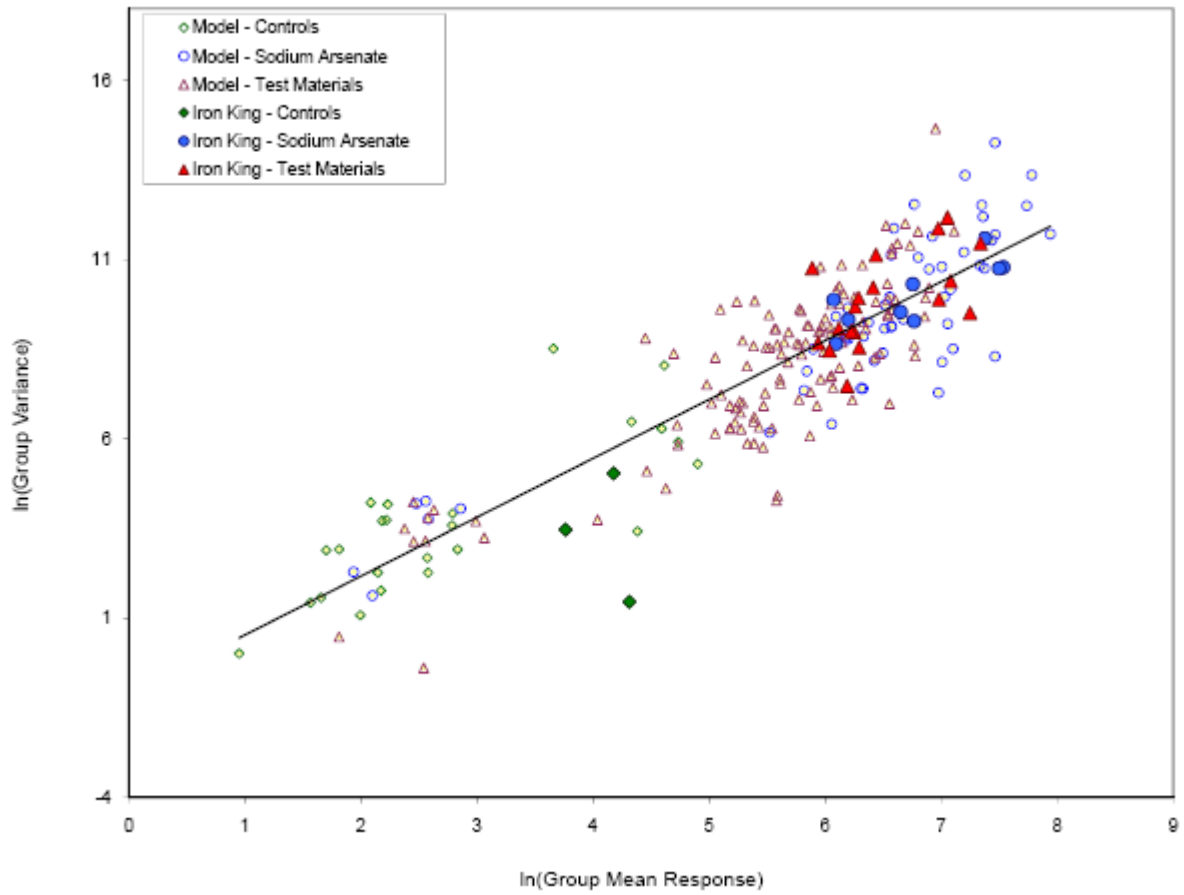
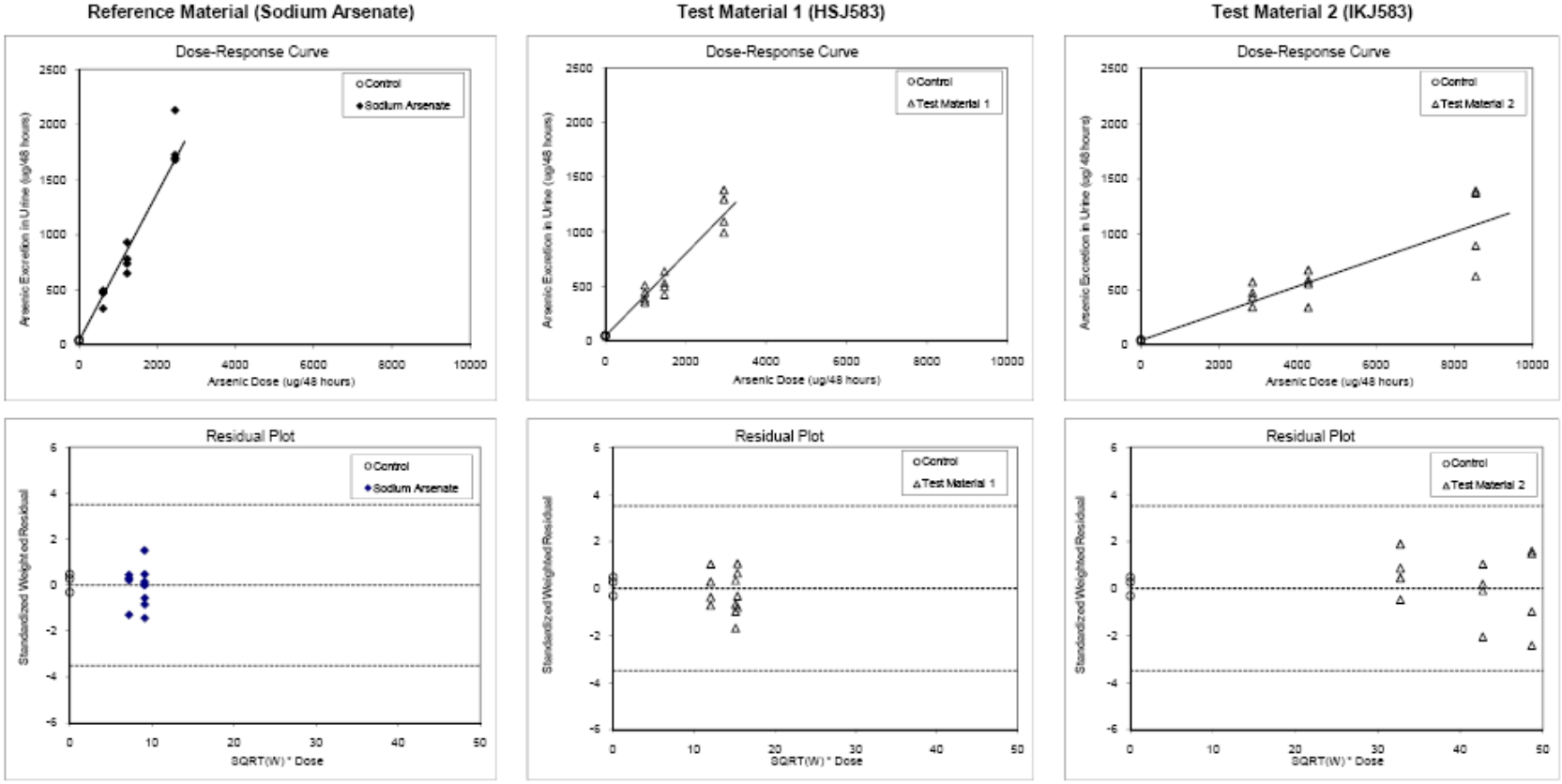


FIGURE 4-2. Iron King Urinary Excretion of Arsenic: Days 5/6



Summary of Fitting[a]

Parameter	Estimate	Standard Error
a	41.1	27.0
b_r	0.67	0.04
b_{t1}	0.38	0.03
b_{t2}	0.12	0.01
Covariance (b_r,b_{t1})	0.2870	--
Covariance (b_r,b_{t2})	0.2967	--
Degrees of Freedom	36	--

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

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

ANOVA

Source	SSE	DF	MSE
Fit	937.74	3	312.58
Error	45.75	35	1.31
Total	983.49	38	25.88

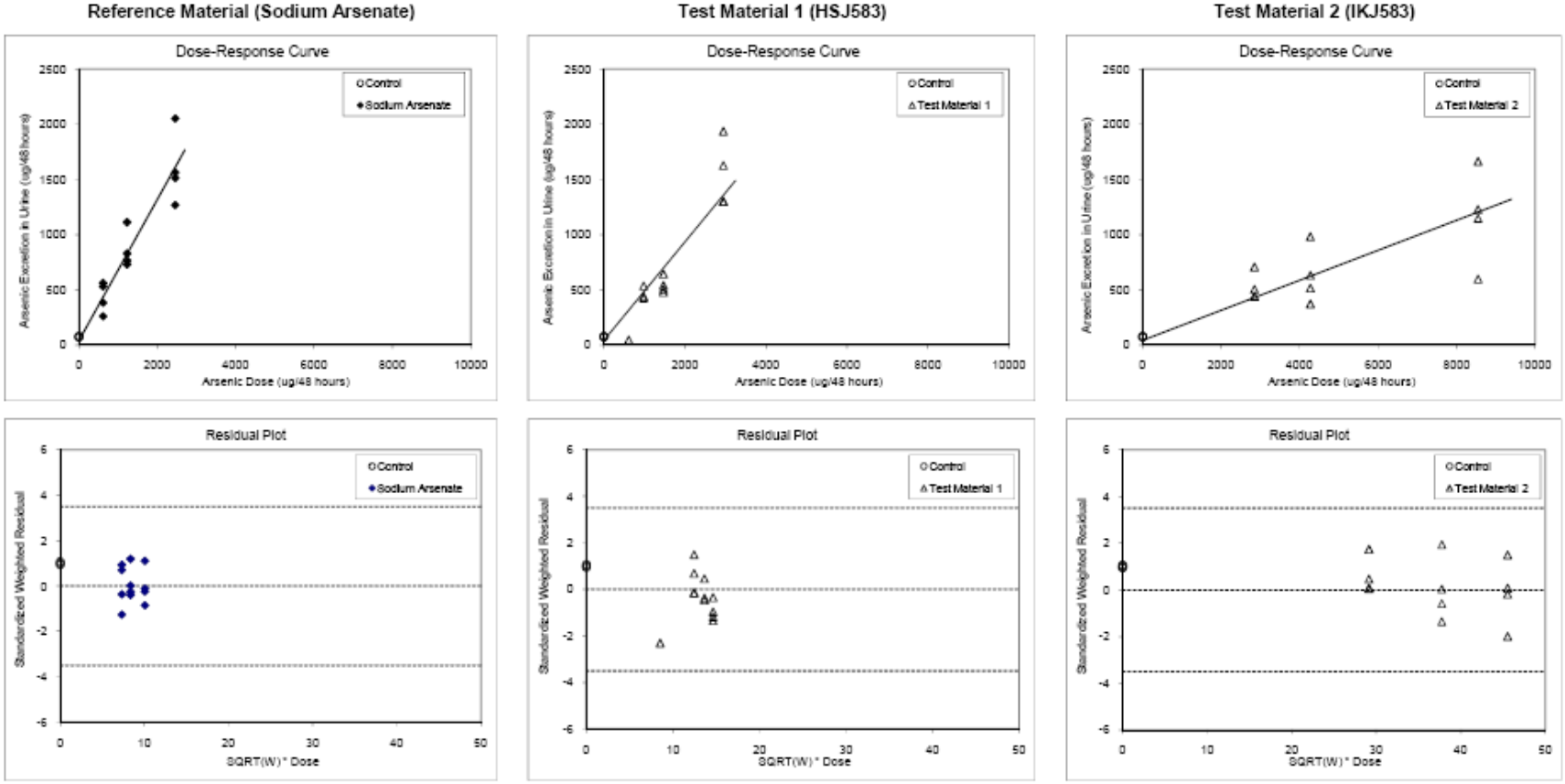
Statistic	Estimate
F	239.116
p	< 0.001
Adjusted R^2	0.9495

RBA and Uncertainty

	Test Material 1	Test Material 2
RBA	0.57	0.16
Lower bound[b]	0.50	0.16
Upper bound[b]	0.65	0.21
Standard Error[b]	0.045	0.015

[b] Calculated using Fieller's theorem

FIGURE 4-3. Iron King Urinary Excretion of Arsenic: Days 9/10



Summary of Fitting[a]

Parameter	Estimate	SE
a	42.3	37.7
b_r	0.64	0.05
b_{t1}	0.45	0.04
b_{t2}	0.14	0.01
Covariance (b_r,b_{t1})	0.3372	--
Covariance (b_r,b_{t2})	0.2877	--
Degrees of Freedom	36	--

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

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

ANOVA

Source	SSE	DF	MSE
Fit	866.64	3	288.88
Error	101.90	35	2.91
Total	968.54	38	25.49

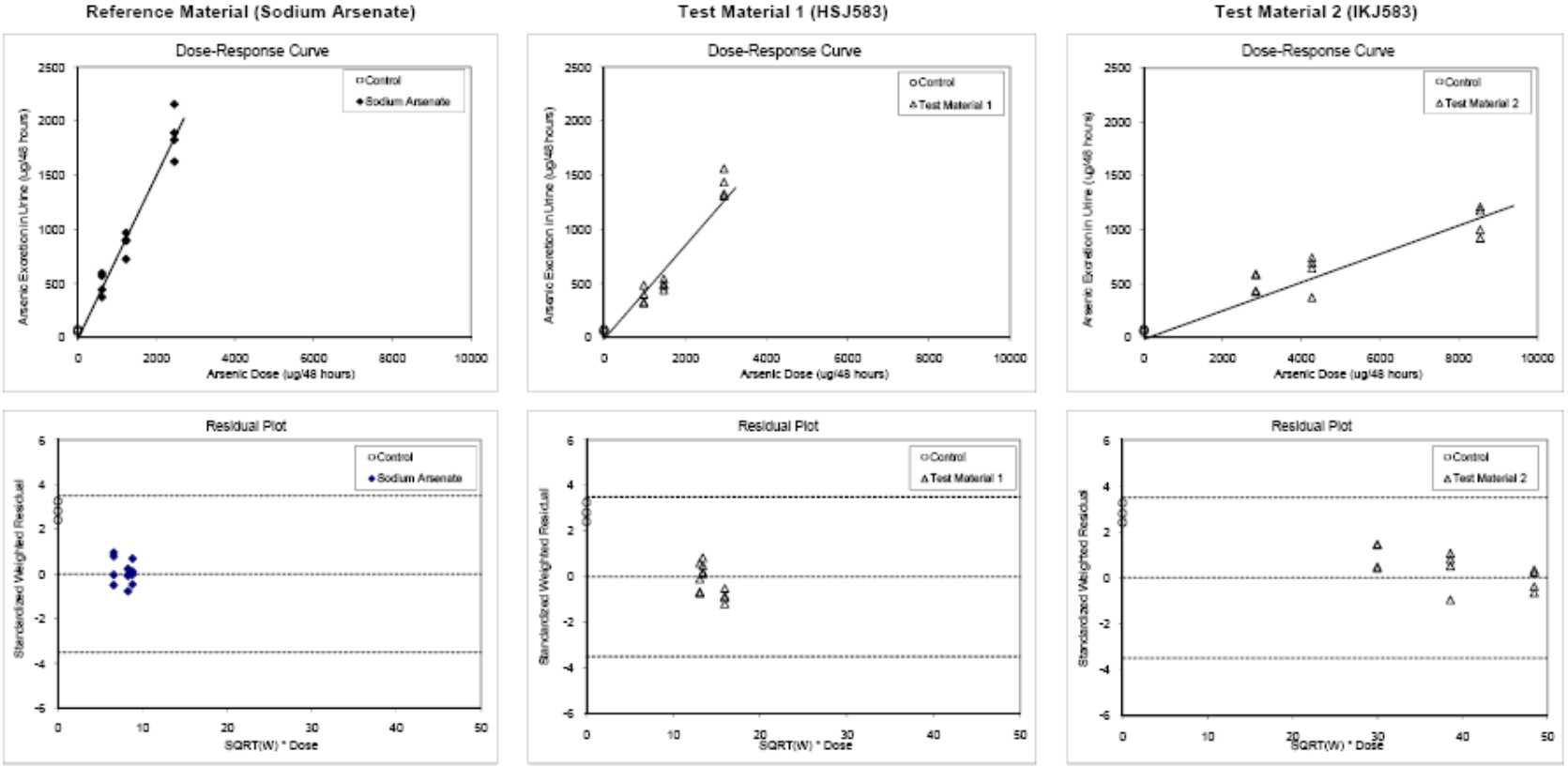
Statistic	Estimate
F	99.224
p	< 0.001
Adjusted R^2	0.8858

RBA and Uncertainty

	Test Material 1	Test Material 2
RBA	0.70	0.21
Lower bound[b]	0.59	0.18
Upper bound[b]	0.82	0.25
Standard Error[b]	0.066	0.022

[b] Calculated using Fieller's theorem

FIGURE 4-4. Iron King Urinary Excretion of Arsenic: Days 12/13



Summary of Fitting[a]

Parameter	Estimate	SE
a	-17.3	42.3
b_r	0.76	0.04
b_{t1}	0.43	0.03
b_{t2}	0.13	0.01
Covariance (b_r,b_{t1})	0.4835	--
Covariance (b_r,b_{t2})	0.4574	--
Degrees of Freedom	36	--

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

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

ANOVA

Source	SSE	DF	MSE
Fit	1044.09	3	348.03
Error	112.11	35	3.20
Total	1156.20	38	30.43

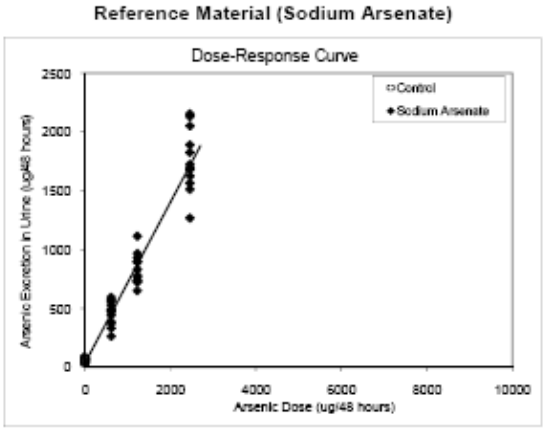
Statistic	Estimate
F	108.649
p	< 0.001
Adjusted R^2	0.8947

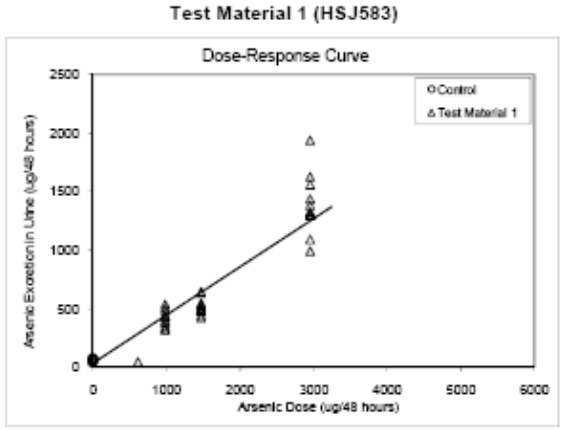
RBA and Uncertainty

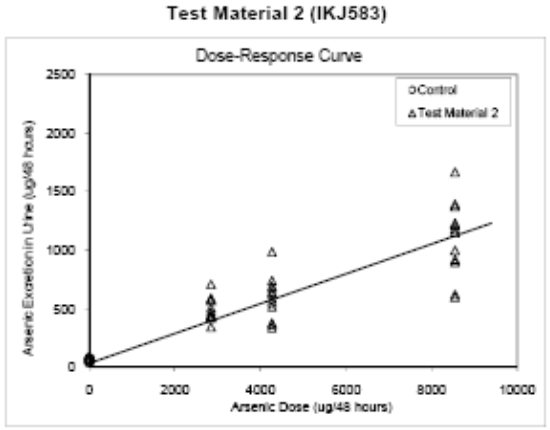
	Test Material 1	Test Material 2
RBA	0.57	0.17
Lower bound[b]	0.51	0.16
Upper bound[b]	0.63	0.19
Standard Error[b]	0.036	0.012

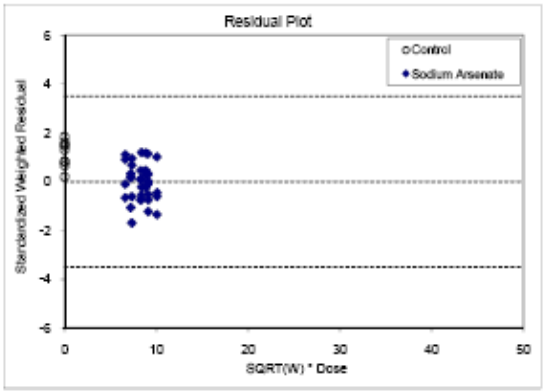
[b] Calculated using Fieller's theorem

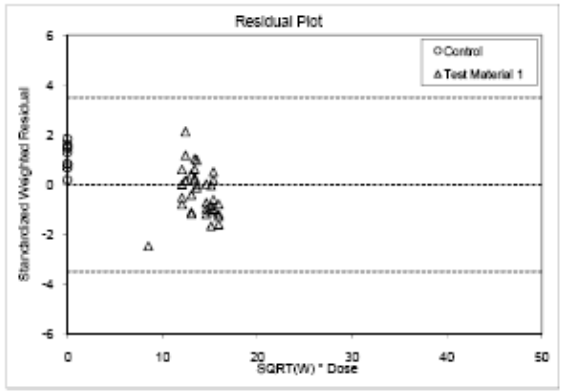
FIGURE 4-5. Iron King Urinary Excretion of Arsenic: All Days

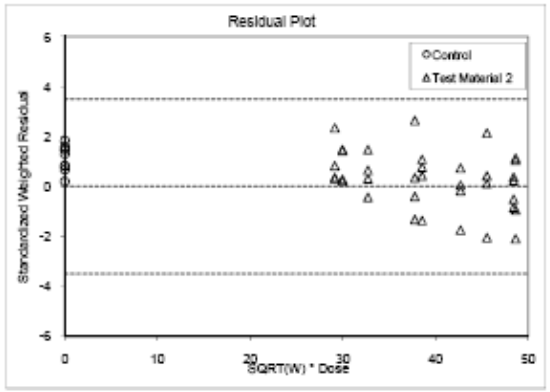












Summary of Fitting[a]

Parameter	Estimate	SE
a	33.4	19.7
b_r	0.68	0.02
b_{t1}	0.41	0.02
b_{t2}	0.13	0.01
Covariance (b_r,b_{t1})	0.3493	--
Covariance (b_r,b_{t2})	0.3250	--
Degrees of Freedom	114	--

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

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

ANOVA

Source	SSE	DF	MSE
Fit	2790.43	3	930.14
Error	215.50	113	1.91
Total	3005.93	116	25.91

Statistic	Estimate
F	487.725
p	< 0.001
Adjusted R^2	0.9264

RBA and Uncertainty

	Test Material 1	Test Material 2
RBA	0.60	0.19
Lower bound[b]	0.56	0.17
Upper bound[b]	0.65	0.20
Standard Error[b]	0.027	0.009

[b] Calculated using Fieller's theorem

APPENDIX A: Group Assignments for the Iron King Arsenic RBA Study November 2009

Swine Number	Group	Treatment	Actual Arsenic Dose [a] µg/kg bw-day
604 613 615 638	1	NaAs	25
611 626 635 641	2	NaAs	50
603 605 628 631	3	NaAs	100
619 633 636 643	4	TM1	40
616 622 627 629	5	TM1	60
602 602 607 609 623	66	TM1	120
606 624 625 639	7	TM2	116
601 610 620 637	8	TM2	175
614 630 632 634	9	TM2	349
608 612 640	10	Control	0

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

APPENDIX B: Body Weights

Group	Swine No.	Weight													
		Day -5 11/3/09	**Group** MBW	**Day -1** 11/8/09	**Group** MBW	**Day 2** 11/11/09	**Group** MBW	**Day 5** 11/14/09	**Group** MBW	**Day 8** 11/17/09	**Group** MBW	**Day 11** 11/20/09	**Group** MBW	**Day 14** 11/23/09	**Group** MBW
1 NaAs	604	8.9		9.2		9.6		10.2		10.5		11.8		12.5	
	613	8.2		8.1		8.4		8.9		9.5		10		10.3	
	615	7.8		8.8		9		9.3		10.2		11.1		11.7	
	638	9.2	8.53 ±0.64	9.1	8.80 ±0.50	9.7	9.18 ±0.60	10.3	9.68 ±0.68	11.1	10.33 ±0.67	11.8	11.18 ±0.85	12.3	11.70 ±0.99
2 NaAs	611	7.7		7.5		8.4		9		9.7		10.2		10.7	
	626	9		9.1		9.3		10.2		10.8		11.7		12.5	
	635	8.4		8.4		9.2		9.7		10.5		11.2		11.5	
	641	8.2	8.33 ±0.54	8.3	8.33 ±0.66	8.9	8.95 ±0.40	9.5	9.60 ±0.50	10.3	10.33 ±0.46	11	11.03 ±0.62	11.7	11.60 ±0.74
3 NaAs	603	8.5		8.5		9.1		9.6		10.4		11.3		11.9	
	605	8.5		8.5		8.8		9.5		10.2		11.2		11.8	
	628	9		9		9.6		10.2		11		11.4		12.4	
	631	9.1	8.78 ±0.32	9.6	8.90 ±0.52	10.5	9.50 ±0.74	11	10.08 ±0.69	11.7	10.83 ±0.68	12.8	11.68 ±0.75	13.3	12.35 ±0.69
4 TM1	619	9		9		9.8		10		10.8		11.6		12.1	
	633	7.9		8.3		9		9.6		10.2		10.8		11.1	
	636	9		9.1		9.6		9.9		11		10.3		11.2	
	643	8.2	8.53 ±0.56	8	8.60 ±0.54	8.8	9.30 ±0.48	9.4	9.73 ±0.28	10	10.50 ±0.48	10.9	10.90 ±0.54	11.4	11.45 ±0.45
5 TM1	616	8.8		8.8		9.2		10.1		10.6		11.4		12.1	
	622	8.9		9		9.3		9.9		10.5		10.9		11.3	
	627	7.6		7.9		8.7		9.1		9.9		10.4		10.8	
	629	8	8.33 ±0.63	7.9	8.40 ±0.58	8.4	8.90 ±0.42	8.9	9.50 ±0.59	9.5	10.13 ±0.52	10.1	10.70 ±0.57	10.7	11.23 ±0.64
6 TM1	602	8.4		8		8.9		9.4		10		11		11.8	
	607	8		8		8.7		9.1		9.7		10.7		11.6	
	609	7.9		7.7		7.8		8.8		9.4		10.1		10.7	
	623	9.3	8.40 ±0.64	9.1	8.20 ±0.62	9.8	8.80 ±0.82	10.5	9.45 ±0.74	11	10.03 ±0.69	12.1	10.98 ±0.84	12.6	11.68 ±0.78
7 TM2	606	9.2		9.2		9.6		10.3		11		11.9		12.6	
	624	8.4		8.1		8.5		9.1		9.8		10.7		10.9	
	625	8.8		8.7		8.5		9.4		10.4		11.5		11.8	
	639	9.5	8.98 ±0.48	9.3	8.83 ±0.55	9.8	9.10 ±0.70	10.1	9.73 ±0.57	10.3	10.38 ±0.49	10.8	11.23 ±0.57	11.5	11.70 ±0.71
8 TM2	601	8.3		8.4		8.5		9.3		10.1		10.9		11.4	
	610	8.6		8.1		8.9		9.3		9.8		11.1		11.7	
	620	9.4		8.7		8.7		8.9		9.7		10.3		10.9	
	637	8.3	8.65 ±0.52	8.1	8.33 ±0.29	8.3	8.60 ±0.26	8.8	9.08 ±0.26	9.5	9.78 ±0.25	10.5	10.70 ±0.37	10.7	11.18 ±0.46

Group	Swine No.	Weight													
		Day -5 11/3/09	**Group** MBW	**Day -1** 11/8/09	**Group** MBW	**Day 2** 11/11/09	**Group** MBW	**Day 5** 11/14/09	**Group** MBW	**Day 8** 11/17/09	**Group** MBW	**Day 11** 11/20/09	**Group** MBW	**Day 14** 11/23/09	**Group** MBW
9 TM2	614	9.1		8.5		9.3		10		10.8		11.7		12.2	
	630	8.7		8.8		9.5		10		10.6		11.8		12.3	
	632	8.4		8.2		8.5		9.1		9.6		10.5		11.2	
	634	8.7	8.73 ±0.29	8.4	8.48 ±0.25	8.5	8.95 ±0.53	9.3	9.60 ±0.47	10.1	10.28 ±0.54	10.8	11.20 ±0.65	11.6	11.83 ±0.52
10 Control	608	9.4		9		9.7		10.3		11.1		11.9		12.6	
	612	7.7		7.8		8.3		9.1		9,6		10.4		11	
	640	8.4	8.55 ±0.70	8.7	8.48 ±0.51	9.2	8.93 ±0.64	9.9	9.65 ±0.55	10.6	10.60 ±0.50	11.6	11.18 ±0.69	12.1	11.83 ±0.68

Group MBW = means and standard deviations of each group's body weight.

APPENDIX C: Typical Feed Composition

Purina TestDiet® 5TXP: Porcine Grower Purified Diet with Low Lead [a]

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

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

[a] This special purified diet was originally developed for lead RBA studies.
[b] Based on the latest ingredient analysis information. Since nutrient composition of natural ingredients varies, analysis will differ accordingly. Nutrients expressed as percent of ration on an As Fed basis except where otherwise indicated.
[c] Energy (kcal/gm) - Sum of decimal fractions of protein, fat and carbohydrate x 4,9,4 kcal/gm respectively.

APPENDIX D: Urinary Volumes and Urinary Arsenic Analytical Results for Iron King Study Samples

Group	Material	Collection Period (days)	Sample ID	Swine Number	Urinary As concentration (µg/L)	Urine Volume (mL)
1	NaAs	5/6	IK-109	604	280	1180
1	NaAs	5/6	IK-146	613	380	1240
1	NaAs	5/6	IK-126	615	220	2240
1	NaAs	5/6	IK-102	638	140	3420
1	NaAs	9/10	IK-193	604	150	1740
1	NaAs	9/10	IK-149	613	150	2560
1	NaAs	9/10	IK-178	615	190	2960
1	NaAs	9/10	IK-148	638	150	3540
1	NaAs	12/13	IK-212	604	150	2470
1	NaAs	12/13	IK-206	613	210	2100
1	NaAs	12/13	IK-228	615	140	4240
1	NaAs	12/13	IK-204	638	82	6940
2	NaAs	5/6	IK-112	611	495	1490
2	NaAs	5/6	IK-147	626	330	2360
2	NaAs	5/6	IK-128	635	290	2240
2	NaAs	5/6	IK-116	641	240	3880
2	NaAs	9/10	IK-174	611	404	2055
2	NaAs	9/10	IK-160	626	220	3480
2	NaAs	9/10	IK-185	635	435	1680
2	NaAs	9/10	IK-166	641	230	4840
2	NaAs	12/13	IK-227	611	240	3720
2	NaAs	12/13	IK-235	626	150	6440
2	NaAs	12/13	IK-226	635	340	2125
2	NaAs	12/13	IK-224	641	180	4980
3	NaAs	5/6	IK-118	603	960	1750
3	NaAs	5/6	IK-139	605	575	3000
3	NaAs	5/6	IK-127	628	300	5660
3	NaAs	5/6	IK-103	631	1300	1640
3	NaAs	9/10	IK-151	603	990	1580
3	NaAs	9/10	IK-158	605	488	3100
3	NaAs	9/10	IK-190	628	170	7460
3	NaAs	9/10	IK-163	631	950	2160
3	NaAs	12/13	IK-239	603	700	2700
3	NaAs	12/13	IK-208	605	290	5600
3	NaAs	12/13	IK-236	628	230	7940
3	NaAs	12/13	IK-240	631	1100	1960
4	TM1	5/6	IK-108	619	57	8880
4	TM1	5/6	IK-105	633	400	1100
4	TM1	5/6	IK-124	636	730	480
4	TM1	5/6	IK-120	643	140	2720
4	TM1	9/10	IK-172	619	72	7440
4	TM1	9/10	IK-155	633	260	1640
4	TM1	9/10	IK-150	636	140	320

Group	Material	Collection Period (days)	Sample ID	Swine Number	Urinary As concentration (µg/L)	Urine Volume (mL)
4	TM1	9/10	IK-173	643	54	8110
4	TM1	12/13	IK-220	619	47	10260
4	TM1	12/13	IK-215	633	290	1100
4	TM1	12/13	IK-232	636	492	660
4	TM1	12/13	IK-229	643	96	4130
5	TM1	5/6	IK-142	616	84	7560
5	TM1	5/6	IK-143	622	150	3300
5	TM1	5/6	IK-123	627	89	4720
5	TM1	5/6	IK-122	629	90	5860
5	TM1	9/10	IK-183	616	160	4015
5	TM1	9/10	IK-167	622	180	3000
5	TM1	9/10	IK-177	627	110	4600
5	TM1	9/10	IK-176	629	96	4980
5	TM1	12/13	IK-195	616	110	3990
5	TM1	12/13	IK-197	622	120	4000
5	TM1	12/13	IK-209	627	70	7020
5	TM1	12/13	IK-203	629	87	6220
6	TM1	5/6	IK-137	602	77	16860
6	TM1	5/6	IK-125	607	960	1440
6	TM1	5/6	IK-144	609	2600	420
6	TM1	5/6	IK-101	623	566	1750
6	TM1	9/10	IK-159	602	160	8130
6	TM1	9/10	IK-188	607	461	2820
6	TM1	9/10	IK-168	609	3400	570
6	TM1	9/10	IK-189	623	720	2260
6	TM1	12/13	IK-221	602	130	11040
6	TM1	12/13	IK-222	607	370	3590
6	TM1	12/13	IK-237	609	3000	520
6	TM1	12/13	IK-200	623	423	3090
7	TM2	5/6	IK-121	606	94	6060
7	TM2	5/6	IK-113	624	446	970
7	TM2	5/6	IK-135	625	67	7050
7	TM2	5/6	IK-115	639	100	3440
7	TM2	9/10	IK-186	606	81	8740
7	TM2	9/10	IK-184	624	190	2660
7	TM2	9/10	IK-165	625	57	7740
7	TM2	9/10	IK-171	639	80	5490
7	TM2	12/13	IK-234	606	66	8800
7	TM2	12/13	IK-233	624	100	5870
7	TM2	12/13	IK-199	625	66	6560
7	TM2	12/13	IK-214	639	72	5880
8	TM2	5/6	IK-117	601	89	7610
8	TM2	5/6	IK-131	610	320	1060
8	TM2	5/6	IK-130	620	730	800
8	TM2	5/6	IK-119	637	210	2640
8	TM2	9/10	IK-157	601	100	6310
8	TM2	9/10	IK-191	610	180	2075
8	TM2	9/10	IK-152	620	543	950
8	TM2	9/10	IK-156	637	390	2520

Group	Material	Collection Period (days)	Sample ID	Swine Number	Urinary As concentration (µg/L)	Urine Volume (mL)
8	TM2	12/13	IK-213	601	110	5820
8	TM2	12/13	IK-207	610	250	1480
8	TM2	12/13	IK-196	620	840	880
8	TM2	12/13	IK-238	637	150	4610
9	TM2	5/6	IK-107	614	580	2400
9	TM2	5/6	IK-106	630	230	2700
9	TM2	5/6	IK-111	632	700	1960
9	TM2	5/6	IK-134	634	390	2300
9	TM2	9/10	IK-164	614	517	3220
9	TM2	9/10	IK-162	630	360	3190
9	TM2	9/10	IK-181	632	640	1920
9	TM2	9/10	IK-175	634	390	1530
9	TM2	12/13	IK-219	614	290	3440
9	TM2	12/13	IK-231	630	250	4840
9	TM2	12/13	IK-230	632	512	2300
9	TM2	12/13	IK-198	634	451	2040
10	Control	5/6	IK-129	608	51	880
10	Control	5/6	IK-104	612	46	800
10	Control	5/6	IK-138	640	43	1110
10	Control	9/10	IK-179	608	45	1710
10	Control	9/10	IK-192	612	52	1400
10	Control	9/10	IK-154	640	57	1310
10	Control	12/13	IK-217	608	43	1810
10	Control	12/13	IK-225	612	72	900
10	Control	12/13	IK-205	640	39	1360

APPENDIX E: Analytical Results for Quality Control Samples

TABLE E-1. Blind Duplicate Samples

Blind Duplicate Sample ID	Sample Type	Swine Number	Urine Collection Days	Original Sample Concentration (µg/L)	Duplicate Concentration (µg/L)	RPD
IK-114	Urine	611	6/7	495	506	2%
IK-133	Urine	609	6/7	2600	2500	4%
IK-136	Urine	601	6/7	89	85	5%
IK-161	Urine	612	9/10	52	51	2%
IK-170	Urine	625	9/10	57	58	2%
IK-187	Urine	613	9/10	150	160	6%
IK-201	Urine	614	12/13	290	280	4%
IK-210	Urine	643	12/13	96	100	4%
IK-211	Urine	602	12/13	130	130	0%

RPD = relative percent difference.

TABLE E-2. Laboratory Spikes

Spike Sample ID	Sample Type	Original Sample Concentration (ppb)	Added Spike Concentration (ppb)	Measured Sample concentration (ppb)	Recovered Spike (ppb)	Recovery
IK-110	Urine	140	200	320	180	90%
IK-120	Urine	140	200	330	190	95%
IK-130	Urine	730	200	880	150	75%
IK-140	Urine	52	200	240	188	94%
IK-150	Urine	140	200	330	190	95%
IK-160	Urine	220	200	413	193	97%
IK-170	Urine	58	200	250	192	96%
IK-180	Urine	436	200	700	264	132%
IK-190	Urine	170	200	360	190	95%
IK-200	Urine	423	200	700	277	139%
IK-210	Urine	100	200	300	200	100%
IK-220	Urine	4747	200	250	203	102%
IK-230	Urine	512	200	790	278	139%
IK-240	Urine	1100	200	1300	200	100%
IK-276	Feed	<0.25	55.9	56	55.7	100%
IK-277	Water	<0.05	9.9	11	11	110%

TABLE E-3. Laboratory Duplicates

Duplicate Sample ID	Sample Type	Original Sample Concentration (ppb)	Duplicate Concentration (ppb)	RPD	Absolute Difference
IK-105	Urine	400	400	0%	0
IK-115IK-115	Urine	100	100	0%	0
IK-125	Urine	960	1000	4%	40
IK-135	Urine	67	67	0%	0
IK-145	Urine	70	68	3%	2
IK-155	Urine	260	280	7%	20
IK-165	Urine	57	58	2%	1
IK-175	Urine	390	436	11%	46
IK-185	Urine	435	486	11%	51
IK-195	Urine	110	120	9%	10
IK-206	Urine	210	210	0%	0
IK-215	Urine	290	280	4%	10
IK-225	Urine	72	74	3%	2
IK-235	Urine	150	150	0%	0
IK-273	Feed	<0.25	<0.25	0%	0
IK-277	Water	<0.05	<0.05	0%	0

RPD = relative percent difference.

TABLE E-4. Laboratory Quality Control Standards

Sample ID	Measured Arsenic Concentration (ppb)	Detection Limit (ppb)	Reference Material ID	Certified Mean ± Standard Deviation	Recovery
QC 1	200	10	NIST 2670a-H	220 ± 10	91%
QC-2	210	10	NIST 2670a-H	220 ± 10	95%
QC-3	210	10	NIST 2670a-H	220 ± 10	95%
QC-4	230	10	NIST 2670a-H	220 ± 10	105%
QC-5	210	10	NIST 2670a-H	220 ± 10	95%
QC-6	220	10	NIST 2670a-H	220 ± 10	100%
QC-7	<5	5	NIST 2670a-L	3	83%
QC-8	57	1	NIST 1643e	58.98 ± 0.7	97%
QC-9	7.5	0.2	NIST 1566b	7.65 ± 0.65	98%

TABLE E-5. Performance Evaluation Samples

Sample ID	PE ID	PE Standard	PE Concentration	Sample Concentration	Adjusted Concentration	RPD
IK-140	ctrl	Control Urine	0	52	2	
IK-218	ctrl	Control Urine	0	39	0	0%
IK-141	mma20	Dimethyl arsenic acid	20	64	14	34%
IK-180	mma400	Dimethyl arsenic acid	400	436	386	4%
IK-216	mma100	Dimethyl arsenic acid	100	180	130	26%
IK-145	dma20	Disodium methylarsenate	20	70	20	1%
IK-169	dma100	Disodium methylarsenate	100	170	120	18%
IK-223	dma400	Disodium methylarsenate	400	462	412	3%
IK-110	as5.100	Sodium arsenate	100	140	90	10%
IK-182	as5.20	Sodium arsenate	20	64	14	34%
IK-202	as5.400	Sodium arsenate	400	408	358	11%
IK-132	as3.400	Sodium arsenite	400	414	364	9%
IK-153	as3.100	Sodium arsenite	100	130	80	22%
IK 194	as3.20	Sodium arsenite	20	60	10	65%

PE = performance evaluation. Sample concentration adjusted by subtracting mean of background arsenic (~50 ug/L) from sample concentration.
RPD = relative percent difference.

TABLE E-6. Blanks

Sample ID	Measured Arsenic Concentration (ppb)	Detection Limit (ppb)
Blank-1	<1	1
Blank-2	<1	1
Blank-3	<1	1
Blank-4	<1	1
Blank-5	<1	1
Blank-6	<1	1
Blank-7	<1	1
Blank-8	<0.5	0.5
Blank-9	<0.1	0.1

FIGURE E-1. Urinary Arsenic Blind Duplicates

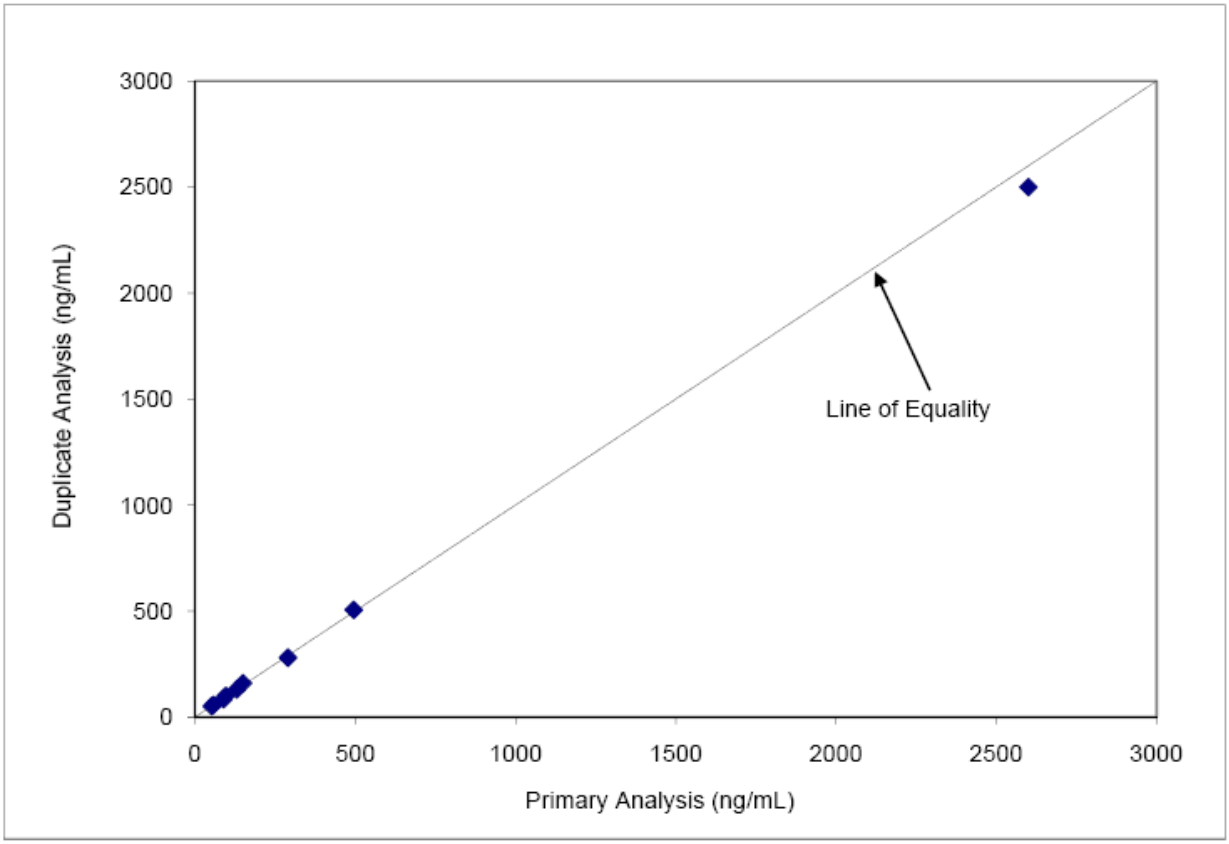
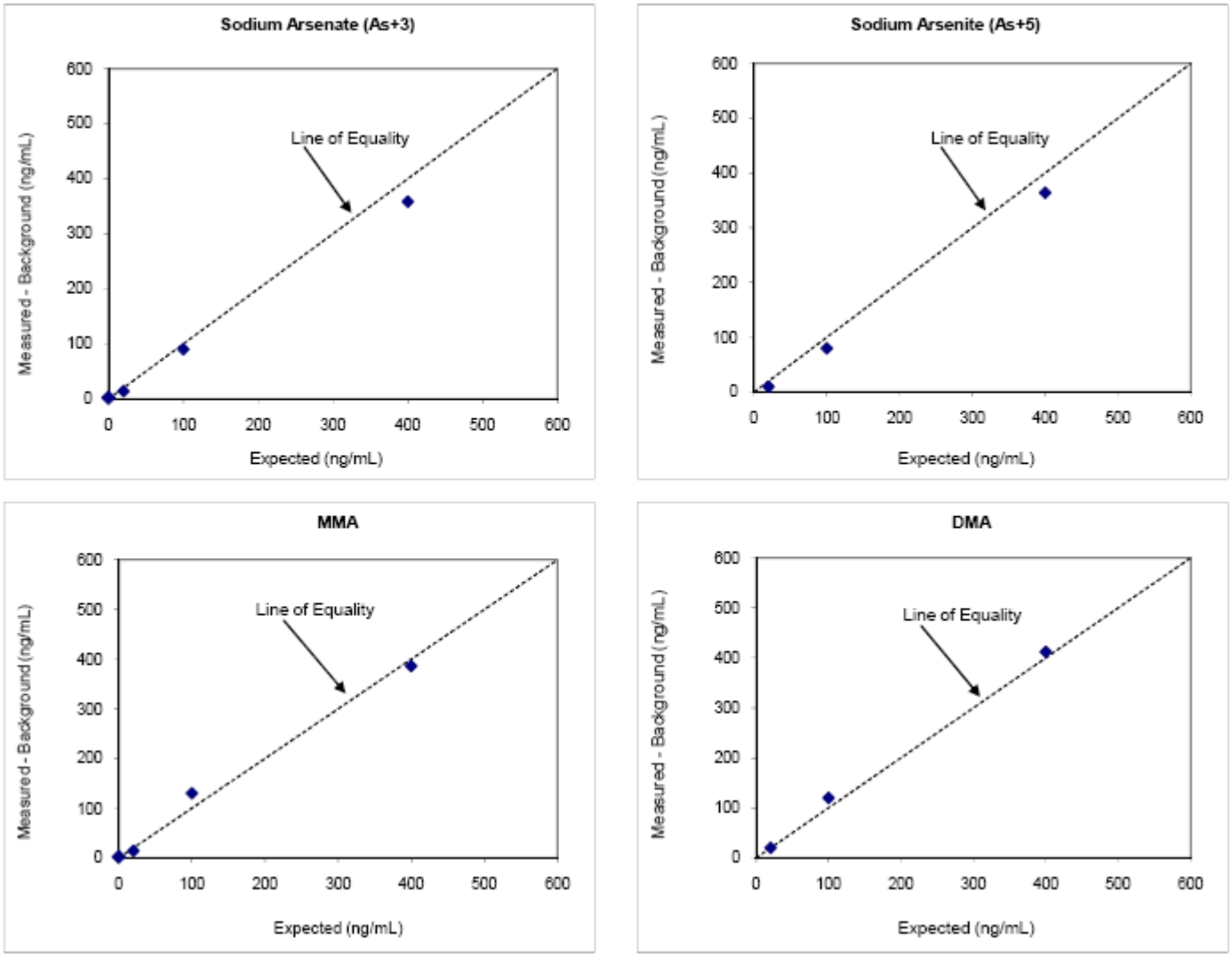


FIGURE E-2. Performance Evaluation Samples



SRC TR-09-254

RELATIVE BIOAVAILABILITY OF ARSENIC IN A MOHR ORCHARD SOIL

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EXECUTIVE SUMMARY

A study using juvenile swine as test animals was performed to measure the gastrointestinal absorption of arsenic from a Mohr Orchard soil sample. The soil sample was collected from the Mohr Orchard site located in Lehigh County, Pennsylvania. The property was historically largely utilized as orchards and currently consists of farmland, woodland, residential, commercial, and industrial properties. The arsenic concentration of the Mohr Orchard soil sample is 340±4.5 mg/kg (mean±SD).

The relative oral bioavailability of arsenic was assessed by comparing the absorption of arsenic from the Mohr Orchard soil ("test material") to that of sodium arsenate. Groups of four swine were given oral doses of sodium arsenate or the test material twice a day for 14 days. Three non-treated swine served as a control.

The amount of arsenic absorbed by each animal was evaluated by measuring the amount of arsenic excreted in the urine (collected over 48-hour periods beginning on days 6, 9, and 12). The urinary excretion fraction (UEF) is the ratio of the amount excreted per 48 hours divided by the dose given per 48 hours. UEF was calculated for the test material and the sodium arsenate using simultaneous weighted linear regression. The relative bioavailability (RBA) of arsenic in each test material compared to sodium arsenate was calculated as follows:

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

Estimated RBA values (mean and 90% confidence interval) are shown below:

Estimated RBA for Mohr Orchard Soil

Measurement Interval	Estimated RBA (90% Confidence Interval)
Days 6/7	0.50 (0.46–0.55)
Days 9/10	0.54 (0.49–0.59)
Days 12/13	0.56 (0.50–0.63)
All Days	0.53 (0.51–0.57)

The best fit point estimate RBA for the Mohr Orchard soil sample is 53%.

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ACRONYMS AND ABBREVIATIONS

ABA	Absolute bioavailability
AF_o	Oral absorption fraction
As^{+3}	Trivalent inorganic arsenic
As^{+5}	Pentavalent inorganic arsenic
°C	Degrees Celsius
D	Ingested dose
DMA	Dimethyl arsenic
g	Gram
GLP	Good Laboratory Practices
kg	Kilogram
K_u	Fraction of absorbed arsenic which is excreted in urine
mL	Milliliter
MMA	Monomethyl arsenic
N	Number of data points
NAA	Neutron activation analysis
NaAs	Sodium arsenate
NERL	National Exposure Research Laboratory
NIST	National Institute of Standards and Technology
NRCC	National Research Council of Canada
QC	Quality control
RBA	Relative bioavailability
ref	Reference material
RfD	Reference dose
RPD	Relative percent difference
SD	Standard deviation
SF	Slope factor
SRM	Standard reference material
TM	Test material
UEF	Urinary excretion fraction
µg	Microgram
µm	Micrometer
USEPA	United States Environmental Protection Agency
XRF	X-ray fluorescence

1.0 INTRODUCTION

1.1 Overview of Bioavailability

Reliable analysis of the potential hazard to humans from ingestion of a chemical depends upon accurate information on a number of key parameters, including the concentration of the chemical in environmental media (*e.g.*, soil, dust, water, food, air, paint), intake rates of each medium, and the rate and extent of absorption ("bioavailability") of the chemical by the body from each ingested medium. The amount of a chemical that actually enters the body from an ingested medium depends on the physical-chemical properties of the chemical and of the medium. For example, some metals in soil may exist, at least in part, as poorly water-soluble minerals, and may also exist inside particles of inert matrix such as rock or slag of variable size, shape, and association. These chemical and physical properties may influence (usually decrease) the absorption (bioavailability) of the metals when ingested. Thus, equal ingested doses of different forms of a chemical in different media may not be of equal health concern.

Bioavailability of a chemical in a particular medium may be expressed either in absolute terms (absolute bioavailability) or in relative terms (relative bioavailability):

Absolute bioavailability (ABA) is the ratio of the amount of the chemical absorbed to the amount ingested:

$$ABA = \frac{Absorbed\ \ Dose}{Ingested\ \ Dose}$$

This ratio is also referred to as the oral absorption fraction (AF_o).

Relative bioavailability (RBA) is the ratio of the AF_o of the chemical present in some test material (*test*) to the AF_o of the chemical in some appropriate reference material (*e.g.*, either the chemical dissolved in water or a solid form that is expected to fully dissolve in the stomach) (*ref*):

$$RBA(test\ vs.\ ref) = \frac{AF_o(test)}{AF_o(ref)}$$

For example, if 100 micrograms (μg) of a chemical (*e.g.*, arsenic) dissolved in drinking water were ingested and a total of 50 μg were absorbed into the body, the AF_o would be 50/100,

or 0.50 (50%). Likewise, if 100 µg of a chemical contained in soil were ingested and 30 µg were absorbed into the body, the AF_o for this chemical in soil would be 30/100, or 0.30 (30%). If the chemical dissolved in water were used as the frame of reference for describing the relative amount of the same chemical absorbed from soil, the RBA would be 0.30/0.50, or 0.60 (60%).

For additional discussion about the concept and application of bioavailability, see Gibaldi and Perrier (1982), Goodman *et al.* (1990), and Klaassen *et al.* (1996).

1.2 Using RBA Data to Improve Risk Calculations

When reliable data are available on the RBA of a chemical in a site medium (*e.g.*, soil), the information can be used to improve the accuracy of exposure and risk calculations at that site. RBA data can be used to adjust default oral toxicity values (reference dose and slope factor) to account for differences in absorption between the chemical ingested in water and the chemical ingested in site media, assuming the toxicity factors are based on a readily soluble form of the chemical. For non-cancer effects, the default reference dose ($RfD_{default}$) can be adjusted ($RfD_{adjusted}$) as follows:

$$RfD_{adjusted} = \frac{RfD_{default}}{RBA}$$

For potential carcinogenic effects, the default slope factor ($SF_{default}$) can be adjusted ($SF_{adjusted}$) as follows:

$$SF_{adjusted} = SF_{default} \cdot RBA$$

Alternatively, it is also acceptable to adjust the dose (rather than the toxicity factors) as follows:

$$Dose_{adjusted} = Dose_{default} \cdot RBA$$

This dose adjustment is mathematically equivalent to adjusting the toxicity factors as described above.

1.3 Purpose of this Study

The objective of this study was to use juvenile swine as a test system in order to determine the RBA of arsenic in a Mohr Orchard soil sample compared to a soluble form of arsenic (sodium arsenate).

2.0 STUDY DESIGN

The test material and a reference material (sodium arsenate, NaAs) were administered to groups of four juvenile swine at three different dose levels for 14 days. The study included a non-treated group of three animals to serve as a control for determining background arsenic levels. Study details are presented in Table 2-1. All doses were administered orally. The study was performed as nearly as possible within the spirit and guidelines of Good Laboratory Practices (GLP: 40 CFR 792).

Table 2-1. Study Design and Dosing Information

Group	Group name abbreviation	Dose material administered	As concentration of material (µg/g or µg/µL)	Number swine in group	Arsenic Dose		
					Target (µg/kg BW-day)	Actual [a] (µg/kg BW-day)	Actual [b] (µg-day)
1	NaAs	Sodium Arsenate	2	4	25	29	308
2	NaAs	Sodium Arsenate	10	4	50	62	620
3	NaAs	Sodium Arsenate	10	4	100	130	1240
4	TM1	Mohr Orchard Soil	340	4	40	52	493
5	TM1	Mohr Orchard Soil	340	4	60	72	738
6	TM1	Mohr Orchard Soil	340	4	120	153	1476
7	Control	None (negative control)	0	3	0	0	0

[a] Calculated as the administered daily dose divided by the measured or extrapolated daily body weight, averaged over days 0–14 for each animal and each group.
[b] Calculated as the mass of soil or sodium arsenate solution administered times the concentration of the soil or sodium arsenate solution.

Doses were administered in two equal portions given at 9:00 AM and 3:00 PM each day. Doses were held constant based on the expected mean weight during the exposure interval (14 days).

2.1 Test Materials

2.1.1 Sample Description

The former Mohr Orchard site is located in Lehigh County, Pennsylvania and consists of farmland, woodland, residential, commercial, and industrial properties. Historically, large portions of the site were utilized as orchards and arsenical pesticides were commonly used to control pests.

2.1.2 Sample Preparation and Analysis

Soil was collected from two, 200-square foot grids that were located next to one another on county property. These areas had arsenic concentrations >100 ppm (as identified *in situ* using X-ray fluorescence [XRF] technology). The soil material was collected into 2-gallon buckets, homogenized, and placed into large plastic bags for storage. Upon receipt of soil at EPA's Office of Research and Development, National Exposure Research Laboratory (NERL), soil was air-dried on drying trays for 4 days at 40°C. Soil was then sieved to remove plant material, rocks and large chunks of aggregated soil, and finally screened to <250 µm. Soil was then passed through a riffler 5 times and 200 gram aliquots were collected in pre-cleaned 250 mL high-density polyethylene bottles for the study.

Soil metal concentrations were determined by neutron activation analysis (NAA). Two subsamples of the Mohr Orchard soil were analyzed in duplicate. The arsenic concentration of the Mohr Orchard soil sample is 340±4.5 mg/kg (mean±SD).

X-ray absorption spectroscopy was conducted on the test material to characterize the arsenic mineralogy (Miller and Scheckel, 2012).

2.2 Experimental Animals

Juvenile swine were selected for use because they are considered to be a good physiological model for gastrointestinal absorption in children (Casteel *et al.*, 1996; Weis and LaVelle, 1991). The animals were intact males of the Pig Improvement Corporation genetically defined Line 26, and were purchased from Chinn Farms, Clarence, Missouri.

The number of animals purchased for the study was several more than required by the protocol. These animals were purchased at an age of about 5–6 weeks (weaning occurs at age

3 weeks) and housed in individual stainless steel cages. The animals were then held under quarantine for one week to observe their health before beginning exposure to dosing materials. Each animal was examined by a certified veterinary clinician (swine specialist) and any animals that appeared to be in poor health during this quarantine period were excluded from the study. To minimize weight variations among animals and groups, extra animals most different in body weight (either heavier or lighter) five days prior to exposure (day 5) were also excluded from the study. The remaining animals were assigned to dose groups at random (group assignments are presented in Appendix A).

When exposure began (day zero), the animals were about 6–7 weeks old. The animals were weighed at the beginning of the study and every three days during the course of the study. In each study, the rate of weight gain was comparable in all dosing groups. Body weight data are presented in Appendix B.

All animals were examined daily by an attending veterinarian while on the study and were subjected to detailed examination at necropsy by a certified veterinary pathologist in order to assess overall animal health.

2.3 Diet

Animals were weaned onto standard swine chow (made at the University of Missouri Animal Science Feed Mill). The feed was nutritionally complete (NRC, 1988). The ingredients of the feed are presented in Appendix C. Arsenic concentration in a randomly selected feed sample measured 0.1 μg/g.

Prior to the start of dosing and throughout the dosing period, each day every animal was given an amount of feed equal to 4.0% of the mean body weight of all animals on study. Feed amounts were adjusted every three days, when animals were weighed. Feed was administered in two equal portions, at 11:00 AM and 5:00 PM daily.

Drinking water was provided *ad libitum* via self-activated watering nozzles within each cage. Arsenic concentration of 5 water samples from randomly selected drinking water nozzles were <1 μg/L.

2.4 Dosing

Animals were exposed to dosing materials (sodium arsenate or sieved test material) for 14 days, with the dose for each day being administered in two equal portions beginning at 9:00 AM and 3:00 PM (two hours before feeding). Swine were dosed two hours before feeding to ensure that they were in a semi-fasted state. To facilitate dose administration, dosing materials were placed in a small depression in a ball of dough consisting of moistened feed (typically about 5 g) and the dough was pinched shut. This was then placed in the feeder at dosing time.

Target arsenic doses (expressed as µg of arsenic per kg of body weight per day) for animals in each group were determined in the study design (Table 2-1). The daily mass of arsenic administered (either as sodium arsenate or as sieved test material) to animals in each group was calculated by multiplying the target dose (µg/kg-day) for that group by the anticipated average weight of the animals (kg) over the course of the study:

$$Mass\ (\mu g / day) = Dose\ (\mu g / kg - day) \cdot Average\ Body\ Weight\ (kg)$$

The average body weight expected during the course of the study was estimated by measuring the average body weight of all animals one day before the study began, and then assuming an average weight gain of 0.5 kg/day during the study. After completion of the study, the true mean body weight was calculated using the actual body weights (measured every three days during the study), and the resulting true mean body weight was used to calculate the actual doses achieved. Any missed or late doses were recorded and the actual doses adjusted accordingly. Actual doses (µg arsenic per day) for each group are shown in Table 2-1.

2.5 Collection and Preservation of Urine Samples

Samples of urine were collected from each animal for 48-hour periods on days 6 to 7 (U-1), 9 to 10 (U-2), and 12 to 13 (U-3) of the study. Collection began at 8:00 AM and ended 48 hours later. The urine was collected in a plastic bucket placed beneath each cage, which was emptied into a plastic storage bottle. Aluminum screens were placed under the cages to minimize contamination with feces or spilled food. Due to the length of the collection period, collection containers were emptied periodically (typically twice daily) into a separate plastic bottles to ensure that there was no loss of sample due to overflow.

At the end of each collection period, the total urine volume for each animal was measured (Appendix D) and three 60-mL portions were removed and acidified with 0.6 mL concentrated nitric acid. All samples were refrigerated. Two of the aliquots were archived and one aliquot was sent for arsenic analysis (refrigeration was maintained until arsenic analysis).

2.6 Arsenic Analysis

Urine samples were assigned random chain-of-custody tag numbers and submitted to the analytical laboratory for analysis in a blind fashion. The samples were analyzed for arsenic by L. E. T., Inc., (Columbia, Missouri). In brief, 25-mL samples of urine were digested by refluxing and then heating to dryness in the presence of magnesium nitrate and concentrated nitric acid. Following magnesium nitrate digestion, samples were transferred to a muffle furnace and ashed at 500°C. The digested and ashed residue was dissolved in hydrochloric acid and analyzed by the hydride generation technique using a PerkinElmer 3100 atomic absorption spectrometer. Previous tests of this method established that each of the different forms of arsenic that may occur in urine, including trivalent inorganic arsenic (As^{+3}), pentavalent inorganic arsenic (As^{+5}), monomethyl arsenic (MMA), and dimethyl arsenic (DMA) are all recovered with high efficiency.

Analytical results for the urine samples are presented in Appendix D.

2.7 Quality Control

A number of quality control (QC) steps were taken during this project to evaluate the accuracy of the analytical procedures. The results for QC samples are presented in Appendix E and are summarized below.

Blind Duplicates (Sample Preparation Replicates)

A random selection of about 10% of all urine samples generated during the study were prepared for laboratory analysis in duplicate (*i.e.*, two separate subsamples of urine were digested) and submitted to the laboratory in a blind fashion. Results are shown in Appendix E (see Table E-1 and Figure E-1). There was generally good agreement between results for the duplicate pairs.

Spike Recovery

During arsenic analysis, one feed sample and every tenth urine sample was spiked with known amounts of arsenic (sodium arsenate) and the recovery of the added arsenic was measured. Results show that mean arsenic concentrations recovered from spiked samples were generally within 10% of actual arsenic concentrations (see Appendix E, Table E-2).

Laboratory Duplicates

During arsenic analysis, every tenth sample was analyzed in duplicate. Duplicate results for urine samples typically agreed within 10% relative percent difference (RPD) (see Appendix E, Table E-3). The duplicate water sample was below the detection limit. A duplicate analysis of a feed sample matched the original feed sample concentration (0.1 µg/g).

Laboratory Control Standards

National Institute of Standards and Technology (NIST) Standard Reference Materials® (SRM), for which a certified concentration of specific analytes has been established, were tested periodically during sample analysis (NIST, 2003). Recovery of arsenic from these standards was generally good and within the acceptable range (see Appendix E, Table E-4 and Figure E-2).

Blanks

Blank samples run along with each batch of samples (n=8). Blanks never yielded a measurable level of arsenic (see Appendix E, Table E-5).

Summary of QC Results

Based on the results of all of the QC samples and steps described above, it is concluded that the analytical results are of sufficient quality for derivation of reliable estimates of arsenic absorption from the test materials.

3.0 DATA ANALYSIS

3.1 Overview

Figure 3-1 shows a conceptual model for the toxicokinetic fate of ingested arsenic. Key points of this model are as follows:

- In most animals (including humans), absorbed arsenic is excreted mainly in the urine over the course of several days. Thus, the urinary excretion fraction (UEF), defined as

the amount excreted in the urine divided by the amount given, is usually a reasonable approximation of the AF_o or ABA. However, this ratio will underestimate total absorption, because some absorbed arsenic is excreted in the feces via the bile, and some absorbed arsenic enters tissue compartments (*e.g.*, skin, hair) from which it is cleared very slowly or not at all. Thus, the urinary excretion fraction should not be equated with the absolute absorption fraction.

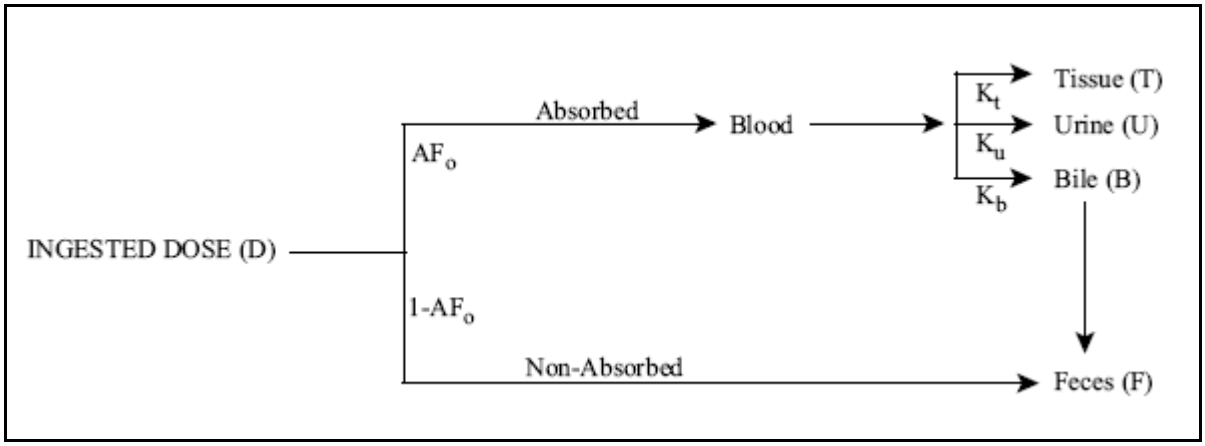
- The RBA of two orally administered materials (*i.e.*, a test material and reference material) can be calculated from the ratio of the urinary excretion fraction of the two materials. This calculation is independent of the extent of tissue binding and of biliary excretion:

$$RBA(\text{test vs ref}) = \frac{AF_o(\text{test})}{AF_o(\text{ref})} = \frac{AF_o(\text{test}) \cdot K_u}{AF_o(\text{ref}) \cdot K_u} = \frac{UEF(\text{test})}{UEF(\text{ref})}$$

where:

D = ingested dose (μg)
K_u = fraction of absorbed arsenic that is excreted in the urine

Figure 3-1. Conceptual Model for Arsenic Toxicokinetics



where:

D = ingested dose
AF_o = oral absorption fraction
K_t = fraction of absorbed arsenic that is retained in tissues
K_u = fraction of absorbed arsenic that is excreted in urine
K_b = fraction of absorbed arsenic that is excreted in bile

Basic Equations

Amount absorbed (μg) $= D \times AF_o$

Amount excreted in urine (μg) $= Amount\ absorbed \times K_u$
$= D \times AF_o \times K_u$

Urinary excretion fraction (UEF) $= Amount\ excreted\ /\ Amount\ ingested$
$= (D \times AF_o \times K_u) / D$
$= AF_o \times K_u$

Relative bioavailability (x vs. y) $= UEF(x) / UEF(y)$
$= AF_o(x) \times K_u / (AFo(y) \times K_u)$
$= UEF(x) / UEF(y)$

Based on the conceptual model above, the basic method used to estimate the RBA of arsenic in a particular test material compared to arsenic in a reference material (sodium arsenate) is as follows:

1. Plot the amount of arsenic excreted in the urine (μg per 48 hours) as a function of the administered amount of arsenic (μg per 48 hours), both for reference material and for test material.
2. Find the best fit linear regression line through the each data set. The slope of each line (μg per 48 hours excreted per μg per 48 hours ingested) is the best estimate of the UEF for each material.
3. Calculate RBA for each test material as the ratio of the UEF for test material compared to UEF for reference material:

$$RBA(test\ vs\ ref) = \frac{UEF(test)}{UEF(ref)}$$

A detailed description of the curve-fitting methods and rationale and the methods used to quantify uncertainty in the arsenic RBA estimates for a test material are summarized below. All model fitting was performed in Microsoft Excel® using matrix functions.

3.2 Dose-Response Model

Simultaneous Regression

The techniques used to derive linear regression fits to the dose-response data are based on the methods recommended by Finney (1978). As noted by Finney (1978), when the data to be analyzed consist of two dose-response curves (the reference material and the test material), it is obvious that both curves must have the same intercept, since there is no difference between the curves when the dose is zero. This requirement is achieved by combining the two dose response equations into one and solving for the parameters simultaneously, as follows:

Separate Models:
$$\mu_r(i) = a + b_r \cdot x_r(i)$$
$$\mu_t(i) = a + b_t \cdot x_t(i)$$

Combined Model:
$$\mu(i) = a + b_r \cdot x_r(i) + b_t \cdot x_t(i)$$

where: $\mu(i)$ indicates the expected mean response of animals exposed at dose $x(i)$, and the subscripts r and t refer to reference and test material, respectively. The coefficients of this combined model are derived using multivariate regression, with the understanding that the combined data set is restricted to cases in which one (or both) of x_r and x_t are zero (Finney, 1978).

Weighted Regression

Regression analysis based on ordinary least squares assumes that the variance of the responses is independent of the dose and/or the response (Draper and Smith, 1998). It has previously been shown that this assumption is generally not satisfied in swine-based RBA studies, where there is a tendency toward increasing variance in response as a function of increasing dose (heteroscedasticity) (USEPA, 2007). One method for dealing with heteroscedasticity is through the use of weighted least squares regression (Draper and Smith, 1998). In this approach, each observation in a group of animals is assigned a weight that is inversely proportional to the variance of the response in that group:

$$w_i = \frac{1}{\sigma_i^2}$$

where:

w_i = weight assigned to all data points in dose group *i*
σ_i^2 = variance of responses in animals in dose group *i*

When the distributions of responses at each dose level are normal, weighted regression is equivalent to the maximum likelihood method.

There are several alternative strategies for assigning weights. The method used in this study estimates the value of σ_i^2 using an "external" variance model based on an analysis of the relationship between variance and mean response using data consolidated across many different swine-based arsenic RBA studies. The data used to derive the variance model are shown in Figure 3-2. As seen, log-variance increases as an approximately linear function of log-mean response:

$$\ln(s_i^2) = k1 + k2 \cdot \ln(\overline{y}_i)$$

where:

s_i^2 = observed variance of responses of animals in dose group *i*
$\overline{y}_i$ = mean observed response of animals in dose group *i*

Based on these data, values of *k*1 and *k*2 were derived using ordinary least squares minimization. The resulting values were -1.10 for *k*1 and 1.64 for *k*2.

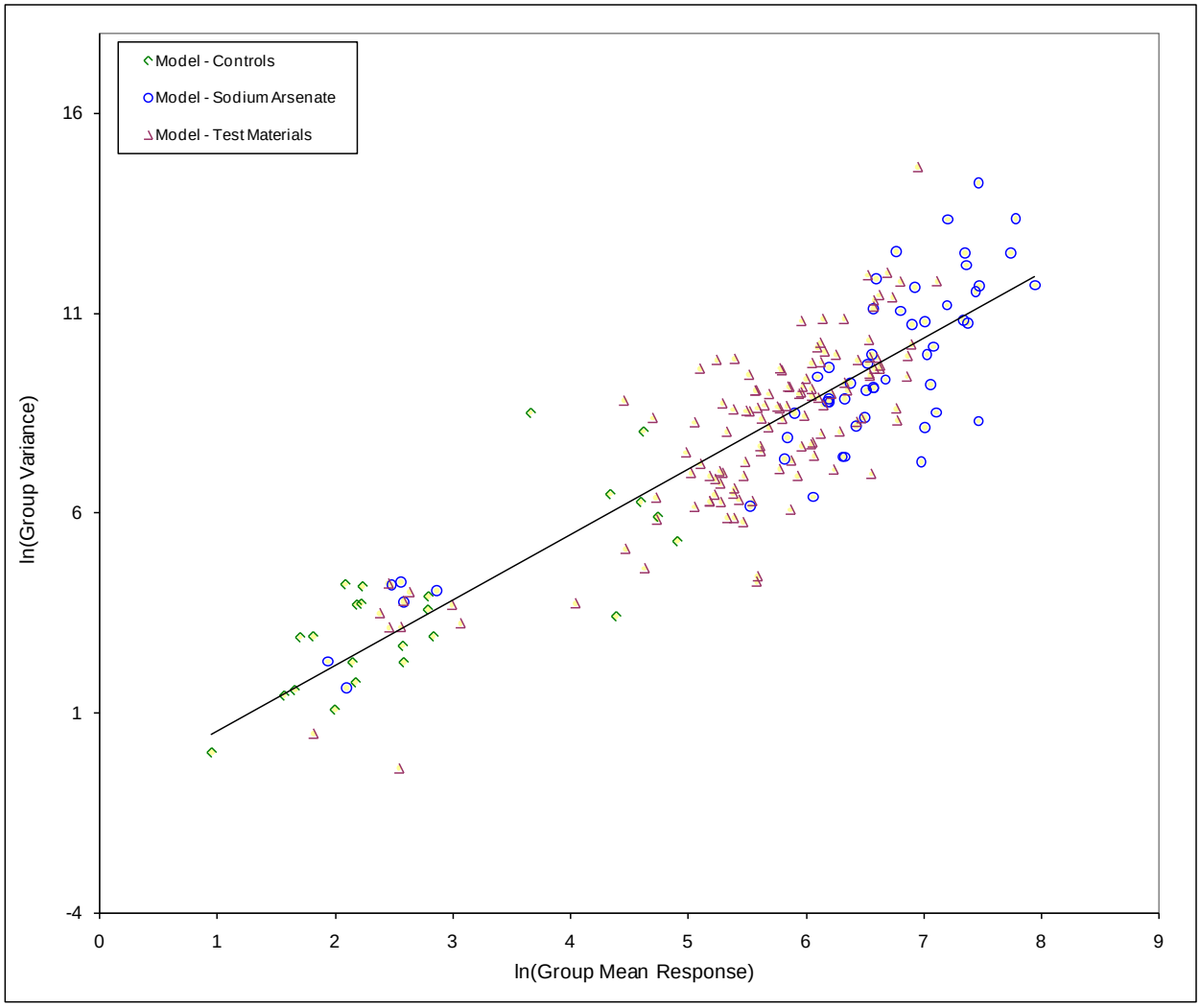
Goodness-of-Fit

The goodness-of-fit of each dose-response model was assessed using the F test statistic and the adjusted coefficient of multiple determination (Adj R^2) as described by Draper and Smith (1998). A fit is considered acceptable if the p-value is less than 0.05.

Assessment of Outliers

In biological assays, it is not uncommon to note the occurrence of individual measured responses that appear atypical compared to the responses from other animals in the same dose group. In this study, responses that yielded standardized weighted residuals greater than 3.5 or less than -3.5 were considered to be potential outliers (Canavos, 1984). Such a data point was encountered in the data set for this study. Therefore, RBA values were calculated both for all the data (outliers included) and without the outlier, and the result with the outlier excluded was used as the preferred estimate.

Figure 3-2. Urinary Arsenic Variance Model



3.3 Calculation of RBA Estimates

The arsenic RBA values were calculated as the ratio of the slope term for the test material data set (b_t) and the reference material data set (b_r):

$$RBA = \frac{b_t}{b_r}$$

The uncertainly range about the RBA ratio was calculated using Fieller's Theorem as described by Finney (1978).

4.0 RESULTS

4.1 Clinical Signs

The doses of arsenic administered in this study are below a level that is expected to cause toxicological responses in swine. No clinical signs of arsenic-induced toxicity were noted in any of the animals used in the studies.

4.2 Dosing Deviations

There were no missed doses during this study. Swine 565 was slow to consume his dough balls on days 2, 3, and 4. This was noted during the study but the final dose amount was not affected by the late consumption.

4.3 Background Arsenic Excretion

Measured values for urinary arsenic excretion (mean and standard deviation) for control animals from days 6 to 13 are shown in Table 4-1.

Table 4-1. Background Urinary Arsenic

Sample ID	Swine Number	Collection Period (days)	Arsenic concentration in urine (µg/L)	Arsenic mass in urine (µg/48 hours)
MO-235	564	6/7	35	51.1
MO-155	564	9/10	46	68.1
MO-187	564	12/13	41	59
MO-227	570	6/7	19	35.3
MO-154	570	9/10	21	50.4
MO-204	570	12/13	26	60.3
MO-236	571	6/7	38	54
MO-149	571	9/10	23	61.4
MO-188	571	12/13	45	84.6

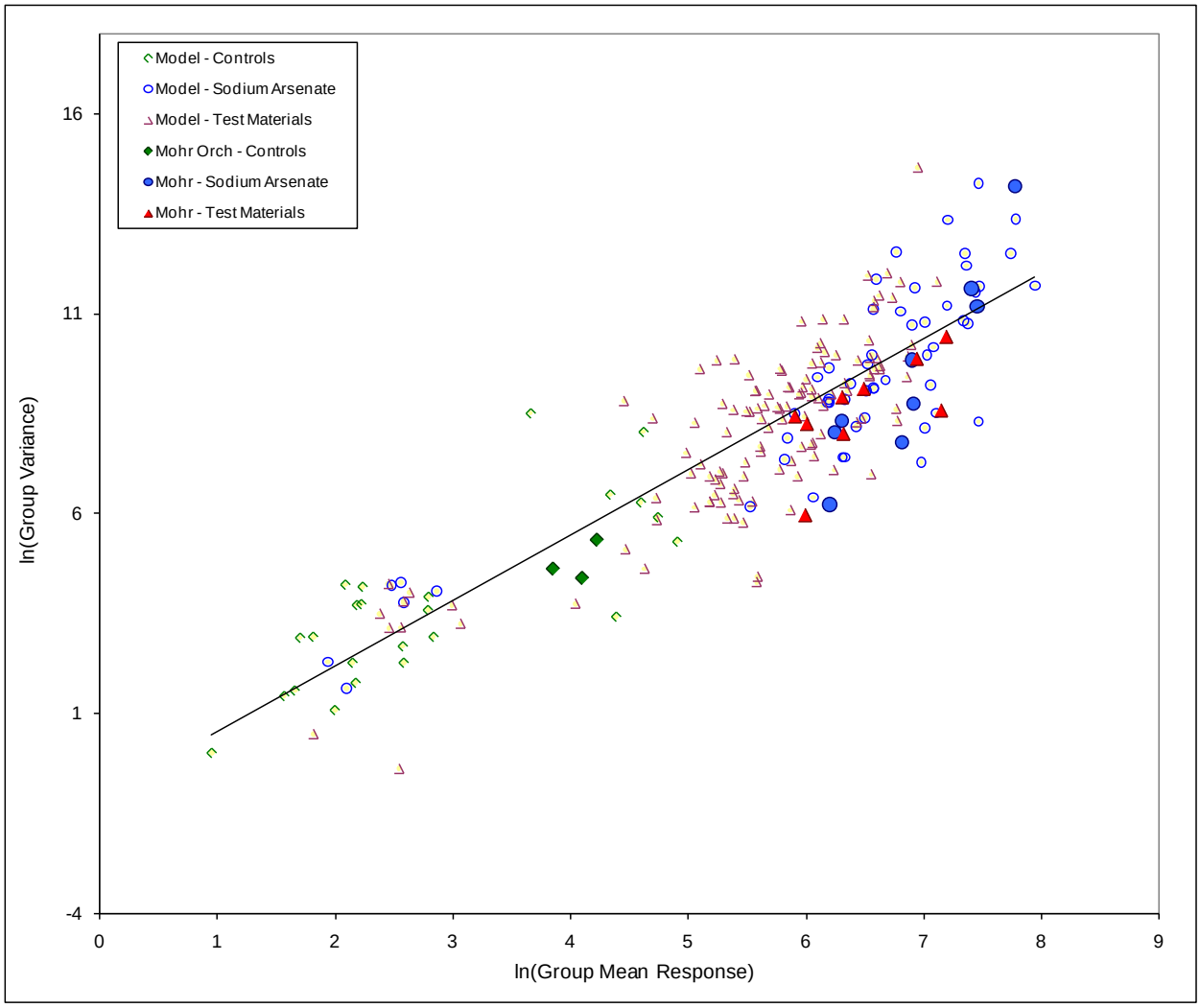
Mean urinary arsenic concentration was 32.6±10.6 µg/L. The values shown are representative of endogenous background levels in food and water and support the view that the animals were not exposed to any significant exogenous sources of arsenic throughout the study.

4.4 Urinary Arsenic Variance

As discussed in Section 3.2, the urinary arsenic dose-response data are analyzed using weighted least squares regression and the weights are assigned using an “external” variance model. To ensure that the variance model was valid, the variance values from each of dose

groups were superimposed on the historic data set (Figure 4-1). As seen, the variance of the urinary arsenic data from this study is consistent with the data used to generate the variance model.

Figure 4-1. Mohr Orchard Data Compared to Urinary Arsenic Variance Model

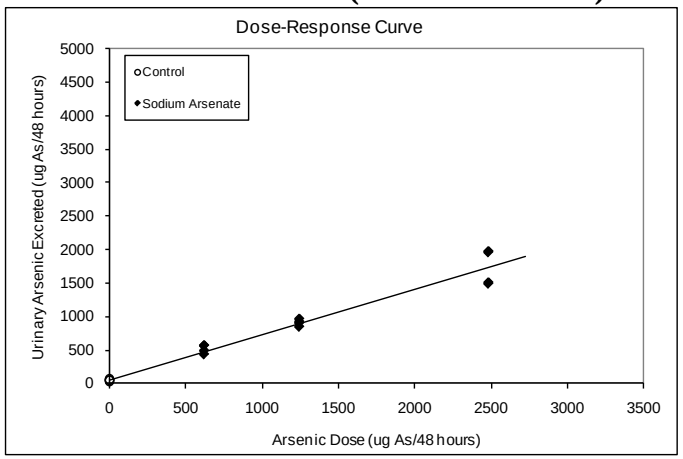


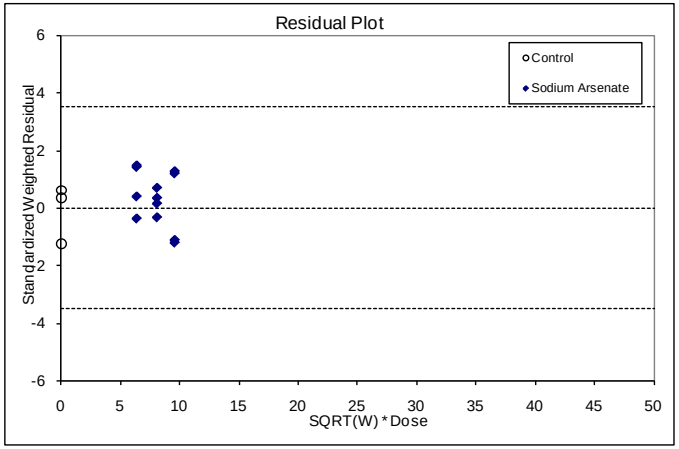
4.5 Dose-Response Modeling

The dose-response data for arsenic in urine were initially modeled using all of the data, and an outlier was identified as discussed in Section 3.2. Initial modeling results are shown in Figures 4-2 through 4-5. Based on this analysis, data for swine 574 on day 9/10 were excluded from the final evaluation for arsenic RBA. Final regression fittings are shown in Figures 4-6 through 4-9.

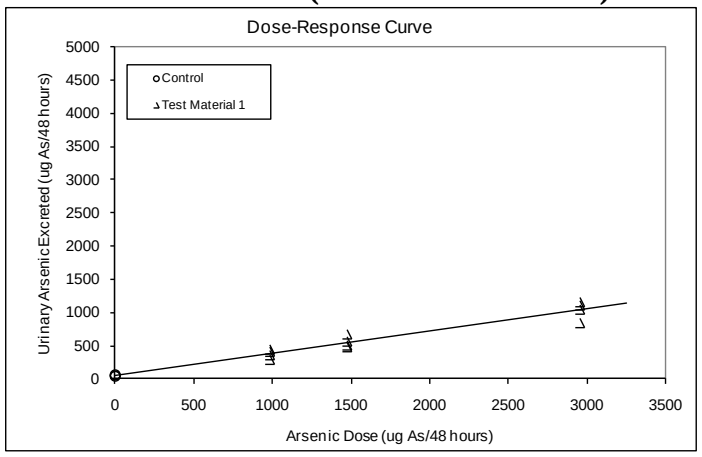
Figure 4-2. Mohr Orchard Urinary Excretion of Arsenic: Days 6/7 (All Data)

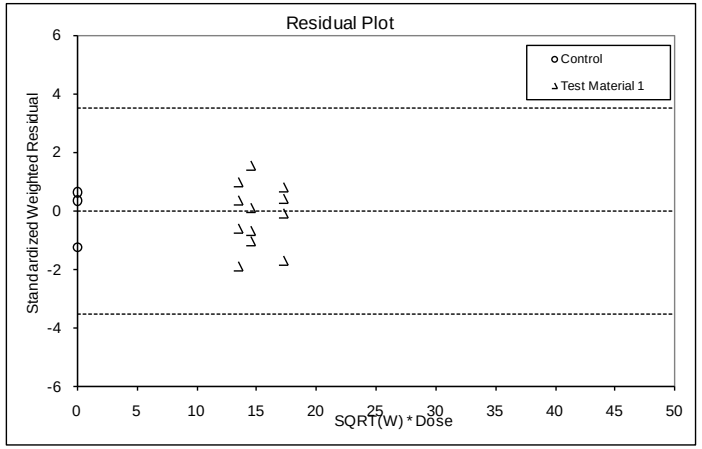
Reference Material (Sodium Arsenate)





Test Material 1 (Mohr Orchard TM1)





Summary of Fitting [a]

Parameter	Estimate	Standard Error
a	47.7	18.8
b_r	0.67	0.03
b_{t1}	0.34	0.02
Covariance (b_r,b_{t1})	0.3723	–
Degrees of Freedom	25	–

[a] $y = a + b_r*x_r + b_{t1}*x_{t1}$
where r = Reference Material, t1 = Test Material 1

ANOVA

Source	SSE	DF	MSE
Fit	623.58	2	311.79
Error	14.21	24	0.59
Total	637.79	26	24.53

Statistic	Estimate
F	526.616

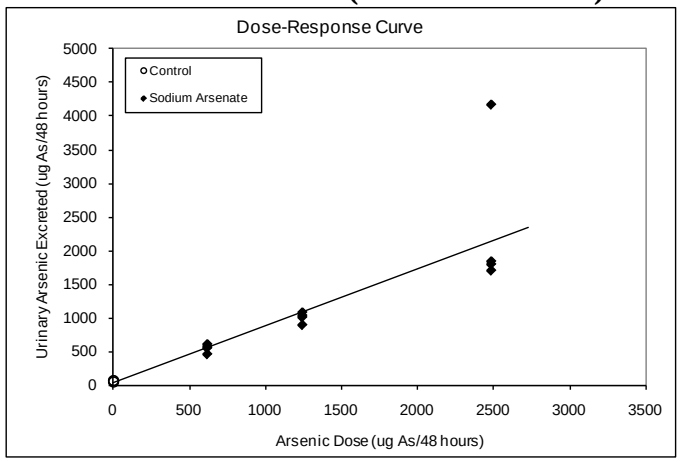
RBA and Uncertainty

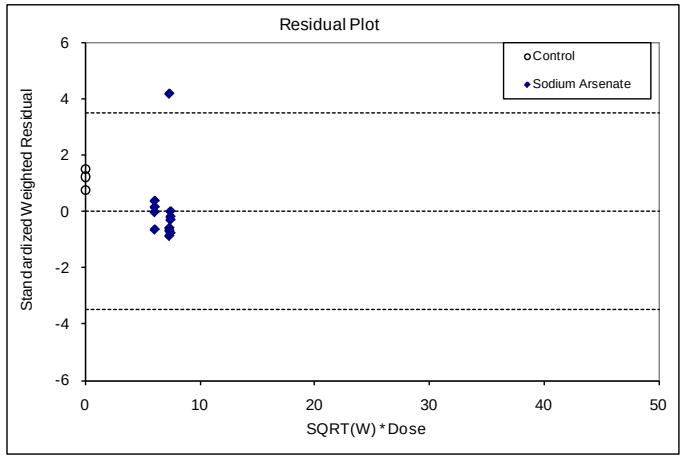
	Test Material 1
RBA	0.50
Lower bound [c]	0.46
Upper bound [c]	0.55
Standard Error [c]	0.027

[c] 90% confidence interval calculated using Fieller's theorem

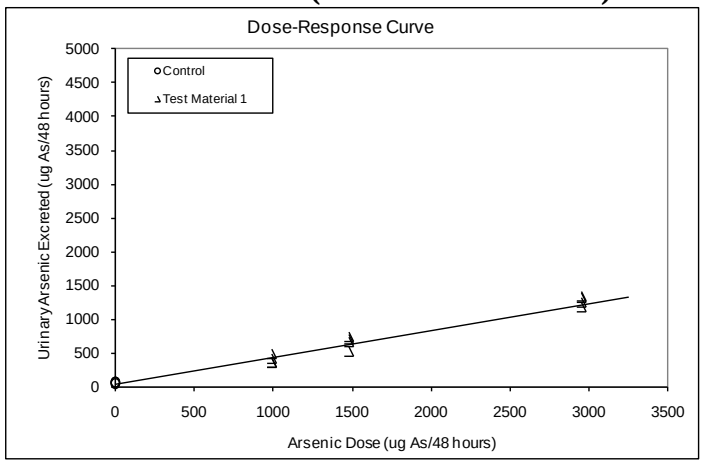
Figure 4-3. Mohr Orchard Urinary Excretion of Arsenic: Days 9/10 (All Data)

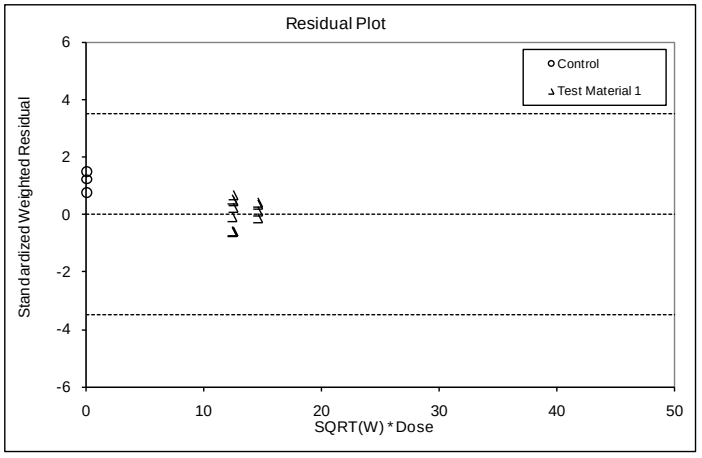
Reference Material (Sodium Arsenate)





Test Material 1 (Mohr Orchard TM1)





Summary of Fitting [a]

Parameter	Estimate	SE
a	32.0	38.9
b_r	0.84	0.07
b_{t1}	0.40	0.04
Covariance (b_r,b_{t1})	0.2500	–
Degrees of Freedom	25	–

[a] $y = a + b_r*x_r + b_{t1}*x_{t1}$
where r = Reference Material, t1 = Test Material 1

ANOVA

Source	SSE	DF	MSE
Fit	683.86	2	341.93
Error	56.92	24	2.37
Total	740.78	26	28.49

Statistic	Estimate
F	144.179
p	<0.001
Adjusted R^2	0.9168

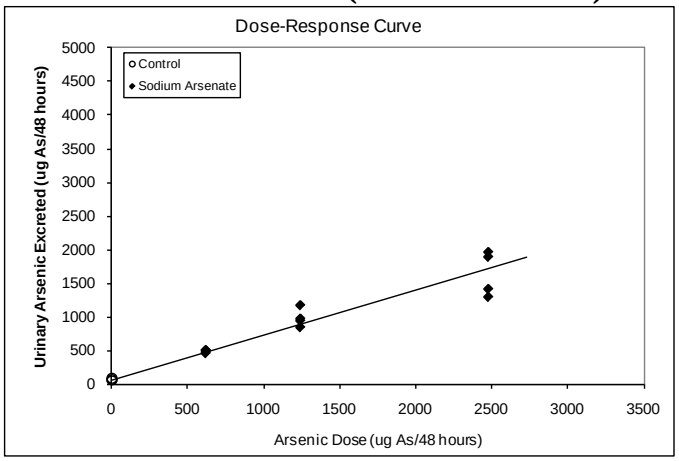
RBA and Uncertainty

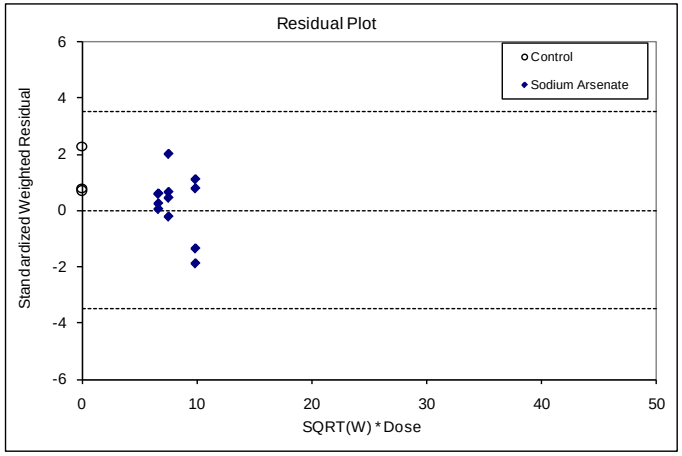
	Test Material 1
RBA	0.47
Lower bound [c]	0.39
Upper bound [c]	0.57
Standard Error [c]	0.053

[c] 90% confidence interval calculated using Fieller's theorem

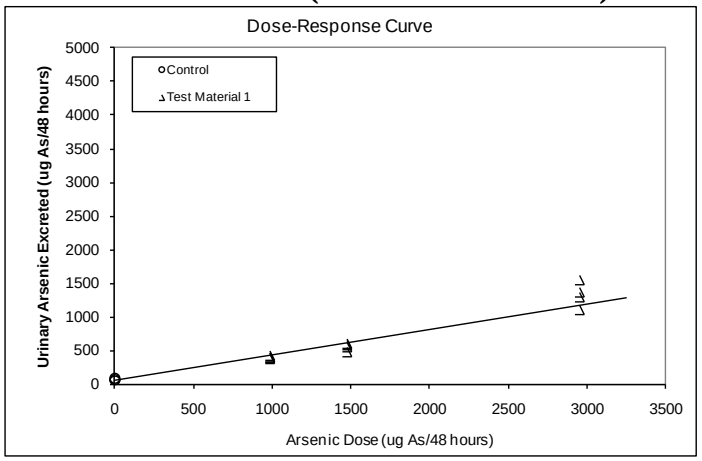
Figure 4-4. Mohr Orchard Urinary Excretion of Arsenic: Days 12/13 (All Data)

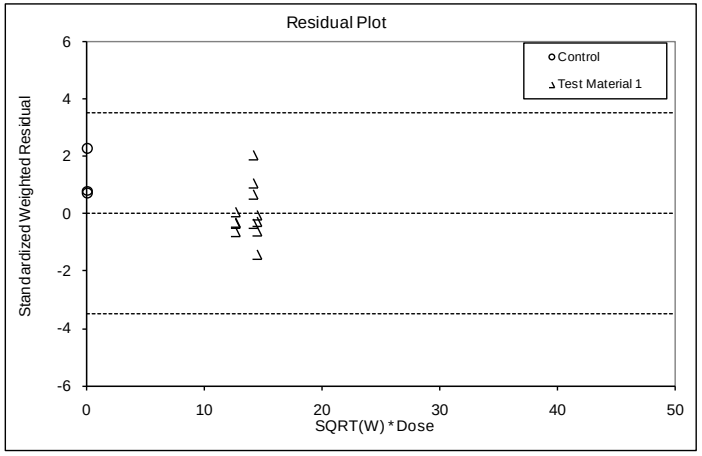
Reference Material (Sodium Arsenate)





Test Material 1 (Mohr Orchard TM1)





Summary of Fitting [a]

Parameter	Estimate	SE
a	47.4	22.8
b_r	0.68	0.03
b_{t1}	0.38	0.02
Covariance (b_r,b_{t1})	0.2729	–
Degrees of Freedom	25	–

[a] $y = a + b_r*x_r + b_{t1}*x_{t1}$
where r = Reference Material, t1 = Test Material 1

ANOVA

Source	SSE	DF	MSE
Fit	600.95	2	300.48
Error	22.09	24	0.92
Total	623.04	26	23.96

Statistic	Estimate
F	326.507
p	<0.001
Adjusted R^2	0.9616

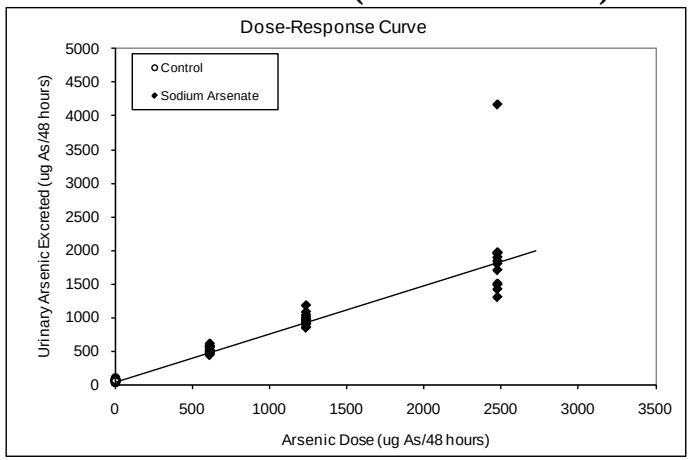
RBA and Uncertainty

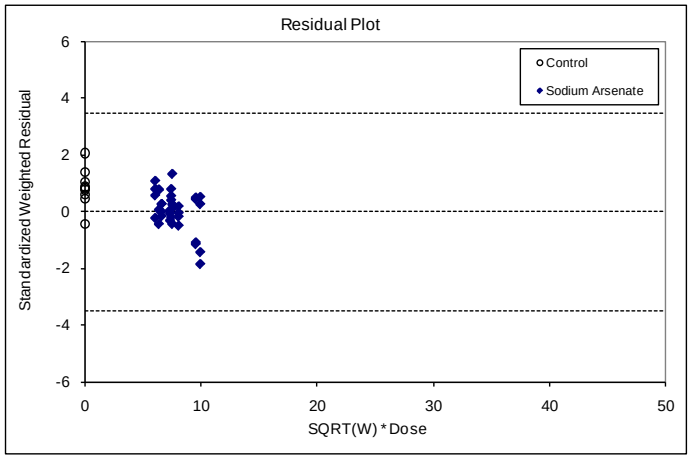
	Test Material 1
RBA	0.56
Lower bound [c]	0.50
Upper bound [c]	0.63
Standard Error [c]	0.037

[c] 90% confidence interval calculated using Fieller's theorem

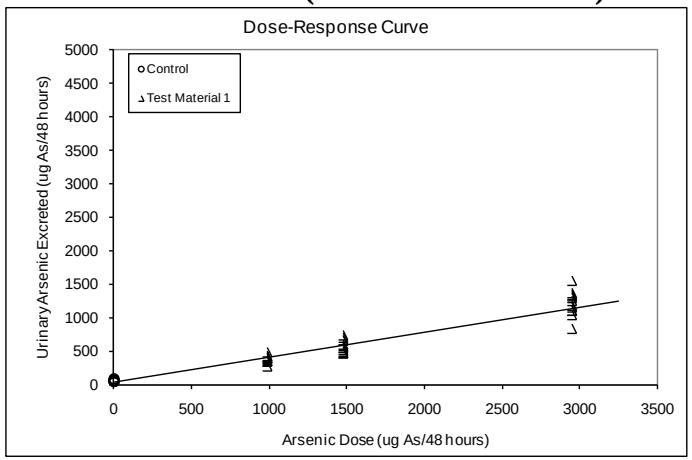
Figure 4-5. Mohr Orchard Urinary Excretion of Arsenic: All Days (Outlier Excluded)

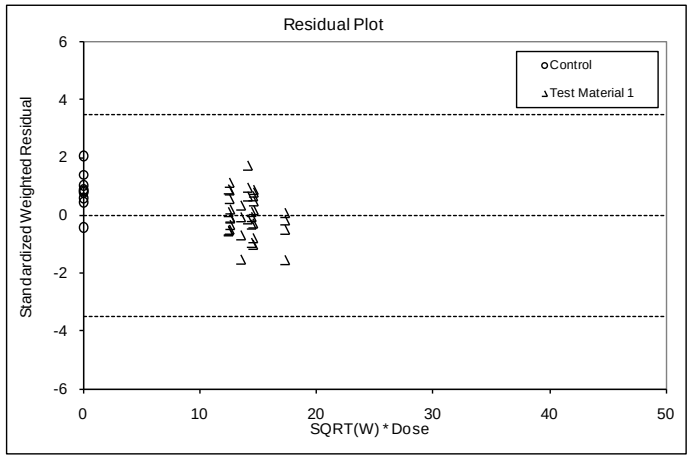
Reference Material (Sodium Arsenate)





Test Material 1 (Mohr Orchard TM1)





Summary of Fitting [a]

Parameter	Estimate	SE
a	41.9	16.9
b_r	0.72	0.03
b_{t1}	0.37	0.02
Covariance (b_r,b_{t1})	0.3052	–
Degrees of Freedom	79	–

[a] $y = a + b_r*x_r + b_{t1}*x_{t1}$
where r = Reference Material, t1 = Test Material 1

ANOVA

Source	SSE	DF	MSE
Fit	1894.87	2	947.44
Error	106.46	78	1.36
Total	2001.33	80	25.02

Statistic	Estimate
F	694.188
p	<0.001
Adjusted R^2	0.9454

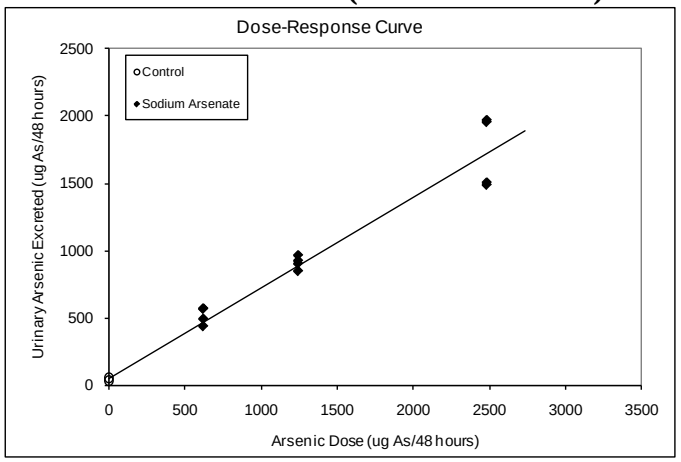
RBA and Uncertainty

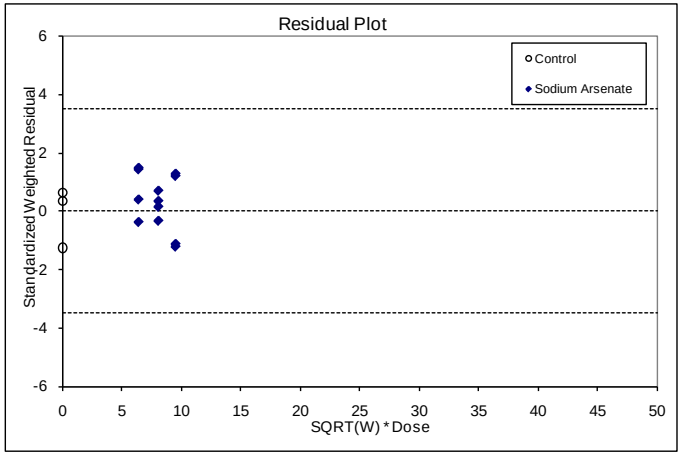
	Test Material 1
RBA	0.52
Lower bound [c]	0.48
Upper bound [c]	0.56
Standard Error [c]	0.025

[c] 90% confidence interval calculated using Fieller's theorem

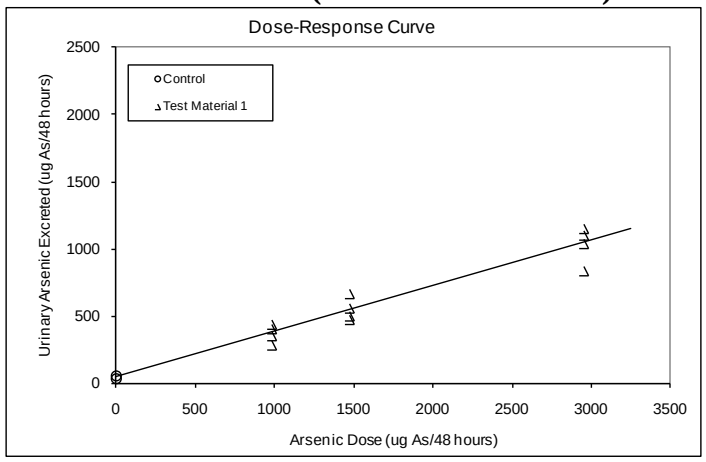
Figure 4-6. Mohr Orchard Urinary Excretion of Arsenic: Days 6/7 (Outlier Excluded)

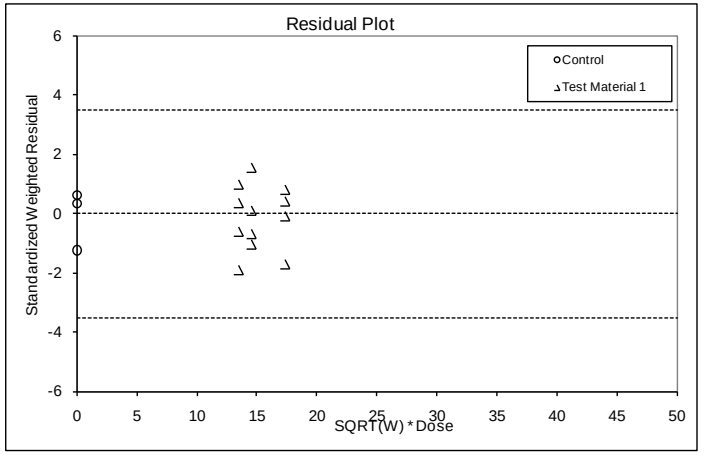
Reference Material (Sodium Arsenate)





Test Material 1 (Mohr Orchard TM1)





Summary of Fitting [a]

Parameter	Estimate	Standard Error
a	47.7	18.8
b_r	0.67	0.03
b_{t1}	0.34	0.02
Covariance (b_r,b_{t1})	0.3723	–
Degrees of Freedom	25	–

[a] $y = a + b_r*x_r + b_{t1}*x_{t1}$
where r = Reference Material, t1 = Test Material 1

ANOVA

Source	SSE	DF	MSE
Fit	623.58	2	311.79
Error	14.21	24	0.59
Total	637.79	26	24.53

Statistic	Estimate
F	526.616
p	<0.001
Adjusted R^2	0.9759

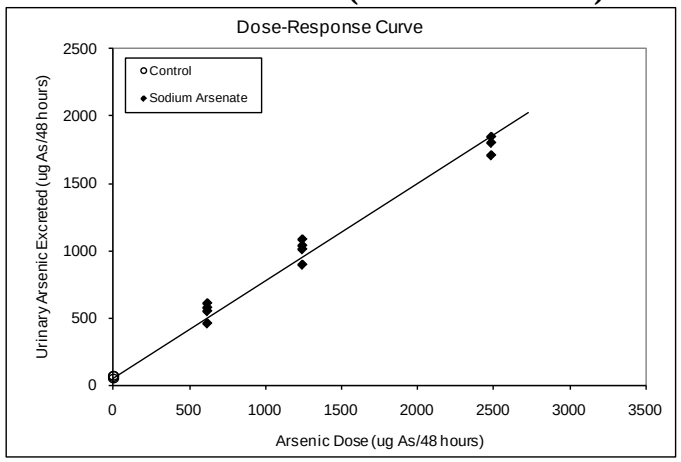
RBA and Uncertainty

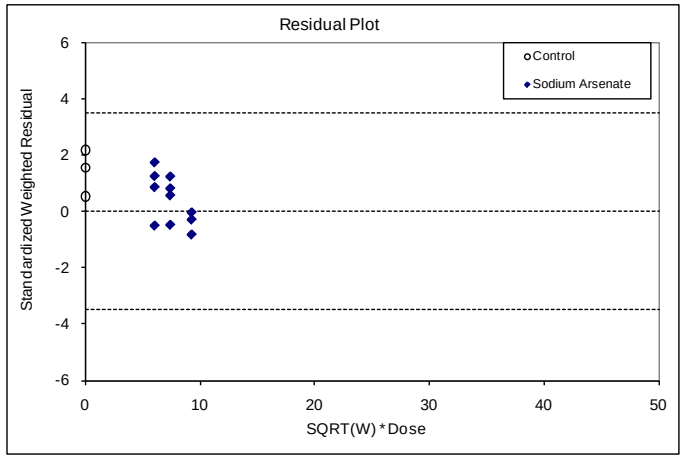
	Test Material 1
RBA	0.50
Lower bound [c]	0.46
Upper bound [c]	0.55
Standard Error [c]	0.027

[c] 90% confidence interval calculated using Fieller's theorem

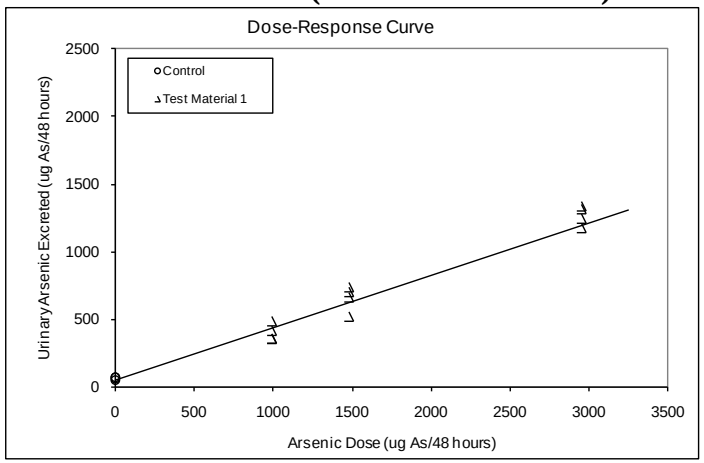
Figure 4-7. Mohr Orchard Urinary Excretion of Arsenic: Days 9/10 (Outlier Excluded)

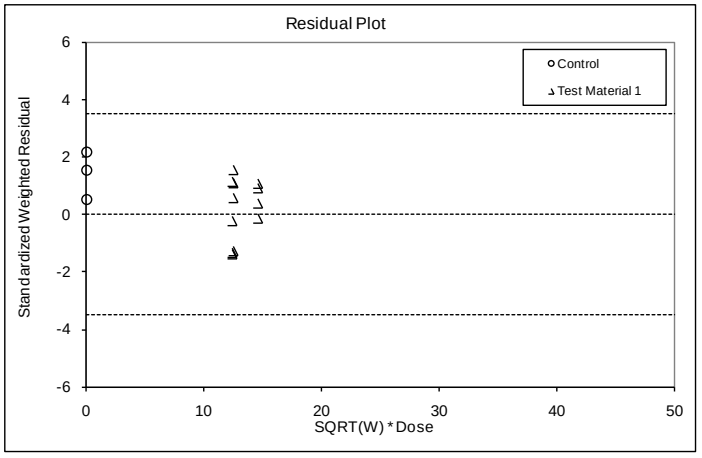
Reference Material (Sodium Arsenate)





Test Material 1 (Mohr Orchard TM1)





Summary of Fitting [a]

Parameter	Estimate	SE
a	44.6	16.8
b_r	0.73	0.03
b_{t1}	0.39	0.02
Covariance (b_r,b_{t1})	0.2503	–
Degrees of Freedom	24	–

[a] $y = a + b_r*x_r + b_{t1}*x_{t1}$
where r = Reference Material, t1 = Test Material 1

ANOVA

Source	SSE	DF	MSE
Fit	590.41	2	295.20
Error	12.51	23	0.54
Total	602.92	25	24.12

Statistic	Estimate
F	542.559
p	<0.001
Adjusted R^2	0.9774

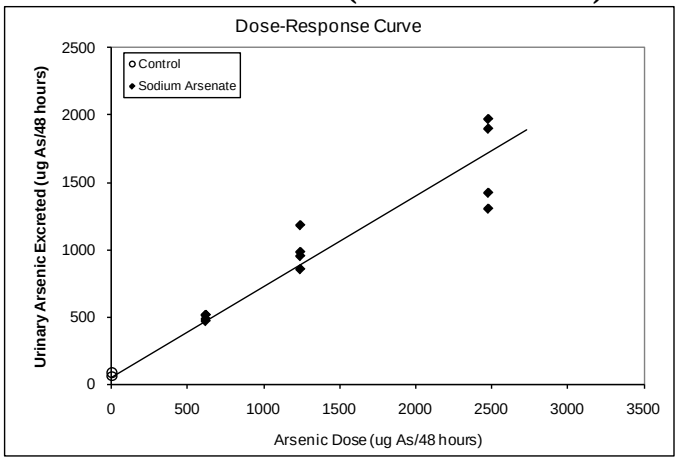
RBA and Uncertainty

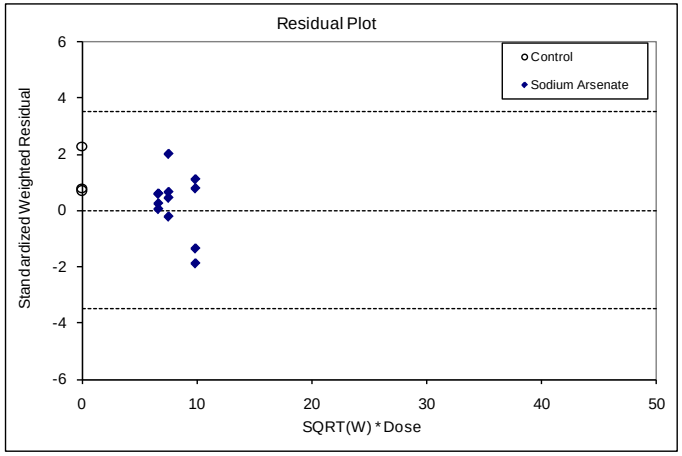
	Test Material 1
RBA	0.54
Lower bound [c]	0.49
Upper bound [c]	0.59
Standard Error [c]	0.027

[c] 90% confidence interval calculated using Fieller's theorem

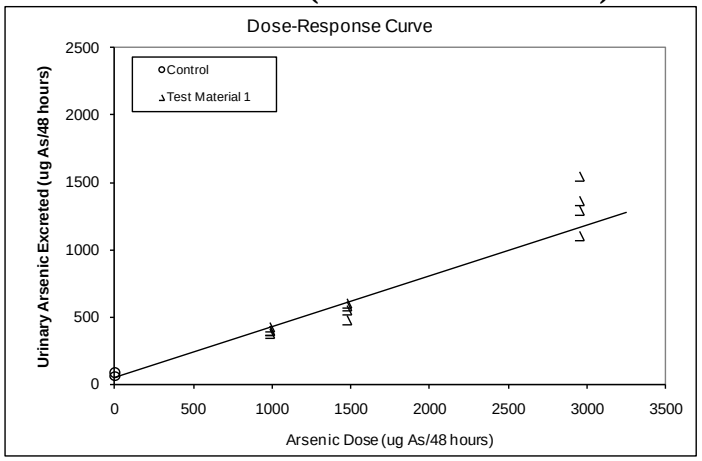
Figure 4-8. Mohr Orchard Urinary Excretion of Arsenic: Days 12/13 (Outlier Excluded)

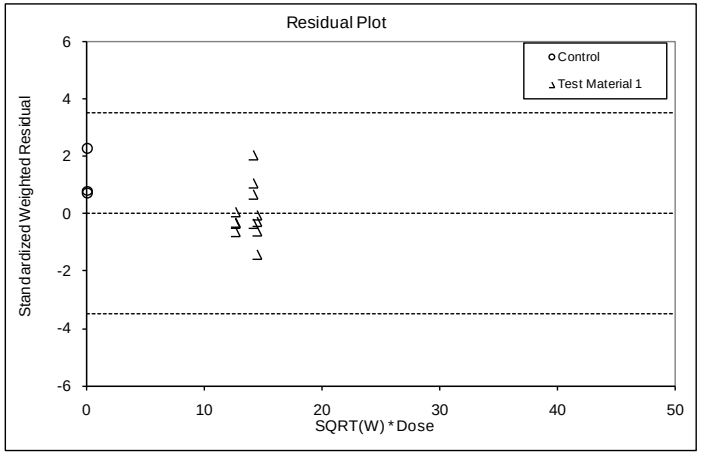
Reference Material (Sodium Arsenate)





Test Material 1 (Mohr Orchard TM1)





Summary of Fitting [a]

Parameter	Estimate	SE
a	47.4	22.8
b_r	0.68	0.03
b_{t1}	0.38	0.02
Covariance (b_r,b_{t1})	0.2729	–
Degrees of Freedom	25	–

[a] $y = a + b_r*x_r + b_{t1}*x_{t1}$
where r = Reference Material, t1 = Test Material 1

ANOVA

Source	SSE	DF	MSE
Fit	600.95	2	300.48
Error	22.09	24	0.92
Total	623.04	26	23.96

Statistic	Estimate
F	326.507
p	<0.001
Adjusted R^2	0.9616

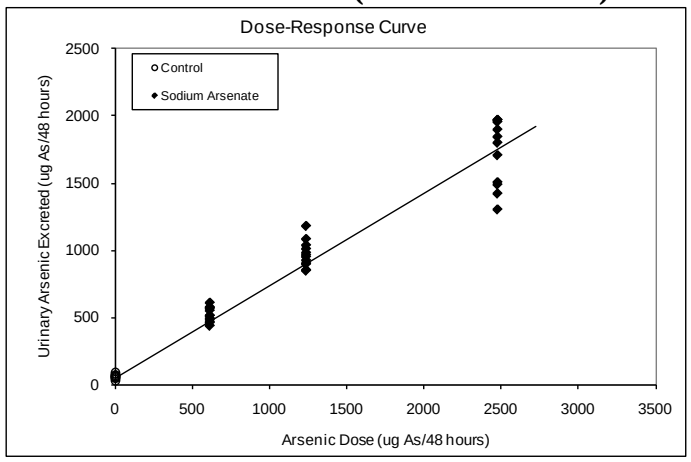
RBA and Uncertainty

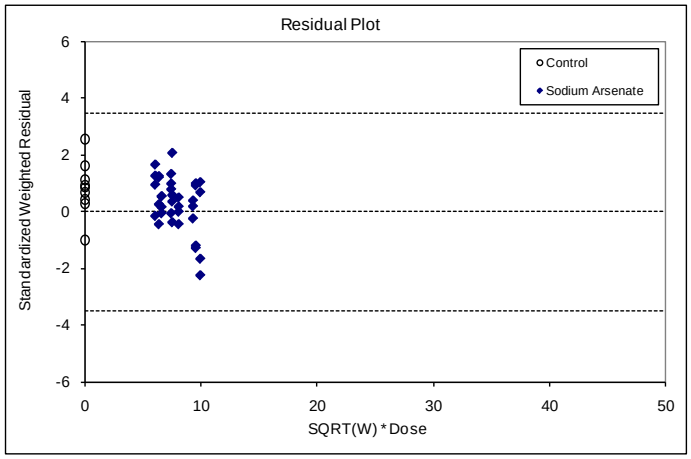
	Test Material 1
RBA	0.56
Lower bound [c]	0.50
Upper bound [c]	0.63
Standard Error [c]	0.037

[c] 90% confidence interval calculated using Fieller's theorem

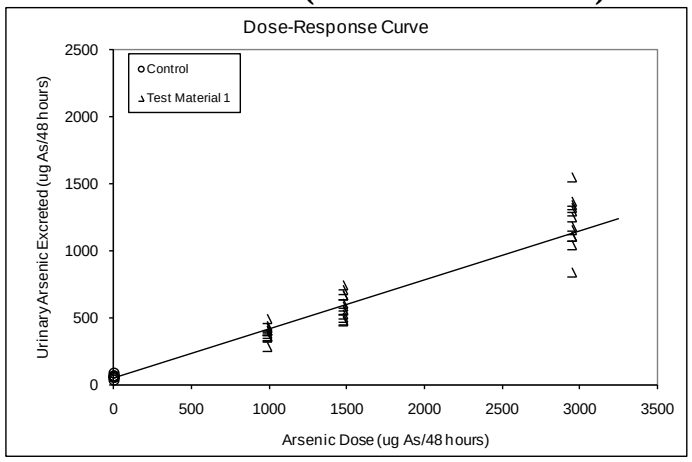
Figure 4-9. Mohr Orchard Urinary Excretion of Arsenic: All Days (Outlier Excluded)

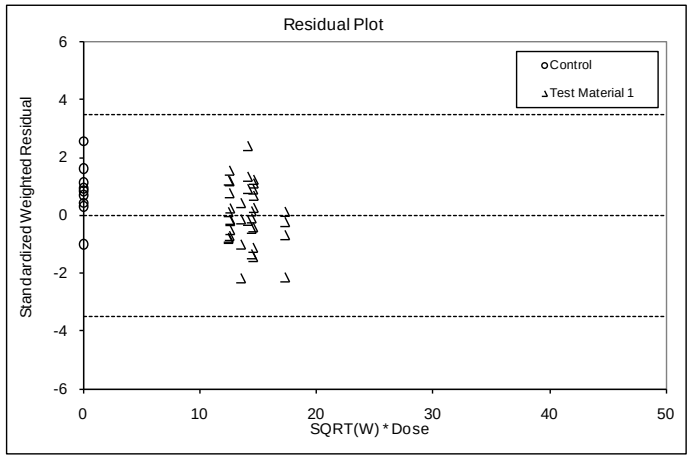
Reference Material (Sodium Arsenate)





Test Material 1 (Mohr Orchard TM1)





Summary of Fitting [a]

Parameter	Estimate	SE
a	46.4	11.4
b_r	0.69	0.02
b_{t1}	0.37	0.01
Covariance (b_r,b_{t1})	0.3045	–
Degrees of Freedom	78	–

[a] $y = a + b_r*x_r + b_{t1}*x_{t1}$
where r = Reference Material, t1 = Test Material 1

ANOVA

Source	SSE	DF	MSE
Fit	1819.76	2	909.88
Error	55.41	77	0.72
Total	1875.17	79	23.74

Statistic	Estimate
F	1264.308
p	<0.001
Adjusted R^2	0.9697

RBA and Uncertainty

	Test Material 1
RBA	0.53
Lower bound [c]	0.51
Upper bound [c]	0.57
Standard Error [c]	0.018

[c] 90% confidence interval calculated using Fieller's theorem

After exclusion of the outlier, all of the dose-response curves were approximately linear, with the slope of the best-fit straight line being equal to the best estimate of the UEF. The resulting slopes (UEF estimates) for the final fittings of the test material and corresponding reference material are shown below in Table 4-2:

Table 4-2. UEF Estimates

Measurement Interval	Outliers Excluded	Slopes (UEF Estimates)	
		b_r	b_{t1}
Days 6/7		0.67	0.34
Days 9/10	0	0.73	0.39
Days 12/13	1	0.68	0.38
All Days	0	0.69	0.37

b_r = slope for reference material dose-response
b_{t1} = slope for test material dose-response

4.6 Calculated RBA Values

Estimated RBA values (mean and 90% confidence interval) are shown below in Table 4-3:

Table 4-3. Estimated RBA for Mohr Orchard Soil

Measurement Interval	Estimated RBA (90% Confidence Interval)
Days 6/7	0.50 (0.46–0.55)
Days 9/10	0.54 (0.49–0.59)
Days 12/13	0.56 (0.50–0.63)
All Days	0.53 (0.51–0.57)

The best fit point estimate RBA for the Mohr Orchard soil sample is 53%.

4.7 Uncertainty

The bioavailability estimates above are subject to uncertainty that arises from several different sources. One source of uncertainty is the inherent biological variability between different animals in a dose group, which in turn causes variability in the amount of arsenic absorbed by the exposed animals. The between-animal variability results in statistical uncertainty in the best-fit dose-response curves and, hence, uncertainty in the calculated values of RBA.

Such statistical uncertainty is accounted for by the statistical models used above and is characterized by the uncertainty range around the RBA estimates.

However, there is also uncertainty in the extrapolation of RBA values measured in juvenile swine to young children or adults, and this uncertainty is not included in the statistical confidence bounds above. Even though the immature swine is believed to be a useful and meaningful animal model for gastrointestinal absorption in humans, it is possible that there are differences in physiological parameters that may influence RBA; therefore, RBA values in swine may not be identical to values in children. In addition, RBA may depend on the amount and type of food in the stomach, since the presence of food can influence stomach pH, holding time, and possibly other factors that may influence solubilization of arsenic. RBA values measured in this study are based on animals that have little or no food in their stomach at the time of exposure and, hence, are likely to yield high-end values of RBA. Thus, these RBA values may be somewhat conservative for humans who ingest the site soils along with food. The magnitude of this bias is not known.

5.0 REFERENCES

Canavos, C. G. 1984. Applied Probability and Statistical Methods. Little, Brown and Co.: Boston, MA.

Casteel, S. W., Cowart, R. P., Weis, C. P., Henningsen, G. M., Hoffman, E., Brattin, W. J., Starost, M. F., Payne, J. T., Stockham, S. L., Becker, S. V., and Turk, J. R.. 1996. A swine model for determining the bioavailability of lead from contaminated media. In: Advances in Swine in Biomedical Research. Tumbleson and Schook, eds., Volume 2, Plenum Press, New York. pp. 637–646.

Draper, N. R. and Smith, H. 1998. Applied Regression Analysis. 3rd ed. John Wiley & Sons: New York, NY.

Finney, D. J. 1978. Statistical Method in Biological Assay. 3rd ed. Charles Griffin and Co., London, England.

Gibaldi, M. and Perrier, D. 1982. Pharmacokinetics. 2nd ed. Marcel Dekker, Inc.: New York, NY. pp. 294–297.

Goodman, A. G., Rall, T. W., Nies, A. S., and Taylor, P. 1990. The Pharmacological Basis of Therapeutics. 8th ed. Pergamon Press, Inc.: Elmsford, NY. pp. 5–21.

Klaassen, C.D., Amdur, M.O., and Doull, J. 1996. Cassarett and Doull's Toxicology: The Basic Science of Poisons. McGraw-Hill, Inc.: New York, NY. pp. 190.

Miller, B.W. and Scheckel, K.G. 2012. Technical Review Workgroup for Metals and Asbestos: Bioavailability Committee. Mineralogical Report. XAS Data and Linear Combination Fitting Results. Available at: http://epa.gov/superfund/bioavailability/guidance.htm

NIST. 2003. Certificate of Analysis, Standard Reference Material® 2710 – Montana Soil, Highly Elevated Trace Element Concentrations. National Institute of Standards & Technology, Gaithersburg, MD. Certificate Issue Date: July 18, 2003.

NRC. 1988. Nutrient requirements of swine. A report of the Committee on Animal Nutrition. National Research Council. National Academy Press: Washington, DC.

USEPA. 2007. Estimation of Relative Bioavailability of Lead in Soil and Soil-Like Materials by *In Vivo* and *In Vitro* Methods OSWER9285.7-77. Office of Solid Waste and Emergency Response, Washington DC, USA.

Weis, C.P. and LaVelle, J.M. 1991. Characteristics to consider when choosing an animal model for the study of lead bioavailability. In: The Proceedings of the International Symposium on the Bioavailability and Dietary Uptake of Lead. Sci. Technol. Lett. 3: 113–119.

APPENDIX A: GROUP ASSIGNMENTS

Table A-1. Group Assignments for the Mohr Orchard Arsenic Study

Swine number	Group	Treatment	Target arsenic dose µg/kg-day
552 554 561 572	1	NaAs	25
551 553 566 573	2	NaAs	50
555 560 563 574	3	NaAs	100
557 575 576 579	4	TM1	40
559 565 568 578	5	TM1	60
556 562 569 577	6	TM1	120
564 570 571	7	Control	0

APPENDIX B: BODY WEIGHTS

Table B-1. Body Weights

Group	Swine number	Weight (kg)													
		Day 5 8/12/09	Group MBW	Day 1 8/16/09	Group MBW	Day 2 8/19/09	Group MBW	Day 5 8/22/09	Group MBW	Day 8 8/25/09	Group MBW	Day 11 8/28/09	Group MBW	Day 14 8/31/09	Group MBW
1 NaAs 25	552	8.9		9.2		10		10.3		10.8		11.4		12.2	
	554	9.7		10		10.4		10.9		11.7		12.3		12.8	
	561	7.8		8		8.7		9.3		9.7		10.4		11	
	572	9	8.85	9.1	9.08	9.8	9.73	10.3	10.20	11.1	10.83	11.8	11.48	12.4	12.10
2 NaAs 50	551	9.3		9.6		10.2		10.6		11.2		11.8		12.5	
	553	7.6		7.9		8.2		8.5		9.1		9.7		10.2	
	566	7.8		8.4		8.6		9.2		9.9		10.5		11.2	
	573	8.7	8.35	9.3	8.80	9.6	9.15	10.1	9.60	10.8	10.25	11.4	10.85	12	11.48
3 NaAs 100	555	7.5		7.9		8.3		8.7		9.1		9.8		10.6	
	560	8.2		8.4		8.9		9.3		10.1		10.6		11.3	
	563	7.5		7.9		8.4		9		9.3		10		10.8	
	574	8.1	7.83	8.8	8.25	9.2	8.70	9.6	9.15	10.6	9.78	11.2	10.40	11.9	11.15
4 TM1 40	557	8.2		8.4		9		9.5		10.1		10.9		11.7	
	575	7.6		8.2		8.5		8.8		9.5		10.2		11	
	576	6.6		7.2		7.5		8		8.8		9.5		10.2	
	579	8.1	7.63	8.6	8.10	9	8.50	9.2	8.88	9.9	9.58	10.5	10.28	11.2	11.03
5 TM1 60	559	8		9.2		9.8		10.3		10.8		11.5		12.2	
	565	8.1		8.5		9		9.2		10.1		10.6		11.2	
	568	7.7		8.2		8.7		9.2		9.8		10.4		11.2	
	578	9.3	8.28	9.6	8.88	10.3	9.45	10.8	9.88	11.3	10.50	12.1	11.15	12.8	11.85
6 TM1 120	556	8.5		8.9		9.7		10.2		10.9		11.7		12.6	
	562	6.7		7.2		7.6		7.9		8.4		9.2		10	
	569	7.9		8.6		9.2		9.6		10.4		11.1		11.9	
	577	7.5	7.65	7.8	8.13	8.5	8.75	9	9.18	9.6	9.83	10.4	10.60	11.2	11.43
7 Control 0	564	7.9		8.3		8.2		8.7		9.5		10.2		10.7	
	570	7.7		8.5		8.9		9.5		10.2		10.8		11.2	
	571	8.7	8.10	9.6	8.80	9.9	9.00	10.3	9.50	11	10.23	11.8	10.93	12.6	11.50

APPENDIX C: URINE VOLUMES AND URINARY ARSENIC ANALYTICAL RESULTS FOR STUDY SAMPLES

Table C-1. Typical Feed Composition: Purina TestDiet® 5TXP: Porcine Grower Purified Diet with Low Lead [1]

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

NUTRITIONAL PROFILE [2]				
Protein, %	**21**	**Fat, %**		**3.5**
Arginine, %	1.42	Cholesterol, ppm		0
Histidine, %	0.61	Linoleic Acid, %		1.95
Isoleucine, %	1.14	Linolenic Acid, %		0.03
Leucine, %	1.95	Arachidonic Acid, %		0
Lysine, %	1.56	Omega-3 Fatty Acids, %		0.03
Methionine, %	0.49	Total Saturated Fatty Acids, %		0.43
Cystine, %	0.23	Total Monounsaturated Fatty Acids, %		0.82
Phenylalanine, %	1.22	Polyunsaturated Fatty Acids, %		1.98
Tyrosine, %	1.03			
Threonine, %	0.88			
Tryptophan, %	0.32	**Fiber (max), %**		**6.8**
Valine, %	1.16			
Alanine, %	0.95	**Carbohydrates, %**		**62.2**
Aspartic Acid, %	2.33			
Glutamic Acid, %	4.96	**Energy (kcal/g) [3]**		**3.62**
Glycine, %	0.79	*From:*	*kcal*	*%*
Proline, %	1.83	Protein	0.84	23.1
Serine, %	1.25	Fat (ether extract)	0.315	8.7
Taurine, %	0	Carbohydrates	2.487	68.3
Minerals		**Vitamins**		
Calcium, %	0.8	Vitamin A, IU/g		1.7
Phosphorus, %	0.72	Vitamin 0-3 (added), IU/g		0.2
Phosphorus (available), %	0.4	Vitamin E, IU/kg		11
Potassium, %	0.27	Vitamin K (as menadione), ppm		0.52
Magnesium, %	0.04	Thiamin Hydrochloride, ppm		1
Sodium, %	0.3	Ribonavin, ppm		3.1
Chlorine, %	0.31	Niacin, ppm		13
Fluorine, ppm	0	Pantothenic Acid, ppm		9
Iron, ppm	82	Folic Acid, ppm		0.3
Zinc, ppm	84	Pyridoxine, ppm		1.7
Manganese, ppm	3	Biotin, ppm		0.1
Copper, ppm	4.9	Vitamin B-12, mcg/kg		15
Cobalt, ppm	0.1	Choline Chloride, ppm		410
Iodine, ppm	0.15	Ascorbic Acid, ppm		0
Chromium, ppm	0			
Molybdenum, ppm	0.01			
Selenium, ppm	0.26			

[1]This special purified diet was originally developed for lead RBA studies.
[2] Based on the latest ingredient analysis information. Since nutrient composition of natural ingredients varies, analysis will differ accordingly. Nutrients expressed as percent of ration on an As Fed basis except where otherwise indicated.
[3] Energy (kcal/gm) – Sum of decimal fractions of protein, fat, and carbohydrate × 4,9,4 kcal/gm respectively.

APPENDIX D: ANALYTICAL RESULTS FOR QUALITY CONTROL SAMPLES

Table D-1. Urinary Arsenic Analytical Results and Urine Volumes for Study Sample

Group	Material	Collection period (days)	Sample ID	Swine number	Urine_As (µg/L)	Urine volume (µL)
1	NaAs	06/07	MO-126	561	69	8200
1	NaAs	06/07	MO-128	552	29	19640
1	NaAs	06/07	MO-130	554	400	1230
1	NaAs	06/07	MO-135	572	560	780
1	NaAs	12/13	MO-171	561	79	6120
1	NaAs	12/13	MO-182	572	470	1090
1	NaAs	12/13	MO-186	554	270	1730
1	NaAs	12/13	MO-192	552	53	9670
1	NaAs	09/10	MO-146	572	550	1000
1	NaAs	09/10	MO-148	552	53	11480
1	NaAs	09/10	MO-150	561	76	7580
1	NaAs	09/10	MO-168	554	280	1640
2	NaAs	06/07	MO-105	566	140	6440
2	NaAs	06/07	MO-106	551	280	3300
2	NaAs	06/07	MO-109	553	206	4680
2	NaAs	06/07	MO-113	573	730	1160
2	NaAs	12/13	MO-174	553	190	5000
2	NaAs	12/13	MO-183	551	440	2680
2	NaAs	12/13	MO-191	573	300	2840
2	NaAs	12/13	MO-195	566	190	5160
2	NaAs	09/10	MO-137	573	710	1260
2	NaAs	09/10	MO-144	551	370	2800
2	NaAs	09/10	MO-147	553	200	5410
2	NaAs	09/10	MO-151	566	130	7760
3	NaAs	06/07	MO-108	574	1600	1230
3	NaAs	06/07	MO-110	560	590	2550
3	NaAs	06/07	MO-125	555	630	2360
3	NaAs	06/07	MO-132	563	760	2570
3	NaAs	12/13	MO-172	574	1200	1640
3	NaAs	12/13	MO-176	560	600	3160
3	NaAs	12/13	MO-177	555	710	2000
3	NaAs	12/13	MO-193	563	470	2770
3	NaAs	09/10	MO-140	560	620	2900
3	NaAs	09/10	MO-156	555	690	2670
3	NaAs	09/10	MO-162	574	1200	3480
3	NaAs	09/10	MO-164	563	580	2940
4	TM1	06/07	MO-111	579	81	3460
4	TM1	06/07	MO-119	557	150	2680
4	TM1	06/07	MO-120	576	140	2500
4	TM1	06/07	MO-122	575	45	9680
4	TM1	12/13	MO-199	576	130	3060
4	TM1	12/13	MO-200	575	55	7740
4	TM1	12/13	MO-201	557	140	2860
4	TM1	12/13	MO-202	579	76	4970
4	TM1	09/10	MO-142	579	83	4340
4	TM1	09/10	MO-157	575	51	9580
4	TM1	09/10	MO-163	557	160	2610
4	TM1	09/10	MO-165	576	120	2980

Table D-1. Urinary Arsenic Analytical Results and Urine Volumes for Study Sample

Group	Material	Collection period (days)	Sample ID	Swine number	Urine_As (µg/L)	Urine volume (µL)
5	TM1	06/07	MO-107	565	140	4750
5	TM1	06/07	MO-115	578	230	2420
5	TM1	06/07	MO-123	559	48	9820
5	TM1	06/07	MO-131	568	190	2620
5	TM1	12/13	MO-170	565	66	8820
5	TM1	12/13	MO-179	559	44	10870
5	TM1	12/13	MO-180	578	230	2620
5	TM1	12/13	MO-190	568	100	5520
5	TM1	09/10	MO-141	559	49	10660
5	TM1	09/10	MO-152	568	120	5540
5	TM1	09/10	MO-158	578	250	2960
5	TM1	09/10	MO-161	565	81	8700
6	TM1	06/07	MO-103	562	370	2980
6	TM1	06/07	MO-114	569	73	11450
6	TM1	06/07	MO-118	556	210	4950
6	TM1	06/07	MO-228	577	300	3840
6	TM1	12/13	MO-181	569	86	15020
6	TM1	12/13	MO-189	556	420	3680
6	TM1	12/13	MO-197	562	310	4400
6	TM1	12/13	MO-198	577	280	3940
6	TM1	09/10	MO-139	562	380	3100
6	TM1	09/10	MO-145	556	540	2440
6	TM1	09/10	MO-166	577	280	4780
6	TM1	09/10	MO-167	569	110	11340
7	Control	06/07	MO-227	570	19	1860
7	Control	06/07	MO-235	564	35	1460
7	Control	06/07	MO-236	571	38	1420
7	Control	12/13	MO-187	564	41	1440
7	Control	12/13	MO-188	571	45	1880
7	Control	12/13	MO-204	570	26	2320
7	Control	09/10	MO-149	571	23	2670
7	Control	09/10	MO-154	570	21	2400
7	Control	09/10	MO-155	564	46	1480

APPENDIX E: ANALYTICAL RESULTS FOR QUALITY CONTROL SAMPLES

Table E-1. Blind Duplicate Samples

Blind duplicate sample ID	Sample type	Swine number	Urine collection days	Original sample concentration (µg/L)	Duplicate concentration (µg/L)	RPD (%)
MO-175	Urine	551	12/13	440	390	12
MO-223	Urine	556	06/07	210	217	3
MO-138	Urine	560	09/10	620	610	2
MO-153	Urine	571	09/10	23	21	9
MO-136	Urine	572	09/10	550	570	4
MO-231	Urine	573	06/07	730	780	7
MO-194	Urine	576	12/13	130	130	0
MO-173	Urine	577	12/13	280	290	4
MO-224	Urine	578	06/07	230	228	4

RPD = relative percent difference

Table E-2. Laboratory Spikes

Spike sample ID	Sample type	Original sample concentration (ppb)	Added spike concentration (ppb)	Measured sample concentration (ppb)	Recovered spike (ppb)	Recovery (%)
MO-114	Urine	73	200	280	207	104
MO-128	Urine	29	200	240	211	106
MO-140	Urine	620	200	790	170	85
MO-150	Urine	76	200	290	214	107
MO-160	Urine	110	200	310	200	100
MO-170	Urine	66	200	270	204	102
MO-180	Urine	230	200	424	194	97
MO-190	Urine	100	200	300	200	100
MO-200	Urine	55	200	280	225	113
MO-204	Urine	26	200	240	214	107
MO-227	Urine	19	200	220	201	101
MO-273	Feed	<1	100	100	100	100

Table E-3. Laboratory Duplicates

Duplicate sample ID	Sample type	Original sample concentration (ppb)	Duplicate concentration (ppb)	RPD (%)	Absolute difference
MO-108	Urine	1600	1600	0	0
MO-120	Urine	140	150	7	10
MO-133	PE Sample	130	120	8	10
MO-145	Urine	540	580	7	40
MO-155	Urine	46	41	11	5
MO-165	Urine	120	120	0	0
MO-175	Urine	390	390	0	0
MO-185	PE Sample	55	54	2	1
MO-195	Urine	190	180	5	10
MO-202	Urine	76	78	3	2
MO-236	Urine	38	39	3	1
MO-269	Feed	0.1	0.1	0	0
MO-271	Water	<1	<1	0	0

RPD = relative percent difference; PE = performance evaluation

Table E-4. Laboratory Quality Control Standards

Sample ID	Measured arsenic concentration (ppb)	Detection limit (ppb)	Reference material ID	Certified mean[a]	Recovery (%)
QC-1	3	3	NIST 2670a-L	3	100
QC-2	240	10	NIST 2670a-H	220 ± 10	109
QC-3	230	10	NIST 2670a-H	220 ± 10	105
QC-4	5	3	NIST 2670a-L	3	167
QC-5	220	10	NIST 2670a-H	220 ± 10	100
QC-6	250	10	NIST 2670a-H	220 ± 10	114
QC-7	60	1	NIST 1643e	58.98 ±0.7	102
QC-8	7.4	0.1	NIST 1566b	7.65 ± 0.65	97

[a]mean or mean ± SD

Table E-5. Blanks

Sample ID	Measured arsenic concentration (ppb)	Detection limit (ppb)
Blank-1	<1	1
Blank-2	<1	1
Blank-3	<1	1
Blank-4	<1	1
Blank-5	<1	1
Blank-6	<1	1
Blank-7	<1	1
Blank-8	<0.1	0.1

Figure E-1. Urinary Arsenic Blind Duplicates

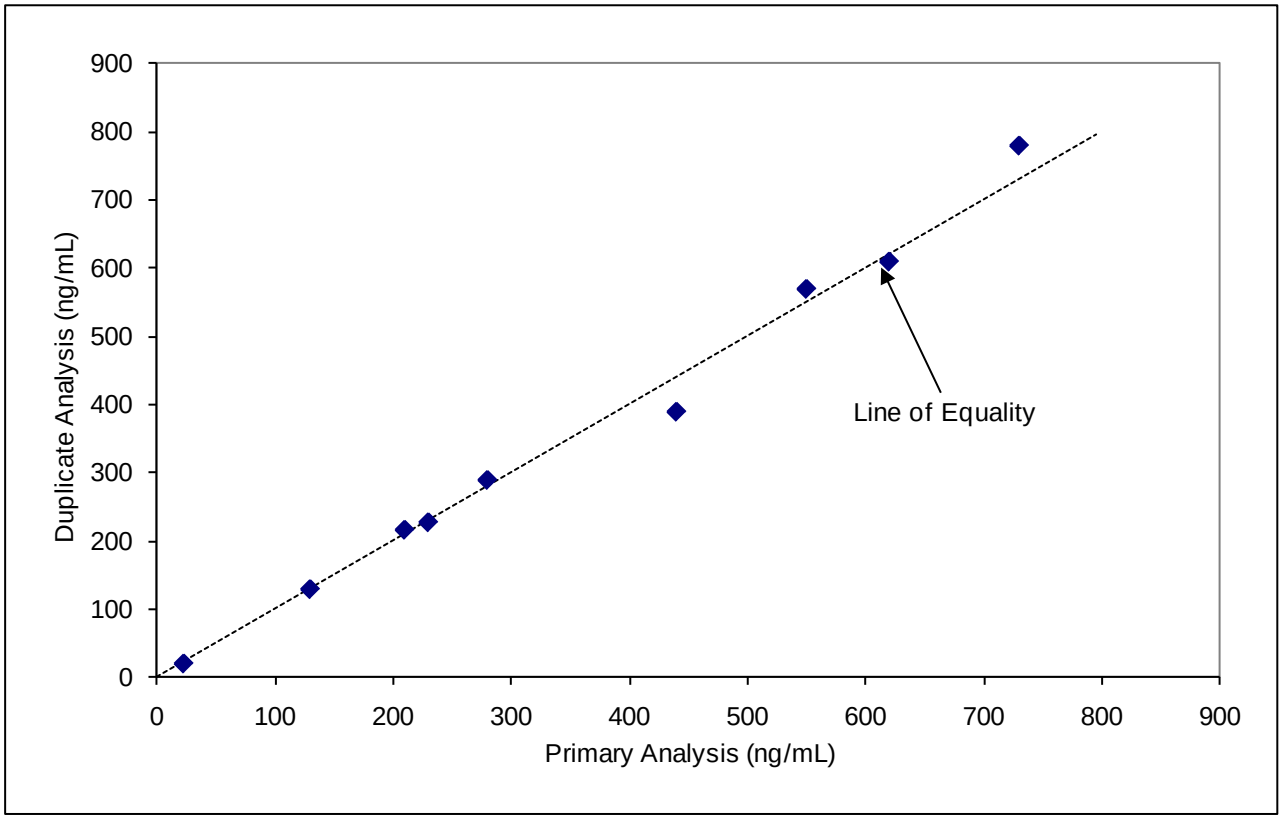
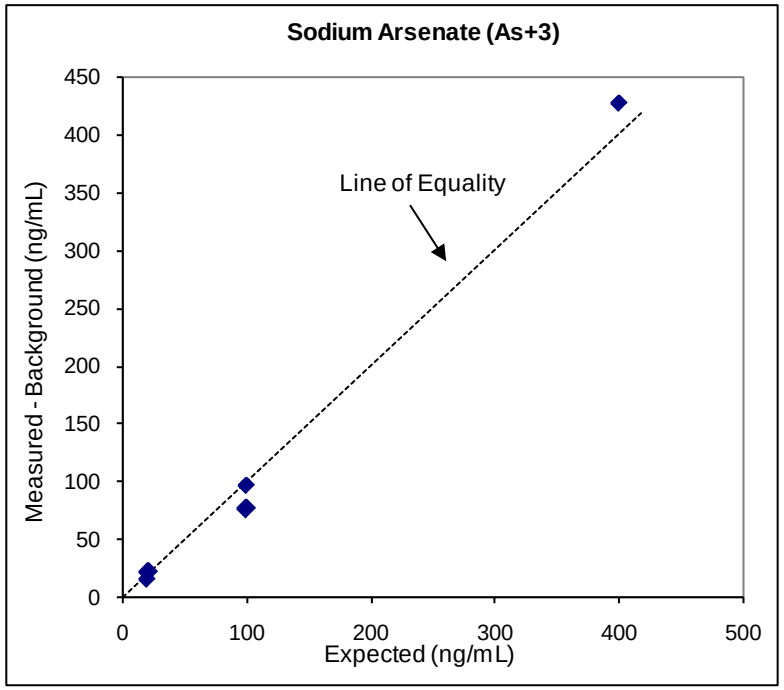
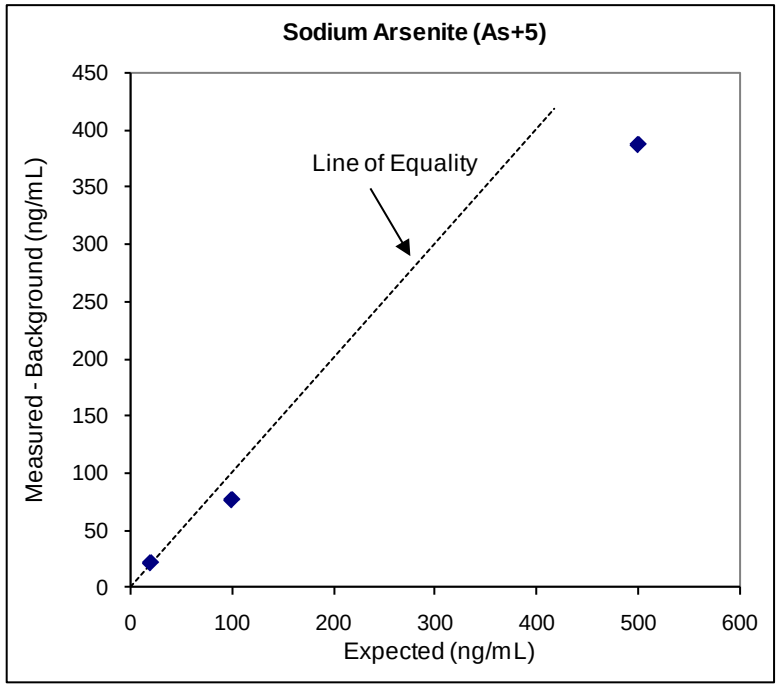
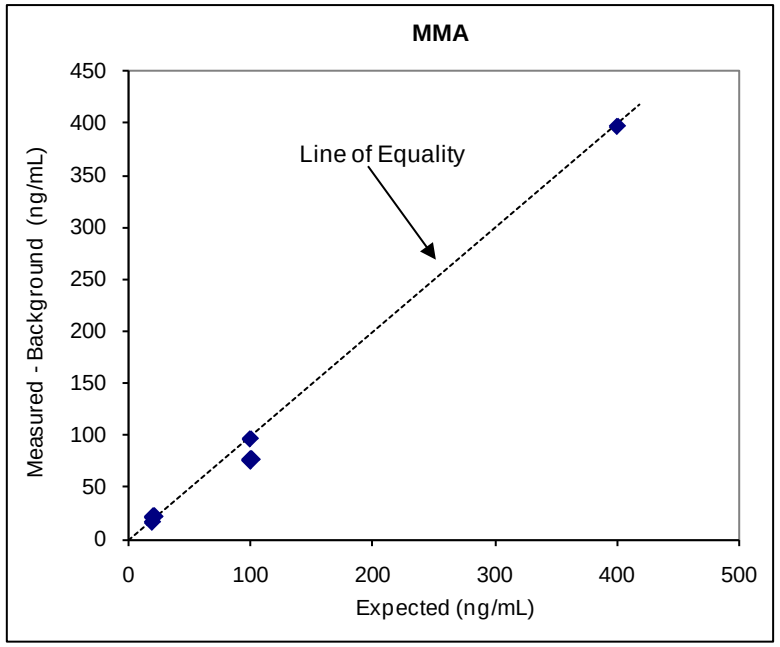
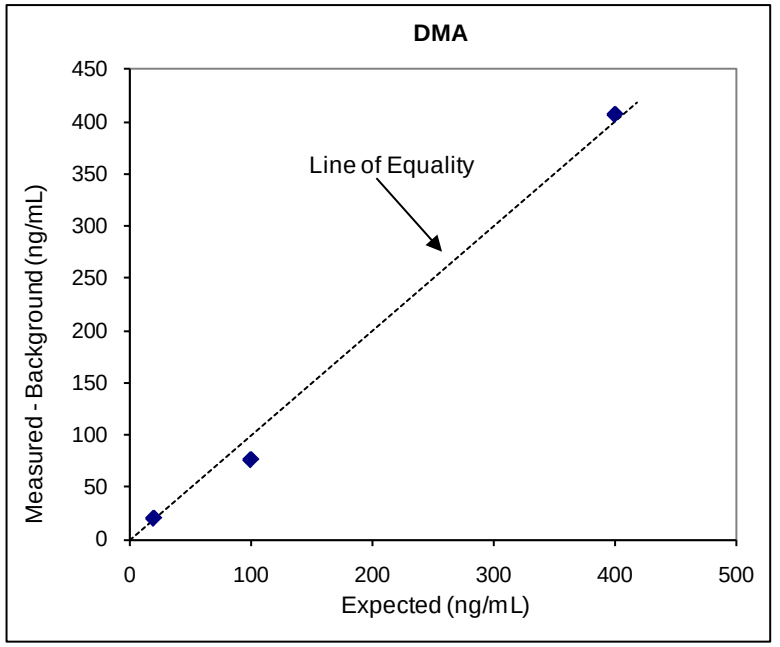


Figure E-2. Performance Evaluation Samples









RELATIVE BIOAVAILABILITY OF ARSENIC IN NIST SRM 2710 (MONTANA SOIL)

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EXECUTIVE SUMMARY

A study using juvenile swine as test animals was performed to measure the gastrointestinal absorption of arsenic from a sample of National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 2710. This is a soil from Montana that is contaminated by mine tailings deposits. The relative bioavailability of arsenic was assessed by comparing the absorption of arsenic from NIST SRM 2710 to that of sodium arsenate. Groups of four swine were given oral doses of sodium arsenate or the test soil twice a day for 14 days. A group of three non-treated swine served as a control.

The amount of arsenic absorbed by each animal was evaluated by measuring the amount of arsenic excreted in the urine (collected over 48-hour periods beginning on days 6, 9, and 12). The urinary excretion fraction (UEF) (the ratio of the amount excreted per 48 hours divided by the dose given per 48 hours) was calculated for both the test soil and sodium arsenate using simultaneous weighted linear regression analysis. The relative bioavailability (RBA) of arsenic in the test soil compared to sodium arsenate was calculated as follows:

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

The results are summarized below:

Time Interval	Estimated RBA (90% Confidence Interval)
Days 6/7	0.41 (0.36 - 0.47)
Days 9/10	0.42 (0.39 - 0.47)
Days 12/13	0.50 (0.40 - 0.62)
All Days	0.44 (0.40 - 0.48)

Arsenic in NIST SRM 2710 is absorbed about 44% as well as arsenic from sodium arsenate.

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ACRONYMS AND ABBREVIATIONS

ABA	Absolute bioavailability
AF_o	Oral absorption fraction
As+3	Trivalent inorganic arsenic
As+5	Pentavalent inorganic arsenic
DMA	Dimethyl arsenic
D	Ingested dose
g	Gram
GLP	Good Laboratory Practices
kg	Kilogram
K_u	Fraction of absorbed arsenic which is excreted in urine
mL	Milliliter
MMA	Monomethyl arsenic
N	Number of data points
NIST	National Institute of Standards and Technology
QA	Quality assurance
RBA	Relative bioavailability
ref	Reference material
RfD	Reference dose
SD	Standard deviation
SF	Slope factor
SRM	Standard reference material
test	Test material
UEF	Urinary excretion fraction
USEPA	United States Environmental Protection Agency
µg	Microgram
µm	Micrometer
°C	Degrees Celsius

1.0 INTRODUCTION

1.1 Overview of Bioavailability

Analysis of the potential hazard to humans from ingestion of a chemical depends upon accurate information on a number of key parameters, including the concentration of the chemical in environmental media (e.g., soil, dust, water, food, air, paint), intake rates of each medium, and the rate and extent of absorption ("bioavailability") of the chemical by the body from each ingested medium. Bioavailability is a measure of the amount of chemical that is absorbed by the body from an ingested medium. The amount of bioavailable chemical depends on the physical-chemical properties of the chemical and of the medium. For example, some metals in soil may exist, at least in part, as poorly water-soluble minerals, and may also exist inside particles of inert matrix such as rock or slag of variable size, shape, and association. These chemical and physical properties may influence (usually decrease) the bioavailability of the metals when ingested. Thus, equal ingested doses of different forms of a chemical in different media may not be of equal health concern.

Bioavailability of a chemical in a particular medium may be expressed either in absolute terms (absolute bioavailability) or in relative terms (relative bioavailability):

> Absolute bioavailability (ABA) is the ratio of the amount of the chemical absorbed to the amount ingested:
>
> $$ABA = \frac{Absorbed\ Dose}{Ingested\ Dose}$$
>
> This ratio is also referred to as the oral absorption fraction (AF_o).
>
> Relative bioavailability (RBA) is the ratio of the AF_o of the chemical present in some test material (*test*) to the AF_o of the chemical in some appropriate reference material (e.g., either the chemical dissolved in water or a solid form that is expected to fully dissolve in the stomach) (*ref*):
>
> $$RBA(test\ vs\ ref) = \frac{AF_o(test)}{AF_o(ref)}$$

For example, if 100 micrograms (µg) of a chemical (e.g., arsenic) dissolved in drinking water were ingested and a total of 50 µg were absorbed into the body, the AF_o would be 50/100, or 0.50 (50%). Likewise, if 100 µg of a chemical contained in soil were ingested and 30 µg were absorbed into the body, the AF_o for this chemical in soil would be 30/100, or 0.30 (30%). If the chemical dissolved in water were used as the frame of reference for describing the relative amount of the same chemical absorbed from soil, the RBA would be 0.30/0.50, or 0.60 (60%).

For additional discussion about the concept and application of bioavailability, see Gibaldi and Perrier (1982), Goodman et al. (1990), and/or Klaassen et al. (1996).

1.2 Using Bioavailability Data to Improve Risk Calculations

When reliable data are available on the bioavailability of a chemical in a site medium (e.g., soil), this information can be used to improve the accuracy of exposure and risk calculations at that site. For example, the basic equation for estimating the site-specific ABA of a test soil is as follows:

$$ABA_{soil} = ABA_{soluble} \cdot RBA_{soil}$$

where:

ABA_{soil}	=	Absolute bioavailability of the chemical in soil ingested by a human
$ABA_{soluble}$	=	Absolute bioavailability of some dissolved or fully soluble form of the chemical in children
RBA_{soil}	=	Relative bioavailability of the chemical in soil as measured in swine

Available bioavailability data can be used to adjust default oral toxicity values (reference dose and slope factor) to account for differences in absorption between the chemical ingested in water and the chemical ingested in site media, assuming the toxicity factors are based on a readily soluble form of the chemical. For non-cancer effects, the default reference dose ($RfD_{default}$) can be adjusted ($RfD_{adjusted}$) as follows:

$$RfD_{adjusted} = \frac{RfD_{default}}{RBA}$$

For potential carcinogenic effects, the default slope factor ($SF_{default}$) can be adjusted ($SF_{adjusted}$) as follows:

$$SF_{adjusted} = SF_{default} \cdot RBA$$

Alternatively, it is also acceptable to adjust the dose (rather than the toxicity factors) as follows:

$$Dose_{adjusted} = Dose_{default} \cdot RBA$$

This dose adjustment is mathematically equivalent to adjusting the toxicity factors as described above.

1.3 Purpose of this Study

The objective of this study was to use juvenile swine as a test system in order to determine the RBA of arsenic in a sample of National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 2710 compared to a soluble form of arsenic (sodium arsenate).

2.0 STUDY DESIGN

This investigation of arsenic RBA was performed according to the basic design presented in Table 2-1. The study investigated arsenic absorption from sodium arsenate (NaAs) and a test material (TM1). Each material was administered to groups of four animals at one or two different dose levels for 14 days (a detailed schedule is presented in Appendix A, Table A-1). Additionally, the study included a non-treated group of two animals to serve as a control for determining background arsenic levels. All doses were administered orally.

The study design was based on the standardized study protocol for measuring lead relative bioavailability (USEPA 2007) using the juvenile swine model. The basic model for estimating arsenic RBA differed from lead in that the urinary excretion fraction (UEF) of arsenic administered in test material and in reference material (sodium arsenate) was measured, and the ratio of the two UEF values then calculated:

RBA(test material) = UEF(test material) / UEF(sodium arsenate)

The UEF for each material (test soil, sodium arsenate) was estimated by plotting the mass of arsenic excreted by each animal as a function of the dose administered, and then fitting a linear regression line to the combined data. The process of deriving the best fit linear regression were fit using simultaneous weighted linear regression.

The study was performed as nearly as possible within the spirit and guidelines of Good Laboratory Practices (GLP: 40 CFR 792).

2.1 Test Materials

2.1.1 Sample Description

The test soil used in this investigation was a sample of NIST SRM 2710. The sample consists of soil collected from the top 4 inches of pasture land along Silver Bow Creek near Butte, Montana. The soil is a native Montana soil that has been contaminated with mine tailings deposits. The collection site is approximately 6.5 miles south of settling ponds that feed the creek. The creek periodically floods, depositing mine tailings with high concentrations of copper, manganese, and zinc at the collection site (NIST 2003).

2.1.2 Sample Preparation

NIST SRM 2710 was prepared by air drying in an oven for three days at room temperature. The material was then passed over a vibrating 2 mm screen to remove plant material, rocks, and large chunks of aggregated soil. Material remaining on the screen was deaggregated and rescreened. The combined material passing the screen was ground in a ball mill to pass a 74 micrometer (μm) screen, radiation sterilized, and blended for 24 hours to achieve a high degree of homogeneity (NIST 2003). This prepared soil as provided by NIST was used as-is for the bioavailability study, without further preparation.

2.1.3 Arsenic Concentration

The certified concentration value for arsenic in NIST SRM 2710 is 626 ± 38 mg/kg (NIST 2003). This value is a weighted mean of results from two independent analytical methods, hydride generation atomic absorption spectrometry and radiochemical neutron activation analysis – mixed acid digestion. The stated uncertainties include allowances for measurement imprecision, material variability, and differences among analytical methods (NIST 2003). Certified values of additional elements are shown in Table A-2 of Appendix A.

2.2 Experimental Animals

Juvenile swine were selected for use in this study because they are considered to be a good physiological model for gastrointestinal absorption in children (Weis and LaVelle 1991, Casteel et al. 1996). The animals were intact males of the Pig Improvement Corporation genetically defined Line 26, and were purchased from Chinn Farms, Clarence, Missouri.

The number of animals purchased for the study was several more than required by the protocol. These animals were purchased at an age of about 5-6 weeks (weaning occurs at age 3 weeks) and housed in individual stainless steel cages. The animals were then held under quarantine for one week to observe their health before beginning exposure to dosing materials. Each animal was examined by a certified veterinary clinician (swine specialist) and any animals that appeared to be in poor health during this quarantine period were excluded from the study. To minimize weight variations among animals and groups, extra animals most different in body weight (either heavier or lighter) five days prior to exposure (day -5) were also excluded from the study. The remaining animals were assigned to dose groups at random (group assignments are presented in Appendix A, Table A-3).

When exposure began (day zero), the animals were about 6-7 weeks old and weighed an average of about 9.3 kilograms (kg). The animals were weighed every three days during the course of the study. On average, animals gained about 0.26 kg/day and the rate of weight gain was comparable in all dosing groups, ranging from 0.23 to 0.32 kg/day. These body weight data are presented in Appendix A, Table A-4, and summarized in Figure 2-1.

All animals were examined daily by an attending veterinarian while on study and were subjected to detailed examination at necropsy by a certified veterinary pathologist in order to assess overall animal health.

2.3 Diet

Animals were weaned onto standard pig chow (purchased from MFA Inc., Columbia, Missouri) by the supplier. The animals were gradually transitioned from the MFA feed to a special purified diet originally developed for lead RBA studies (purchased from Purina TestDiet®, Richmond, IN) several days before dosing began, and this feed was maintained for the duration of the study. The feed was nutritionally complete and met all requirements of the National Institutes of Health–National Research Council (NRC 1988); the ingredients and nutritional profile of the feed are presented in Table 2-2.

Prior to the start of dosing, each day every animal was given an amount of feed equal to 4.0% of the mean body weight of all animals on study. After dosing began (beginning with the evening feeding of Day 1), the amount of feed per day was reduced to 3.5% of the mean body weight to encourage consumption of the dose materials. Feed amounts were adjusted every three days, when animals were weighed. Feed was administered in two equal portions at 11:00 AM and 5:00 PM daily. Analysis of random feed samples indicated that the arsenic levels did not exceed 0.1 μg/g.

Drinking water was provided *ad libitum* via self-activated watering nozzles within each cage. Analysis of samples from randomly selected drinking water nozzles indicated the arsenic concentrations were below a level of detection (1 μg/L).

2.4 Dosing

The protocol for exposing animals to arsenic is shown in Table 2-1. Animals were exposed to dosing materials (sodium arsenate or test soil) for 14 days, with the dose for each day being administered in two equal portions beginning at 9:00 AM and 3:00 PM (two hours before feeding). To facilitate dose administration, dosing materials were placed in a small depression in a ball of dough consisting of moistened TestDiet® feed (typically about 5 g) and the dough was pinched shut. This was then placed in the feeder at dosing time.

Occasionally, some animals did not consume their entire dose. In these instances, the missed doses were estimated and recorded and the time-weighted average dose calculation for each animal was adjusted downward accordingly (see Appendix A, Table A-4). Doses that were consumed late are noted in Table A-5, although no dose adjustments are required in these cases.

Administered amounts of dose materials were held constant throughout the study and were determined using the expected mean body weight during the exposure interval (14 days). The expected mean body weight was estimated as the mean of the actual measured weights on day -1 and the predicted weights for day 14, which were extrapolated from the day -1 weights assuming a weight gain of 1.5 kg every 3 days. The resulting estimated mean body weight was 12.86 kg.

After completion of the study, body weights were estimated by interpolation for those days when measurements were not collected. The actual administered doses were then calculated for each day and averaged across all days. The actual mean doses for each dosing group are included in Table 2-1; the actual daily doses administered to each animal are presented in Appendix A, Table A-4.

2.5 Collection and Preservation of Urine Samples

Samples of urine were collected from each animal for 48-hour periods on days 6 to 7 (U-1), 9 to 10 (U-2), and 12 to 13 (U-3) of the study. Collection began at 9:00 AM and ended 48 hours later. The urine was collected in a plastic bucket placed beneath each cage, which was emptied into a plastic storage bottle. Aluminum screens were placed under the cages to minimize contamination with feces, spilled food, or other debris. Due to the length of the collection

period, collection containers were emptied periodically (typically twice daily) into a separate plastic bottles to ensure that there was no loss of sample due to overflow.

At the end of each collection period, the total urine volume for each animal was measured (see Appendix A, Table A-6) and three 60-milliliter (mL) portions were removed and acidified with 0.6 mL concentrated nitric acid[1]. All samples were refrigerated. Two of the aliquots were archived in the refrigerator and one aliquot was sent for arsenic analysis (refrigeration was maintained until arsenic analysis).

2.6 Arsenic Analysis

Urine samples were assigned random chain-of-custody tag numbers and submitted to the analytical laboratory for analysis in a blind fashion; the samples were analyzed for arsenic by L. E. T., Inc., (Columbia, Missouri). In brief, 25 mL samples of urine were digested by refluxing and then heating to dryness in the presence of magnesium nitrate and concentrated nitric acid. Following magnesium nitrate digestion, samples were transferred to a muffle furnace and ashed at 500°C. The digested and ashed residue was dissolved in hydrochloric acid and analyzed by the hydride generation technique using a PerkinElmer 3100 atomic absorption spectrometer. Preliminary tests of this method established that each of the different forms of arsenic that may occur in urine, including trivalent inorganic arsenic (As+3), pentavalent inorganic arsenic (As+5), monomethyl arsenic (MMA), and dimethyl arsenic (DMA), are all recovered with high efficiency.

Urine analytical results are presented in Appendix A, Table A-7. All responses below the quantitation limit were evaluated at one-half the quantitation limit. Quality assurance samples are described in the following section (2.7).

2.7 Quality Assurance

A number of quality assurance (QA) steps were taken during this project to evaluate the accuracy of the analytical procedures. The results for quality assurance samples are presented in Appendix A, Table A-8, and are summarized below.

Blind Duplicates (Sample Preparation Replicates)

A random selection of about 20% of all urine samples generated during the study were prepared for laboratory analysis in duplicate (i.e., two separate subsamples of urine were prepared for analysis) and submitted to the laboratory in a blind fashion. The results for the blind duplicates are shown in Figure 2-2. As seen, there was good agreement between results for the duplicate pairs in all cases.

[1] Urine samples EP3-1-134 and EP3-1-160 (pigs 312 and 318 from group 2, U-2 urine collection) were inadvertently combined into a single sample prior to analysis. Thus, results for these two samples were excluded from the data evaluation.

Performance Evaluation Samples

A number of Performance Evaluation (PE) samples (urines of known arsenic concentration) were submitted to the laboratory in a blind fashion. The PE samples included several different concentrations each of four different types of arsenic (As+3, As+5, MMA, and DMA). The results for the PE samples are shown in Figure 2-3. As seen, there was good recovery of the arsenic in all cases.

Spike Recovery

During arsenic analysis, every tenth sample was spiked with known amounts of arsenic (sodium arsenate) and the recovery of the added arsenic was measured. Arsenic recovery for individual samples ranged from 95% to 106%, with an average of 103 ± 3.3% (N = 10).

Laboratory Duplicates

During arsenic analysis, every tenth sample was analyzed in duplicate. All duplicate results (N = 12) agreed within ±1 times the detection limit or less than 10% relative percent difference (RPD).

Laboratory Control Standards

Laboratory control standards (samples of reference materials for which a certified concentration of specific analytes has been established) were tested periodically during sample analysis. Results are summarized below:

Standard	Description	Certified Mean ± SD (ng/mL)	Mean (ng/mL)	Range (ng/mL)	SD (ng/mL)	Mean % Recovery	N
NIST 2670a-H	Freeze-dried human urine, spiked	220 +/-10	237	230 - 240	5.8	108%	3
NIST 2670a-L	Freeze-dried human urine, unspiked	3*	4	3 - 5	1.4	133%	2
NIST 1640	Natural fresh water (unspiked)	26.7 +/-0.41	26	--	--	97%	1
NIST 1566b	Freeze-dried oyster tissue	7.65 +/-0.65	7.5	--	--	98%	1

*Note that the arsenic concentration in NIST 2670a-L as provided by NIST is a reference value, not a certified value. Reference values are non-certified values that are the best estimate of the true value but do not meet the NIST criteria for certification.
SD = Standard deviation
N = Number of samples analyzed

Recovery of arsenic from these standards was generally good and within the acceptable range.

Blanks

Blank samples run along with each batch of samples never yielded a measurable level of arsenic (N = 7).

Based on the results of all of the quality assurance samples and steps described above, it is concluded that the analytical results are of sufficient quality for derivation of reliable estimates of arsenic absorption from the test materials.

3.0 DATA ANALYSIS

3.1 Overview

Figure 3-1 shows a conceptual model for the toxicokinetic fate of ingested arsenic. Key points of this model are as follows:

- In most animals (including humans), absorbed arsenic is excreted mainly in the urine over the course of several days. Thus, the urinary excretion fraction (UEF), defined as the amount excreted in the urine divided by the amount given, is usually a reasonable approximation of the AF_o or ABA. However, this ratio will underestimate total absorption, because some absorbed arsenic is excreted in the feces via the bile, and some absorbed arsenic enters tissue compartments (e.g., skin, hair) from which it is cleared very slowly or not at all. Thus, the urinary excretion fraction should not be equated with the absolute absorption fraction.

- The RBA of two orally administered materials (i.e., a test material and reference material) can be calculated from the ratio of the urinary excretion fraction of the two materials. This calculation is independent of the extent of tissue binding and of biliary excretion:

$$RBA(test\ vs\ ref) = \frac{AF_o(test)}{AF_o(ref)} = \frac{D \cdot AF_o(test) \cdot K_u}{D \cdot AF_o(ref) \cdot K_u} = \frac{UEF(test)}{UEF(ref)}$$

  where:

  D = Ingested dose (μg)

  K_u = Fraction of absorbed arsenic that is excreted in the urine

Based on the conceptual model above, the basic method used to estimate the RBA of arsenic in a particular test material compared to arsenic in a reference material (sodium arsenate) is as follows:

1. Plot the amount of arsenic excreted in the urine (μg/day) as a function of the administered amount of arsenic (μg/day), both for reference material (sodium arsenate) and for test material.

2. Find the best fit linear regression line through each data set. The slope of each line (μg/day excreted per μg/day ingested) is the best estimate of the urinary excretion fraction (UEF) for each material.

3. Calculate RBA for each test material as the ratio of the UEF for test material compared to UEF for reference material:

$$RBA(test\ vs\ ref) = \frac{UEF(test)}{UEF(ref)}$$

A detailed description of the curve-fitting methods and rationale and the methods used to quantify uncertainty in the arsenic RBA estimates for a test material are summarized below. All model fitting was performed in Microsoft Excel® using matrix functions.

3.2 Dose-Response Model

Simultaneous Regression

The techniques used to derive linear regression fits to the dose-response data are based on the methods recommended by Finney (1978). As noted by Finney (1978), when the data to be analyzed consist of two dose-response curves (the reference material and the test material), it is obvious that both curves must have the same intercept, since there is no difference between the curves when the dose is zero. This requirement is achieved by combining the two dose response equations into one and solving for the parameters simultaneously, as follows:

Separate Models:

$$\mu_r(i) = a + b_r \cdot x_r(i)$$

$$\mu_t(i) = a + b_t \cdot x_t(i)$$

Combined Model

$$\mu(i) = a + b_r \cdot x_r(i) + b_t \cdot x_t(i)$$

where $\mu(i)$ indicates the expected mean response of animals exposed at dose $x(i)$, and the subscripts r and t refer to reference and test material, respectively. The coefficients of this combined model are derived using multivariate regression, with the understanding that the combined data set is restricted to cases in which one (or both) of x_r and x_t are zero (Finney, 1978).

Weighted Regression

Regression analysis based on ordinary least squares assumes that the variance of the responses is independent of the dose and/or the response (Draper and Smith 1998). This assumption is generally not satisfied in swine-based RBA studies, where there is a tendency toward increasing variance in response as a function of increasing dose (heteroscedasticity). One method for dealing with heteroscedasticity is through the use of weighted least squares regression (Draper and Smith 1998). In this approach, each observation in a group of animals is assigned a weight that is inversely proportional to the variance of the response in that group:

$$w_i = \frac{1}{\sigma_i^2}$$

where:

w_i = weight assigned to all data points in dose group *i*

σ_i^2 = variance of responses in animals in dose group *i*

When the distributions of responses at each dose level are normal, weighted regression is equivalent to the maximum likelihood method.

There are several alternative strategies for assigning weights. The method used in this study estimates the value of σ_i^2 using an "external" variance model based on an analysis of the relationship between variance and mean response using data consolidated across many different swine-based arsenic RBA studies. Log-variance increases as an approximately linear function of log-mean response:

$$\ln(s_i^2) = k1 + k2 \cdot \ln(\bar{y}_i)$$

where:

s_i^2 = observed variance of responses of animals in dose group *i*
$\bar{y}_i$ = mean observed response of animals in dose group *i*

Goodness of Fit

The goodness-of-fit of each dose-response model was assessed using the F test statistic and the adjusted coefficient of multiple determination (Adj R^2) as described by Draper and Smith (1998). A fit is considered acceptable if the p-value is less than 0.05.

Assessment of Outliers

In biological assays, it is not uncommon to note the occurrence of individual measured responses that appear atypical compared to the responses from other animals in the same dose group. In this study, responses that yielded standardized weighted residuals greater than 3.5 or less than -3.5 were considered to be potential outliers (Canavos 1984). When such data points were encountered in a data set, the RBA values were calculated both with and without the potential outlier(s) excluded, and the result with the outlier(s) excluded was used as the preferred estimate.

3.3 Calculation of RBA Estimates

The arsenic RBA values were calculated as the ratio of the slope term for the test material data set (b_t) and the reference material data set (b_r):

$$RBA = \frac{b_t}{b_r}$$

The uncertainly range about the RBA ratio was calculated using Fieller's Theorem as described by Finney (1978).

4.0 RESULTS

4.1 Clinical Signs

The doses of arsenic administered in this study are below a level that is expected to cause toxicological responses in swine. No clinical signs of arsenic-induced toxicity were noted in any of the animals used in the study.

However, four animals exhibited signs of illness (e.g., gastrointestinal distress, elevated temperature) in the early days of the study and were treated with 1 cubic centimeter Naxcel, an injectable antibiotic given for transient illness. Pigs 319 (group 6) and 308 (group 3) were treated for three days beginning on day 0 and day 1, respectively, and pigs 318 (group 2) and 309 (group 4) were treated for one day on day 1. Symptoms promptly went away and the animals were retained on study.

4.2 Background Arsenic Excretion

The urinary excretion results for control animals from days 6-13 ranged from 2.2 to 11.1 μg/48 hours with a mean of 6.2. These values are representative of endogenous background levels in food and water and support the view that the animals were not exposed to any significant exogenous sources of arsenic throughout the study.

4.3 Dose-Response Patterns

Urinary Arsenic Variance

Discussed in Section 3.2, the urinary arsenic dose-response data are analyzed using weighted least squares regression and the weights are assigned using an "external" variance model. The data used to derive the variance model are shown in Figure 4-1. This data was gathered from previous RBA studies on swine. Based on these data, values of $k1$ and $k2$ were derived using ordinary least squares minimization. The resulting values were -1.10 for $k1$ and 1.64 for $k2$.

Superimposed on Figure 4-1 is the variance data from this study (as indicated by the solid symbols) on top of the historic data set (open symbols). As seen, the variance of the urinary arsenic data from this study is consistent with the data used to generate the variance model.

Urinary Arsenic

The dose-response data for arsenic in urine were modeled using a linear equation (see Section 3.2). The results of these fittings are shown in Figures 4-2 (days 6/7), 4-3 (days 9/10), 4-4 (days 12/13), and 4-5 (all days combined)[2]. One outlier was identified in the fittings, from group 6 on

[2] Urine samples EP3-1-134 and EP3-1-160 (pigs 312 and 318 from group 2, U-2 urine collection) were inadvertently combined into a single sample prior to analysis. Thus, results for these two samples were excluded from the data evaluation.

days 6/7. This outlier was excluded from the final evaluation for arsenic RBA; see Figures 4-6 (days 6/7) and 4-7 (all days combined) for the revised fittings.

4.4 Calculated RBA Values

As seen in Figures 4-2 through 4-7, all of the dose-response curves are approximately linear, with the slope of the best-fit straight line being equal to the best estimate of the UEF. The following table summarizes the resulting slopes (outliers excluded when applicable):

Time Interval	Outliers Excluded[a]	Slope (UEF Estimate)	
		b_r	b_{t1}
Days 6/7	1	0.83	0.34
Days 9/10	0	0.82	0.35
Days 12/13	0	0.81	0.40
All Days	1	0.82	0.36

[a] As indicated in Figures 4-2 and 4-5
b_r = slope term for the reference material data set
b_{t1} = slope term for the Test Material 1 data set

As discussed previously (Section 3), the relative bioavailability of arsenic in a specific test material is calculated as follows:

$$RBA(test\ vs\ ref) = \frac{UEF(test)}{UEF(ref)} = \frac{b_t}{b_r}$$

The following table summarizes the estimated RBA values:

Time Interval	Estimated RBA (90% Confidence Interval)
Days 6/7	0.41 (0.36 - 0.47)
Days 9/10	0.42 (0.39 - 0.47)
Days 12/13	0.50 (0.40 - 0.62)
All Days	0.44 (0.40 - 0.48)

As shown, using sodium arsenate as a relative frame of reference, the RBA estimate is approximately 44% for NIST SRM 2710.

4.5 Uncertainty

The bioavailability estimates above are subject to uncertainty that arises from several different sources. One source of uncertainty is the inherent biological variability between different animals in a dose group, which in turn causes variability in the amount of arsenic absorbed by the exposed animals. This between-animal variability in response results in statistical uncertainty in the best-fit dose-response curves and, hence, uncertainty in the calculated values of RBA. Such statistical uncertainty is accounted for by the statistical models used above and is characterized by the uncertainty range around the RBA estimates.

However, there is also uncertainty in the extrapolation of RBA values measured in juvenile swine to young children or adults, and this uncertainty is not included in the statistical confidence bounds above. Even though the immature swine is believed to be a useful and meaningful animal model for gastrointestinal absorption in children, it is possible that there are differences in physiological parameters that may influence RBA and, so, RBA values in swine may not be identical to values in children. In addition, RBA may depend on the amount and type of food in the stomach, since the presence of food can influence stomach pH, holding time, and possibly other factors that may influence solubilization of arsenic. In this regard, it is important to recall that RBA values measured in this study are based on animals that have little or no food in their stomach at the time of exposure and, hence, are likely to yield high-end values of RBA. Thus, these RBA values may be somewhat conservative for humans who ingest the site soils along with food. The magnitude of this bias is not known.

5.0 REFERENCES

Canavos, C. G. 1984. Applied Probability and Statistical Methods. Little, Brown and Co., Boston.

Casteel, S. W., R. P. Cowart, C. P. Weis, G. M. Henningsen, E. Hoffman, W. J. Brattin, M. F. Starost, J. T. Payne, S. L. Stockham, S. V. Becker, and J. R. Turk. 1996. A swine model for determining the bioavailability of lead from contaminated media. In: Advances in Swine in Biomedical Research. Tumbleson and Schook, eds. Vol 2, Plenum Press, New York. Pp. 637-46.

Draper, N. R., and H. Smith. 1998. Applied Regression Analysis (3rd Edition). John Wiley & Sons, New York.

Finney, D. J. 1978. Statistical Method in Biological Assay (3rd Edition). Charles Griffin and Co., London.

Gibaldi, M., and Perrier, D. 1982. Pharmacokinetics (2nd edition), pp 294-297. Marcel Dekker, Inc, NY, NY.

Goodman, A.G., Rall, T.W., Nies, A.S., and Taylor, P. 1990. The Pharmacological Basis of Therapeutics (8th ed.), pp. 5-21. Pergamon Press, Inc. Elmsford, NY.

Klaassen, C.D., Amdur, M.O., and Doull, J. (eds). 1996. Cassarett and Doull's Toxicology: The Basic Science of Poisons, pp. 190. McGraw-Hill, Inc. NY, NY.

NIST. 2003. Certificate of Analysis, Standard Reference Material® 2710 – Montana Soil, Highly Elevated Trace Element Concentrations. National Institute of Standards & Technology, Gaithersburg, MD. Certificate Issue Date: July 18, 2003.

NRC. 1988. Nutrient requirements of swine. A report of the Committee on Animal Nutrition. National Research Council. National Academy Press, Washington, DC.

USEPA. 2007. Estimation of Relative Bioavailability of Lead in Soil and Soil-Like Materials by *In Vivo* and *In Vitro* Methods OSWER9285.7-77. Office of Solid Waste and Emergency Response, Washington DC, USA.

Weis, C.P., and LaVelle, J.M. 1991. Characteristics to consider when choosing an animal model for the study of lead bioavailability. In: The proceedings of the international symposium on the bioavailability and dietary uptake of lead. Science and Technology Letters 3:113-119.

TABLES AND FIGURES

TABLE 2-1 DOSING PROTOCOL

Group	Number of Animals	Dose Material Administered	Arsenic Dose (µg/kg-day)	
			Target	Actual [a]
1	3	Control	0	0.0
2	4	Sodium Arsenate	25	24.1
3	4	Sodium Arsenate	50	47.5
4	4	Sodium Arsenate	100	95.9
5	4	Test Material 1	60	58.2
6	4	Test Material 1	120	114.5

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

Doses were administered in two equal portions given at 9:00 AM and 3:00 PM each day. Doses were held constant based on a body weight of 12.86 kg, the expected mean weight during the exposure interval (14 days).

TABLE 2-2 TYPICAL FEED COMPOSITION

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

INGREDIENTS

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

NUTRITIONAL PROFILE[2]

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

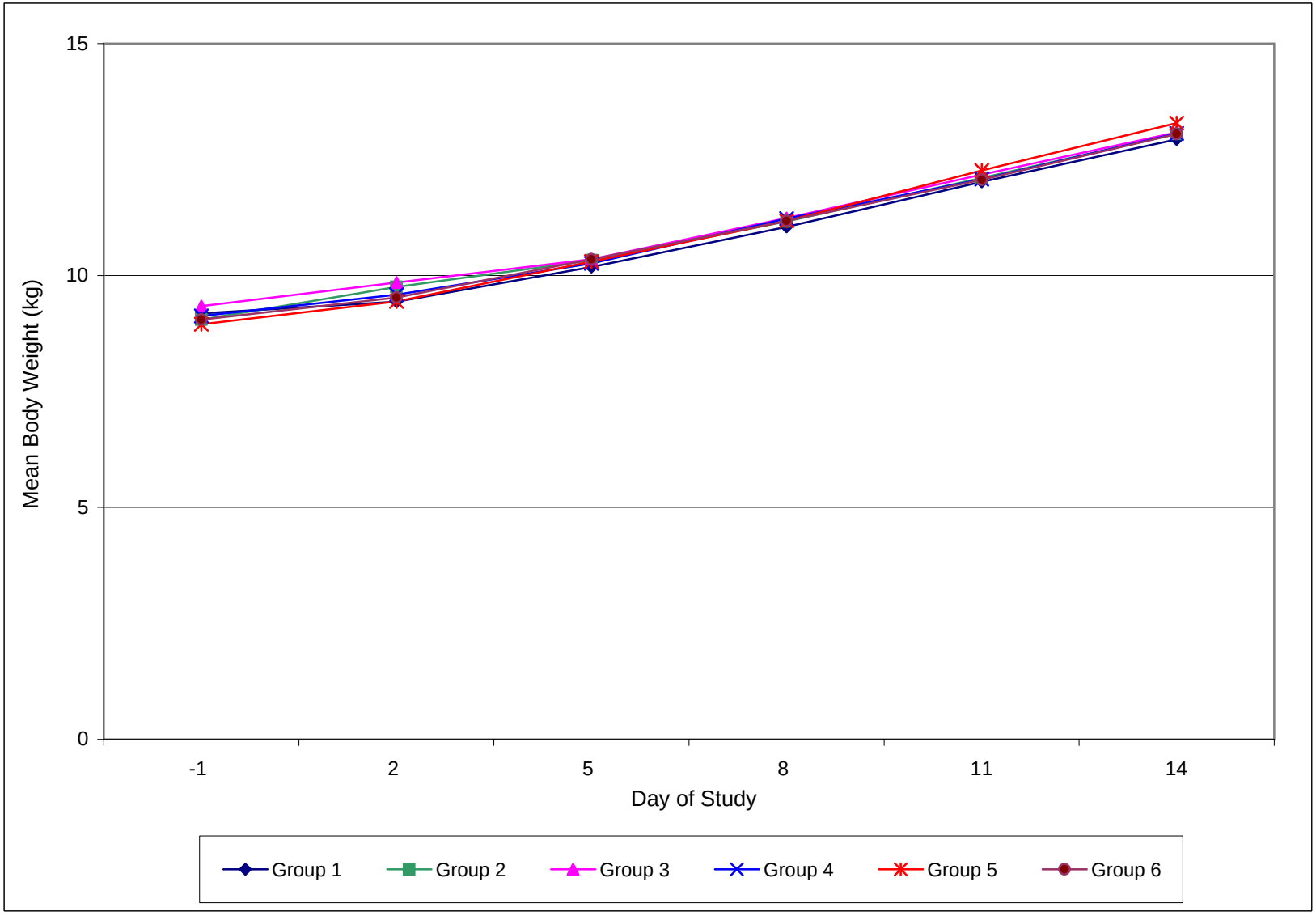
FOOTNOTES

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

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

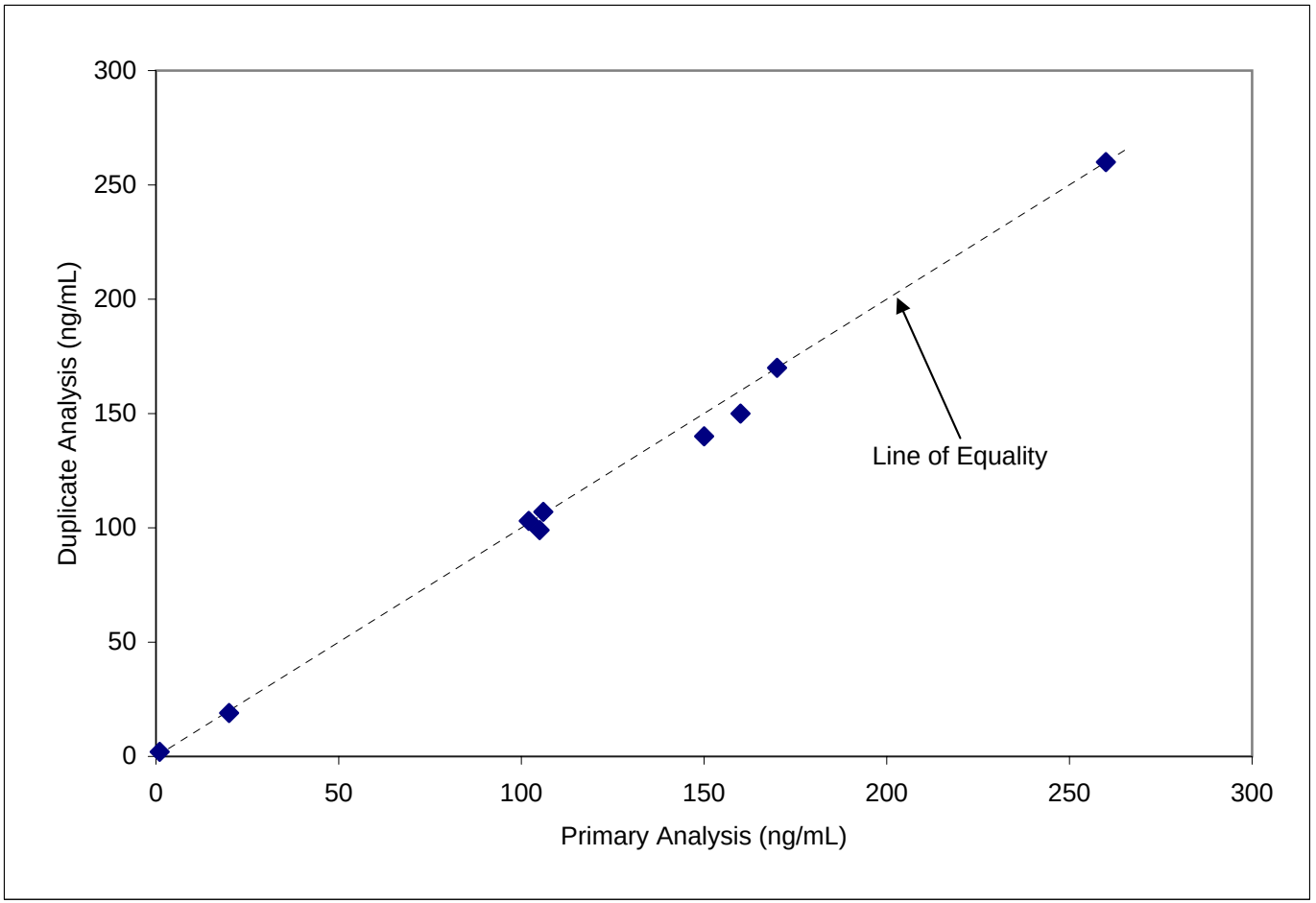
[3] Energy (kcal/gm) - Sum of decimal fractions of protein, fat and carbohydrate x 4,9,4 kcal/gm respectively.

FIGURE 2-1 BODY WEIGHT GAIN



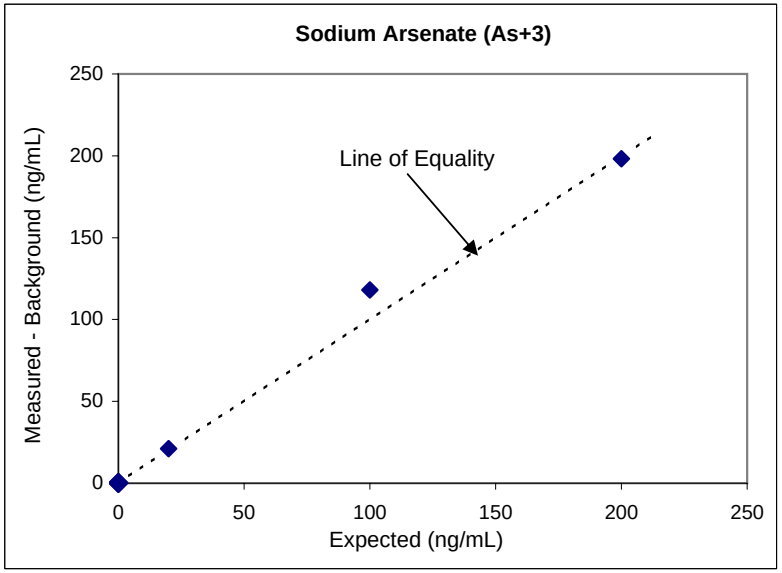
1_NIST1 Weights.xls (Fig2-1_group weights)

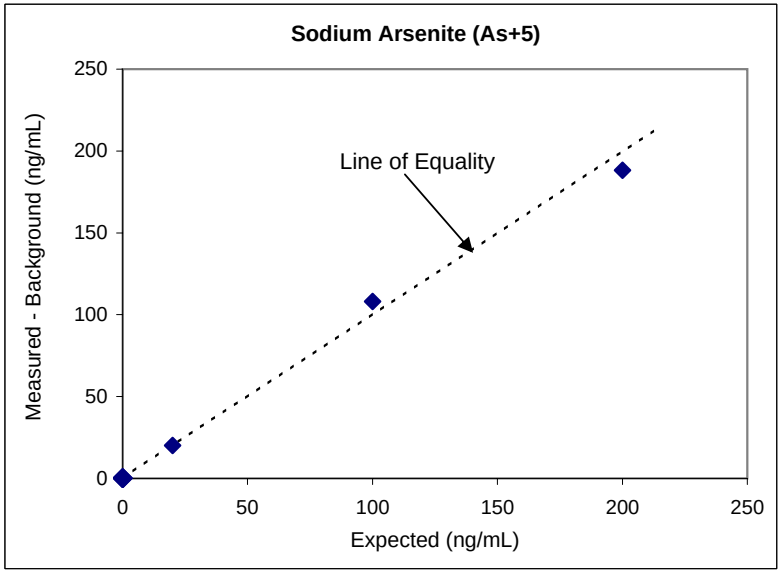
FIGURE 2-2 URINARY ARSENIC BLIND DUPLICATES

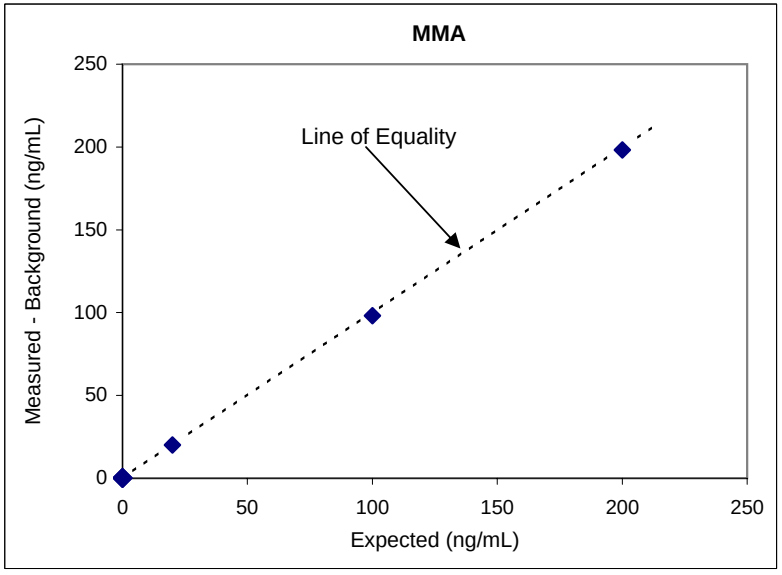


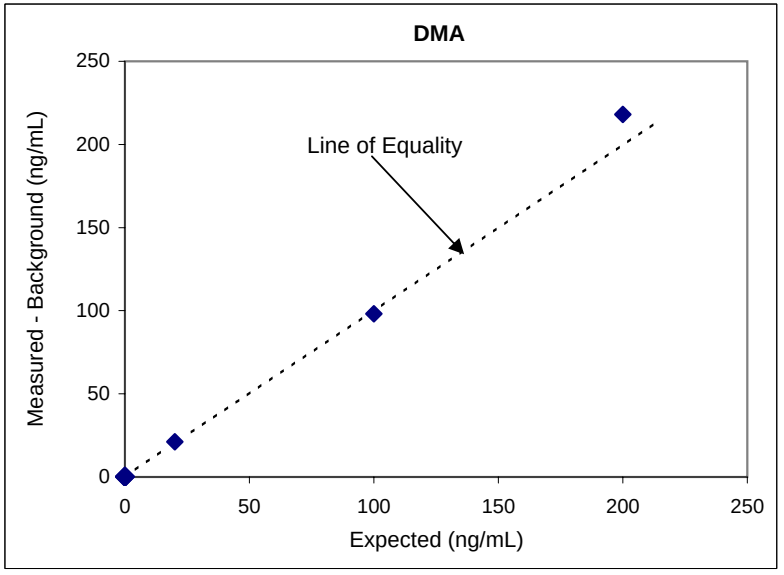
4_NIST1 Lab QC analysis.xls (Fig2-2_BlindDupes)

FIGURE 2-3 PERFORMANCE EVALUATION SAMPLES



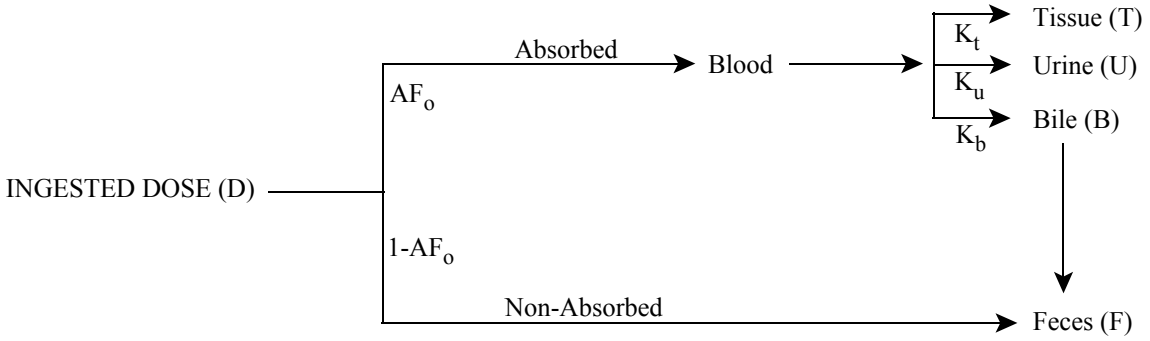






4_NIST1 Lab QC analysis.xls (Fig2-3_PE)

Figure 3-1. Conceptual Model for Arsenic Toxicokinetics



where:

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

BASIC EQUATIONS:

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

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

$= D \times AF_o \times K_u$

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

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

$= AF_o \times K_u$

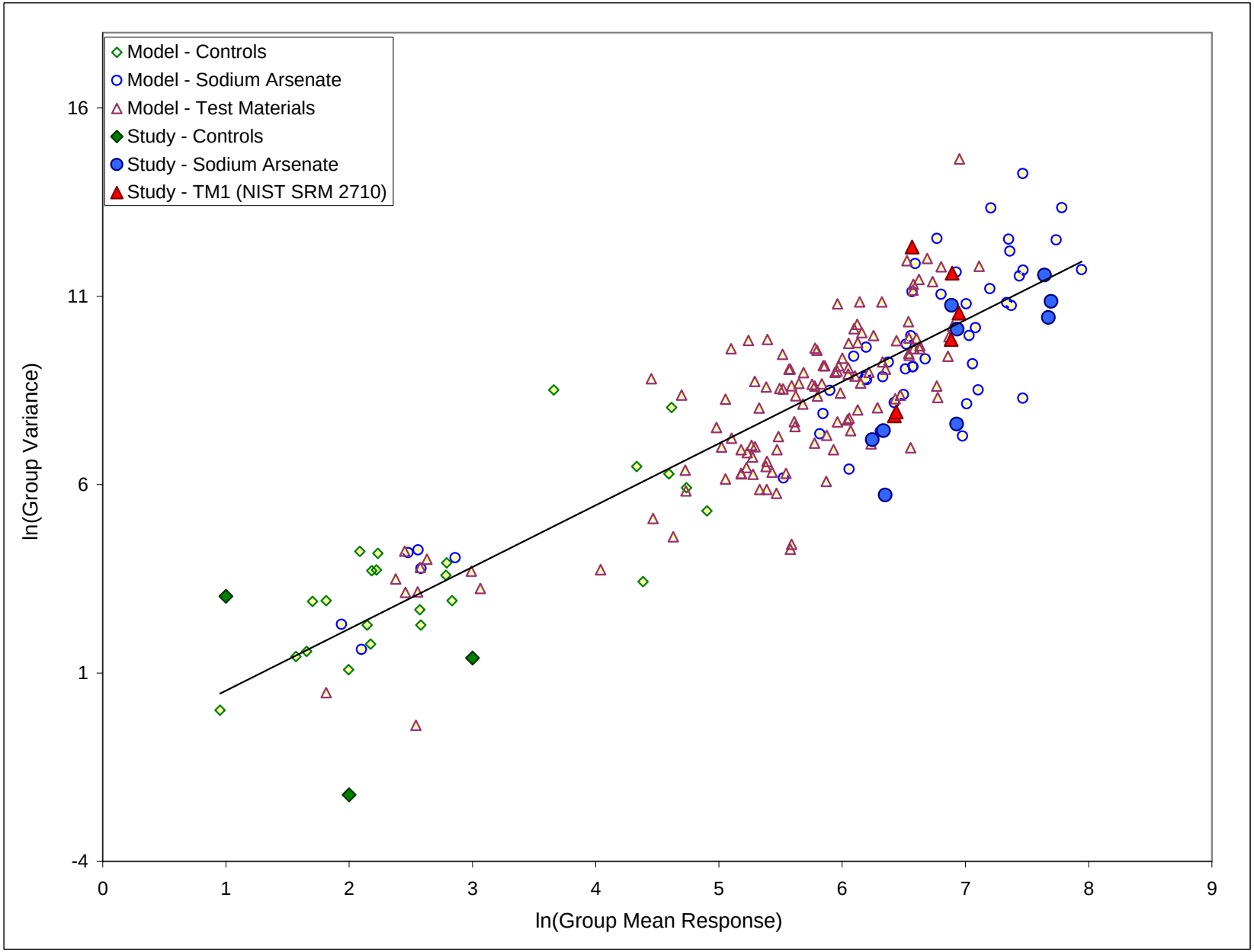
Relative Bioavailability (x vs. y) $= UEF(x) / UEF(y)$

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

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

Fig 3-1_Toxicokinetics.wpd

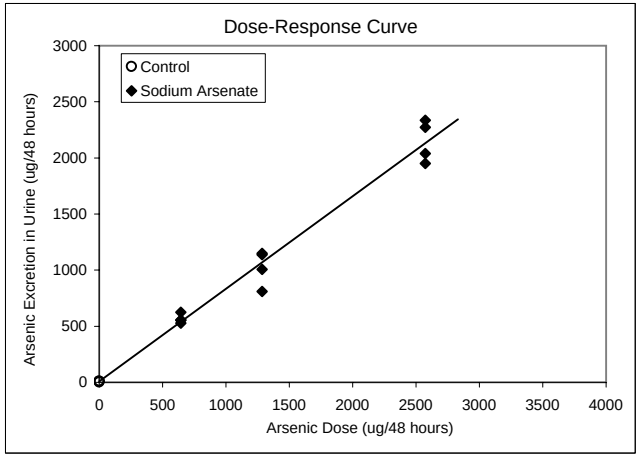
FIGURE 4-1 URINARY ARSENIC VARIANCE MODEL



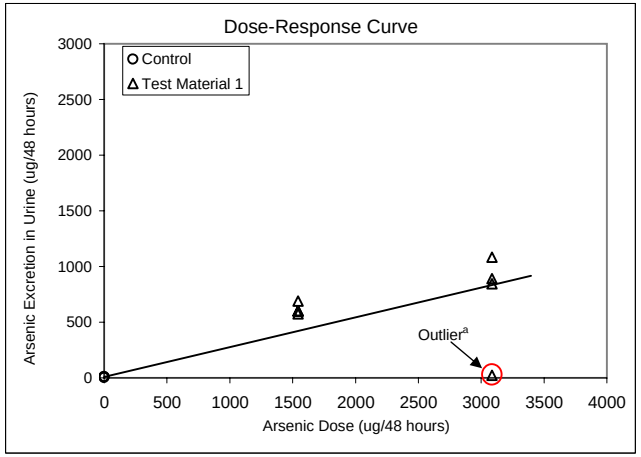
3_NIST1 Variance.xls (Fig 4-1)

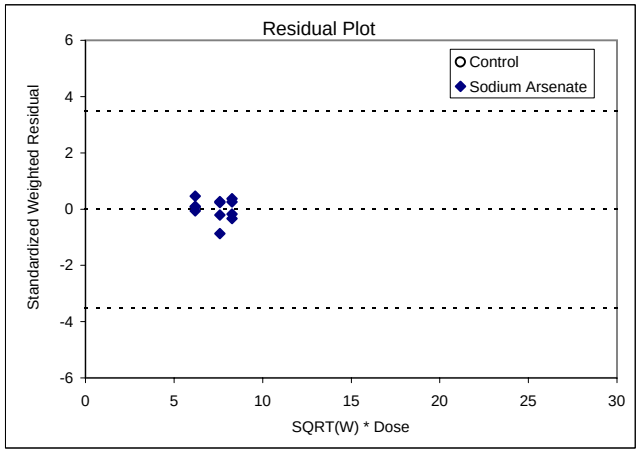
FIGURE 4-2 URINARY EXCRETION OF ARSENIC: Days 6/7 (All Data)

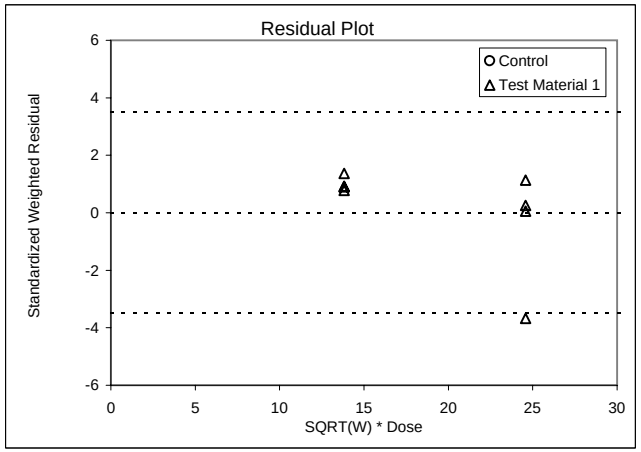
Reference Material (Sodium Arsenate)



Test Material 1 (NIST SRM 2710)







[a] Note that the data from this figure were refitted with the outlier excluded (see Figure 4-6); this outlier was excluded from the final evaluation for arsenic F

Summary of Fitting[b]

Parameter	Estimate	Standard Error
a	7.3	3.1
b_r	0.83	0.07
b_{t1}	0.27	0.03
Covariance (b_r,b_{t1})	0.0013	--
Degrees of Freedom	21	--

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

where r = Reference Material, t1 = Test Material 1

ANOVA

Source	MSE
Fit	337.45
Error	3.43
Total	33.79

Statistic	Estimate
F	98.508
p	< 0.001
Adjusted R^2	0.8986

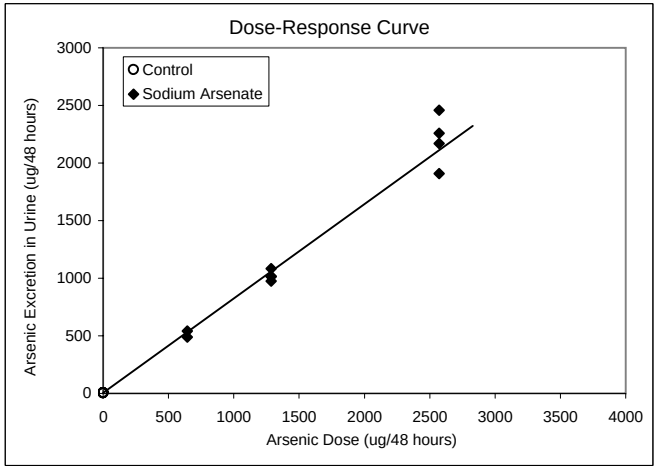
RBA and Uncertainty

	Test Material 1
RBA	0.32
Lower bound[c]	0.25
Upper bound[c]	0.42
Standard Error[c]	0.049

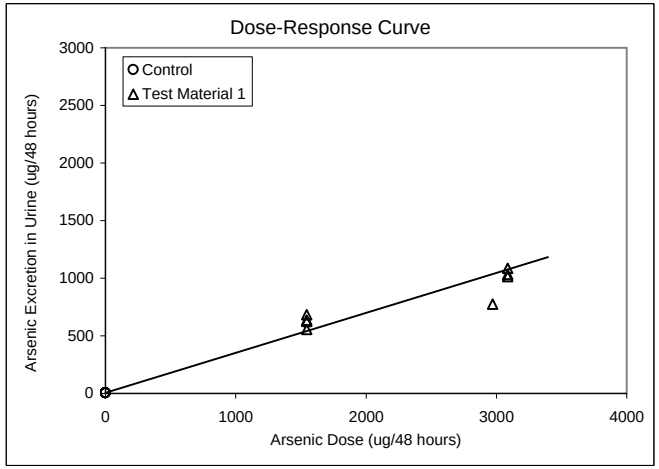
[c] Calculated using Fieller's theorem

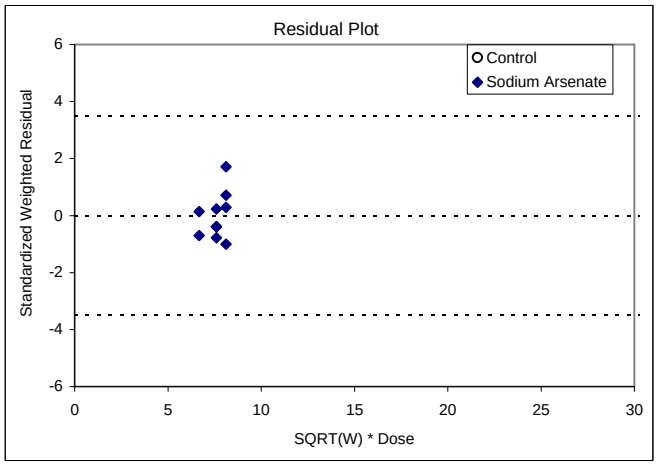
FIGURE 4-3 URINARY EXCRETION OF ARSENIC: Days 9/10 (All Data)

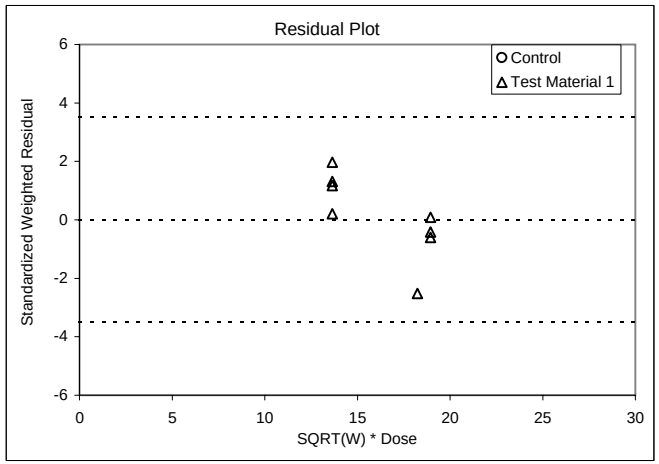
Reference Material (Sodium Arsenate)



Test Material 1 (NIST SRM 2710)







Summary of Fitting[a]

Parameter	Estimate	SE
a	5.6	0.9
b_r	0.82	0.03
b_{t1}	0.35	0.01
Covariance (b_r,b_{t1})	0.0007	--
Degrees of Freedom	19	--

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

where r = Reference Material, t1 = Test Material 1

ANOVA

Source	MSE
Fit	324.90
Error	0.46
Total	32.90

Statistic	Estimate
F	713.819
p	< 0.001
Adjusted R^2	0.9862

RBA and Uncertainty

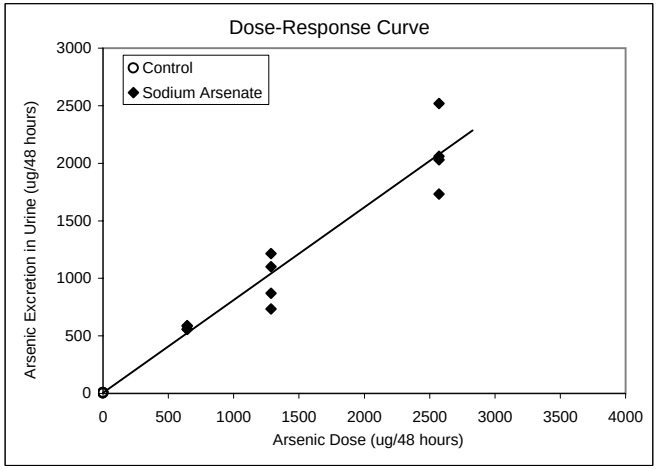
	Test Material 1
RBA	0.42
Lower bound[b]	0.39
Upper bound[b]	0.47
Standard Error[b]	0.023

[b] Calculated using Fieller's theorem

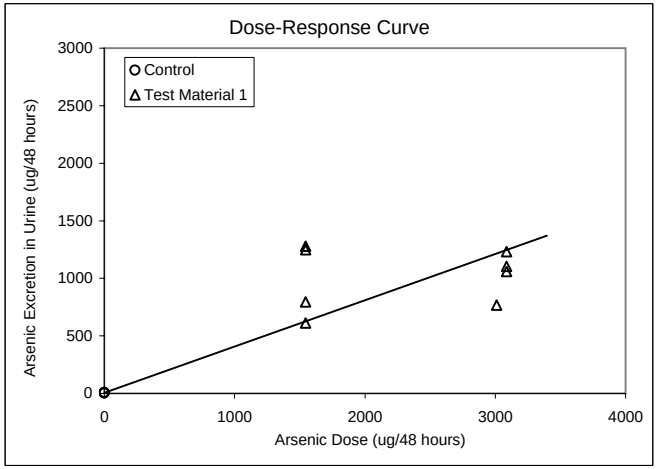
2_NIST1 RBA Calcs_all data.xls (Graph_2)

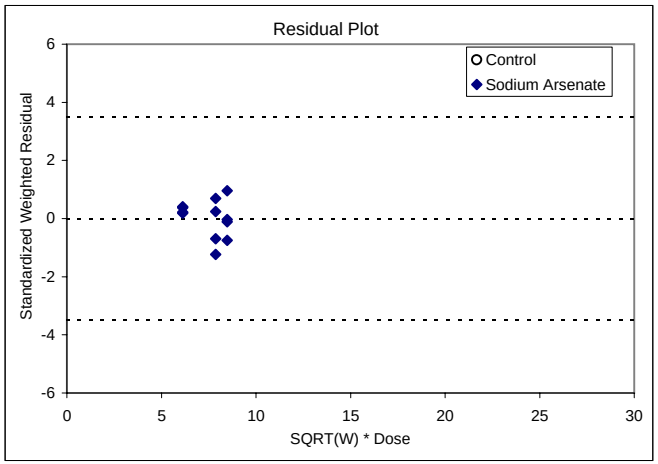
FIGURE 4-4 URINARY EXCRETION OF ARSENIC: Days 12/13 (All Data)

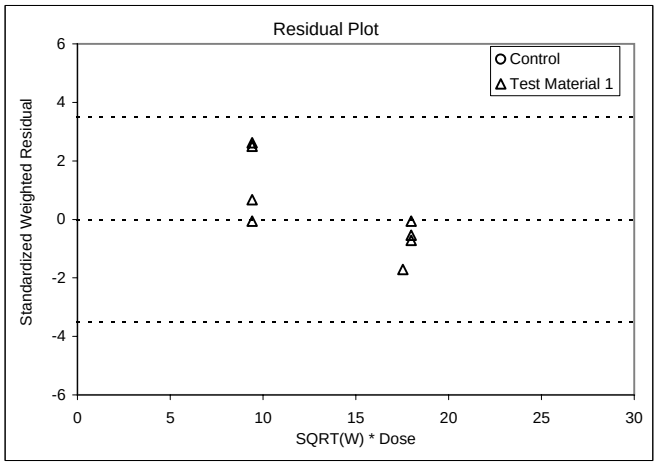
Reference Material (Sodium Arsenate)



Test Material 1 (NIST SRM 2710)







Summary of Fitting[a]

Parameter	Estimate	SE
a	5.9	2.3
b_r	0.81	0.06
b_{t1}	0.40	0.04
Covariance (b_r,b_{t1})	0.0006	--
Degrees of Freedom	21	--

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

where r = Reference Material, t1 = Test Material 1

ANOVA

Source	MSE
Fit	353.82
Error	2.58
Total	34.52

Statistic	Estimate
F	136.884
p	< 0.001
Adjusted R^2	0.9251

RBA and Uncertainty

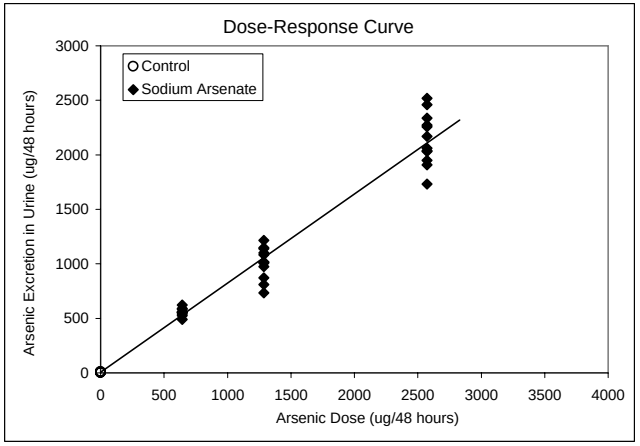
	Test Material 1
RBA	0.50
Lower bound[b]	0.40
Upper bound[b]	0.62
Standard Error[b]	0.062

[b] Calculated using Fieller's theorem

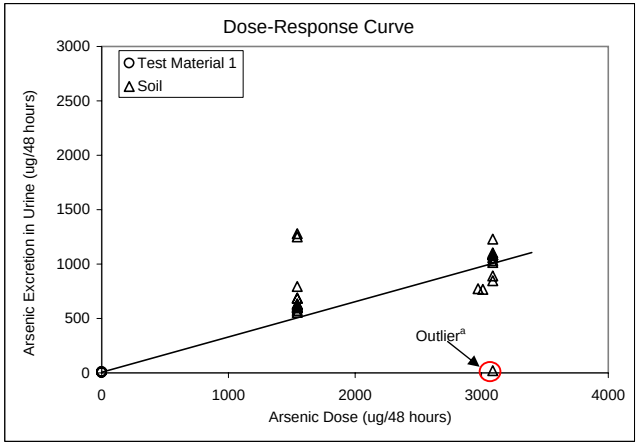
2_NIST1 RBA Calcs_all data.xls (Graph_3)

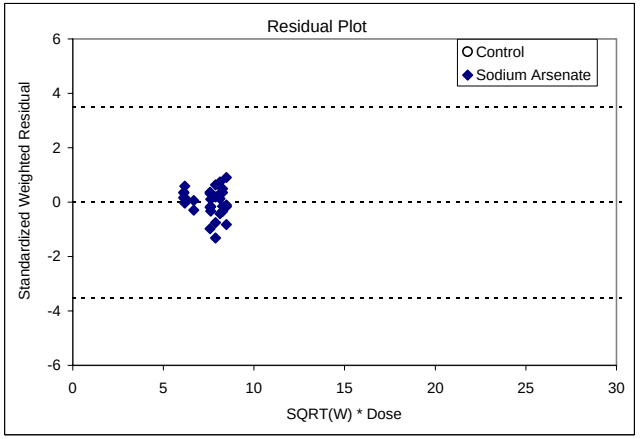
FIGURE 4-5 URINARY EXCRETION OF ARSENIC: All Days (All Data)

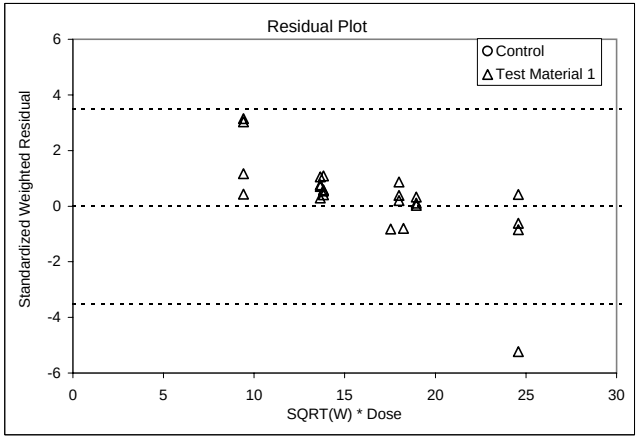
Reference Material (Sodium Arsenate)



Test Material 1 (NIST SRM 2710)







[a] Note that the data from this figure were refitted with the outlier excluded (see Figure 4-7); this outlier was excluded from the final evaluation for arsenic RBA.

Summary of Fitting[b]

Parameter	Estimate	SE
a	6.1	1.3
b_r	0.82	0.03
b_{t1}	0.32	0.02
Covariance (b_r,b_{t1})	0.0008	--
Degrees of Freedom	65	--

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

where r = Reference Material, t1 = Test Material 1

ANOVA

Source	MSE
Fit	1006.11
Error	2.34
Total	32.76

Statistic	Estimate
F	429.067
p	< 0.001
Adjusted R^2	0.9284

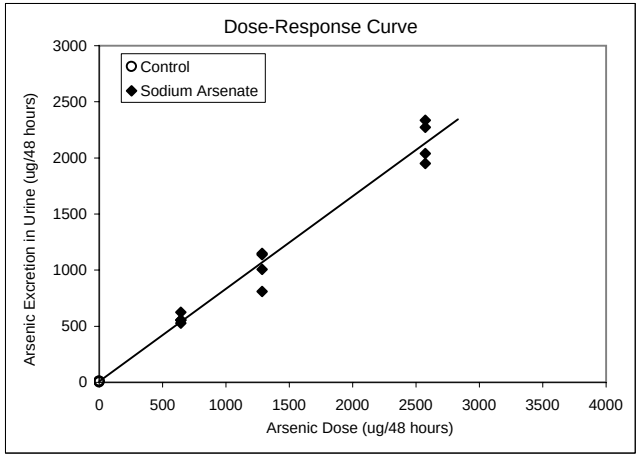
RBA and Uncertainty

	Test Material 1
RBA	0.40
Lower bound[c]	0.35
Upper bound[c]	0.45
Standard Error[c]	0.028

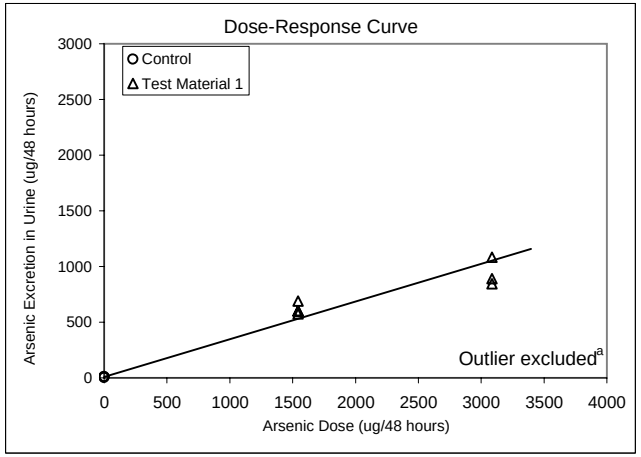
[c] Calculated using Fieller's theorem

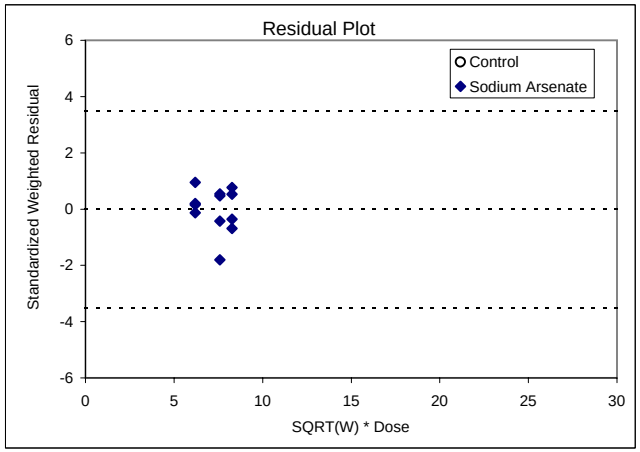
FIGURE 4-6 URINARY EXCRETION OF ARSENIC: Days 6/7 (Outliers Excluded)

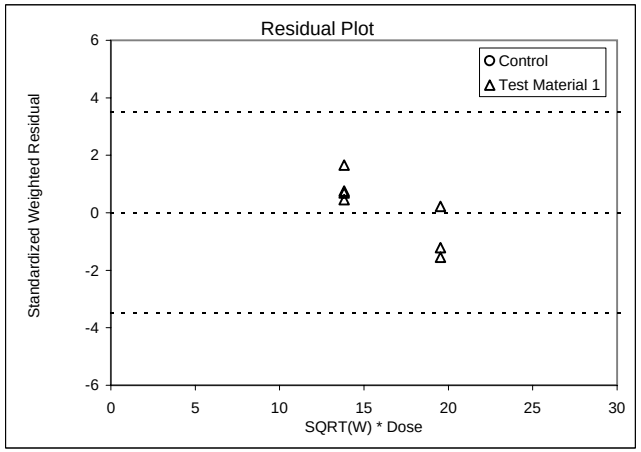
Reference Material (Sodium Arsenate)



Test Material 1 (NIST SRM 2710)







[a] The outlier was identified in the initial fitting (see Figure 4-2); the data are plotted here (Figure 4-6) with the outlier excluded. These results, with the outlier excluded, were used in the final evaluation for arsenic RBA.

Summary of Fitting[b]

Parameter	Estimate	Standard Error
a	7.2	1.5
b_r	0.83	0.04
b_{t1}	0.34	0.02
Covariance (b_r,b_{t1})	0.0011	--
Degrees of Freedom	20	--

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

where r = Reference Material, t1 = Test Material 1

ANOVA

Source	MSE
Fit	333.25
Error	0.81
Total	32.47

Statistic	Estimate
F	411.586
p	< 0.001
Adjusted R^2	0.9751

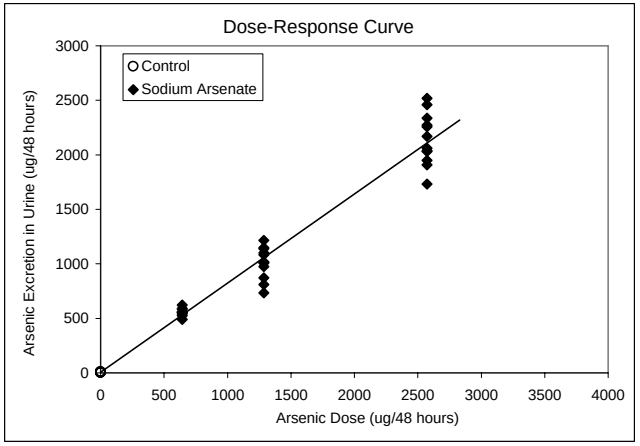
RBA and Uncertainty

	Test Material 1
RBA	0.41
Lower bound[c]	0.36
Upper bound[c]	0.47
Standard Error[c]	0.030

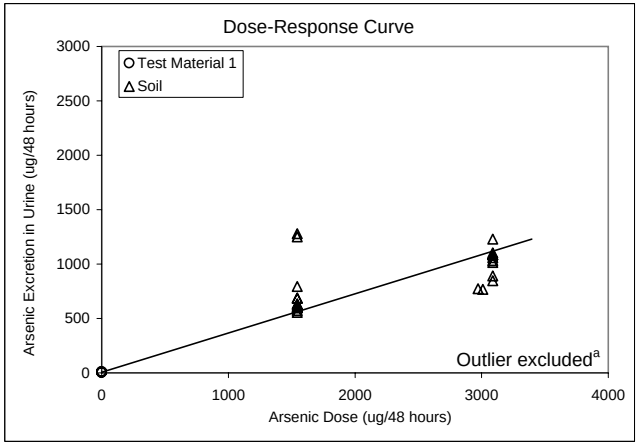
[c] Calculated using Fieller's theorem

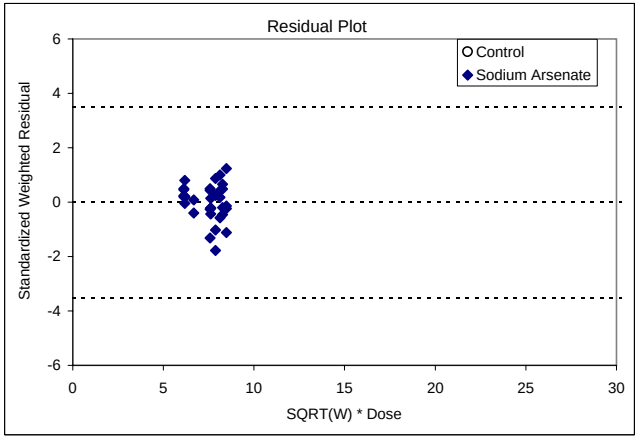
FIGURE 4-7 URINARY EXCRETION OF ARSENIC: All Days (Outliers Excluded)

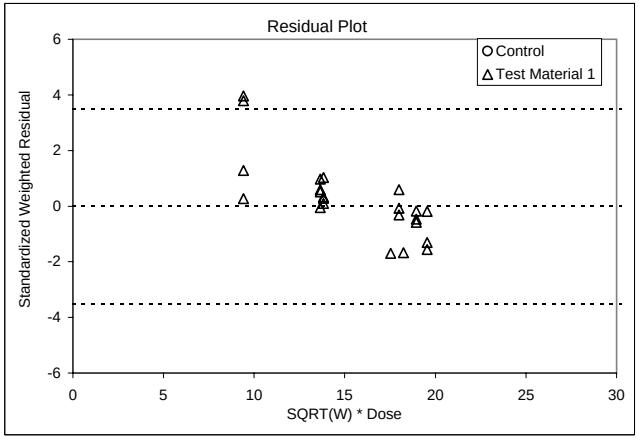
Reference Material (Sodium Arsenate)



Test Material 1 (NIST SRM 2710)







[a] The outlier was identified in the initial fitting (see Figure 4-5); the data are plotted here (Figure 4-7) with the outlier excluded. These results, with the outlier excluded, were used in the final evaluation for arsenic RBA.

Summary of Fitting[b]

Parameter	Estimate	SE
a	6.1	1.0
b_r	0.82	0.03
b_{t1}	0.36	0.01
Covariance (b_r,b_{t1})	0.0008	--
Degrees of Freedom	64	--

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

where r = Reference Material, t1 = Test Material 1

ANOVA

Source	MSE
Fit	1010.24
Error	1.27
Total	32.32

Statistic	Estimate
F	794.939
p	< 0.001
Adjusted R^2	0.9607

RBA and Uncertainty

	Test Material 1
RBA	0.44
Lower bound[c]	0.40
Upper bound[c]	0.48
Standard Error[c]	0.023

[c] Calculated using Fieller's theorem

APPENDIX A

DETAILED RESULTS

TABLE A-1 SCHEDULE

Study Day	Day	Date	Cull Pigs/ Assign Dose Group	Feed Special Diet	Weigh	Dose Preparation	Dose Administration	Urine Collection[a]	Sacrifice/ Necropsy
-6	Tuesday	04/10/07							
-5	Wednesday	04/11/07	Cull Pigs		X				
-4	Thursday	04/12/07		transition					
-3	Friday	04/13/07	Assign Dose Groups	transition					
-2	Saturday	04/14/07		transition					
-1	Sunday	04/15/07		transition	X	X			
0	Monday	04/16/07		X			X		
1	Tuesday	04/17/07		X			X		
2	Wednesday	04/18/07		X	X		X		
3	Thursday	04/19/07		X		X	X		
4	Friday	04/20/07		X			X		
5	Saturday	04/21/07		X	X		X		
6	Sunday	04/22/07		X			X	U-1	
7	Monday	04/23/07		X			X		
8	Tuesday	04/24/07		X	X	X	X		
9	Wednesday	04/25/07		X			X	U-2	
10	Thursday	04/26/07		X			X		
11	Friday	04/27/07		X	X		X		
12	Saturday	04/28/07		X			X	U-3	
13	Sunday	04/29/07		X			X		
14	Monday	04/30/07		X	X				X

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

TABLE A-2 CERTIFIED VALUES

Element	Mass Fraction (%)
Aluminum	6.44 ± 0.08
Calcium	1.25 ± 0.03
Iron	3.38 ± 0.10
Magnesium	0.853 ± 0.042
Manganese	1.01 ± 0.04
Phosphorus	0.106 ± 0.015
Potassium	2.11 ± 0.11
Silicon	28.97 ± 0.18
Sodium	1.14 ± 0.06
Sulfur	0.240 ± 0.006
Titanium	0.283 ± 0.010

Element	Mass Fraction (mg/kg)
Antimony	38.4 ± 3
Arsenic	626 ± 38
Barium	707 ± 51
Cadmium	21.8 ± 0.2
Copper	2950 ± 130
Lead	5532 ± 80
Mercury	32.6 ± 1.8
Nickel	14.3 ± 1.0
Silver	35.3 ± 1.5
Vanadium	76.6 ± 2.3
Zinc	6952 ± 91

Source: NIST, 2003

TABLE A-3 GROUP ASSIGNMENTS

Pig Number	Dose Group	Material Administered	Target Dose of Arsenic (µg/kg-day)
317 320 326	1	Control	0
304 312 318 327	2	Sodium Arsenate	25
308 310 314 315	3	Sodium Arsenate	50
302 305 309 313	4	Sodium Arsenate	100
301 311 321 328	5	Test Material 1	60
303 306 307 319	6	Test Material 1	120

TABLE A-4 BODY WEIGHTS AND ACTUAL ADMINISTERED DOSES, BY DAY

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

Group	Pig #	Day -1		Day 0		Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7		Day 8		Day 9		Day 10		Day 11		Day 12		Day 13		Day 14		Days 0-14
		BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	Mean As Dose (µg/kg-d)
1	317	9.4	0.00	9.5	0.00	9.6	0.00	9.7	0.00	10.0	0.00	10.3	0.00	10.6	0.00	10.7	0.00	10.9	0.00	11.1	0.00	11.4	0.00	11.8	0.00	12.1	0.00	12.3	0.00	12.6	0.00	12.8	0.00	0.00
1	320	8.9	0.00	8.9	0.00	8.9	0.00	8.9	0.00	9.2	0.00	9.5	0.00	9.8	0.00	10.1	0.00	10.5	0.00	10.8	0.00	11.1	0.00	11.4	0.00	11.8	0.00	12.1	0.00	12.4	0.00	12.7	0.00	0.00
1	326	9.4	0.00	9.5	0.00	9.6	0.00	9.7	0.00	9.9	0.00	10.0	0.00	10.2	0.00	10.6	0.00	10.9	0.00	11.3	0.00	11.6	0.00	11.9	0.00	12.2	0.00	12.6	0.00	13.0	0.00	13.4	0.00	0.00
2	304	8.8	0.00	9.0	35.60	9.3	34.70	9.5	33.85	9.7	33.21	9.9	32.59	10.1	32.00	10.3	31.17	10.6	30.39	10.9	29.64	11.2	28.75	11.5	27.92	11.9	27.14	12.1	26.54	12.4	25.97	12.7	25.42	30.92
2	312	8.5	0.00	8.7	37.10	8.9	36.20	9.1	35.34	9.4	34.33	9.6	33.38	9.9	32.48	10.2	31.53	10.5	30.63	10.8	29.78	11.1	28.93	11.4	28.13	11.8	27.37	12.1	26.61	12.4	25.90	12.8	25.22	31.54
2	318	9.4	0.00	9.6	33.56	9.8	32.76	10.1	32.00	10.2	31.53	10.4	31.07	10.5	30.63	10.8	29.78	11.1	28.97	11.4	28.21	11.7	27.45	12.0	26.72	12.4	26.04	12.7	25.35	13.0	24.70	13.4	24.09	29.39
2	327	9.6	0.00	9.9	32.65	10.1	31.84	10.4	31.07	10.5	30.58	10.7	30.10	10.9	29.64	11.1	28.97	11.4	28.33	11.6	27.72	11.9	27.06	12.2	26.43	12.5	25.83	12.8	25.16	13.1	24.52	13.5	23.91	28.71
3	308	9.8	0.00	9.9	65.07	10.0	48.16	10.2	63.36	10.3	62.24	10.5	61.16	10.7	60.11	11.0	58.38	11.3	56.75	11.7	55.21	12.0	53.82	12.3	52.50	12.6	51.25	12.8	50.12	13.1	49.03	13.4	48.00	56.46
3	310	9.6	0.00	9.7	66.30	9.8	65.63	9.9	64.96	10.0	64.10	10.2	63.26	10.3	62.44	10.6	60.58	10.9	58.82	11.3	57.17	11.6	55.60	11.9	54.12	12.2	52.72	12.5	51.59	12.7	50.51	13.0	49.47	59.57
3	314	8.5	0.00	8.7	73.78	9.0	71.59	9.3	69.53	9.4	68.18	9.6	66.88	9.8	65.63	10.1	63.78	10.4	62.04	10.7	60.39	11.0	58.38	11.4	56.50	11.8	54.74	12.1	53.08	12.5	51.52	12.9	50.05	63.04
3	315	9.6	0.00	9.7	66.08	9.9	64.86	10.1	63.68	10.3	62.64	10.4	61.64	10.6	60.67	10.9	59.19	11.1	57.77	11.4	56.42	11.7	55.13	11.9	53.90	12.2	52.72	12.5	51.45	12.8	50.25	13.1	49.10	58.64
4	302	8.6	0.00	8.7	147.57	8.8	72.81	9.0	143.72	9.2	140.58	9.4	137.57	9.6	134.69	9.9	129.93	10.3	125.49	10.6	121.35	10.8	119.10	11.0	116.94	11.2	114.85	11.6	110.89	12.0	107.19	12.4	103.73	122.96
4	305	9.0	0.00	9.2	140.32	9.3	137.82	9.5	135.40	9.7	133.30	9.8	131.26	10.0	129.28	10.2	125.90	10.5	122.70	10.8	119.66	11.0	116.58	11.3	113.66	11.6	110.89	11.9	108.09	12.2	105.43	12.5	102.90	124.34
4	309	9.0	0.00	9.1	141.87	9.2	140.07	9.3	138.31	9.6	134.46	9.8	130.81	10.1	127.36	10.4	123.29	10.8	119.47	11.1	115.88	11.4	112.83	11.7	109.94	12.0	107.19	12.3	104.72	12.6	102.36	12.9	100.10	123.22
4	313	10.0	0.00	10.2	126.52	10.4	123.88	10.6	121.35	10.9	118.19	11.2	115.19	11.5	112.34	11.8	109.16	12.1	106.16	12.5	103.32	12.8	100.49	13.2	97.82	13.5	95.28	13.8	92.99	14.2	90.80	14.5	88.71	109.05
5	301	8.6	0.00	8.7	88.37	8.9	86.56	9.1	84.81	9.4	82.10	9.7	79.57	10.0	77.18	10.2	75.66	10.4	74.21	10.6	72.81	10.9	70.70	11.2	68.70	11.6	66.82	11.9	65.13	12.2	63.52	12.5	61.99	75.99
5	311	9.4	0.00	9.5	81.24	9.6	76.37	9.7	79.57	10.1	76.79	10.4	74.21	10.8	71.79	11.0	69.95	11.3	68.20	11.6	66.53	12.1	63.96	12.5	61.58	13.0	59.37	13.4	57.67	13.8	56.06	14.2	54.54	69.41
5	321	9.0	0.00	9.1	84.66	9.2	83.59	9.4	82.54	9.7	79.98	10.0	77.57	10.3	75.30	10.6	73.04	10.9	70.91	11.2	68.91	11.6	66.72	11.9	64.67	12.3	62.75	12.6	61.09	13.0	59.52	13.3	58.03	72.93
5	328	8.9	0.00	9.1	84.81	9.4	82.54	9.6	80.39	9.8	78.75	10.0	77.18	10.2	75.66	10.6	73.04	10.9	70.59	11.3	68.30	11.6	66.53	11.9	64.86	12.2	63.26	12.6	61.50	12.9	59.83	13.3	58.25	72.57
6	303	9.1	0.00	9.3	166.87	9.4	164.21	9.6	157.59	9.9	156.71	10.2	152.08	10.5	147.71	10.8	143.59	11.1	139.69	11.4	122.40	11.6	129.55	11.9	123.40	12.2	120.69	12.5	120.72	12.8	117.73	13.1	117.83	142.26
6	306	9.2	0.00	9.2	167.48	9.3	166.27	9.4	165.09	9.6	161.35	9.8	157.77	10.0	154.36	10.3	150.59	10.5	147.01	10.8	143.59	11.1	139.69	11.4	136.00	11.7	132.49	12.0	129.17	12.3	126.01	12.6	122.99	149.31
6	307	8.8	0.00	9.0	171.83	9.2	167.48	9.5	163.34	9.7	159.40	9.9	155.65	10.2	152.08	10.4	148.18	10.7	144.48	11.0	140.96	11.3	136.60	11.7	132.49	12.0	128.63	12.3	125.15	12.7	121.86	13.0	118.74	147.43
6	319	9.2	0.00	9.4	82.25	9.6	161.35	9.8	158.31	10.1	152.83	10.5	147.71	10.8	142.92	11.1	139.27	11.4	135.80	11.7	132.49	11.9	129.53	12.2	126.69	12.5	123.98	12.8	120.43	13.2	117.08	13.6	113.92	133.70

Missed Doses:

Day 0 - Pig 319 did not eat entire PM dose (ate approximately 0%). Daily dose adjusted to 50%.
Day 1 - Pig 302 did not eat entire AM dose (ate approximately 0%). Daily dose adjusted to 50%.
Day 1 - Pig 308 did not eat entire AM dose (ate approximately 50%). Daily dose adjusted to 75%.
Day 1 - Pig 311 did not eat entire AM or PM dose (ate approximately 95% of each). Daily dose adjusted to 95%.
Day 2 - Pig 303 did not eat entire PM dose (ate approximately 95%). Daily dose adjusted to 97.5%.
Day 8 - Pig 303 did not eat entire PM dose (ate approximately 80%). Daily dose adjusted to 90%.
Day 9 - Pig 303 did not eat entire PM dose (ate approximately 95%). Daily dose adjusted to 97.5%.
Day 10 - Pig 303 did not eat entire AM or PM dose (ate approximately 95% of each). Daily dose adjusted to 95%.
Day 11 - Pig 303 did not eat entire AM or PM dose (ate approximately 95% of each). Daily dose adjusted to 95%.
Day 12 - Pig 303 did not eat entire PM dose (ate approximately 95%). Daily dose adjusted to 97.5%.
Day 13 - Pig 303 did not eat entire PM dose (ate approximately 95%). Daily dose adjusted to 97.5%.

Instances of late consumption of doses are shown in Table A-5 (no adjustments necessary).

TABLE A-5 LATE DOSE CONSUMPTION

Study Day	Pig	Notes
Day 0	303	PM dose was finished between 3 PM and 5 PM.
	307	AM dose was finished by 2 PM; 80% of PM dose was eaten at dosing; dose was finished by 5:30 PM.
	311	PM dose was finished with PM feeding.
	317	PM dose was finished with PM feeding.
Day 1	302	AM and PM doses were finished overnight.
	303	AM dose was finished by 3 PM.
	306	AM dose was finished by 3 PM.
	308	AM and PM doses were finished overnight.
	311	95% of AM dose was eaten by 3 PM.*
	317	AM dose was finished by Noon.
	320	PM dose was finished by 5 AM.
Day 2	303	AM dose was finished by 3 PM; 95% of PM dose was eaten by 6 PM.*
	310	PM dose was finished by 5 PM.
	311	AM dose was finished by 3 PM; 75% of PM dose was eaten by PM feeding; dose was finished overnight.
Day 3	303	AM dose was finished by 11 AM.
	310	75% of PM dose was eaten at dosing; dose was finished overnight.
	311	95% of PM dose was eaten by 6 PM; dose was finished by 10 PM.
Day 4	302	AM dose was finished by 3 PM; 80% of PM dose was eaten by 5 PM; dose was finished overnight.
	303	AM dose was finished by 3 PM; PM dose was finished by 5 PM.
	310	AM dose was finished by 3 PM; 90% of PM dose was eaten by 5 PM.*
Day 5	301	PM dose was eaten at 4:30 PM (doughball had been caught up in the feeder).
	303	AM dose was finished by 11:30 AM; PM dose was finished by 4:30 PM.
	310	AM dose was finished by 11:30 AM; PM dose was finished by 4:30 PM.
Day 6	303	AM dose was finished by 11 AM.
	310	AM dose was finished by 11 AM.
Day 7	303	AM dose was finished by 3 PM; PM dose was finished by 5 PM.
	310	PM dose was finished by 4:30 PM.
Day 8	303	AM dose was finished by 3 PM; PM dose was finished by 5 PM.
Day 9	303	AM dose was finished by 3 PM; 80% of PM dose was eaten by 5 PM; 95% was eaten by the next morning.*
Day 10	303	AM dose was finished by 3 PM; 80% of PM dose was eaten by 5 PM; 95% was eaten by the next morning.*
Day 11	303	AM dose was finished by 3 PM; 80% of PM dose was eaten by 5 PM; 95% was eaten by the next morning.*
Day 12	303	AM dose was finished by 3 PM; 50% of PM dose was eaten by 5 PM; 95% was eaten by the next morning.*
	310	AM dose was finished by 3 PM.
Day 13	303	AM dose was finished by 3 PM; 50% of PM dose was eaten by 5 PM; 95% was eaten by the next morning.*

*Incomplete dose is accounted for in Table A-4.
See Table A-4 for missed doses.

TABLE A-6 URINE VOLUMES

Group	Pig Number	Urine Collection[a]		
		U-1 Days 6-7	**U-2** Days 9-10	**U-3** Days 12-13
1	317	4320	5320	7140
	320	5530	5580	7480
	326	1860	1990	1990
2	304	8360	7115	6270
	312	5260	10690	4660
	318	8560	6220	8860
	327	32800	24460	19820
3	308	3960	2850	2900
	310	7630	6092	4900
	314	10840	8470	7140
	315	3150	3760	3960
4	302	4840	5780	5640
	305	11470	10850	10500
	309	14600	9460	10850
	313	8420	6100	4560
5	301	7180	6620	6000
	311	13570	13040	16200
	321	5580	4510	4030
	328	4920	3800	4740
6	303	5840	4840	4800
	306	5940	5740	5860
	307	19030	16700	16480
	319	18800	7920	10800

Units = milliliters

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

TABLE A-7 URINARY ARSENIC ANALYTICAL RESULTS FOR STUDY SAMPLES

Sample Number	Tag Number	Pig Number	Group	Material Administered	Urine Collection Days	48-hr Dose (ug/48hr)	48-hr BWAdj Dose (ug/kg-48hr)	Reported As Conc (ng/mL)	DL	AdjConc* (ng/mL)	Urine Volume (mL)	Total Excreted (ug/48hrs)
EP3-1-320-U1	EP3-1-107	320	1	Control	6/7	0	0	2	1	2	5530	11
EP3-1-326-U1	EP3-1-106	326	1	Control	6/7	0	0	4.5	1	4.5	1860	8
EP3-1-317-U1	EP3-1-131	317	1	Control	6/7	0	0	<1	1	0.5	4320	2
EP3-1-304-U1	EP3-1-111	304	2	Sodium Arsenate	6/7	643.15	61.56	63	1	63	8360	527
EP3-1-312-U1	EP3-1-102	312	2	Sodium Arsenate	6/7	643.15	62.15	105	1	105	5260	552
EP3-1-318-U1	EP3-1-125	318	2	Sodium Arsenate	6/7	643.15	58.75	65	1	65	8560	556
EP3-1-327-U1	EP3-1-126	327	2	Sodium Arsenate	6/7	643.15	57.3	19	1	19	32800	623
EP3-1-308-U1	EP3-1-120	308	3	Sodium Arsenate	6/7	1286.3	115.13	290	4	290	3960	1148
EP3-1-315-U1	EP3-1-118	315	3	Sodium Arsenate	6/7	1286.3	116.95	320	4	320	3150	1008
EP3-1-310-U1	EP3-1-113	310	3	Sodium Arsenate	6/7	1286.3	119.4	106	1	106	7630	809
EP3-1-314-U1	EP3-1-101	314	3	Sodium Arsenate	6/7	1286.3	125.82	105	1	105	10840	1138
EP3-1-302-U1	EP3-1-127	302	4	Sodium Arsenate	6/7	2572.61	255.42	421	4	421	4840	2038
EP3-1-305-U1	EP3-1-114	305	4	Sodium Arsenate	6/7	2572.61	248.6	170	2	170	11470	1950
EP3-1-309-U1	EP3-1-122	309	4	Sodium Arsenate	6/7	2572.61	242.76	160	2	160	14600	2336
EP3-1-313-U1	EP3-1-115	313	4	Sodium Arsenate	6/7	2572.61	215.32	270	4	270	8420	2273
EP3-1-321-U1	EP3-1-104	321	5	Test Material 1	6/7	1543.57	143.95	103	1	103	5580	575
EP3-1-311-U1	EP3-1-116	311	5	Test Material 1	6/7	1543.57	138.15	44	1	44	13570	597
EP3-1-301-U1	EP3-1-110	301	5	Test Material 1	6/7	1543.57	149.87	84	1	84	7180	603
EP3-1-328-U1	EP3-1-129	328	5	Test Material 1	6/7	1543.57	143.63	140	2	140	4920	689
EP3-1-303-U1	EP3-1-128	303	6	Test Material 1	6/7	3087.13	283.28	3.7	1	3.7	5840	22
EP3-1-306-U1	EP3-1-119	306	6	Test Material 1	6/7	3087.13	297.6	150	2	150	5940	891
EP3-1-307-U1	EP3-1-105	307	6	Test Material 1	6/7	3087.13	292.67	57	1	57	19030	1085
EP3-1-319-U1	EP3-1-123	319	6	Test Material 1	6/7	3087.13	275.07	45	1	45	18800	846
EP3-1-320-U2	EP3-1-140	320	1	Control	9/10	0	0	1	1	1	5580	6
EP3-1-326-U2	EP3-1-138	326	1	Control	9/10	0	0	3	1	3	1990	6
EP3-1-317-U2	EP3-1-150	317	1	Control	9/10	0	0	1	1	1	5320	5
EP3-1-327-U2	EP3-1-137	327	2	Sodium Arsenate	9/10	643.15	53.49	20	1	20	24460	489
EP3-1-304-U2	EP3-1-158	304	2	Sodium Arsenate	9/10	643.15	56.68	76	1	76	7115	541
EP3-1-315-U2	EP3-1-151	315	3	Sodium Arsenate	9/10	1286.3	109.02	270	4	270	3760	1015
EP3-1-308-U2	EP3-1-149	308	3	Sodium Arsenate	9/10	1286.3	106.32	380	4	380	2850	1083
EP3-1-310-U2	EP3-1-148	310	3	Sodium Arsenate	9/10	1286.3	109.73	160	2	160	6092	975
EP3-1-314-U2	EP3-1-143	314	3	Sodium Arsenate	9/10	1286.3	114.88	120	1	120	8470	1016
EP3-1-302-U2	EP3-1-159	302	4	Sodium Arsenate	9/10	2572.61	236.04	330	4	330	5780	1907
EP3-1-309-U2	EP3-1-157	309	4	Sodium Arsenate	9/10	2572.61	222.77	260	4	260	9460	2460
EP3-1-313-U2	EP3-1-133	313	4	Sodium Arsenate	9/10	2572.61	198.31	370	4	370	6100	2257
EP3-1-305-U2	EP3-1-152	305	4	Sodium Arsenate	9/10	2572.61	230.25	200	2	200	10850	2170
EP3-1-328-U2	EP3-1-135	328	5	Test Material 1	9/10	1543.57	131.39	180	2	180	3800	684
EP3-1-301-U2	EP3-1-161	301	5	Test Material 1	9/10	1543.57	139.4	84	1	84	6620	556
EP3-1-311-U2	EP3-1-155	311	5	Test Material 1	9/10	1543.57	125.54	48	1	48	13040	626
EP3-1-321-U2	EP3-1-145	321	5	Test Material 1	9/10	1543.57	131.4	141	1	141	4510	636
EP3-1-319-U2	EP3-1-139	319	6	Test Material 1	9/10	3087.13	256.22	128	1	128	7920	1014
EP3-1-307-U2	EP3-1-147	307	6	Test Material 1	9/10	3087.13	269.09	65	1	65	16700	1086
EP3-1-303-U2	EP3-1-141	303	6	Test Material 1	9/10	2971.36	252.95	160	2	160	4840	774
EP3-1-306-U2	EP3-1-146	306	6	Test Material 1	9/10	3087.13	275.69	180	2	180	5740	1033
EP3-1-317-U3	EP3-1-176	317	1	Control	12/13	0	0	<1	1	0.5	7140	4
EP3-1-320-U3	EP3-1-163	320	1	Control	12/13	0	0	1	1	1	7480	7
EP3-1-326-U3	EP3-1-178	326	1	Control	12/13	0	0	3.2	1	3.2	1990	6
EP3-1-312-U3	EP3-1-186	312	2	Sodium Arsenate	12/13	643.15	52.51	120	1	120	4660	559
EP3-1-318-U3	EP3-1-166	318	2	Sodium Arsenate	12/13	643.15	50.06	66	1	66	8860	585
EP3-1-327-U3	EP3-1-177	327	2	Sodium Arsenate	12/13	643.15	49.67	28	1	28	19820	555
EP3-1-304-U3	EP3-1-165	304	2	Sodium Arsenate	12/13	643.15	52.51	94	1	94	6270	589
EP3-1-308-U3	EP3-1-171	308	3	Sodium Arsenate	12/13	1286.3	99.15	380	4	380	2900	1102
EP3-1-310-U3	EP3-1-192	310	3	Sodium Arsenate	12/13	1286.3	102.1	150	2	150	4900	735
EP3-1-314-U3	EP3-1-167	314	3	Sodium Arsenate	12/13	1286.3	104.6	170	2	170	7140	1214
EP3-1-315-U3	EP3-1-190	315	3	Sodium Arsenate	12/13	1286.3	101.7	220	4	220	3960	871
EP3-1-309-U3	EP3-1-164	309	4	Sodium Arsenate	12/13	2572.61	207.08	190	2	190	10850	2062
EP3-1-313-U3	EP3-1-170	313	4	Sodium Arsenate	12/13	2572.61	183.78	380	4	380	4560	1733
EP3-1-305-U3	EP3-1-175	305	4	Sodium Arsenate	12/13	2572.61	213.53	240	4	240	10500	2520
EP3-1-302-U3	EP3-1-183	302	4	Sodium Arsenate	12/13	2572.61	218.08	360	4	360	5640	2030
EP3-1-311-U3	EP3-1-173	311	5	Test Material 1	12/13	1543.57	113.73	49	1	49	16200	794
EP3-1-321-U3	EP3-1-169	321	5	Test Material 1	12/13	1543.57	120.61	310	4	310	4030	1249
EP3-1-328-U3	EP3-1-174	328	5	Test Material 1	12/13	1543.57	121.32	270	4	270	4740	1280
EP3-1-301-U3	EP3-1-168	301	5	Test Material 1	12/13	1543.57	128.65	102	1	102	6000	612
EP3-1-319-U3	EP3-1-189	319	6	Test Material 1	12/13	3087.13	237.52	98	1	98	10800	1058
EP3-1-303-U3	EP3-1-179	303	6	Test Material 1	12/13	3009.95	238.45	160	2	160	4800	768
EP3-1-306-U3	EP3-1-185	306	6	Test Material 1	12/13	3087.13	255.17	210	4	210	5860	1231
EP3-1-307-U3	EP3-1-182	307	6	Test Material 1	12/13	3087.13	247.01	67	1	67	16480	1104

NOTE: Urine samples EP3-1-134 and EP3-1-160 were inadvertently combined into a single sample prior to analysis. Thus, results shown here represent the mean concentration of the two samples combined.

EP3-1-312-U2	EP3-1-134	312	2	Sodium Arsenate	9/10	643.15	57.05	60	1	60	10690	641
EP3-1-318-U2	EP3-1-160	318	2	Sodium Arsenate	9/10	643.15	54.17	61	1	61	6220	379

*Non-detects taken at one-half the detection limit.

TABLE A-8 ARSENIC ANALYTICAL RESULTS FOR QUALITY CONTROL SAMPLES

Blind Duplicates

Tag Number	Reported As Conc	DL	Units	Pig Number	Original Pig #	Group	Event/Day
EP3-1-130	99	1	Ng/ml	2312	312	2	U1
EP3-1-112	107	1	Ng/ml	2310	310	3	U1
EP3-1-108	140	2	Ng/ml	2306	306	6	U1
EP3-1-136	2	1	Ng/ml	2317	317	1	U2
EP3-1-154	19	1	Ng/ml	2327	327	2	U2
EP3-1-156	260	4	Ng/ml	2309	309	4	U2
EP3-1-191	170	2	Ng/ml	2314	314	3	U3
EP3-1-172	103	1	Ng/ml	2301	301	5	U3
EP3-1-162	150	2	Ng/ml	2303	303	6	U3

Performance Evaluation Samples

Tag Number	Reported As Conc	DL	Units	QC Sample	Nominal PE Conc
EP3-1-181	2	1	Ng/ml	Control Urine	0
EP3-1-103	2	1	Ng/ml	Control Urine	0
EP3-1-184	200	4	Ng/ml	Sodium arsenate	200
EP3-1-132	23	1	Ng/ml	Sodium arsenate	20
EP3-1-124	120	2	Ng/ml	Sodium arsenate	100
EP3-1-180	22	1	Ng/ml	Sodium arsenite	20
EP3-1-153	110	2	Ng/ml	Sodium arsenite	100
EP3-1-121	190	2	Ng/ml	Sodium arsenite	200
EP3-1-187	100	2	Ng/ml	Dimethyl arsenic acid	100
EP3-1-144	200	4	Ng/ml	Dimethyl arsenic acid	200
EP3-1-117	22	1	Ng/ml	Dimethyl arsenic acid	20
EP3-1-188	220	4	Ng/ml	Disodium methylarsenate	200
EP3-1-142	100	2	Ng/ml	Disodium methylarsenate	100
EP3-1-109	23	1	Ng/ml	Disodium methylarsenate	20

Laboratory Spikes

Tag Number	Spiked As Conc	DL	Units	Nominal Spike Amount
EP3-1-110	290	4	Ng/ml	200
EP3-1-120	489	4	Ng/ml	200
EP3-1-130	310	4	Ng/ml	200
EP3-1-140	210	4	Ng/ml	200
EP3-1-150	210	4	Ng/ml	200
EP3-1-160	270	4	Ng/ml	200
EP3-1-170	589	4	Ng/ml	200
EP3-1-180	230	4	Ng/ml	200
EP3-1-186	330	4	Ng/ml	200
EP3-1-192	360	4	Ng/ml	200
EP3-1-409	10	0.2	mcg/g	9.96
EP3-1-412	39	1	Ng/ml	40

Laboratory Duplicates

Tag Number	Duplicate As Conc	DL	Units
EP3-1-105	57	1	Ng/ml
EP3-1-115	270	4	Ng/ml
EP3-1-125	65	1	Ng/ml
EP3-1-135	180	2	Ng/ml
EP3-1-145	140	1	Ng/ml
EP3-1-155	50	1	Ng/ml
EP3-1-165	93	1	Ng/ml
EP3-1-175	220	4	Ng/ml
EP3-1-183	370	4	Ng/ml
EP3-1-189	97	1	Ng/ml
EP3-1-407	0.06	0.05	mcg/g
EP3-1-410	<1	1	Ng/ml

Laboratory Control Standards

Tag Number	Reported As Conc	DL	Units	SRMID	Certified Mean
QC-1	5	3	Ng/ml	NIST 2670a-L	3
QC-2	<3	3	Ng/ml	NIST 2670a-L	3
QC-3	240	10	Ng/ml	NIST 2670a-H	220 ± 10
QC-4	230	10	Ng/ml	NIST 2670a-H	220 ± 10
QC-5	240	10	Ng/ml	NIST 2670a-H	220 ± 10
EP3-1-1566	7.5	0.1	mcg/g	NIST 1566b	7.65 ± 0.65
EP3-1-415	26	1	Ng/ml	NIST 1640	26.7 ± 0.41

Blanks

Tag Number	Reported As Conc	DL	Units
Blank-1	<1	1	Ng/ml
Blank-2	<1	1	Ng/ml
Blank-3	<1	1	Ng/ml
Blank-4	<1	1	Ng/ml
Blank-5	<1	1	Ng/ml
Blank-6	<0.05	0.05	mcg/g
Blank-7	<1	1	Ng/ml

SRC TR-09-0951

RELATIVE BIOAVAILABILITY OF ARSENIC AND LEAD IN THE NIST 2710A SOIL STANDARD

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EXECUTIVE SUMMARY

A study using juvenile swine as test animals was performed to measure the gastrointestinal absorption of arsenic and lead from a sample of NIST 2710a soil. NIST 2710a is a National Institute of Standards and Technology (NIST) certified standard reference material consisting of contaminated Montana soil collected near Silver Bow Creek that is blended with lead oxide. Arsenic and lead concentrations (mean±SD) of the soil are 1540±100 mg/kg and 5520±30 mg/kg, respectively.

The relative oral bioavailability of arsenic and lead in NIST 2710a was assessed by comparing the absorption of arsenic or lead from NIST 2710a ("test material") to that of a reference material, either sodium arsenate or lead acetate. Groups of five swine were given oral doses of a reference material or the test material twice a day for 14 days. A group of three non-treated swine served as a control for both the arsenic and lead test groups.

The amount of arsenic absorbed by each animal was evaluated by measuring the amount of arsenic excreted in the urine (collected over 48-hour periods beginning on days 6, 9, and 12). The urinary excretion fraction (UEF) is the ratio of the amount excreted per 48 hours divided by the dose given per 48 hours. UEF was calculated for each test material and the sodium arsenate using simultaneous weighted linear regression. The relative bioavailability (RBA) of arsenic in the test material compared to sodium arsenate was calculated as follows:

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

The amount of lead absorbed by each animal was evaluated by measuring the amount of lead in the blood (measured on days 0, 2, 4, 8, 11, and 15) and the amount of lead in liver, kidney, and bone (measured on day 15 at study termination). Because equal absorbed doses of lead will produce equal responses in tissue concentrations regardless of the source or nature of the ingested lead, the RBA of a test material is calculated as the ratio of doses (test material and reference material) that produce equal increases in lead concentration in the body compartment. Thus, the basic data reduction task to calculate a lead RBA for the test material was to fit mathematical equations to the dose-response data for both the test material and the reference material, and then solve the equations to find the ratio of doses that would be expected to yield equal responses.

Estimated arsenic RBA values (mean and 90% confidence interval) are as follows:

Collection Interval	Estimated Arsenic RBA (90% Confidence Interval)
Days 6/7	0.43 (0.39–0.47)
Days 9/10	0.41 (0.37–0.44)
Days 12/13	0.42 (0.38–0.46)
All Days	**0.42 (0.40–0.44)**

Estimated lead RBA values (mean and 90% confidence interval) are as follows:

Measurement Endpoint	Estimated Lead RBA (90% Confidence Interval)
Blood Lead AUC	0.49 (0.38–0.68)
Liver Lead	0.75 (0.57–0.99)
Kidney Lead	0.52 (0.38–0.71)
Femur Lead	0.53 (0.44–0.63)
Point Estimate	**0.57 (0.39–0.84)**

The best fit point estimates for arsenic and lead RBAs for the NIST 2710a soil are 42 and 57% for arsenic and lead, respectively.

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ACRONYMS AND ABBREVIATIONS

ABA	Absolute bioavailability
AUC	Area under the curve
AF_o	Oral absorption fraction
As^{+3}	Trivalent inorganic arsenic
As^{+5}	Pentavalent inorganic arsenic
CDC	Centers for Disease Control and Prevention
D	Ingested dose
DMA	Dimethyl arsenic
EDTA	Ethylenediaminetetra-acetic acid
g	Gram
GLP	Good Laboratory Practices
ICP MS	Inductively coupled plasma mass spectrometry
kg	Kilogram
K_u	Fraction of absorbed arsenic which is excreted in urine
mL	Milliliter
MMA	Monomethyl arsenic
N	Number of data points
ng	Nanogram
NIST	National Institute of Standards and Technology
PE	Performance evaluation
ppb	Parts per billion
ppm	Parts per million
QC	Quality control
RBA	Relative bioavailability
ref	Reference material
RfD	Reference dose
RPD	Relative percent difference
SD	Standard deviation
SF	Slope factor
SRM	Standard reference material
TM	Test material
UEF	Urinary excretion fraction
USEPA	United States Environmental Protection Agency
µg	Microgram
µm	Micrometer
°C	Degrees Celsius
°F	Degrees Fareinheit

1.0 INTRODUCTION

1.1 Overview of Bioavailability

Reliable analysis of the potential hazard to humans from ingestion of a chemical depends upon accurate information on a number of key parameters, including the concentration of the chemical in environmental media (e.g., soil, dust, water, food, air, paint), intake rates of each medium, and the rate and extent of absorption ("bioavailability") of the chemical by the body from each ingested medium. The amount of a chemical that actually enters the body from an ingested medium depends on the physical-chemical properties of the chemical and of the medium. For example, some metals in soil may exist, at least in part, as poorly water-soluble minerals, and may also exist inside particles of inert matrix such as rock or slag of variable size, shape, and association. These chemical and physical properties may influence (usually decrease) the absorption (bioavailability) of the metals when ingested. Thus, equal ingested doses of different forms of a chemical in different media may not be of equal health concern.

Bioavailability of a chemical in a particular medium may be expressed either in absolute terms (absolute bioavailability) or in relative terms (relative bioavailability):

> Absolute bioavailability (ABA) is the ratio of the amount of the chemical absorbed to the amount ingested:
>
> $$ABA = \frac{Absorbed\ \ Dose}{Ingested\ \ Dose}$$
>
> This ratio is also referred to as the oral absorption fraction (AF_o).
>
> Relative bioavailability (RBA) is the ratio of the AF_o of the chemical present in some test material (*test*) to the AF_o of the chemical in some appropriate reference material (*ref*), such as the chemical dissolved in water or a solid form that is expected to fully dissolve in the stomach:
>
> $$RBA(test\ vs\ ref) = \frac{AF_o(test)}{AF_o(ref)}$$

For example, if 100 micrograms (μg) of a chemical dissolved in drinking water were ingested and a total of 50 μg were absorbed into the body, the AF_o would be 50/100, or 0.50 (50%). Likewise, if 100 μg of the same chemical contained in soil were ingested and 30 μg were absorbed into the body, the AF_o for this chemical in soil would be 30/100, or 0.30 (30%). If the chemical dissolved in water were used as the frame of reference for describing the relative bioavailability of the same chemical in soil, the RBA would be 0.30/0.50, or 0.60 (60%).

For additional discussion about the concept and application of bioavailability, see Gibaldi and Perrier (1982), Goodman et al. (1990), and/or Klaassen et al. (1996).

1.2 Using RBA Data to Improve Risk Calculations

When reliable data are available on the RBA of a chemical in a site medium (e.g., soil), the information can be used to improve the accuracy of exposure and risk calculations at that site. RBA data can be used to adjust default oral toxicity values (reference dose and slope factor) to account for differences in absorption between the chemical ingested in water and the chemical ingested in site media, assuming the toxicity factors are based on a readily soluble form of the chemical.

1.2.1 Arsenic

For non-cancer effects, the default reference dose ($RfD_{default}$) can be adjusted ($RfD_{adjusted}$) as follows:

$$RfD_{adjusted} = \frac{RfD_{default}}{RBA}$$

For potential carcinogenic effects, the default slope factor ($SF_{default}$) can be adjusted ($SF_{adjusted}$) as follows:

$$SF_{adjusted} = SF_{default} \cdot RBA$$

Alternatively, it is also acceptable to adjust the dose (rather than the toxicity factors) as follows:

$$Dose_{adjusted} = Dose_{default} \cdot RBA$$

This dose adjustment is mathematically equivalent to adjusting the toxicity factors as described above.

1.2.2 Lead

Based on available information on lead absorption in humans and animals, the U.S. Environmental Protection Agency (USEPA) estimates that the absolute bioavailability of lead from water and other fully soluble forms of lead is usually about 50% in children (USEPA 1991) and about 20% in adults (USEPA 2003). Thus, when a reliable site-specific lead RBA value for soil is available, it may be used to estimate a site-specific absolute bioavailability in that soil, as follows:

$$ABA_{soil}\ (child) = 50\% \cdot RBA_{soil}$$

$$ABA_{soil}\ (adult) = 20\% \cdot RBA_{soil}$$

The default lead RBA used by USEPA for lead in soil and dust compared to lead in water is 60% for both children and adults. When the measured RBA in soil or dust at a site is found to be less than 60% compared to some fully soluble form of lead, it may be concluded that exposures to and hazards from lead in these media at that site are probably lower than the typical default

assumptions. If the measured RBA is higher than 60%, absorption of and hazards from lead in these media may be higher than usually assumed.

1.3 Purpose of this Study

The objective of this study was to use juvenile swine as a test system in order to determine the RBA of arsenic and lead in a standard soil reference material (NIST 2710a) compared to soluble forms of arsenic (sodium arsenate) and lead (lead acetate).

2.0 STUDY DESIGN

The test and reference materials were administered to groups of five juvenile swine at three different dose levels for 14 days. The study included a non-treated group of three animals to serve as a control for determining background arsenic and lead levels. Study design details are presented in Table 2-1. All doses were administered orally. The study was performed as nearly as possible within the spirit and guidelines of Good Laboratory Practices (GLP: 40 CFR 792).

Table 2-1. Study Design and Dosing Information

Group	Dose Material Administered	Number of Swine in Group	Arsenic Dose (µg/kg BW-day)		Lead Dose (µg/kg BW-day)	
			Target Dose	Actual Dose[a]	Target Dose	Actual Dose[b]
1	Lead acetate	5	0	0	75	76
2	Lead acetate	5	0	0	150	160
3	Lead acetate	5	0	0	300	314
4	NIST 2710a	5	40	41	143	147
5	NIST 2710a	5	60	62	215	219
6	NIST 2710a	5	120	121	430	440
7	Sodium arsenate	5	25	26	0	0
8	Sodium arsenate	5	50	52	0	0
9	Sodium arsenate	5	100	105	0	0
10	None (negative control)	3	0	0	0	0

[a] Calculated as the administered daily dose divided by the measured or extrapolated daily body weight, averaged over days 6/7, 9/10, and 12/13 for each animal and each group.
[b] Calculated as the administered daily dose divided by the measured or extrapolated daily body weight, averaged over days 0–15 for each animal and each group.
Doses were administered in two equal portions given at 9:00 AM and 3:00 PM each day. Doses were adjusted upwards every 3 days during the exposure interval based on measured group mean weights.

2.1 Test Materials

2.1.1 Sample Description

The test soil used in this investigation was a sample of National Institute of Standards and Technology (NIST) Standard Reference Material® (SRM) 2710a ("NIST 2710a"). NIST 2710a consists of soil collected from land along Silver Bow Creek approximately 5 miles west of Butte,

Montana. The collection site is approximately nine miles east of Anaconda and 6.5 miles south of settling ponds that feed the creek (NIST 2009).

2.1.2 Sample Preparation and Analysis

All preparation and analysis of the bulk material was conducted by NIST, with no further processing before administration to swine. As described in NIST (2009), NIST 2710a was prepared by air drying at room temperature. The material was then deaggregated and sieved to remove coarse (≥2 mm) material. Material remaining on the screen was ground in a ball mill together with enough lead oxide to achieve a 0.55% mass fraction of lead in the final product. The ball-milled batch of soil was transferred to a cross-flow V-blender for mixing. The blended soil was radiation sterilized, then split into containers using a spinning riffler, used to apportion approximately 50 g into each pre-cleaned bottles. Homogeneity assessments were performed on every 100th bottle and results indicated that additional processing was needed to achieve optimum homogeneity. Therefore, material from all bottles was combined, and then ground in batches between stainless steel plates for a time sufficient to produce a powder of which ≥95%, by mass, passed through a 200 mesh (74 μm) sieve. The resulting powder was blended, and 50 g portions were dispensed into bottles using the spinning riffler. Homogeneity assessments on the re-blended material were acceptable.

This prepared soil as provided by NIST was used *as is* for the bioavailability study, without further preparation. The NIST-certified arsenic and lead concentrations of the NIST 2710a sample are 1540±100 mg/kg and 5520±30 mg/kg, respectively.

2.2 Experimental Animals

Juvenile swine were selected for use because they are considered to be a good physiological model for gastrointestinal absorption in children (Weis and LaVelle 1991; Casteel et al. 1996). The animals were intact males of the Pig Improvement Corporation genetically defined Line 26, and were purchased from Chinn Farms, Clarence, Missouri.

The number of animals purchased for the study was several more than required by the protocol. These animals were purchased at an age of about 5–6 weeks (weaning occurs at age 3 weeks) and housed in individual stainless steel cages. The animals were then held under quarantine for one week to observe their health before beginning exposure to dosing materials. Each animal was examined by a certified veterinary clinician (swine specialist) and any animals that appeared to be in poor health during this quarantine period were excluded from the study. To minimize weight variations among animals and groups, extra animals most different in body weight (either heavier or lighter) five days prior to exposure (day-5) were also excluded from the study. The remaining animals were assigned to dose groups at random (group assignments are presented in Appendix A).

When exposure began (day 0), the animals were about 6–7 weeks old. The animals were weighed at the beginning of the study and every three days during the course of the study. In each study, the rate of weight gain was comparable in all dosing groups. Body weight data are presented in Appendix B.

All animals were examined daily by an attending veterinarian while on study and were subjected to detailed examination at necropsy by a certified veterinary pathologist in order to assess overall animal health.

2.3 Diet

Animals were weaned onto standard swine chow (purchased from MFA Inc., Columbia, Missouri) by the supplier. In order to minimize lead exposure from the diet, all animals were gradually transitioned from the MFA feed to a special purified low-lead feed (purchased from TestDiet®, Richmond, Indiana) several days before dosing began, and this feed was maintained for the duration of the study. The feed was nutritionally complete and met all requirements of the National Institutes of Health–National Research Council (NRC 1988). The ingredients and nutritional profile of the feed are presented in Appendix C. Arsenic and lead concentrations in a randomly selected feed sample measured <0.1 μg/g.

Beginning 5 days before the first day of dosing, each animal was given a daily amount of feed equal to 4.0% of the mean body weight of all animals on study. Feed was reduced to 3.7% body weight starting on day 8 of the study. Feed amounts were adjusted every three days, when animals were weighed. Feed was administered in two equal portions, at 11:00 AM and 5:00 PM daily.

Drinking water was provided *ad libitum* via self-activated watering nozzles within each cage. Arsenic and lead concentrations of five water samples from randomly selected drinking water nozzles were <0.6 μg/L.

2.4 Dosing

Animals were exposed to dosing materials for 14 days, with the dose for each day being administered in two equal portions beginning at 9:00 AM and 3:00 PM (two hours before feeding). Swine were dosed two hours before feeding to ensure that they were in a semi-fasted state. To facilitate dose administration, dosing materials were placed in a small depression in a ball of dough consisting of moistened feed (typically about 5 g) and the dough was pinched shut. This was then placed in the feeder at dosing time.

Target arsenic and lead doses (expressed as μg of metal per kg of body weight per day) for animals in each group are shown in the study design (see Table 2-1). The actual administered doses were calculated based on the arsenic content of the material administered and the measured group mean body weights. Specifically, doses of arsenic for the three days following each weighing were based on the group mean body weight adjusted by the addition of 1 kg to account for the expected weight gain over the time interval. After completion of the study, body weights were estimated by interpolation for those days when measurements were not collected and the actual administered doses were calculated for each day and then averaged across all days. The actual mean doses for each dosing group are included in Table 2-1.

2.5 Collection and Preservation of Urine Samples

Samples of urine were collected from each animal for 48-hour periods on days 6 to 7 (U-1), 9 to 10 (U-2), and 12 to 13 (U-3) of the study. Collection began at 8:00 AM and ended 48 hours

later. The urine was collected in a plastic bucket placed beneath each cage, which was emptied into a plastic storage bottle. Aluminum screens were placed under the cages to minimize contamination with feces or spilled food. Due to the length of the collection period, collection containers were emptied periodically (typically twice daily) into separate plastic bottles to ensure that there was no loss of sample due to overflow.

At the end of each collection period, the total urine volume for each animal was measured (see Appendix D) and three 60-mL portions were removed and acidified with 0.6 mL concentrated nitric acid. All samples were refrigerated. Two of the aliquots were archived and one aliquot was sent for arsenic analysis. Refrigeration was maintained until arsenic analysis.

2.6 Collection and Preservation of Blood Samples

Samples of blood were collected from each animal on the first day of exposure (day 0) and on days 2, 4, 8, 11, and 15 following the start of exposure. All blood samples were collected by vena-puncture of the anterior vena cava, and samples were immediately placed in purple-top Vacutainer® tubes containing EDTA (ethylenediaminetetra-acetic acid) as anticoagulant. Blood samples were collected each sampling day beginning at 8:00 AM, approximately one hour before the first of the two daily exposures to lead on the sampling day and 17 hours after the last lead exposure the previous day. This blood collection time was selected because the rate of change in blood lead resulting from the preceding exposures is expected to be relatively small after this interval (LaVelle et al. 1991; Weis et al. 1993), so the exact timing of sample collection relative to the last dosing is not likely to be critical.

2.7 Collection and Preservation of Tissue and Bone Samples

Following collection of the final blood sample on day 15, all animals were humanely euthanized and samples of liver, kidney, and bone (the right femur, defleshed) were removed and stored at -80°C in lead-free plastic bags for lead analysis.

2.8 Preparation and Analysis

All biological samples were assigned random chain-of-custody tag numbers and submitted to the analytical laboratory for analysis in a blind fashion. The samples were analyzed for arsenic or lead by L.E.T., Inc. (Columbia, Missouri).

Subsamples of all the biological samples collected were archived in order to allow for reanalysis and verification of lead or arsenic levels, if needed.

2.8.1 Urine Sample Preparation and Analysis

Urine samples (25 mL) were digested by refluxing and then heating to dryness in the presence of magnesium nitrate and concentrated nitric acid. Following magnesium nitrate digestion, samples were transferred to a muffle furnace and ashed at 500°C. The digested and ashed residue was dissolved in hydrochloric acid and analyzed by the hydride generation technique using a Perkin Elmer 3100 atomic absorption spectrometer. Previous tests of this method established that each of the different forms of arsenic that may occur in urine, including trivalent inorganic arsenic

(As^{+3}), pentavalent inorganic arsenic (As^{+5}), monomethyl arsenic (MMA), and dimethyl arsenic (DMA) are all recovered with high efficiency.

Analytical results for the urine samples are presented in Appendix D.

2.8.2 Blood Sample Preparation

One milliliter of whole blood was removed from the purple-top Vacutainer® tube and added to 9.0 mL of "matrix modifier", a solution recommended by the Centers for Disease Control and Prevention (CDC) for analysis of blood samples for lead. The composition of matrix modifier is 0.2% (v/v) ultrapure nitric acid, 0.5% (v/v) Triton X-100, and 0.2% (w/v) dibasic ammonium phosphate in deionized distilled water.

2.8.3 Liver and Kidney Sample Preparation

One gram of soft tissue (liver or kidney) was placed in a lead-free screw-cap Teflon container with 2 mL of concentrated (70%) nitric acid and heated in an oven to 90°C overnight. After cooling, the digestate was transferred to a clean lead-free 10 mL volumetric flask and diluted to volume with deionized distilled water.

2.8.4 Bone Sample Preparation

The right femur of each animal was defleshed, broken, and dried at 100°C overnight. The dried bones were then placed in a muffle furnace and dry-ashed at 450°C for 48 hours. Following dry ashing, the bone was ground to a fine powder using a lead-free mortar and pestle, and 200 mg was removed and dissolved in 10.0 mL of 1:1 (v:v) concentrated nitric acid/water. After the powdered bone was dissolved and mixed, 1.0 mL of the acid solution was removed and diluted to 10.0 mL in deionized distilled water.

2.8.5 Lead Sample Analysis

Samples of blood, liver, kidney, and bone and other materials (e.g., food, water, reagents, solutions) were analyzed for lead by graphite furnace atomic absorption using a Perkin Elmer Analyst 800 high-performance atomic absorption spectrometer.

All analytical results were reported in units of μg Pb/L (ng/mL) of prepared sample. The quantitation limit was defined as three-times the standard deviation of a set of seven replicates of a low-lead sample (typically about 2–5 μg/L). The standard deviation was approximately 0.3 μg/L, therefore the quantitation limit was approximately 0.9–1.0 μg/L. For prepared blood samples (diluted 1/10), this corresponds to a quantitation limit of 10 μg/L (1 μg/dL). For soft tissues (liver and kidney, diluted 1/10), the corresponding quantitation limit is 10 μg/kg (10 ng/g) wet weight, and for bone (diluted 1/500) the corresponding quantitation limit is 0.5 μg/g (50 ng/g) ashed weight. All responses below the quantitation limit were evaluated at one-half the quantitation limit. Lead analytical results for study samples are presented in Appendix E.

2.9 Quality Control

A number of quality control (QC) steps were taken during this project to evaluate the accuracy of the analytical procedures. The results for QC samples are presented in Appendix F and are summarized below.

Blind Duplicates (Sample Preparation Replicates)

A random selection of about 8% of all urine samples, 9% of all blood samples, and 3 samples each for kidney, liver, and femur samples generated during the study were prepared for laboratory analysis in duplicate and submitted to the laboratory in a blind fashion. Results are shown in Appendix F (see Table F-1 and Figures F-1 and F-2). There was generally good agreement between results for the duplicate pairs.

Spike Recovery

During analysis, one feed and water sample and every tenth urine, blood, bone, or tissue sample was spiked with known amounts of arsenic (sodium arsenate) or lead (lead acetate) and the recovery of the added arsenic or lead was measured. Results (see Table F-2) show that mean arsenic and lead concentrations recovered from spiked samples were typically within 10% of actual concentrations.

Laboratory Duplicates

During analysis, every tenth sample was analyzed in duplicate. Duplicate results for urine and lead samples (see Table F-3) typically agreed within 10% relative percent difference (RPD).

Laboratory Control Standards

Several NIST standard reference materials (SRMs), for which certified concentrations of specific analytes has been established, were tested periodically during sample analysis. Recovery of arsenic and lead from these standards was generally good and within the acceptable range (see Table F-4).

Performance Evaluation Samples for Arsenic

A number of Performance Evaluation (PE) samples (urine samples of known arsenic concentration) were submitted to the laboratory in a blind fashion. The PE samples included varying concentrations (20, 100, or 400 µg/L) each of four different types of arsenic (As^{+3}, As^{+5}, MMA, and DMA). The results for the PE samples are shown in Table F-5 and Figure F-3. All sample results were close to the expected values, indicating that there was good recovery of the arsenic in all cases.

CDC Samples for Lead

The CDC provides a variety of blood lead “check samples” for use in quality assurance programs for blood lead studies. Several CDC check samples of different concentrations were provided to the analytical laboratory in a blind fashion, to be analyzed periodically during blood sample

analysis. The results are summarized in Table F-6 and Figure F-4. Sample results were slightly lower than expected values; however, this same relationship has been observed in lead studies in the past, and therefore the relationship is interpreted as normal and expected for blood lead samples.

Laboratory Blanks

Laboratory blank samples were run along with each batch of samples at a rate of about 10%. Blanks never yielded a measurable level of arsenic (all results <1 µg/L) and only one sample, a blank sample associated with the water samples, yielded a measureable level of lead (Blank-1 = 1 ng/mL). Results are shown in Table F-7.

Summary of QC Results

Based on the results of all of the QC samples and steps described above, it is concluded that the analytical results are of sufficient quality for derivation of reliable estimates of arsenic and lead absorption from the test materials.

3.0 DATA ANALYSIS FOR ARSENIC

3.1 Overview

Figure 3-1 shows a conceptual model for the toxicokinetic fate of ingested arsenic. Key points of this model are as follows:

- In most animals (including humans), absorbed arsenic is excreted mainly in the urine over the course of several days. Thus, the urinary excretion fraction (UEF), defined as the amount excreted in the urine divided by the amount given, is usually a reasonable approximation of the AF_o or ABA. However, this ratio will underestimate total absorption, because some absorbed arsenic is excreted in the feces via the bile, and some absorbed arsenic enters tissue compartments (e.g., skin, hair) from which it is cleared very slowly or not at all. Thus, the UEF should not be equated with the absolute absorption fraction.

- The RBA of two orally administered materials (i.e., a test material and reference material) can be calculated from the ratio of the UEF of the two materials. This calculation is independent of the extent of tissue binding and of biliary excretion:

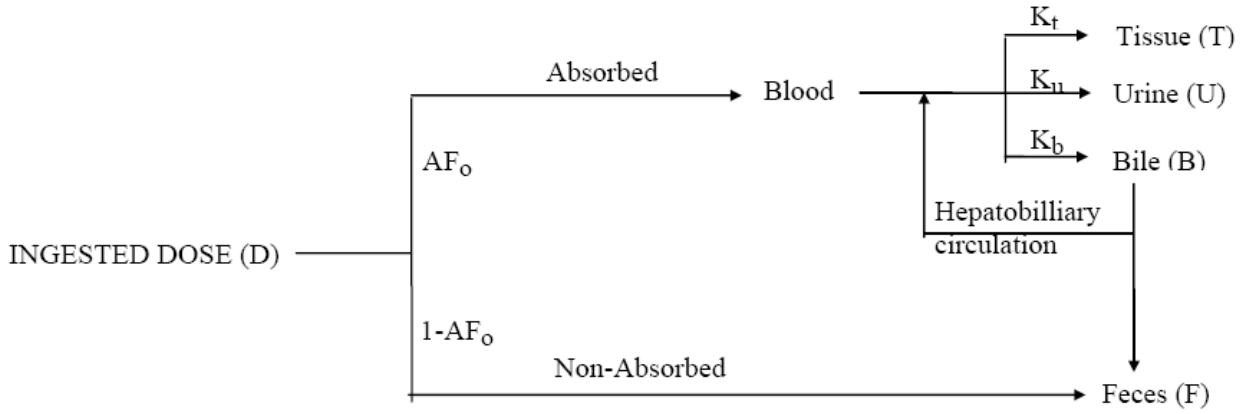
$$RBA(test\ vs\ ref) = \frac{AF_o(test)}{AF_o(ref)} = \frac{D \cdot AF_o(test) \cdot K_u}{D \cdot AF_o(ref) \cdot K_u} = \frac{UEF(test)}{UEF(ref)}$$

where:

D = ingested dose (µg)

K_u = fraction of absorbed arsenic that is excreted in the urine

Figure 3-1. Conceptual Model for Arsenic Toxicokinetics



where:

AF_o = Oral Absorption Fraction
K_t = Fraction of absorbed arsenic which is retained in tissues
K_u = Fraction of absorbed arsenic which is excreted in urine
K_b = Fraction of absorbed arsenic which is excreted in the bile

BASIC EQUATIONS:

Amount in Urine

$$U_{oral} = D \bullet AF_o \bullet K_u$$

Urinary Excretion Fraction (UEF)

$$UEF_{oral} = \frac{U_{oral}}{D_{oral}} = AF_o \bullet K_u$$

Relative Bioavailability

$$RBA_{(x\ vs.\ y)} = \frac{UEF_{x,oral}}{UEF_{y,oral}} = \frac{AF_{o(x)} \bullet K_u}{AF_{o(y)} \bullet K_u} = \frac{AF_{o(x)}}{AF_{o(y)}}$$

Based on the conceptual model above, the basic method used to estimate the RBA of arsenic in a particular test material compared to arsenic in a reference material (sodium arsenate) is as follows:

1. Plot the amount of arsenic excreted in the urine (μg per 48 hours) as a function of the administered amount of arsenic (μg per 48 hours), both for reference material and for test material.

2. Find the best fit linear regression line through each data set. The slope of each line (μg per 48 hours excreted per μg per 48 hours ingested) is the best estimate of the UEF for each material.

3. Calculate RBA for each test material as the ratio of the UEF for test material compared to UEF for reference material:

$$RBA(test\ vs\ ref) = \frac{UEF(test)}{UEF(ref)}$$

A detailed description of the curve-fitting methods and rationale and the methods used to quantify uncertainty in the arsenic RBA estimates for a test material are summarized below. All model fitting was performed in Microsoft Excel® using matrix functions.

3.2 Arsenic Dose-Response Model

Simultaneous Regression

The techniques used to derive linear regression fits to the dose-response data are based on the methods recommended by Finney (1978). As noted by Finney (1978), when the data to be analyzed consist of two dose-response curves (the reference material and the test material), it is obvious that both curves must have the same intercept, since there is no difference between the curves when the dose is zero. This requirement is achieved by combining the two dose response equations into one and solving for the parameters simultaneously, as follows:

Separate models:

$$\mu_r(i) = a + b_r \cdot x_r(i)$$

$$\mu_t(i) = a + b_t \cdot x_t(i)$$

Combined model:

$$\mu(i) = a + b_r \cdot x_r(i) + b_t \cdot x_t(i)$$

where $\mu(i)$ indicates the expected mean response of animals exposed at dose $x(i)$, and the subscripts r and t refer to reference and test material, respectively. The coefficients of this combined model are derived using multivariate regression, with the understanding that the combined data set is restricted to cases in which one (or both) of x_r and x_t are zero (Finney 1978).

Weighted Regression

Regression analysis based on ordinary least squares assumes that the variance of the responses is independent of the dose and/or the response (Draper and Smith 1998). It has previously been shown that this assumption is generally not satisfied in swine-based RBA studies, where there is a tendency toward increasing variance in response as a function of increasing dose (heteroscedasticity) (USEPA 2007). One method for dealing with heteroscedasticity is through the use of weighted least squares regression (Draper and Smith 1998). In this approach, each observation in a group of animals is assigned a weight that is inversely proportional to the variance of the response in that group:

$$w_i = \frac{1}{\sigma_i^2}$$

where:

w_i = weight assigned to all data points in dose group *i*

σ_i^2 = variance of responses in animals in dose group *i*

When the distributions of responses at each dose level are normal, weighted regression is equivalent to the maximum likelihood method.

There are several alternative strategies for assigning weights. The method used in this study estimates the value of σ_i^2 using an "external" variance model based on an analysis of the relationship between variance and mean response using data consolidated across many different swine-based arsenic RBA studies. The data used to derive the variance model are shown in Figure 3-2. As seen, log-variance increases as an approximately linear function of log-mean response:

$$\ln(s_i^2) = k1 + k2 \cdot \ln(\bar{y}_i)$$

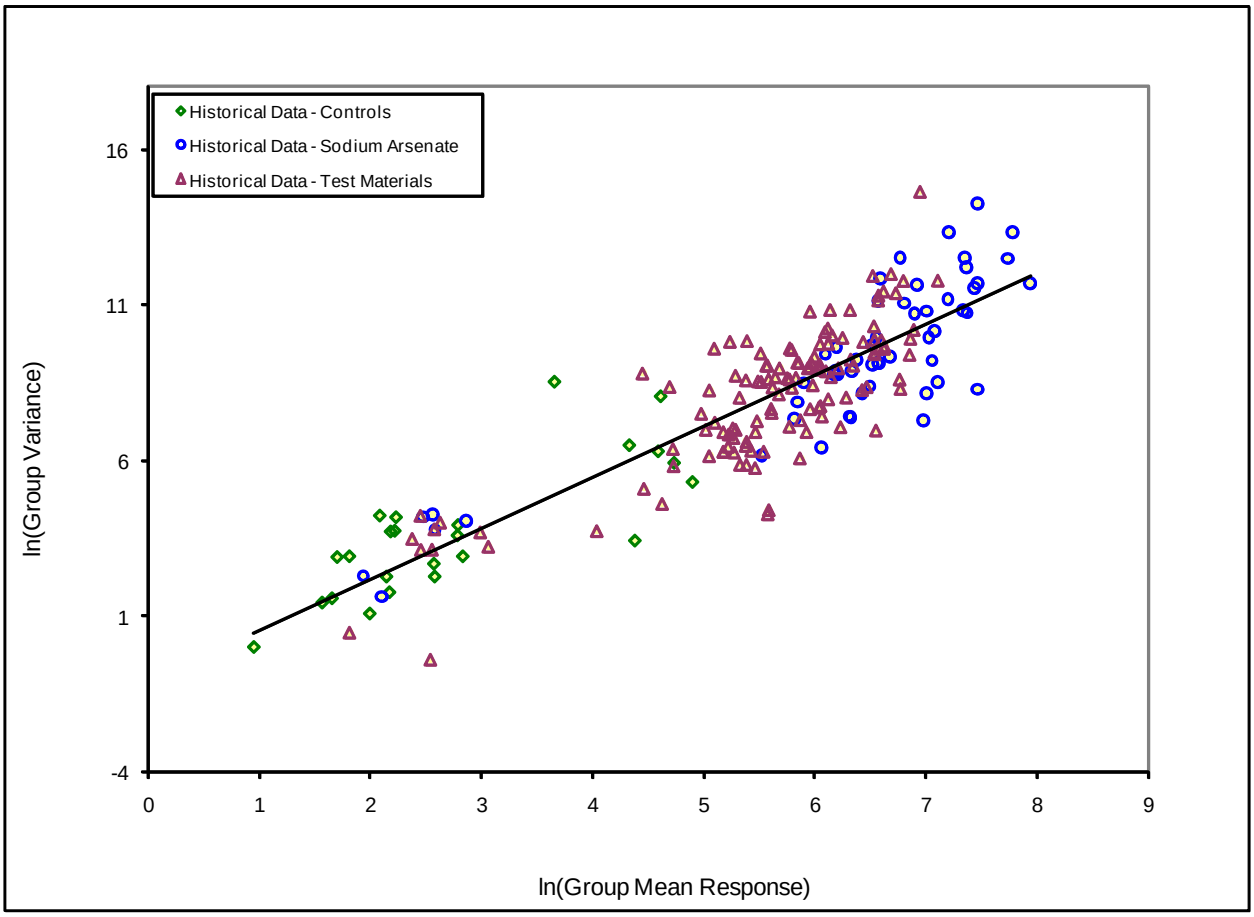
where:

s_i^2 = observed variance of responses of animals in dose group *i*

$\bar{y}_i$ = mean observed response of animals in dose group *i*

Based on these data, values of *k*1 and *k*2 were derived using ordinary least squares minimization. The resulting values were -1.10 for *k*1 and 1.64 for *k*2.

Figure 3-2. Urinary Arsenic Variance Model



Goodness-of-Fit

The goodness-of-fit of each dose-response model was assessed using the F test statistic and the adjusted coefficient of multiple determination (Adj R^2) as described by Draper and Smith (1998). A fit is considered acceptable if the p-value is less than 0.05.

Data Assessment

Arsenic data were assessed in two parts. First, the urine volumes and arsenic concentrations were reviewed. A large volume of urine is typically indicative that a swine spilled its drinking water into the urine collection trays. In these instances, the arsenic concentration in the diluted urine will become very small and difficult to measure with accuracy. Furthermore, because the response of the swine to arsenic dose is calculated from the product of urine concentration and volume, the result becomes highly uncertain when the concentration is multiplied by a volume that is not representative of the total urine volume. For this reason, in cases where total urine volume per 24-hour period was more than 5 liters (more than twice the average urine output of swine) and the measured urine concentration of arsenic was at or below the quantitation limit

(<2 µg/L), the samples were judged to be unreliable and were excluded from the quantitative analysis.

Once samples with a high urine volume to arsenic concentration were removed, the remaining data set was modeled. The modeled data set was then analyzed for individual measured responses that appeared atypical compared to the responses from other animals in the same dose group. Responses that yielded standardized weighted residuals greater than 3.5 or less than -3.5 were considered to be potential outliers (Canavos 1984).

3.3 Calculation of Arsenic RBA Estimates

The arsenic RBA values were calculated as the ratio of the slope term for the test material data set (b_t) and the reference material data set (b_r):

$$RBA = \frac{b_t}{b_r}$$

The uncertainly range about the RBA ratio was calculated using Fieller's Theorem as described by Finney (1978).

4.0 DATA ANALYSIS FOR LEAD

4.1 Overview

The basic approach for measuring lead absorption *in vivo* is to administer an oral dose of lead to test animals and measure the increase in lead level in one or more body compartments (e.g., blood, soft tissue, bone). In order to calculate the RBA value of a test material, the increase in lead in a body compartment is measured both for that test material and a reference material (lead acetate). Because equal absorbed doses of lead (as Pb^{+2}) will produce equal responses (i.e., equal increases in concentration in tissues) regardless of the source or nature of the ingested lead, the RBA of a test material is calculated as the ratio of doses (test material and reference material) that produce equal increases in lead concentration in the body compartment. Thus, the basic data reduction task required to calculate an RBA for a test material is to fit mathematical equations to the dose-response data for both the test material and the reference material, and then solve the equations to find the ratio of doses that would be expected to yield equal responses.

Some biological responses to lead exposure may be non-linear functions of dose (i.e., tending to flatten out or plateau as dose increases). The cause of this non-linearity is uncertain but might be due either to non-linear absorption kinetics and/or to non-linear biological response per unit dose absorbed. However, the principal advantage of the approach described above is that it is not necessary to understand the basis for a non-linear dose response curve (non-linear absorption and/or non-linear biological response) in order to derive valid RBA estimates; the approach yields reliable results for both non-linear and linear responses.

A detailed description of the curve-fitting methods and rationale, along with the methods used to quantify uncertainty in the RBA estimates for the test material, are presented in USEPA (2007) and are summarized below.

4.2 Description of Measurement Endpoints for Lead

Four independent measurement endpoints were evaluated based on the concentration of lead observed in blood, liver, kidney, and bone (femur). For liver, kidney, and bone, the measurement endpoint was simply the concentration in the tissue at the time of sacrifice (day 15). The measurement endpoint used to quantify the blood lead response was the area under the curve (AUC) for blood lead vs. time (days 0–15). 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 blood lead level by day. The AUC was calculated using the trapezoidal rule to estimate the AUC between each time point that a blood lead value was measured:

$$AUC(d_i \text{ to } d_j) = 0.5 \cdot (r_i + r_j) \cdot (d_j - d_i)$$

where:

d = day number

r = response (blood lead value) on day i (r_i) or day j (r_j)

The areas were then summed across all time intervals in the study to yield the final AUC for each animal.

4.3 Lead Dose-Response Models

Basic Equations

Nearly all blood lead AUC data sets can be well-fit using an exponential equation (USEPA 2007) and most tissue (liver, kidney, and bone) lead data can be well-fit using a linear equation, as follows:

Linear (liver, kidney, bone): $\text{Response} = a + b \cdot \text{Dose}$

Exponential (blood lead AUC): $\text{Response} = a + b \cdot [1 - \exp(-c \cdot \text{Dose})]$

Simultaneous Regression

Because the data to be analyzed consist of two dose-response curves for each endpoint and there is no difference between the curves when the dose is zero, both curves for a given endpoint must have the same intercept. This requirement is achieved by combining the two dose response equations into one and solving for the parameters simultaneously, resulting in the following equations:

Linear: $y = a + b_r \cdot x_r + b_t \cdot x_t$

Exponential: $y = a + b \cdot [\ (1-\exp(-c_r \cdot x_r)) + (1-\exp(-c_t \cdot x_t))\ ]$

where:

y = response
x = dose
a, b, c = empirical coefficients for the reference material (r) and test material (t).

All linear model fitting was performed in Microsoft® Office Excel using matrix functions. Exponential model fitting was performed using JMP® version 3.2.2, a commercial software package developed by SAS®.

Weighted Regression

An "external" variance model was used to estimate the value of σ_i^2 for lead based on an analysis of the relationship between variance and mean response using data consolidated across many different swine-based lead RBA studies. The data used to derive the variance models for each endpoint are shown in Figure 4-1. Values of k1 and k2 were derived for each endpoint using ordinary least squares minimization, and the resulting values are shown below:

Endpoint	k1	k2
Blood AUC	-1.3226	1.5516
Liver	-2.6015	2.0999
Kidney	-1.8499	1.9557
Femur	-1.9713	1.6560

Goodness-of-Fit

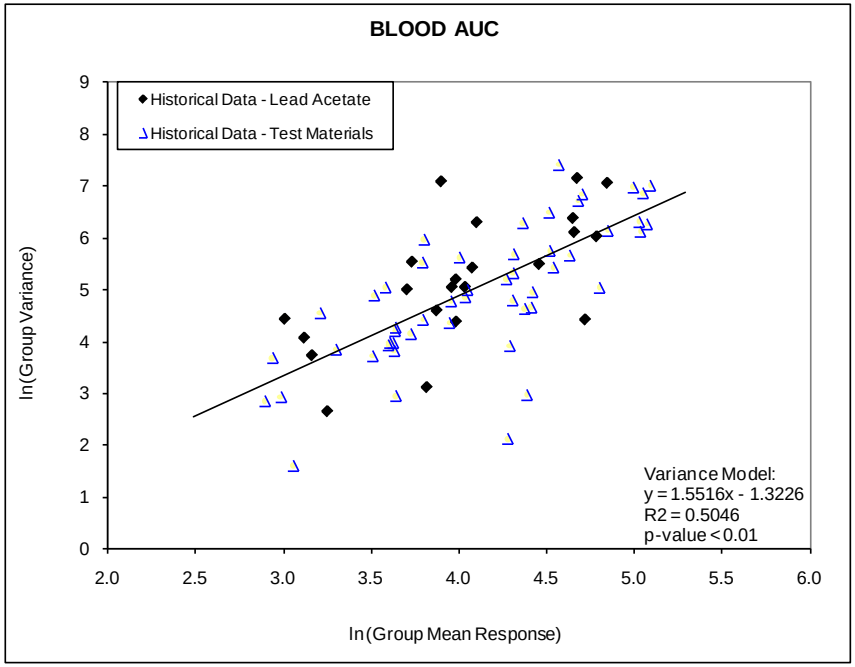
The goodness-of-fit of each dose-response model was assessed using the F test statistic and the adjusted coefficient of multiple determination (Adj R^2) as described by Draper and Smith (1998). A fit is considered acceptable if the p-value is less than 0.05.

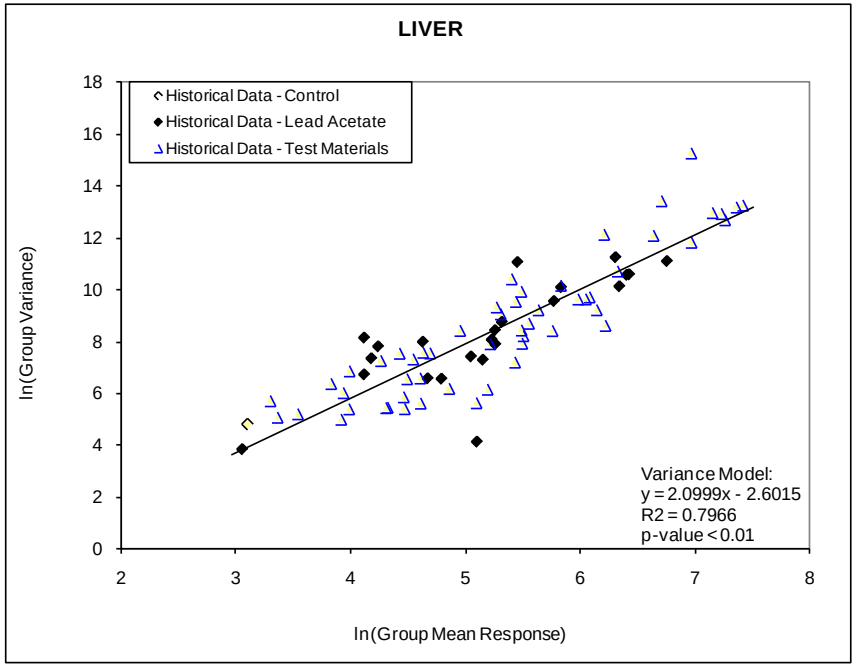
Data Assessment

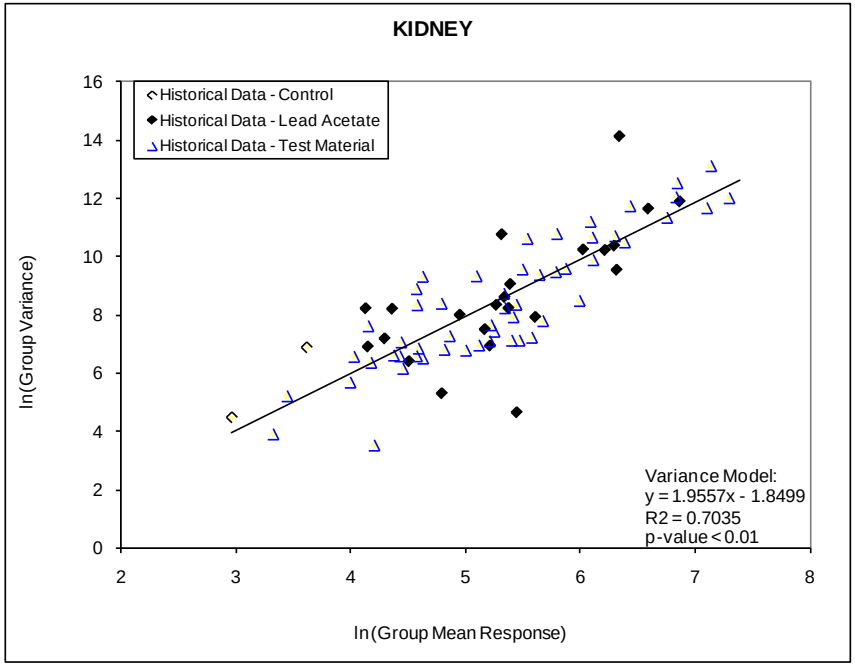
Lead data were assessed in two parts. First, blood lead data were reviewed. Occasionally, blood lead values are obtained that are clearly different than expected. Blood lead values that were more than a factor of 1.5 above or below the group mean for any given day were flagged as potentially unreliable data points. Each data point identified in this way was reviewed and professional judgment was used to decide if the value should be retained or excluded. In order to avoid inappropriate biases, blood lead exclusion designations are restricted to values that are clearly aberrant from a time-course and/or dose-response perspective. Once individual unreliable blood lead data points were removed, AUC was determined and this data set was modeled.

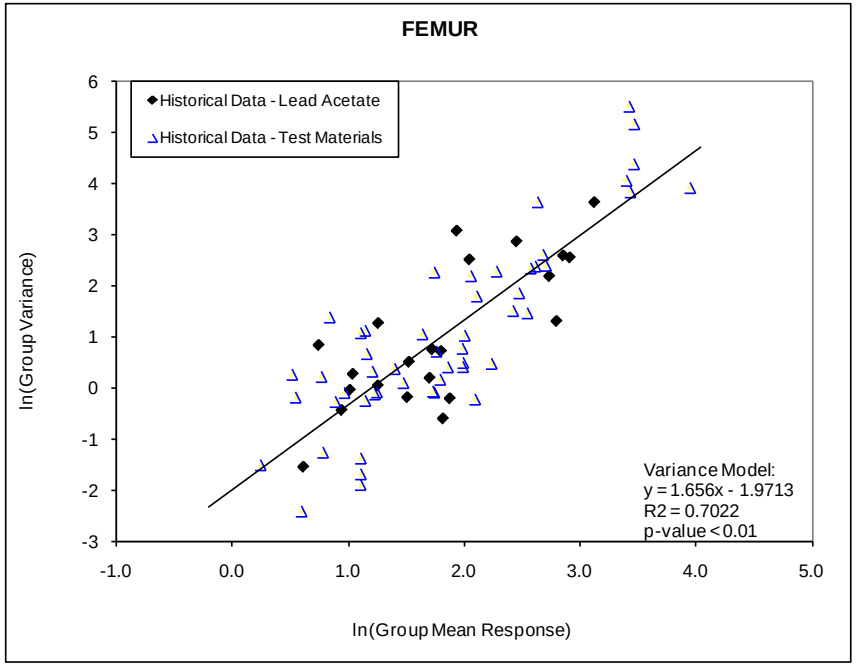
The modeled data set, including AUC, liver, kidney, and femur data was then analyzed for individual measured responses that appeared atypical compared to the responses from other animals in the same dose group. Responses that yielded standardized weighted residuals greater than 3.5 or less than -3.5 were considered to be potential outliers (Canavos 1984).

Figure 4-1. Variance Models for Lead Endpoints









4.4 Calculation of Lead RBA Estimates

Endpoint-Specific RBA Estimates

Lead RBA values were estimated using the basic statistical techniques recommended by Finney (1978). Each endpoint-specific RBA value was calculated as the ratio of a model coefficient for the reference material data set and for the test material data set:

Linear endpoints: $RBA_t = b_t / b_r$

Exponential endpoint: $RBA_t = c_t / c_r$

The uncertainly range about the RBA ratio was calculated using Fieller's Theorem as described by Finney (1978).

RBA Point Estimate

Because there are four independent estimates of RBA (one from each measurement endpoint) for a given test material, the final RBA estimate for a test material involves combining the four endpoint-specific RBA values into a single value (point estimate) and estimating the uncertainty around that point estimate. As described in USEPA (2007), analysis of data from multiple studies suggests that the four endpoint-specific RBA values are all approximately equally reliable (as reflected in the average coefficient of variation in RBA values derived from each endpoint). Therefore, the RBA point estimate for the test material was calculated as the simple mean of all four endpoint-specific RBA values.

The uncertainty bounds around this point estimate were estimated using Monte Carlo simulation. Values for RBA were drawn from the uncertainty distributions for each endpoint with equal frequency. Each endpoint-specific uncertainty distribution was assumed to be normal, with the mean equal to the best estimate of RBA and the standard deviation estimated from Fieller's Theorem (Finney 1978). The uncertainty in the point estimate was characterized as the range from the 5^{th} to the 95^{th} percentile of the mean across endpoints.

5.0 RESULTS

5.1 Clinical Signs

The doses of arsenic and lead administered in this study are below a level that is expected to cause toxicological responses in swine. No clinical signs of toxicity were noted in any of the animals used in the studies. Four swine received 1 cc Naxcel once per day for several days during the study (Table 5-1) to treat a systemic bacterial infection (swine were found with fever $\geq$104°F).

Table 5-1. NAXCEL Treatments

Swine Number	Days of Treatment
647	0–2
659	0–2
649	3–4
646	5–7

5.2 Dosing Deviations

Missed doses are summarized in Table 5-2. Most missed doses occurred on the first four days of dosing and were not specific to any particular group.

Table 5-2. Missed Dose Consumption

Swine Number	Study Day	% Dose Ingested		
		AM	PM	Combined
659	0	100	0	50
649	2	100	0	50
	3	0	0	0
	4	100	50	75
682	2	0	0	0
695	2	0	100	50
	3	100	50	75
	7	100	50	75
657	3	100	50	75
687	3	100	50	75
646	7	100	0	50
656	7	75	50	63

5.3 Background Arsenic and Lead

Measured values for urinary arsenic, tissue, and bone lead levels, and blood lead AUC for control animals are shown in Table 5-3. Urinary arsenic concentration (mean±SD) for all control animals combined across days 6 to 13 was 14.8±9.6 µg/L. Tissue and bone lead levels were typically less than detection limits, and blood lead AUC was 7.5 for all swine (after excluding the outlier for swine 685, day 8; see Table 5-5). The urinary arsenic and blood, bone and tissue lead values observed in the control animals were within the range of typical endogenous background levels reported from other studies (see Figures 3-2 and 4-1). Therefore, the background data support the view that the animals were not exposed to any significant exogenous sources of arsenic or lead throughout the study.

Table 5-3. Background Urinary Arsenic and Blood and Tissue Lead Levels

Analyte	Period of Collection	Measure	Swine Number		
			645	684	685
Arsenic	Days 6 and 7	Total As excreted (µg/48 hours)	10.41	15.9	10.04
	Days 9 and 10	Total As excreted (µg/48 hours)	34	4.46	13.62
	Days 12 and 13	Total As excreted (µg/48 hours)	25.93	5.61	12.8
Lead	Days 0, 2, 4, 8, 11, and 15	Blood AUC	7.5	7.5	7.5
	Day 15	Femur lead (ng/g)	<300	<300	<300
	Day 15	Liver lead (ng/g)	<10	<10	220
	Day 15	Kidney lead (ng/g)	<10	<10	30

5.4 Variance Data

As discussed in Sections 3.2 and 4.3, urinary arsenic and lead endpoint dose-response data are analyzed using weighted least squares regression and the weights are assigned using "external" variance models. To ensure that the variance models are valid, the variance values from each of the dose groups were superimposed on the historic data sets (Figures 5-1 and 5-2). As shown, the variances of the urinary arsenic and lead endpoint data from this study are consistent with the data used to generate the variance model.

Figure 5-1. NIST 2710a Data Compared to Urinary Arsenic Variance Model

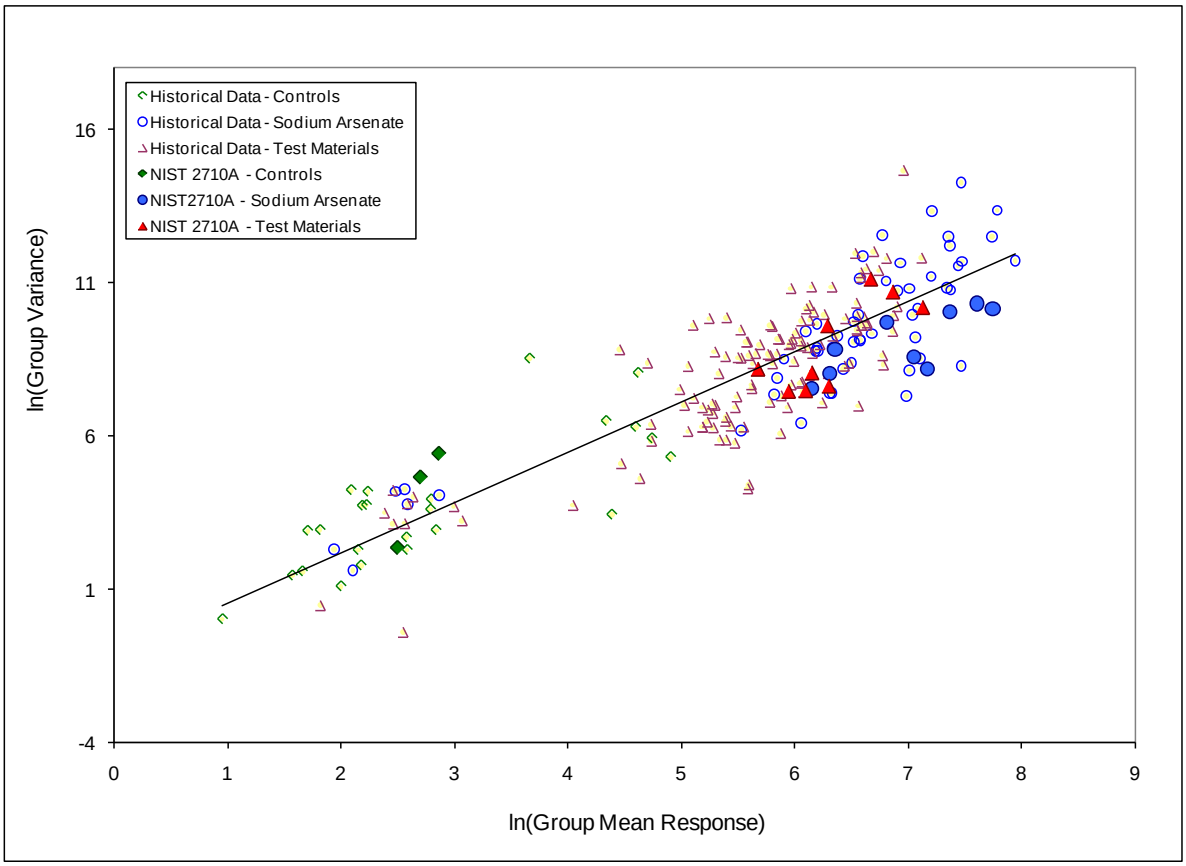
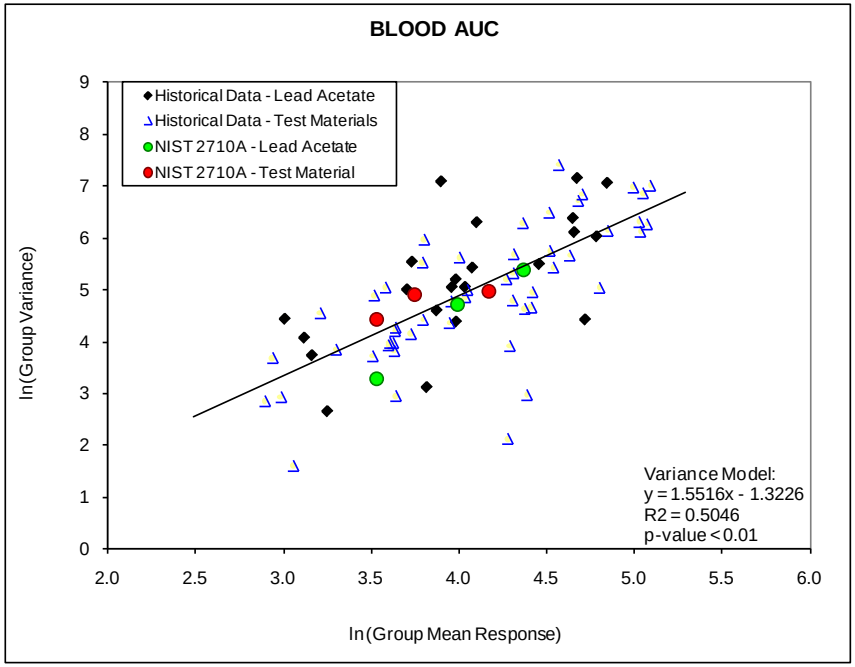
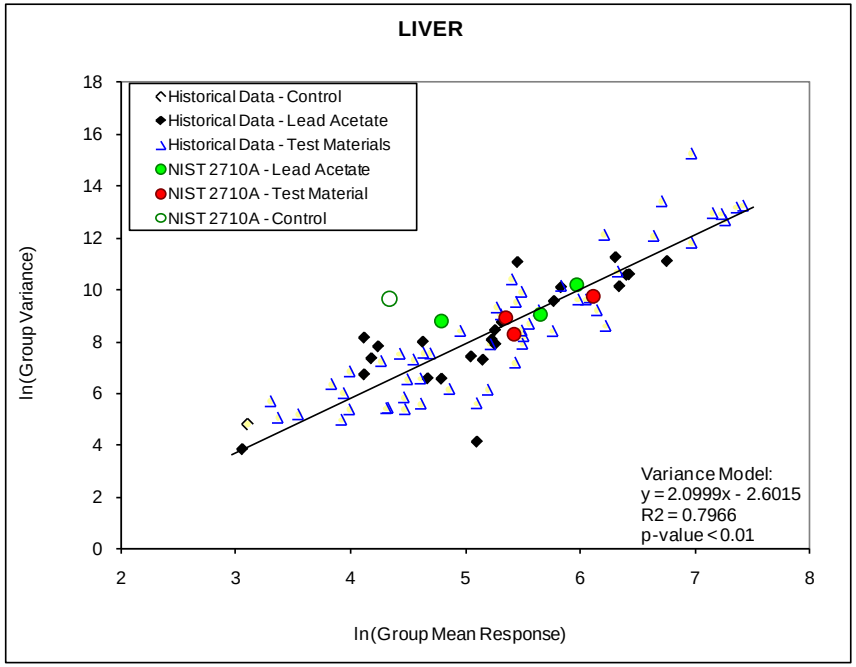
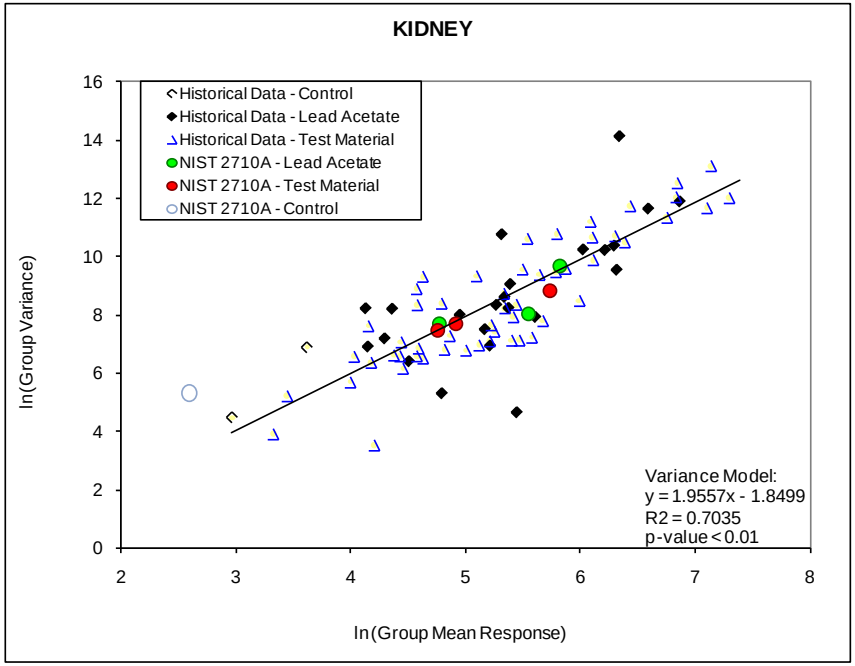
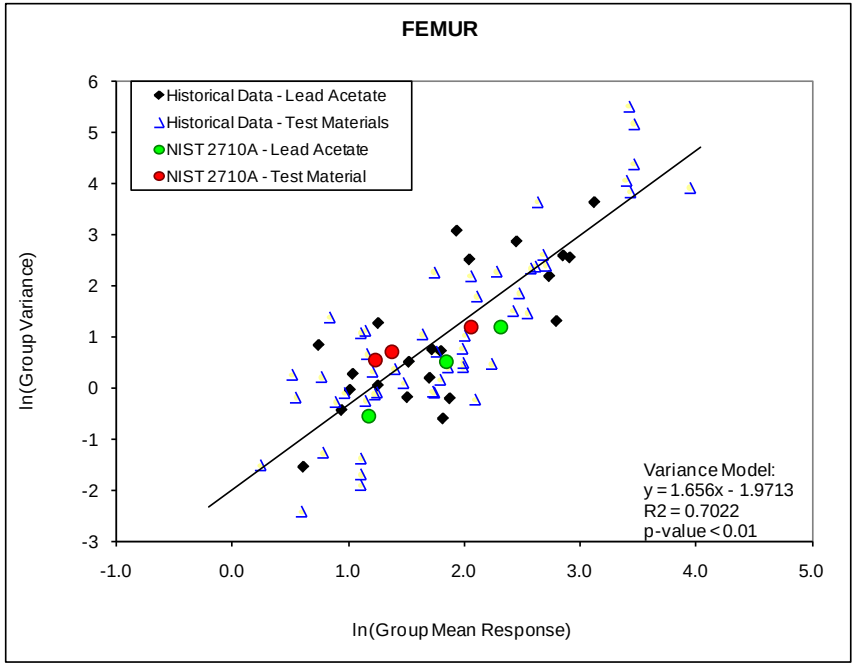


Figure 5-2. NIST 2710a Data Compared to Lead Variance Models









5.5 Dose-Response Modeling

5.5.1 Arsenic

Four urine samples were excluded due to high volume and low arsenic concentrations (see Section 3.2). This included swine 645 (all days) and swine 684 (days 6/7). Both swine were from the control group.

Once samples with a high urine volume to arsenic concentration were removed, the remaining data set was analyzed (Figures 5-3 through 5-6). No samples were identified as outliers (see Section 3.2).

All of the dose-response curves were approximately linear, with the slope of the best fit straight line being equal to the best estimate of the UEF. The resulting slopes (UEF estimates) for the final fittings of the test material and corresponding reference material are shown in Table 5-4.

Table 5-4. Urine Excretion Fraction (UEF) Estimates

Urine Collection Period (days)	Outliers Excluded	Slopes (UEF Estimates)	
		b_r	b_{t1}
Days 6/7	0	0.73	0.31
Days 9/10	0	0.84	0.34
Days 12/13	0	0.86	0.36
All Days	0	0.80	0.34

b_r = slope for reference material (sodium arsenate)
b_{t1} = slope for test material 1 (NIST 2710a)

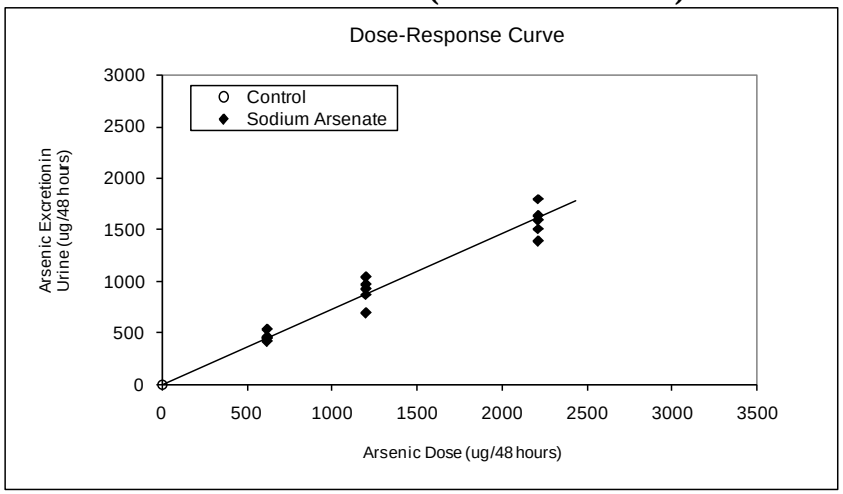
5.5.2 Lead

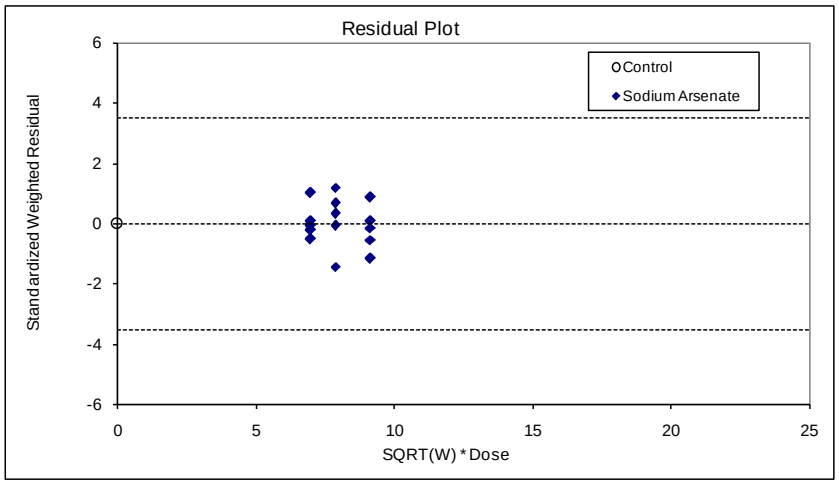
Group mean blood lead data for all swine are plotted by day in Figure 5-7 (Panel A). In this study, three values were judged as unreliable data points as described in Section 4.3 (see Table 5-5). These lead values were excluded from calculations of AUC, and the missing values were replaced by values interpolated from the preceding and following values from the same animal. Figure 5-7 (Panel B) shows the group mean blood lead data plotted by day based on the interpolated values for these three measurements. The AUC determinations for days 0–15 are presented in Table 5-6.

The blood lead AUC data were then modeled using an exponential equation. The results of this fitting are shown in Figure 5-8. The dose-response data for lead in liver, kidney, and bone (measured at sacrifice on day 15) were modeled using a linear equation. The results of these fittings are shown in Figures 5-9a (liver), 5-10 (kidney), and 11 (femur). One outlier was identified in the liver control group (as indicated in Figure 5-9a) and was excluded from the final evaluation for lead RBA (see Figure 5-9b). No other outliers were identified for any of the endpoints.

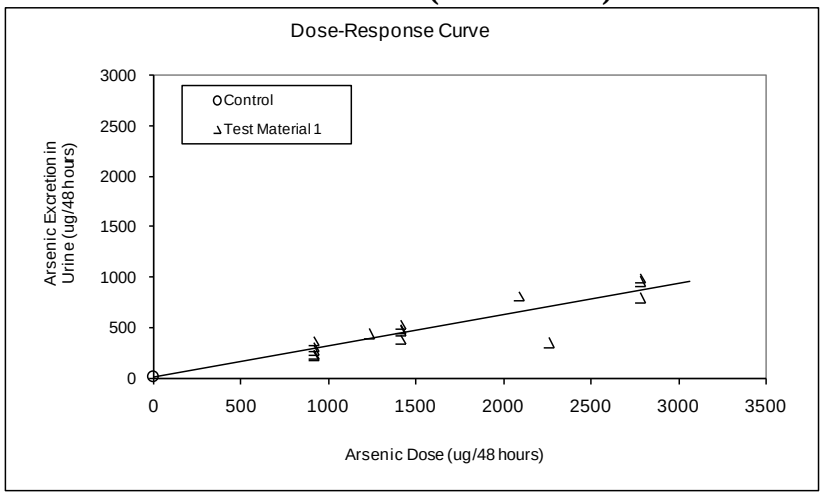
Figure 5-3. NIST 2710a Urinary Excretion of Arsenic: Days 6/7 (All Data)

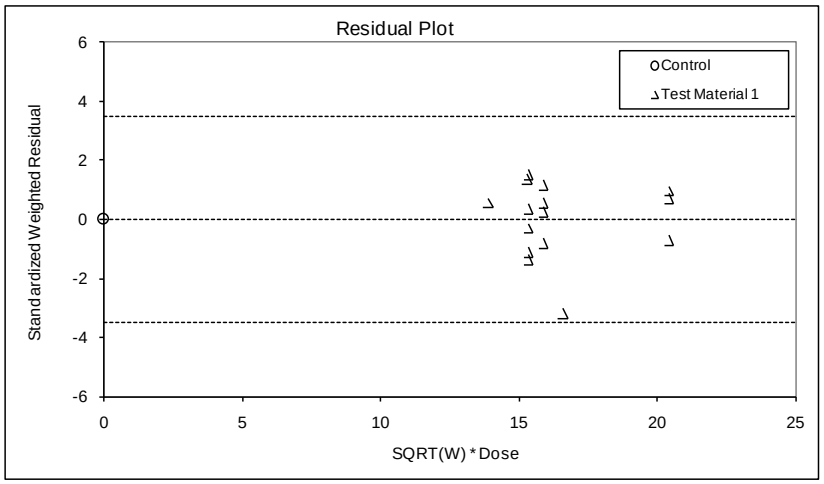
Reference Material (Sodium Arsenate)





Test Material 1 (NIST 2710a)





Summary of Fitting [a]

Parameter	Estimate	Standard Error
a	10.1	3.3
b_r	0.73	0.03
b_{t1}	0.31	0.01
Covariance (b_r,b_{t1})	0.0168	–
Degrees of Freedom	29	–

[a] $y = a + b_r*x_r + b_{t1}*x_{t1} + b_{t2}*x_{t2}$
where r = Reference Material, t1 = Test Material 1

ANOVA

Source	SSE	DF	MSE
Fit	888.35	2	444.18
Error	21.35	28	0.76
Total	909.71	30	30.32

Statistic	Estimate
F	582.478
P	<0.001
Adjusted R^2	0.9749

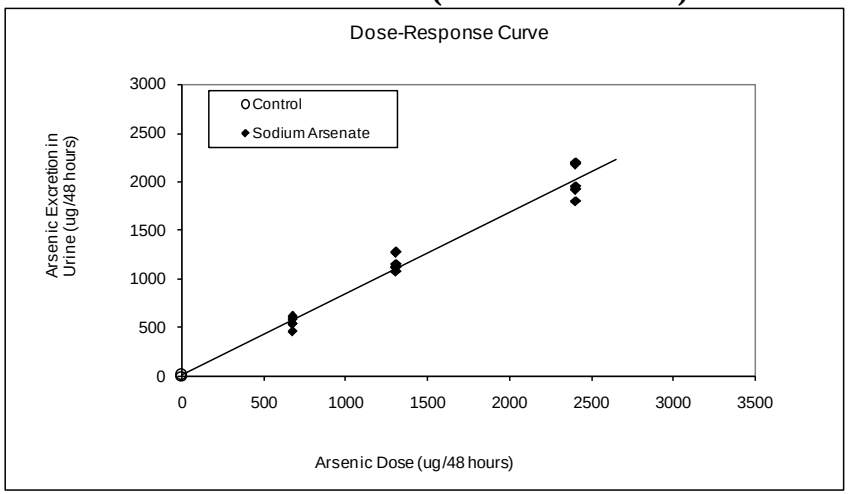
RBA and Uncertainty

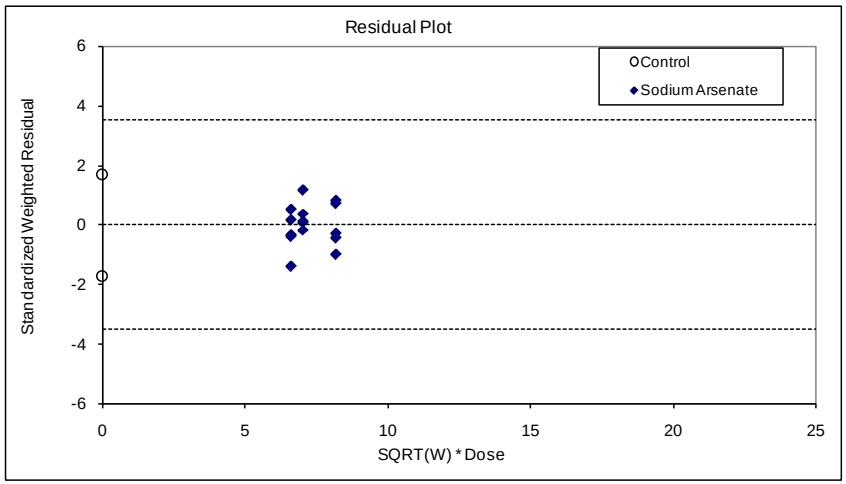
	Test Material 1
RBA	0.43
Lower bound [c]	0.39
Upper bound [c]	0.47
Standard Error [c]	0.025

[c] 90% confidence interval calculated using Fieller's theorem

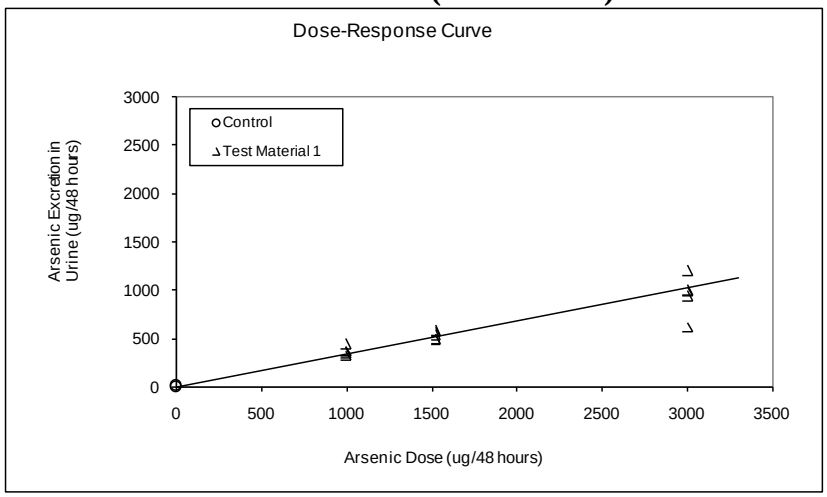
Figure 5-4. NIST 2710a Urinary Excretion of Arsenic: Days 9/10

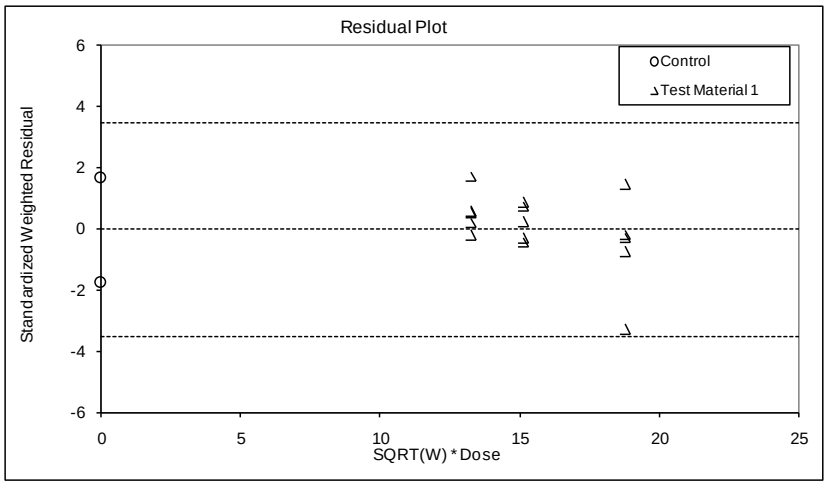
Reference Material (Sodium Arsenate)





Test Material 1 (NIST 2710a)





Summary of Fitting [a]

Parameter	Estimate	Standard Error
a	9.2	2.0
b_r	0.84	0.03
b_{t1}	0.34	0.01
Covariance (b_r,b_{t1})	0.0051	–
Degrees of Freedom	30	–

[a] $y = a + b_r*x_r + b_{t1}*x_{t1} + b_{t2}*x_{t2}$
where r = Reference Material, t1 = Test Material 1

ANOVA

Source	SSE	DF	MSE
Fit	983.49	2	491.75
Error	18.10	29	0.62
Total	1001.60	31	32.31

Statistic	Estimate
F	787.693
P	<0.001
Adjusted R^2	0.9807

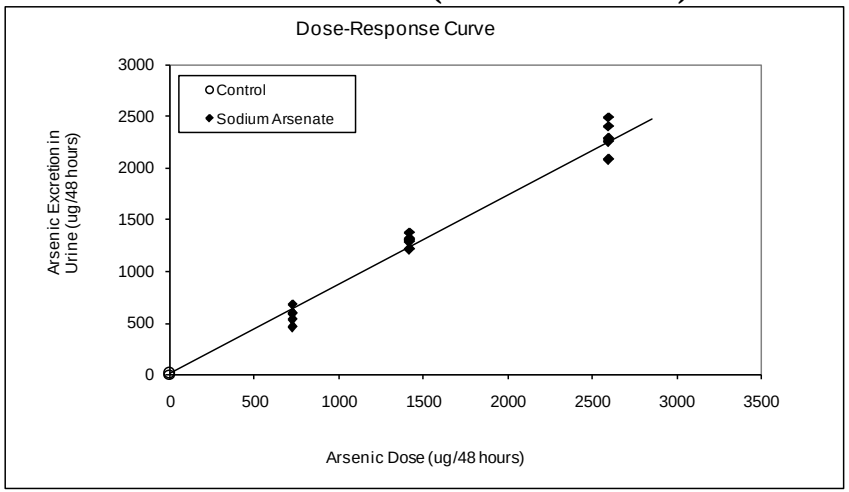
RBA and Uncertainty

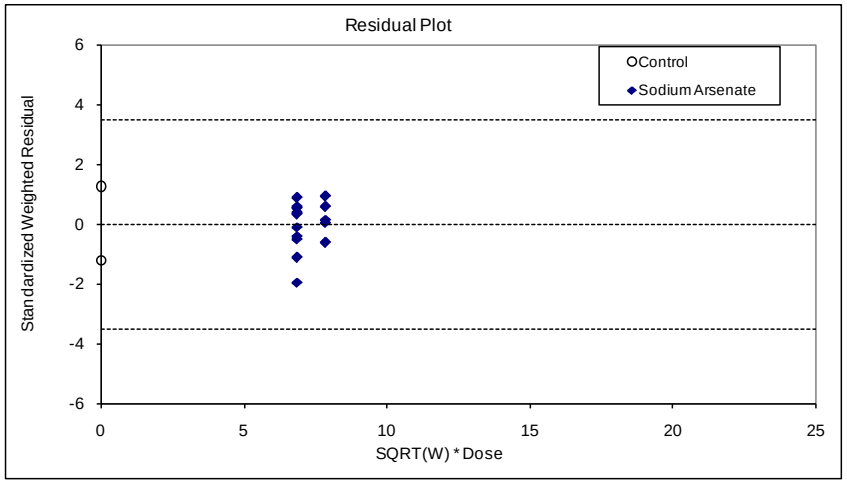
	Test Material 1
RBA	0.41
Lower bound [c]	0.37
Upper bound [c]	0.44
Standard Error [c]	0.021

[c] 90% confidence interval calculated using Fieller's theorem

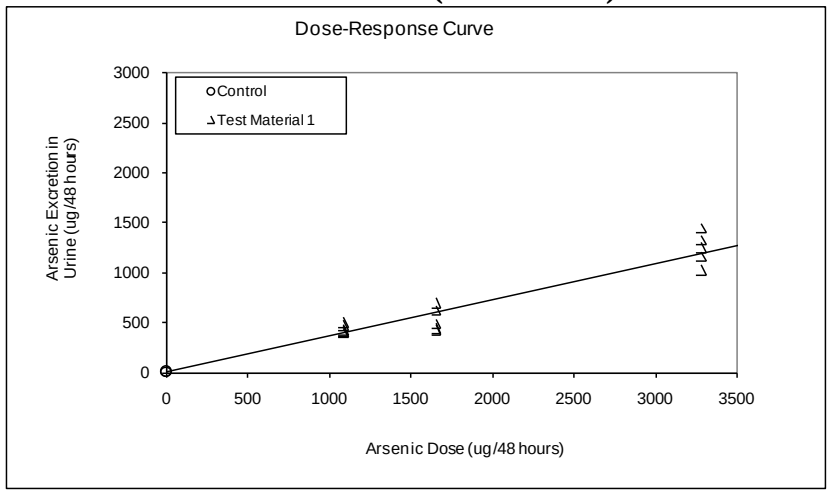
Figure 5-5. NIST 2710a Urinary Excretion of Arsenic: Days 12/13

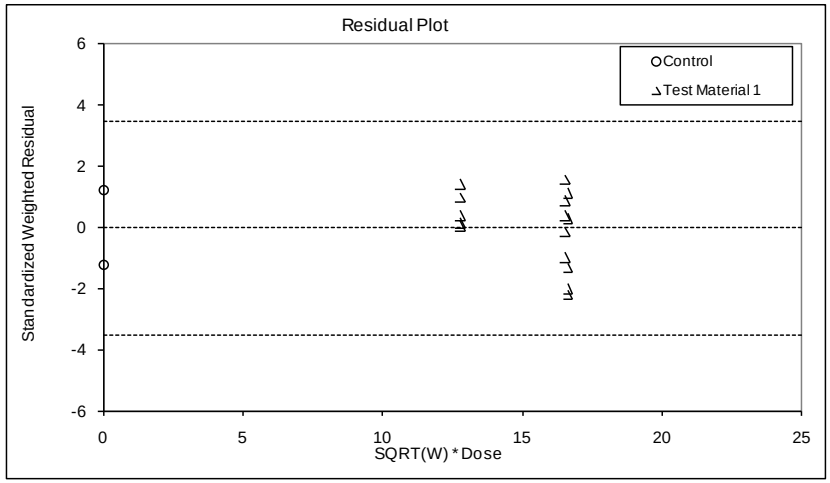
Reference Material (Sodium Arsenate)





Test Material 1 (NIST 2710a)





Summary of Fitting [a]

Parameter	Estimate	Standard Error
a	9.1	2.2
b_r	0.86	0.03
b_{t1}	0.36	0.01
Covariance (b_r,b_{t1})	0.0046	–
Degrees of Freedom	30	–

[a] $y = a + b_r*x_r + b_{t1}*x_{t1} + b_{t2}*x_{t2}$
where r = Reference Material, t1 = Test Material 1

ANOVA

Source	SSE	DF	MSE
Fit	1029.17	2	514.58
Error	21.36	29	0.74
Total	1050.52	31	33.89

Statistic	Estimate
F	698.767
P	<0.001
Adjusted R^2	0.9783

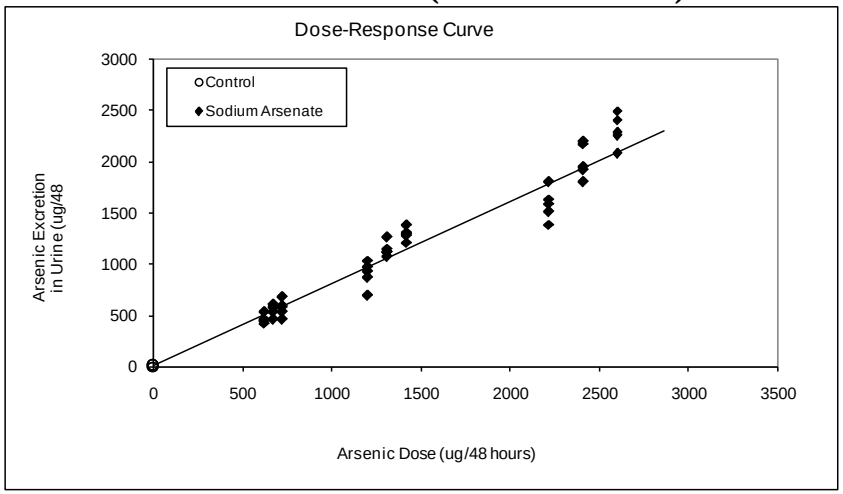
RBA and Uncertainty

	Test Material 1
RBA	0.42
Lower bound [c]	0.38
Upper bound [c]	0.46
Standard Error [c]	0.022

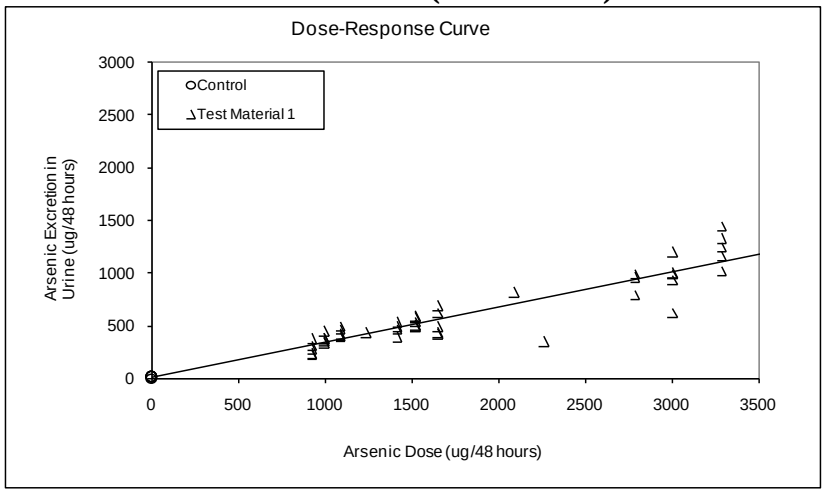
[c] 90% confidence interval calculated using Fieller's theorem

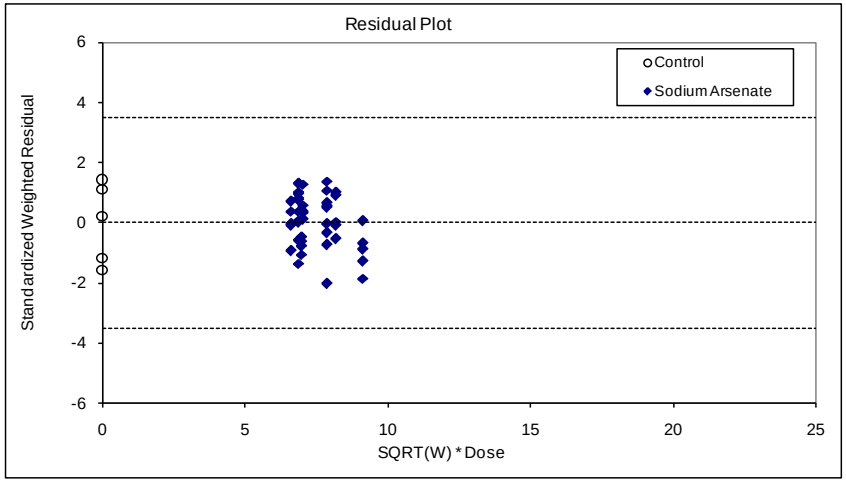
Figure 5-6. NIST 2710a Urinary Excretion of Arsenic: All Days

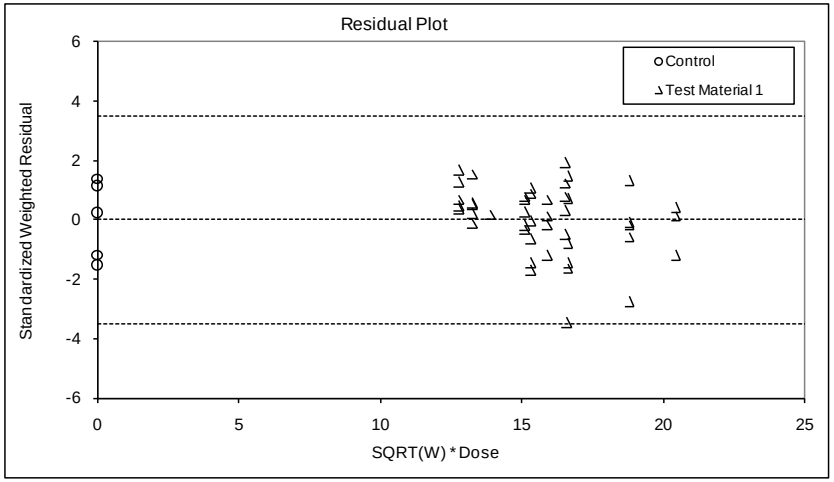
Reference Material (Sodium Arsenate)



Test Material 1 (NIST 2710a)







Summary of Fitting [a]

Parameter	Estimate	Standard Error
a	9.3	1.4
b_r	0.80	0.02
b_{t1}	0.34	0.01
Covariance (b_r,b_{t1})	0.0069	–
Degrees of Freedom	93	–

[a] $y = a + b_r*x_r + b_{t1}*x_{t1} + b_{t2}*x_{t2}$
where r = Reference Material, t1 = Test Material 1

ANOVA

Source	SSE	DF	MSE
Fit	2899.63	2	1449.82
Error	73.60	92	0.80
Total	2973.23	94	31.63

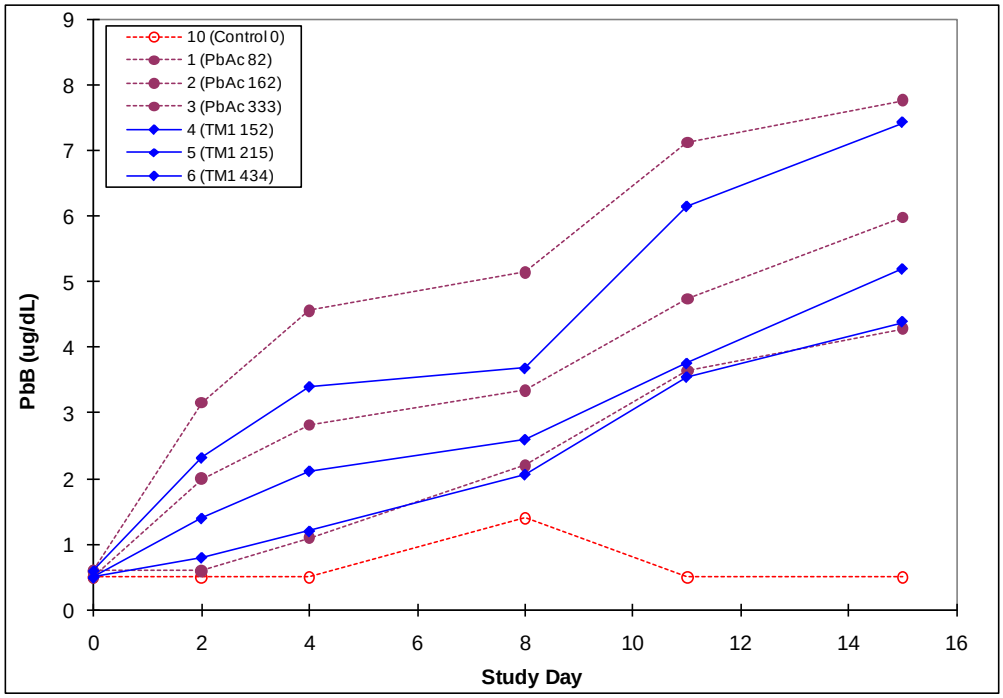
Statistic	Estimate
F	1812.252
P	<0.001
Adjusted R^2	0.9747

RBA and Uncertainty

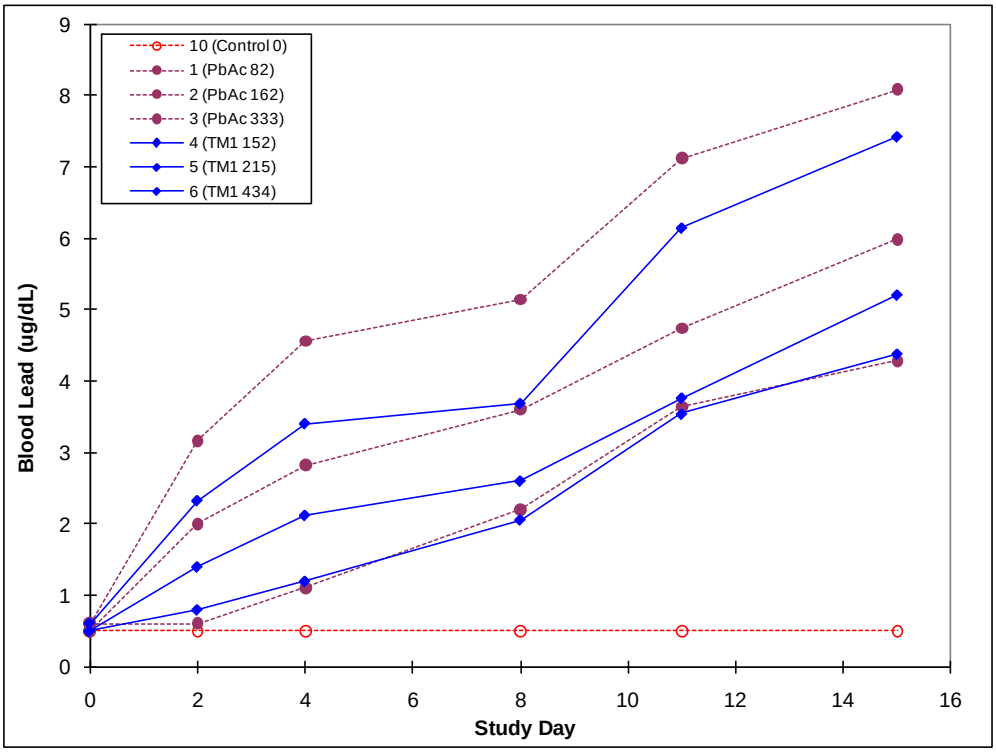
	Test Material 1
RBA	0.42
Lower bound [c]	0.40
Upper bound [c]	0.44
Standard Error [c]	0.014

[c] 90% confidence interval calculated using Fieller's theorem

Figure 5-7. Group Mean Blood Lead by Day



Panel A: All Data



Panel B: Outliers Excluded

Table 5-5. Blood Lead Outlier Identification

Group	Swine Number	Blood Lead by Day (µg/dL)					
		0	2	4	8	11	15
1	664	1	1	1	2	3	4.1
1	669	0.5	0.5	1	2	3.2	3.6
1	682	0.5	0.5	0.5	3	4.9	5.3
1	686	0.5	0.5	1	2	3	3.9
1	692	0.5	0.5	2	2	4.1	4.5
2	648	0.5	2	2	3.3	3.9	5.7
2	658	0.5	3	3	3.5	4.7	5.8
2	662	0.5	1	2	2[a]	4.3	6
2	676	0.5	3	4.1	4.8	6.3	7.3
2	690	0.5	1	3	3.1	4.5	5.1
3	665	0.5	2	2	3.7	6.9	7.5
3	666	1	5.1	7.2	6.2	8.2	9.8
3	667	0.5	3.6	4.4	4.7	6.3	8.5
3	681	0.5	2	3.4	5.8	8.1	7.7
3	691	0.5	3.1	5.8	5.3	6.1	5.3[b]
4	650	0.5	1	2	1	3	5.4
4	657	0.5	0.5	0.5	0.5	2	3.5
4	670	0.5	1	1	3.5	3.8	4.2
4	673	0.5	1	2	3.3	4.1	4
4	687	0.5	0.5	0.5	2	4.8	4.8
5	655	0.5	2	3	4	4.8	6.1
5	674	0.5	2	3.1	3	3.9	5.4
5	677	0.5	2	2	2	3.5	4.5
5	695	0.5	0.5	0.5	3	4.6	6.2
5	697	0.5	0.5	2	1	2	3.8
6	646	1	2	5.4	4.7	7.4	7.3
6	652	0.5	3	3.5	4.2	7.9	7.9
6	654	0.5	3.1	3.1	3.3	4.8	6.6
6	656	0.5	3	2	2	4.2	7.5
6	694	0.5	0.5	3	4.2	6.4	7.8
10	645	0.5	0.5	0.5	0.5	0.5	0.5
10	684	0.5	0.5	0.5	0.5	0.5	0.5
10	685	0.5	0.5	0.5	3.2[c]	0.5	0.5

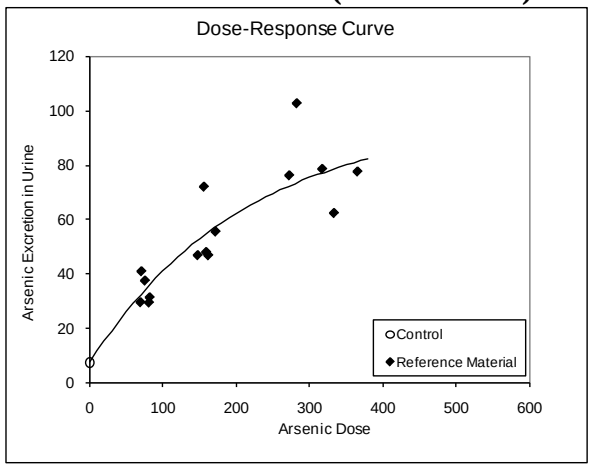
[a] Result was excluded as an outlier; a value of 3.3 was interpolated from previous and following results.
[b] Result was excluded as an outlier; a value of 6.9 was determined by taking the mean increase in daily blood lead levels.
[c] Result was excluded as an outlier; a value of 0.5 was interpolated from previous and following results.

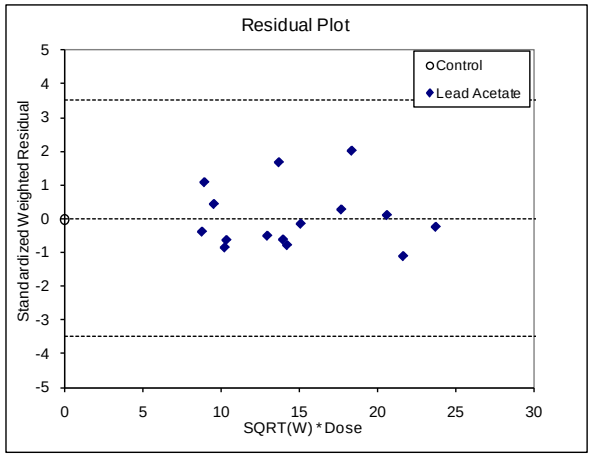
Table 5-6. Area Under Curve Determinations

Group	Swine Number	AUC (µg/dL-days) for Time Interval Shown					AUC Total (µg/dL-days)
		0–2	2–4	4–8	8–11	11–15	
1	664	2.00	2.00	6.00	7.50	14.20	31.70
1	669	1.00	1.50	6.00	7.80	13.60	29.90
1	682	1.00	1.00	7.00	11.85	20.40	41.25
1	686	1.00	1.50	6.00	7.50	13.80	29.80
1	692	1.00	2.50	8.00	9.15	17.20	37.85
2	648	2.50	4.00	10.60	10.80	19.20	47.10
2	658	3.50	6.00	13.00	12.30	21.00	55.80
2	662	1.50	3.00	10.62	11.42	20.60	47.14
2	676	3.50	7.10	17.80	16.65	27.20	72.25
2	690	1.50	4.00	12.20	11.40	19.20	48.30
3	665	2.50	4.00	11.40	15.90	28.80	62.60
3	666	6.10	12.30	26.80	21.60	36.00	102.80
3	667	4.10	8.00	18.20	16.50	29.60	76.40
3	681	2.50	5.40	18.40	20.85	31.60	78.75
3	691	3.60	8.90	22.20	17.10	26.00	77.80
4	650	1.50	3.00	6.00	6.00	16.80	33.30
4	657	1.00	1.00	2.00	3.75	11.00	18.75
4	670	1.50	2.00	9.00	10.95	16.00	39.45
4	673	1.50	3.00	10.60	11.10	16.20	42.40
4	687	1.00	1.00	5.00	10.20	19.20	36.40
5	655	2.50	5.00	14.00	13.20	21.80	56.50
5	674	2.50	5.10	12.20	10.35	18.60	48.75
5	677	2.50	4.00	8.00	8.25	16.00	38.75
5	695	1.00	1.00	7.00	11.40	21.60	42.00
5	697	1.00	2.50	6.00	4.50	11.60	25.60
6	646	3.00	7.40	20.20	18.15	29.40	78.15
6	652	3.50	6.50	15.40	18.15	31.60	75.15
6	654	3.60	6.20	12.80	12.15	22.80	57.55
6	656	3.50	5.00	8.00	9.30	23.40	49.20
6	694	1.00	3.50	14.40	15.90	28.40	63.20
10	645	1.00	1.00	2.00	1.50	2.00	7.50
10	684	1.00	1.00	2.00	1.50	2.00	7.50
10	685	1.00	1.00	2.00	1.50	2.00	7.50

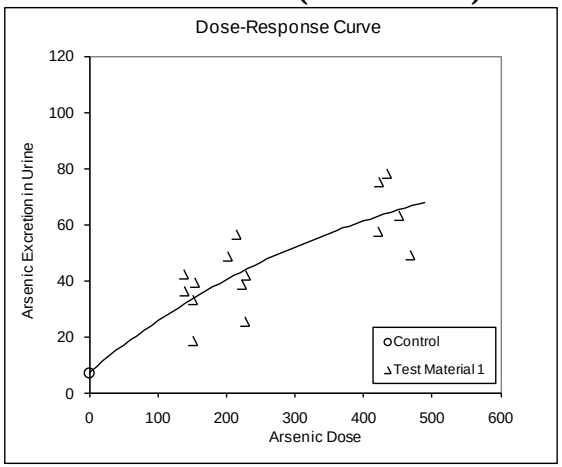
Figure 5-8. Blood Lead AUC Dose-Response

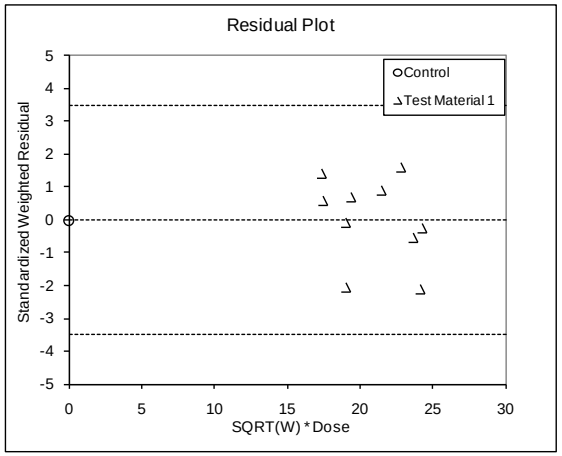
Reference Material (Lead Acetate)





Test Material 1 (NIST 2710a)





Summary of Fitting [a]

Parameter	Estimate	Standard Error
A	7.54E+00	1.38E+00
B	9.00E+01	1.91E+01
c_r	4.69E-03	1.63E-03
c_{t1}	2.28E-03	7.24E-04
Covariance (c_r,c_{t1})	0.9161	–
Degrees of Freedom	29	–

[a] $y = a + b\cdot(1-\exp(-c_r\cdot x_r)) + b\cdot(1-\exp(-c_{t1}\cdot x_{t1}))$
where r = Reference Material, t1 = Test Material 1

ANOVA

Source	MSE
Fit	154.08
Error	0.93
Total	10.50

Statistic	Estimate
F	166.559
P	<0.001
Adjusted R^2	0.9119

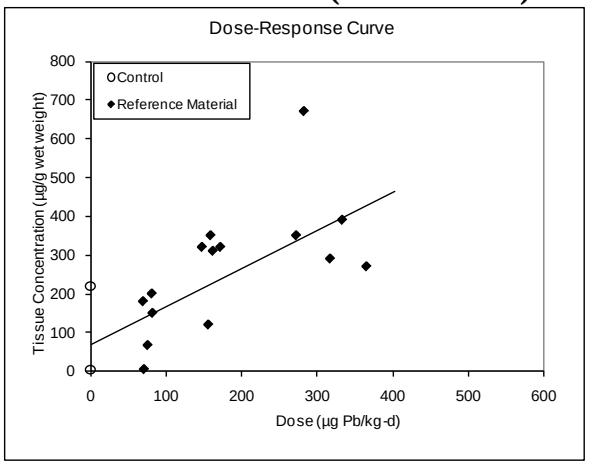
RBA and Uncertainty

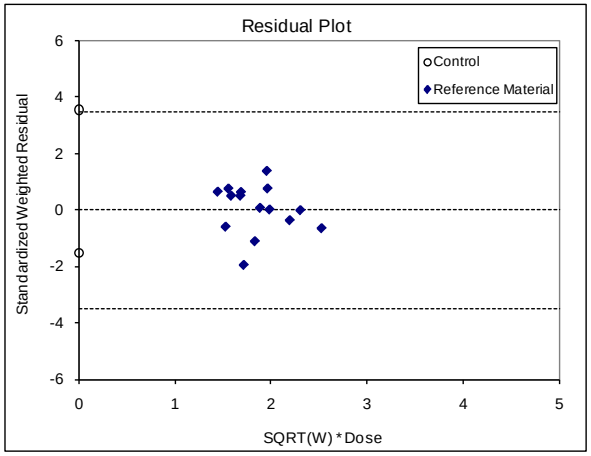
	Test Material 1
RBA	0.49
Lower bound [c]	0.38
Upper bound [c]	0.68
Standard Error [c]	0.065

[c] 90% confidence interval calculated using Fieller's theorem

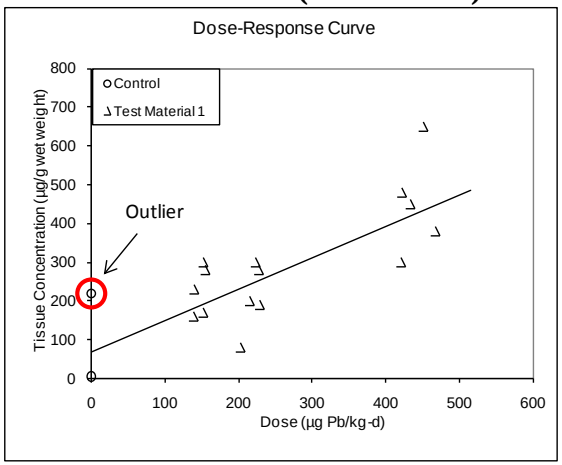
Figure 5-9a. Liver Lead Dose-Response (All Data)

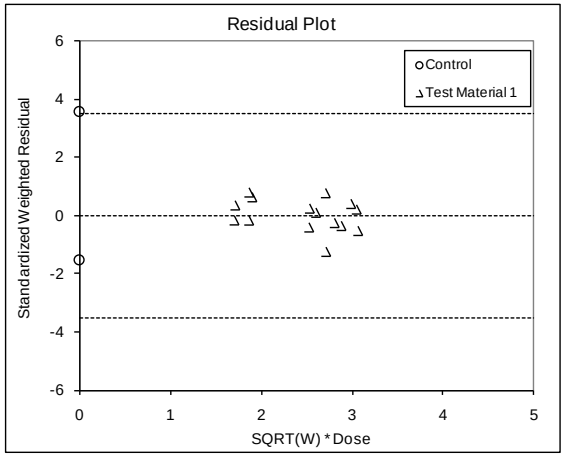
Reference Material (Lead Acetate)





Test Material 1 (NIST 2710a)





Summary of Fitting [a]

Parameter	Estimate	Standard Error
a	6.90E+01	2.24E+01
b_r	9.81E-01	2.80E-01
b_{t1}	8.09E-01	1.96E-01
Covariance (b_r,b_{t1})	0.2611	–
Degrees of Freedom	30	–

[a] $y = a + b_r \cdot x_r + b_{t1} \cdot x_{t1}$

ANOVA

Source	MSE
Fit	33.19
Error	2.85
Total	4.74

Statistic	Estimate
F	11.652
P	<0.001
Adjusted R^2	0.3997

RBA and Uncertainty

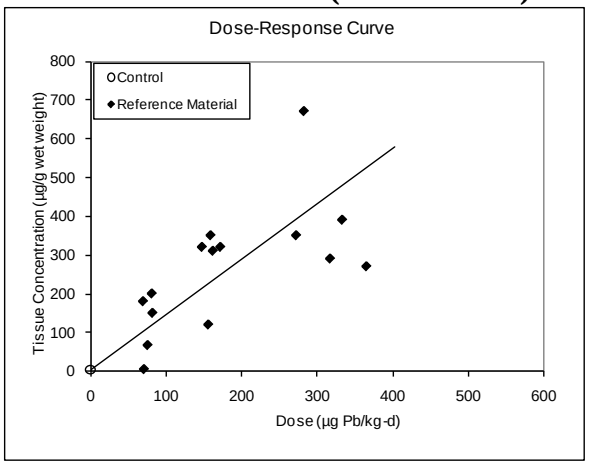
	Test Material 1
RBA	0.83
Lower bound [c]	0.47
Upper bound [c]	1.58
Standard Error [c]	0.266*

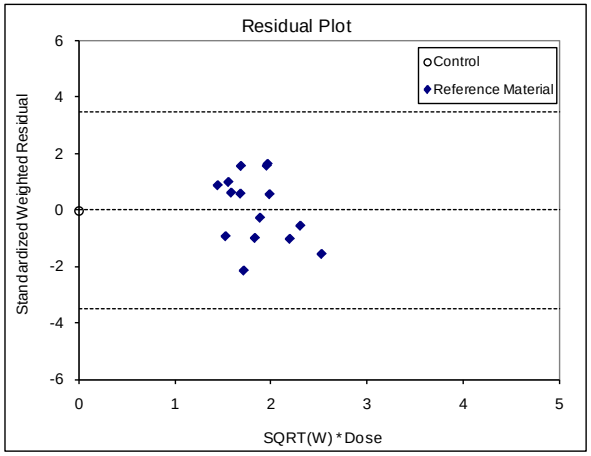
[c] 90% confidence interval calculated using Fieller's theorem

* g ≥0.05, estimate is uncertain

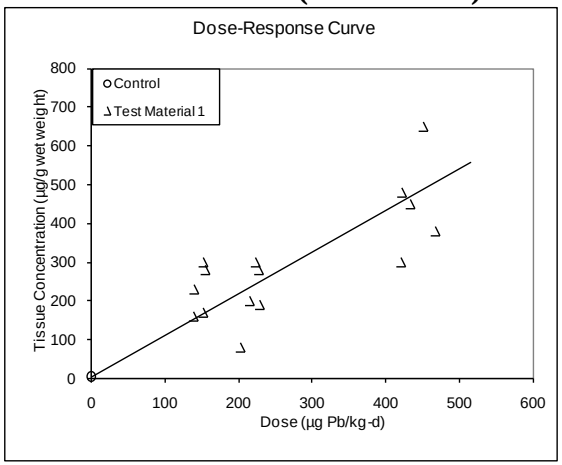
Figure 5-9b. Liver Lead Dose-Response (Outlier Excluded)

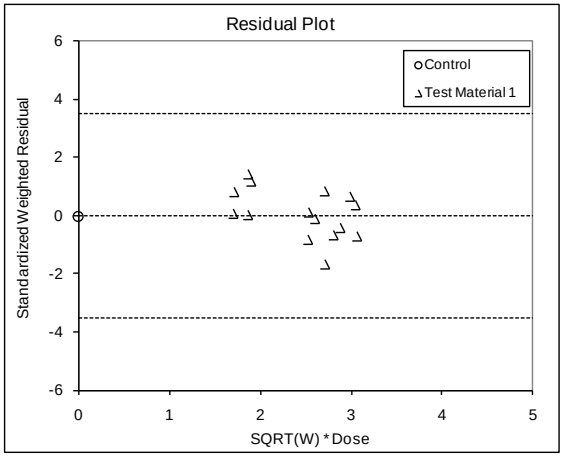
Reference Material (Lead Acetate)





Test Material 1 (NIST 2710a)





Summary of Fitting [a]

Parameter	Estimate	Standard Error
a	5.05E+00	1.23E+00
b_r	1.43E+00	1.62E-01
b_{t1}	1.07E+00	1.21E-01
Covariance (b_r,b_{t1})	0.0022	–
Degrees of Freedom	29	–

[a] $y = a + b_r \cdot x_r + b_{t1} \cdot x_{t1}$

ANOVA

Source	MSE
Fit	107.77
Error	1.38
Total	8.24

Statistic	Estimate
F	78.023
P	<0.001
Adjusted R^2	0.8325

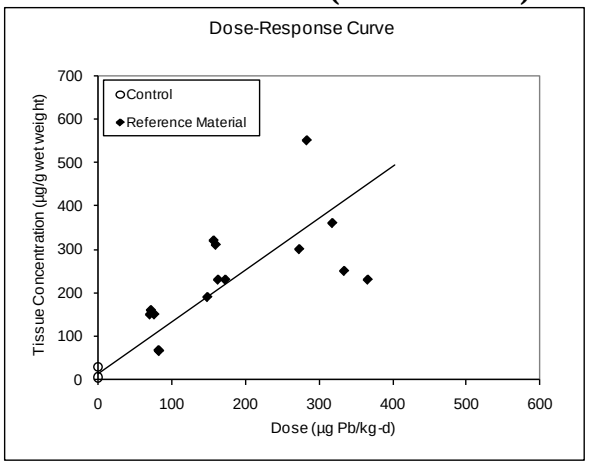
RBA and Uncertainty

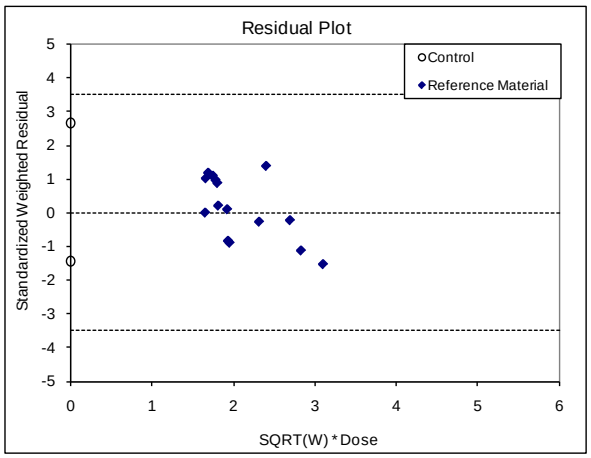
	Test Material 1
RBA	0.75
Lower bound [c]	0.57
Upper bound [c]	0.99
Standard Error [c]	0.120

[c] 90% confidence interval calculated using Fieller's theorem

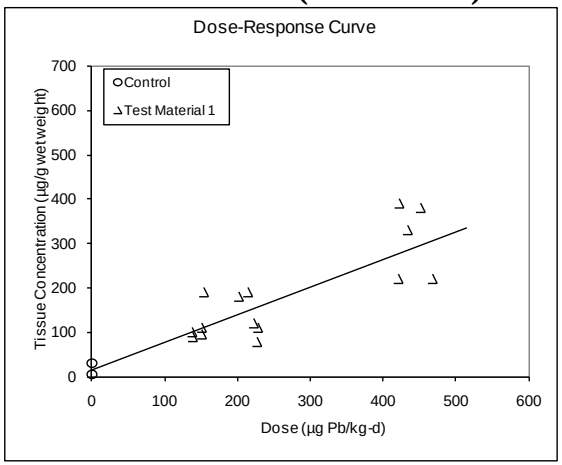
Figure 5-10. Kidney Lead Dose-Response

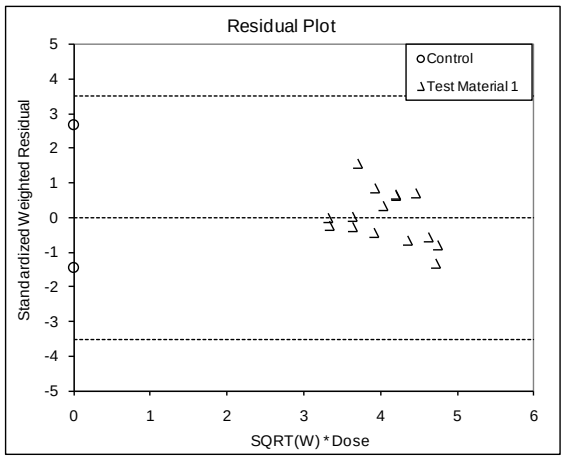
Reference Material (Lead Acetate)





Test Material 1 (NIST 2710a)





Summary of Fitting [a]

Parameter	Estimate	Standard Error
a	1.38E+01	3.60E+00
b_r	1.20E+00	1.54E-01
b_{t1}	6.27E-01	8.11E-02
Covariance (b_r,b_{t1})	0.0281	–
Degrees of Freedom	30	–

[a] $y = a + b_r \cdot x_r + b_{t1} \cdot x_{t1}$

ANOVA

Source	MSE
Fit	92.41
Error	1.58
Total	7.26

Statistic	Estimate
F	58.434
P	<0.001
Adjusted R^2	0.7821

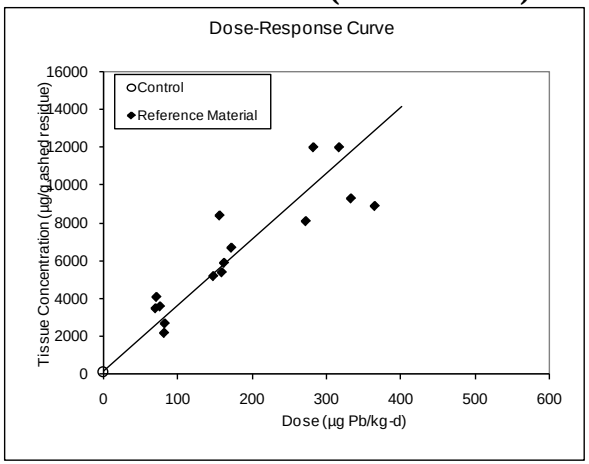
RBA and Uncertainty

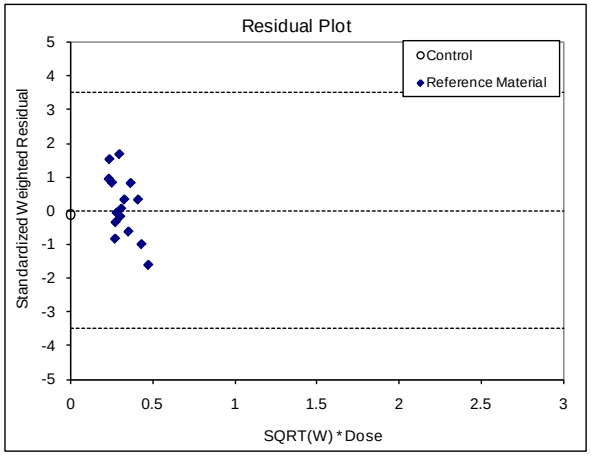
	Test Material 1
RBA	0.52
Lower bound [c]	0.38
Upper bound [c]	0.71
Standard Error [c]	0.094

[c] 90% confidence interval calculated using Fieller's theorem

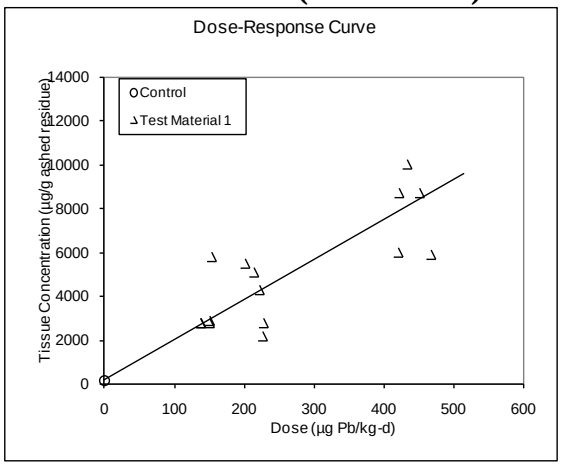
Figure 5-11. Femur Lead Dose-Response

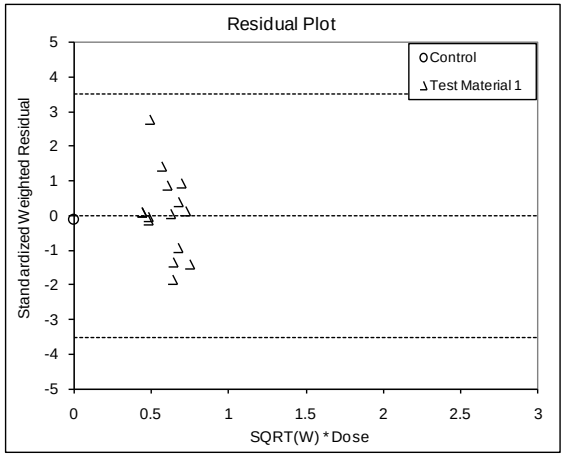
Reference Material (Lead Acetate)





Test Material 1 (NIST 2710a)





Summary of Fitting [a]

Parameter	Estimate	Standard Error
a	1.58E+02	4.52E+01
b_r	3.49E+01	2.61E+00
b_{t1}	1.83E+01	1.43E+00
Covariance (b_r,b_{t1})	0.0130	–
Degrees of Freedom	30	–

[a] $y = a + b_r \cdot x_r + b_{t1} \cdot x_{t1}$

ANOVA

Source	MSE
Fit	1861.27
Error	11.03
Total	126.67

Statistic	Estimate
F	168.808
P	<0.001
Adjusted R^2	0.9130

RBA and Uncertainty

	Test Material 1
RBA	0.53
Lower bound [c]	0.44
Upper bound [c]	0.63
Standard Error [c]	0.057

[c] 90% confidence interval calculated using Fieller's theorem

5.6 Calculated RBA Values

Estimated arsenic and lead RBA values (mean and 90% confidence interval) are shown in Tables 5-7 and 5-8. The best fit point estimate arsenic and lead RBAs for NIST 2710a soil are 42% and 57% for arsenic and lead, respectively.

Table 5-7. Estimated Arsenic RBA for NIST 2710a Soil

Urine Collection Period (days)	Estimated RBA (90% Confidence Interval)
Days 6/7	0.43 (0.39–0.47)
Days 9/10	0.41 (0.37–0.44)
Days 12/13	0.42 (0.38–0.46)
All Days	0.42 (0.40–0.44)

Table 5-8. Estimated Lead RBA for NIST 2710a Soil

Endpoint	Estimated RBA (90% Confidence Interval)
Blood lead AUC	0.49 (0.38–0.68)
Liver lead	0.75 (0.57–0.99)
Kidney lead	0.52 (0.38–0.71)
Femur lead	0.53 (0.44–0.63)
Point estimate	0.57 (0.39–0.84)

5.7 Uncertainty

The bioavailability estimates above are subject to uncertainty that arises from several different sources. One source of uncertainty is the inherent biological variability between different animals in a dose group, which in turn causes variability in the amount of arsenic or lead absorbed by the exposed animals. The between-animal variability results in statistical uncertainty in the best fit dose-response curves and, hence, uncertainty in the calculated values of RBA. Such statistical uncertainty is accounted for by the statistical models used above and is characterized by the uncertainty range around the RBA estimates.

However, there is also uncertainty in the extrapolation of RBA values measured in juvenile swine to young children or adults, and this uncertainty is not included in the statistical confidence bounds above. Even though the immature swine is believed to be a useful and meaningful animal model for gastrointestinal absorption in humans, it is possible that there are differences in physiological parameters that may influence RBA. Therefore, RBA values in swine may not be identical to values in children. In addition, RBA may depend on the amount and type of food in the stomach, since the presence of food can influence stomach pH, holding time, and possibly other factors that may influence solubilization and absorption of arsenic or lead. RBA values measured in this study are based on animals that have little or no food in their

stomach at the time of exposure and, hence, are likely to yield high-end values of RBA. Thus, these RBA values may be somewhat conservative for humans who ingest the site soils along with food. The magnitude of this bias is not known.

6.0 REFERENCES

Canavos, C. G. 1984. Applied Probability and Statistical Methods. Little, Brown and Co., Boston, MA.

Casteel, S. W., R. P. Cowart, C. P. Weis, G. M. Henningsen, E. Hoffman, W. J. Brattin, M. F. Starost, J. T. Payne, S. L. Stockham, S. V. Becker, and J. R. Turk. 1996. A swine model for determining the bioavailability of lead from contaminated media. In: Advances in Swine in Biomedical Research. Tumbleson, M.E. and L.B. Schook (eds.), Volume 2, Plenum Press, New York, NY. pp. 637–46.

Draper, N. R. and H. Smith. 1998. Applied Regression Analysis. 3rd edition. John Wiley & Sons, New York, NY.

Finney, D. J. 1978. Statistical Method in Biological Assay. 3rd edition. Charles Griffin and Co., London, England.

Gibaldi, M. and D. Perrier. 1982. Pharmacokinetics. 2nd edition. Marcel Dekker, Inc., New York, NY. pp. 294–297.

Goodman, A. G., T. W. Rall, A. S. Nies, and P. Taylor. 1990. The Pharmacological Basis of Therapeutics. 8th edition. Pergamon Press, Inc., Elmsford, NY. pp. 5–21.

Klaassen, C. D., M. O. Amdur, and J. Doull (eds.). 1996. Cassarett and Doull's Toxicology: The Basic Science of Poisons. McGraw-Hill, Inc., New York, NY. p. 190.

LaVelle, J. M, R. H. Poppenga, B. J. Thacker, J. P. Giesy, C. Weis, R. Othoudt, and C. Vandervoort. 1991. Bioavailability of lead in mining wastes: An oral intubation study of young swine. *Chem. Spec. Bioavail.* 3: 105–111.

NIST. 2009. Certificate of Analysis, Standard Reference Material® 2710a – Montana I Soil, Highly Elevated Trace Element Concentrations. National Institute of Standards & Technology, Gaithersburg, MD. Certificate Issue Date: April 9, 2009.

NRC. 1988. Nutrient Requirements of Swine. A Report of the Committee on Animal Nutrition. National Research Council. National Academy Press, Washington, DC.

USEPA. 1991. Technical support document on lead. United States Environmental Protection Agency, Environmental Criteria and Assessment Office. ECAO-CIN-757.

USEPA. 2003. Recommendations of the Technical Review Workgroup for Lead for an approach to assessing risks associated with adult exposures to lead in soil. United States Environmental Protection Agency Technical Review Workgroup for Lead. OSWER 9285.7-54, EPA-540-R-03-001. January.

USEPA. 2007. Estimation of Relative Bioavailability of Lead in Soil and Soil-Like Materials by *In Vivo* and *In Vitro* Methods. OSWER9285.7-77. U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response, Washington DC.

Weis, C. P. and J. M. LaVelle. 1991. Characteristics to consider when choosing an animal model for the study of lead bioavailability. In: The Proceedings of the International Symposium on the Bioavailability and Dietary Uptake of Lead. *Science Technology Letters* 3:113–119.

Weis, C. P., Henningsen, G. M., Poppenga, R. H., Thacker, B. J. 1993. Pharmacokinetics of lead in blood of immature swine following acute oral and intravenous exposure [Abstract]. *Toxicologist* 13: 175.

APPENDIX A: GROUP ASSIGNMENTS FOR THE NIST 2710A ARSENIC AND LEAD RBA STUDY – DECEMBER 2009

Table A-1. Group Assignments for the NIST 2710a Arsenic and Lead RBA Study – December 2009

Swine Number	Group	Treatment	Target Arsenic Dose (µg/kg BW-day)	Target Lead Dose (µg/kg BW-day)
664 669 682 686 692	1	PbAc	0	75
648 658 662 676 690	2	PbAc	0	150
665 666 667 681 691	3	PbAc	0	300
650 657 670 673 687	4	TM1	40	143
655 674 677 695 697	5	TM1	60	215
646 652 654 656 694	6	TM1	120	430
668 671 672 679 688	7	NaAs	25	0
647 651 659 663 683	8	NaAs	50	0
649 678 680 689 693	9	NaAs	100	0
645 684 685	10	Control	0	0

APPENDIX B: BODY WEIGHTS

Table B-1. Body Weights

Group	Swine No.	Weight (kg)													
		Day -5 12/2/09	Group MBW	Day -1 12/6/09	Group MBW	Day 2 12/9/09	Group MBW	Day 5 12/12/09	Group MBW	Day 8 12/15/09	Group MBW	Day 11 12/18/09	Group MBW	Day 14 12/21/09	Group MBW
1 PbAc 75	664	7.8		8.9		9.5		10.3		11.0		12.3		13.6	
	669	9.8		10.5		11.2		12.1		13.3		14.4		15.5	
	682	9.2		9.3		10.4		11.1		12.1		13.2		14.6	
	686	8.8		8.7		9.5		10.5		11.3		12.3		13.9	
	692	8.7	8.86±0.73	9.3	9.34±0.70	10.1	10.14±0.71	11.3	11.06±0.71	12.3	12.00±0.91	13.2	13.08±0.86	14.7	14.46±0.74
2 PbAc 150	648	8.4		8.9		9.6		10.7		12.1		13.1		14.2	
	658	8.2		9.2		9.3		10.2		11.1		11.8		13.5	
	662	8.9		9.8		10.9		12.0		12.9		14.2		15.3	
	676	8.7		9.4		10.4		11.1		12.3		13.3		14.7	
	690	8.3	8.50±0.29	9.2	9.30±0.33	10.0	10.04±0.63	11.0	11.00±0.66	12.0	12.08±0.65	13.3	13.14±0.86	14.4	14.42±0.66
3 PbAc 300	665	7.8		8.1		9.1		10.0		11.0		11.6		12.5	
	666	9.1		9.6		10.7		11.5		12.8		14.0		15.1	
	667	9.6		10.4		11.2		12.1		13.3		14.0		15.6	
	681	8.1		8.3		9.3		10.5		11.3		12.4		14.0	
	691	8.0	8.52±0.79	8.6	9.00±0.97	8.8	9.82±1.06	9.2	10.66±1.16	9.6	11.60±1.48	10.0	12.40±1.70	11.2	13.68±1.83
4 TM1 40 (As)	650	7.7		8.6		9.4		10.2		11.1		12.4		13.8	
	657	7.9		8.2		9.2		10.1		10.9		12.2		13.6	
	670	7.7		8.1		9.4		10.1		11.0		11.9		13.5	
	673	9.3		9.9		10.4		11.3		12.2		13.3		14.6	
	687	9.2	8.36±0.82	9.7	8.90±0.85	10.3	9.74±0.56	10.8	10.50±0.53	11.9	11.42±0.59	13.0	12.56±0.58	14.5	14.00±0.51
5 TM1 60 (As)	655	8.5		9.5		10.0		11.0		12.2		13.2		14.8	
	674	8.9		9.7		10.7		12.1		12.7		13.9		15.7	
	677	8.9		8.5		9.8		10.8		11.6		12.9		14.1	
	695	8.0		8.4		9.0		9.7		10.5		11.5		12.8	
	697	8.7	8.60±0.37	9.7	9.16±0.65	9.8	9.86±0.61	10.2	10.76±0.91	11.3	11.66±0.84	12.3	12.76±0.91	13.8	14.24±1.09

Table B-1. Body Weights

Group	Swine No.	Weight (kg)													
		Day -5 12/2/09	Group MBW	Day -1 12/6/09	Group MBW	Day 2 12/9/09	Group MBW	Day 5 12/12/09	Group MBW	Day 8 12/15/09	Group MBW	Day 11 12/18/09	Group MBW	Day 14 12/21/09	Group MBW
6 TM1 120 (As)	646	8.3		8.8		9.6		10.4		11.4		12.5		14.2	
	652	8.9		9.5		10.2		11.1		12.1		13.3		14.9	
	654	8.8		9.3		10.2		11.2		12.3		13.3		14.8	
	656	8.2		8.9		9.3		10.0		10.4		11.7		13.2	
	694	8.5	8.54±0.30	8.6	9.02±0.37	9.5	9.76±0.42	10.3	10.60±0.52	11.4	11.52±0.75	12.6	12.68±0.66	14.0	14.22±0.69
7 NaAs 25	668	8.5		9.4		10.3		11.5		12.5		13.3		15.1	
	671	8.3		8.7		9.6		10.7		11.8		12.8		14.4	
	672	9.2		10.0		10.4		11.3		12.0		13.0		14.1	
	679	8.9		10.1		10.6		11.7		12.7		13.7		15.3	
	688	9.1	8.80±0.39	10.1	9.66±0.61	11.1	10.40±0.54	12.1	11.46±0.52	13.3	12.46±0.59	14.3	13.42±0.60	15.6	14.90±0.63
8 NaAs 50	647	8.1		8.7		9.3		10.1		11.2		12.2		13.9	
	651	8.8		9.8		10.7		11.6		12.7		13.9		15.4	
	659	8.1		9.4		9.9		10.9		12.1		13.0		14.6	
	663	7.9		9.2		10.1		11.0		12.0		13.1		15.0	
	683	9.0	8.38±0.49	9.7	9.36±0.44	10.6	10.12±0.57	11.5	11.02±0.60	12.5	12.10±0.58	13.6	13.16±0.65	15.1	14.80±0.58
9 NaAs 100	649	8.0		8.2		8.8		9.4		10.4		11.2		13.1	
	678	7.9		8.4		9.4		10.0		11.1		12.4		13.7	
	680	8.3		7.9		8.6		8.7		9.6		10.2		11.3	
	689	8.9		9.3		9.7		10.8		11.6		12.5		14.0	
	693	8.9	8.40±0.48	9.7	8.70±0.76	10.6	9.42±0.79	11.4	10.06±1.08	12.5	11.04±1.11	13.6	11.98±1.31	15.1	13.44±1.40
10 Control 0	645	8.7		9.0		9.3		10.0		10.9		11.7		13.1	
	684	8.3		8.6		10.0		10.4		11.4		12.6		14.3	
	685	8.1	8.37±0.31	8.9	8.83±0.21	9.9	9.73±0.38	10.8	10.40±0.40	11.3	11.20±0.26	12.5	12.27±0.49	14.1	13.83±0.64

Group MBW = means and standard deviations of each group's body weight.

APPENDIX C: TYPICAL FEED COMPOSITION

Appendix C. Typical Feed Composition

Purina TestDiet® 5TXP: Porcine Grower Purified Diet with Low Lead[a]

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

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

[a] This special purified diet was originally developed for lead RBA studies.
[b] Based on the latest ingredient analysis information. Since nutrient composition of natural ingredients varies, analysis will differ accordingly. Nutrients expressed as percent of ration on an arsenic fed basis except where otherwise indicated.
[c] Energy (kcal/gm) – sum of decimal fractions of protein, fat, and carbohydrate × 4, 9, and 4 kcal/g, respectively.

APPENDIX D: URINARY ARSENIC ANALYTICAL RESULTS AND URINE VOLUMES FOR NIST 2710A STUDY SAMPLES

Table D-1. Urinary Arsenic Analytical Results and Urine Volumes for NIST 2710a Study Samples

Group	Material	Collection Period (days)	Sample ID	Swine Number	Urinary Arsenic Concentration (µg/L)	Urine Volume (mL)
4	TM1	6/7	NISTa-573	650	69	3320
			NISTa-618	657	59	4090
			NISTa-627	670	28	13380
			NISTa-594	673	31	10200
			NISTa-608	687	34	8350
		9/10	NISTa-646	650	120	3140
			NISTa-667	657	38	10000
			NISTa-642	670	27	12420
			NISTa-666	687	36	12380
			NISTa-669	673	54	6640
		12/13	NISTa-719	650	79	5940
			NISTa-732	657	44	11350
			NISTa-721	670	29	14675
			NISTa-729	673	39	10320
			NISTa-695	687	49	8360
5	TM1	6/7	NISTa-605	655	120	4480
			NISTa-592	674	25	18840
			NISTa-596	677	190	2600
			NISTa-619	695	140	3140
			NISTa-607	697	49	7960
		9/10	NISTa-660	655	140	4220
			NISTa-658	674	60	8200
			NISTa-653	677	150	3880
			NISTa-638	695	160	3140
			NISTa-652	697	69	7860
		12/13	NISTa-694	695	79	5540
			NISTa-733	677	130	4820
			NISTa-736	697	28	15065
			NISTa-722	655	140	4960
			NISTa-710	674	66	7500
6	TM1	6/7	NISTa-611	656	60	5880
			NISTa-600	646	130	6300
			NISTa-621	654	76	10520
			NISTa-583	694	88	10940
			NISTa-599	652	210	4740
		9/10	NISTa-639	646	130	7660
			NISTa-649	652	471	2560
			NISTa-659	654	270	3480
			NISTa-631	656	110	5720
			NISTa-681	694	74	13600
		12/13	NISTa-728	646	180	7400
			NISTa-693	652	464	2700
			NISTa-715	654	240	4860
			NISTa-731	656	160	6400
			NISTa-708	694	93	15585

Table D-1. Urinary Arsenic Analytical Results and Urine Volumes for NIST 2710a Study Samples

Group	Material	Collection Period (days)	Sample ID	Swine Number	Urinary Arsenic Concentration (µg/L)	Urine Volume (mL)
7	NaAs	6/7	NISTa-576	668	210	2580
			NISTa-580	671	110	4280
			NISTa-597	672	130	3280
			NISTa-595	679	95	4720
			NISTa-586	688	95	4820
		9/10	NISTa-663	671	110	4920
			NISTa-628	672	110	4220
			NISTa-650	668	190	3220
			NISTa-680	679	69	8460
			NISTa-641	688	96	5680
		12/13	NISTa-702	668	200	3420
			NISTa-690	671	81	7360
			NISTa-724	672	100	5340
			NISTa-720	679	97	6060
			NISTa-716	688	97	4760
8	NaAs	6/7	NISTa-622	663	190	5480
			NISTa-591	683	230	4045
			NISTa-624	647	85	8260
			NISTa-612	651	170	5720
			NISTa-623	659	300	2920
		9/10	NISTa-647	647	57	22280
			NISTa-634	651	220	5260
			NISTa-635	659	360	3100
			NISTa-630	663	250	4320
			NISTa-668	683	250	4480
		12/13	NISTa-712	651	230	5280
			NISTa-697	647	93	13940
			NISTa-704	659	531	2420
			NISTa-711	663	200	6900
			NISTa-707	683	140	9420
9	NaAs	6/7	NISTa-581	678	100	16360
			NISTa-572	680	380	4740
			NISTa-616	689	240	6640
			NISTa-582	693	450	3360
			NISTa-606	649	310	4480
		9/10	NISTa-636	649	573	3840
			NISTa-656	678	150	12000
			NISTa-655	680	440	4440
			NISTa-665	693	558	3440
			NISTa-675	689	220	9900
		12/13	NISTa-700	649	469	4820
			NISTa-709	678	170	13460
			NISTa-730	680	421	4940
			NISTa-738	689	160	15060
			NISTa-734	693	550	4540

Table D-1. Urinary Arsenic Analytical Results and Urine Volumes for NIST 2710a Study Samples

Group	Material	Collection Period (days)	Sample ID	Swine Number	Urinary Arsenic Concentration (µg/L)	Urine Volume (mL)
10	Control	6/7	NISTa-617	684	1	15900
			NISTa-609	685	2	5020
			NISTa-604	645	0.5	20820
		9/10	NISTa-651	645	2	17000
			NISTa-657	685	3	4540
			NISTa-676	684	0.5	8920
		12/13	NISTa-713	645	1	25930
			NISTa-698	684	1	5610
			NISTa-723	685	2	6400

APPENDIX E: LEAD ANALYTICAL RESULTS FOR NIST 2710A STUDY SAMPLES

Table E-1. Lead Analytical Results for NIST 2710a Study Samples

Group	Material	Sample ID	Collection Day	Swine Number	Sample Type	Lead Concentration	Units
1	PbAc	NISTa-126	0	664	Blood	10	ng/mL
1	PbAc	NISTa-123	0	669	Blood	<10	ng/mL
1	PbAc	NISTa-140	0	682	Blood	<10	ng/mL
1	PbAc	NISTa-141	0	686	Blood	<10	ng/mL
1	PbAc	NISTa-131	0	692	Blood	<10	ng/mL
1	PbAc	NISTa-196	2	664	Blood	10	ng/mL
1	PbAc	NISTa-179	2	669	Blood	<10	ng/mL
1	PbAc	NISTa-195	2	682	Blood	<10	ng/mL
1	PbAc	NISTa-199	2	686	Blood	<10	ng/mL
1	PbAc	NISTa-154	2	692	Blood	<10	ng/mL
1	PbAc	NISTa-209	4	664	Blood	10	ng/mL
1	PbAc	NISTa-259	4	669	Blood	10	ng/mL
1	PbAc	NISTa-226	4	682	Blood	<10	ng/mL
1	PbAc	NISTa-248	4	686	Blood	10	ng/mL
1	PbAc	NISTa-212	4	692	Blood	20	ng/mL
1	PbAc	NISTa-265	8	664	Blood	20	ng/mL
1	PbAc	NISTa-302	8	669	Blood	20	ng/mL
1	PbAc	NISTa-304	8	682	Blood	30	ng/mL
1	PbAc	NISTa-312	8	686	Blood	20	ng/mL
1	PbAc	NISTa-282	8	692	Blood	20	ng/mL
1	PbAc	NISTa-282	8	692	Blood	3000	ng/mL
1	PbAc	NISTa-336	11	664	Blood	30	ng/mL
1	PbAc	NISTa-336	11	664	Blood	31	ng/mL
1	PbAc	NISTa-318	11	669	Blood	32	ng/mL
1	PbAc	NISTa-364	11	682	Blood	49	ng/mL
1	PbAc	NISTa-319	11	686	Blood	30	ng/mL
1	PbAc	NISTa-319	11	686	Blood	30	ng/mL
1	PbAc	NISTa-340	11	692	Blood	41	ng/mL
1	PbAc	NISTa-399	15	664	Blood	41	ng/mL
1	PbAc	NISTa-413	15	669	Blood	36	ng/mL
1	PbAc	NISTa-383	15	682	Blood	53	ng/mL
1	PbAc	NISTa-388	15	686	Blood	39	ng/mL
1	PbAc	NISTa-418	15	692	Blood	45	ng/mL
2	PbAc	NISTa-113	0	648	Blood	<10	ng/mL
2	PbAc	NISTa-146	0	658	Blood	<10	ng/mL
2	PbAc	NISTa-118	0	662	Blood	<10	ng/mL
2	PbAc	NISTa-149	0	676	Blood	<10	ng/mL
2	PbAc	NISTa-119	0	690	Blood	<10	ng/mL
2	PbAc	NISTa-186	2	648	Blood	20	ng/mL
2	PbAc	NISTa-205	2	658	Blood	30	ng/mL
2	PbAc	NISTa-163	2	662	Blood	10	ng/mL
2	PbAc	NISTa-163	2	662	Blood	2500	ng/mL
2	PbAc	NISTa-178	2	676	Blood	30	ng/mL
2	PbAc	NISTa-159	2	690	Blood	10	ng/mL
2	PbAc	NISTa-250	4	648	Blood	20	ng/mL
2	PbAc	NISTa-250	4	648	Blood	20	ng/mL
2	PbAc	NISTa-213	4	658	Blood	30	ng/mL
2	PbAc	NISTa-213	4	658	Blood	2500	ng/mL
2	PbAc	NISTa-249	4	662	Blood	20	ng/mL

Table E-1. Lead Analytical Results for NIST 2710a Study Samples

Group	Material	Sample ID	Collection Day	Swine Number	Sample Type	Lead Concentration	Units
2	PbAc	NISTa-247	4	676	Blood	41	ng/mL
2	PbAc	NISTa-234	4	690	Blood	30	ng/mL
2	PbAc	NISTa-264	8	648	Blood	33	ng/mL
2	PbAc	NISTa-261	8	658	Blood	35	ng/mL
2	PbAc	NISTa-276	8	662	Blood	20	ng/mL
2	PbAc	NISTa-266	8	676	Blood	48	ng/mL
2	PbAc	NISTa-269	8	690	Blood	31	ng/mL
2	PbAc	NISTa-322	11	648	Blood	39	ng/mL
2	PbAc	NISTa-365	11	658	Blood	49	ng/mL
2	PbAc	NISTa-365	11	658	Blood	47	ng/mL
2	PbAc	NISTa-314	11	662	Blood	43	ng/mL
2	PbAc	NISTa-346	11	676	Blood	63	ng/mL
2	PbAc	NISTa-348	11	690	Blood	45	ng/mL
2	PbAc	NISTa-369	15	648	Blood	57	ng/mL
2	PbAc	NISTa-379	15	658	Blood	58	ng/mL
2	PbAc	NISTa-405	15	662	Blood	60	ng/mL
2	PbAc	NISTa-389	15	676	Blood	73	ng/mL
2	PbAc	NISTa-372	15	690	Blood	51	ng/mL
2	PbAc	NISTa-372	15	690	Blood	2550	ng/mL
3	PbAc	NISTa-152	0	665	Blood	<10	ng/mL
3	PbAc	NISTa-106	0	666	Blood	10	ng/mL
3	PbAc	NISTa-106	0	666	Blood	<10	ng/mL
3	PbAc	NISTa-129	0	667	Blood	<10	ng/mL
3	PbAc	NISTa-114	0	681	Blood	<10	ng/mL
3	PbAc	NISTa-108	0	691	Blood	<10	ng/mL
3	PbAc	NISTa-166	2	665	Blood	20	ng/mL
3	PbAc	NISTa-191	2	666	Blood	51	ng/mL
3	PbAc	NISTa-176	2	667	Blood	36	ng/mL
3	PbAc	NISTa-164	2	681	Blood	20	ng/mL
3	PbAc	NISTa-180	2	691	Blood	31	ng/mL
3	PbAc	NISTa-207	4	665	Blood	20	ng/mL
3	PbAc	NISTa-207	4	665	Blood	30	ng/mL
3	PbAc	NISTa-253	4	666	Blood	72	ng/mL
3	PbAc	NISTa-208	4	667	Blood	44	ng/mL
3	PbAc	NISTa-218	4	681	Blood	34	ng/mL
3	PbAc	NISTa-221	4	691	Blood	56	ng/mL
3	PbAc	NISTa-221	4	691	Blood	58	ng/mL
3	PbAc	NISTa-279	8	665	Blood	37	ng/mL
3	PbAc	NISTa-299	8	666	Blood	62	ng/mL
3	PbAc	NISTa-283	8	667	Blood	47	ng/mL
3	PbAc	NISTa-263	8	681	Blood	58	ng/mL
3	PbAc	NISTa-263	8	681	Blood	58	ng/mL
3	PbAc	NISTa-275	8	691	Blood	53	ng/mL
3	PbAc	NISTa-275	8	691	Blood	51	ng/mL
3	PbAc	NISTa-356	11	665	Blood	69	ng/mL
3	PbAc	NISTa-327	11	666	Blood	82	ng/mL
3	PbAc	NISTa-327	11	666	Blood	2400	ng/mL
3	PbAc	NISTa-343	11	667	Blood	63	ng/mL
3	PbAc	NISTa-343	11	667	Blood	2630	ng/mL

Table E-1. Lead Analytical Results for NIST 2710a Study Samples

Group	Material	Sample ID	Collection Day	Swine Number	Sample Type	Lead Concentration	Units
3	PbAc	NISTa-345	11	681	Blood	81	ng/mL
3	PbAc	NISTa-313	11	691	Blood	61	ng/mL
3	PbAc	NISTa-313	11	691	Blood	2400	ng/mL
3	PbAc	NISTa-382	15	665	Blood	75	ng/mL
3	PbAc	NISTa-396	15	666	Blood	98	ng/mL
3	PbAc	NISTa-400	15	667	Blood	85	ng/mL
3	PbAc	NISTa-400	15	667	Blood	2630	ng/mL
3	PbAc	NISTa-384	15	681	Blood	77	ng/mL
3	PbAc	NISTa-403	15	691	Blood	53	ng/mL
4	TM1	NISTa-134	0	650	Blood	<10	ng/mL
4	TM1	NISTa-102	0	657	Blood	<10	ng/mL
4	TM1	NISTa-116	0	670	Blood	<10	ng/mL
4	TM1	NISTa-144	0	673	Blood	<10	ng/mL
4	TM1	NISTa-144	0	673	Blood	2500	ng/mL
4	TM1	NISTa-144	0	673	Blood	<10	ng/mL
4	TM1	NISTa-147	0	687	Blood	<10	ng/mL
4	TM1	NISTa-206	2	650	Blood	10	ng/mL
4	TM1	NISTa-189	2	657	Blood	<10	ng/mL
4	TM1	NISTa-171	2	670	Blood	10	ng/mL
4	TM1	NISTa-169	2	673	Blood	10	ng/mL
4	TM1	NISTa-169	2	673	Blood	10	ng/mL
4	TM1	NISTa-184	2	687	Blood	<10	ng/mL
4	TM1	NISTa-229	4	650	Blood	20	ng/mL
4	TM1	NISTa-229	4	650	Blood	2400	ng/mL
4	TM1	NISTa-220	4	657	Blood	<10	ng/mL
4	TM1	NISTa-255	4	670	Blood	10	ng/mL
4	TM1	NISTa-239	4	673	Blood	20	ng/mL
4	TM1	NISTa-236	4	687	Blood	<10	ng/mL
4	TM1	NISTa-286	8	650	Blood	10	ng/mL
4	TM1	NISTa-310	8	657	Blood	<10	ng/mL
4	TM1	NISTa-294	8	670	Blood	35	ng/mL
4	TM1	NISTa-281	8	673	Blood	33	ng/mL
4	TM1	NISTa-260	8	687	Blood	20	ng/mL
4	TM1	NISTa-347	11	650	Blood	30	ng/mL
4	TM1	NISTa-315	11	657	Blood	20	ng/mL
4	TM1	NISTa-359	11	670	Blood	38	ng/mL
4	TM1	NISTa-359	11	670	Blood	2610	ng/mL
4	TM1	NISTa-361	11	673	Blood	41	ng/mL
4	TM1	NISTa-351	11	687	Blood	48	ng/mL
4	TM1	NISTa-351	11	687	Blood	46	ng/mL
4	TM1	NISTa-368	15	650	Blood	54	ng/mL
4	TM1	NISTa-411	15	657	Blood	35	ng/mL
4	TM1	NISTa-386	15	670	Blood	42	ng/mL
4	TM1	NISTa-386	15	670	Blood	2560	ng/mL
4	TM1	NISTa-404	15	673	Blood	40	ng/mL
4	TM1	NISTa-376	15	687	Blood	48	ng/mL
5	TM1	NISTa-112	0	655	Blood	2400	ng/mL
5	TM1	NISTa-112	0	655	Blood	<10	ng/mL
5	TM1	NISTa-117	0	674	Blood	<10	ng/mL

Table E-1. Lead Analytical Results for NIST 2710a Study Samples

Group	Material	Sample ID	Collection Day	Swine Number	Sample Type	Lead Concentration	Units
5	TM1	NISTa-117	0	674	Blood	<10	ng/mL
5	TM1	NISTa-135	0	677	Blood	<10	ng/mL
5	TM1	NISTa-135	0	677	Blood	<10	ng/mL
5	TM1	NISTa-107	0	695	Blood	<10	ng/mL
5	TM1	NISTa-115	0	697	Blood	<10	ng/mL
5	TM1	NISTa-198	2	655	Blood	20	ng/mL
5	TM1	NISTa-183	2	674	Blood	20	ng/mL
5	TM1	NISTa-172	2	677	Blood	20	ng/mL
5	TM1	NISTa-175	2	695	Blood	2500	ng/mL
5	TM1	NISTa-175	2	695	Blood	<10	ng/mL
5	TM1	NISTa-170	2	697	Blood	<10	ng/mL
5	TM1	NISTa-230	4	655	Blood	30	ng/mL
5	TM1	NISTa-223	4	674	Blood	31	ng/mL
5	TM1	NISTa-245	4	677	Blood	20	ng/mL
5	TM1	NISTa-245	4	677	Blood	2400	ng/mL
5	TM1	NISTa-224	4	695	Blood	<10	ng/mL
5	TM1	NISTa-222	4	697	Blood	20	ng/mL
5	TM1	NISTa-273	8	655	Blood	40	ng/mL
5	TM1	NISTa-272	8	674	Blood	30	ng/mL
5	TM1	NISTa-262	8	677	Blood	20	ng/mL
5	TM1	NISTa-287	8	695	Blood	30	ng/mL
5	TM1	NISTa-280	8	697	Blood	10	ng/mL
5	TM1	NISTa-335	11	655	Blood	48	ng/mL
5	TM1	NISTa-362	11	674	Blood	39	ng/mL
5	TM1	NISTa-330	11	677	Blood	35	ng/mL
5	TM1	NISTa-358	11	695	Blood	46	ng/mL
5	TM1	NISTa-325	11	697	Blood	20	ng/mL
5	TM1	NISTa-416	15	655	Blood	61	ng/mL
5	TM1	NISTa-409	15	674	Blood	54	ng/mL
5	TM1	NISTa-377	15	677	Blood	45	ng/mL
5	TM1	NISTa-366	15	695	Blood	62	ng/mL
5	TM1	NISTa-385	15	697	Blood	38	ng/mL
6	TM1	NISTa-104	0	646	Blood	10	ng/mL
6	TM1	NISTa-145	0	652	Blood	<10	ng/mL
6	TM1	NISTa-150	0	654	Blood	<10	ng/mL
6	TM1	NISTa-150	0	654	Blood	<10	ng/mL
6	TM1	NISTa-142	0	656	Blood	<10	ng/mL
6	TM1	NISTa-139	0	694	Blood	<10	ng/mL
6	TM1	NISTa-202	2	646	Blood	20	ng/mL
6	TM1	NISTa-168	2	652	Blood	30	ng/mL
6	TM1	NISTa-203	2	654	Blood	31	ng/mL
6	TM1	NISTa-158	2	656	Blood	30	ng/mL
6	TM1	NISTa-181	2	694	Blood	<10	ng/mL
6	TM1	NISTa-181	2	694	Blood	10	ng/mL
6	TM1	NISTa-258	4	646	Blood	54	ng/mL
6	TM1	NISTa-258	4	646	Blood	2300	ng/mL
6	TM1	NISTa-244	4	652	Blood	35	ng/mL
6	TM1	NISTa-243	4	654	Blood	31	ng/mL
6	TM1	NISTa-257	4	656	Blood	20	ng/mL

Table E-1. Lead Analytical Results for NIST 2710a Study Samples

Group	Material	Sample ID	Collection Day	Swine Number	Sample Type	Lead Concentration	Units
6	TM1	NISTa-241	4	694	Blood	30	ng/mL
6	TM1	NISTa-307	8	646	Blood	46	ng/mL
6	TM1	NISTa-307	8	646	Blood	47	ng/mL
6	TM1	NISTa-290	8	652	Blood	42	ng/mL
6	TM1	NISTa-267	8	654	Blood	33	ng/mL
6	TM1	NISTa-308	8	656	Blood	20	ng/mL
6	TM1	NISTa-303	8	694	Blood	42	ng/mL
6	TM1	NISTa-331	11	646	Blood	74	ng/mL
6	TM1	NISTa-355	11	652	Blood	79	ng/mL
6	TM1	NISTa-329	11	654	Blood	48	ng/mL
6	TM1	NISTa-360	11	656	Blood	42	ng/mL
6	TM1	NISTa-337	11	694	Blood	64	ng/mL
6	TM1	NISTa-387	15	646	Blood	73	ng/mL
6	TM1	NISTa-371	15	652	Blood	79	ng/mL
6	TM1	NISTa-395	15	654	Blood	66	ng/mL
6	TM1	NISTa-393	15	656	Blood	75	ng/mL
6	TM1	NISTa-393	15	656	Blood	75	ng/mL
6	TM1	NISTa-402	15	694	Blood	78	ng/mL
10	Control	NISTa-111	0	645	Blood	<10	ng/mL
10	Control	NISTa-128	0	684	Blood	3000	ng/mL
10	Control	NISTa-128	0	684	Blood	<10	ng/mL
10	Control	NISTa-130	0	685	Blood	<10	ng/mL
10	Control	NISTa-190	2	645	Blood	<10	ng/mL
10	Control	NISTa-185	2	684	Blood	<10	ng/mL
10	Control	NISTa-174	2	685	Blood	<10	ng/mL
10	Control	NISTa-231	4	645	Blood	<10	ng/mL
10	Control	NISTa-251	4	684	Blood	<10	ng/mL
10	Control	NISTa-238	4	685	Blood	<10	ng/mL
10	Control	NISTa-238	4	685	Blood	<10	ng/mL
10	Control	NISTa-268	8	645	Blood	2400	ng/mL
10	Control	NISTa-268	8	645	Blood	<10	ng/mL
10	Control	NISTa-271	8	684	Blood	<10	ng/mL
10	Control	NISTa-311	8	685	Blood	32	ng/mL
10	Control	NISTa-317	11	645	Blood	<10	ng/mL
10	Control	NISTa-342	11	684	Blood	<10	ng/mL
10	Control	NISTa-326	11	685	Blood	<10	ng/mL
10	Control	NISTa-398	15	645	Blood	<10	ng/mL
10	Control	NISTa-381	15	684	Blood	<10	ng/mL
10	Control	NISTa-381	15	684	Blood	<10	ng/mL
10	Control	NISTa-414	15	685	Blood	2540	ng/mL
10	Control	NISTa-414	15	685	Blood	<10	ng/mL
1	PbAc	NISTa-569	15	664	Femur	2700	ng/g
1	PbAc	NISTa-540	15	669	Femur	3500	ng/g
1	PbAc	NISTa-548	15	682	Femur	4100	ng/g
1	PbAc	NISTa-536	15	686	Femur	2200	ng/g
1	PbAc	NISTa-557	15	692	Femur	3600	ng/g
2	PbAc	NISTa-531	15	648	Femur	5900	ng/g
2	PbAc	NISTa-566	15	658	Femur	6700	ng/g
2	PbAc	NISTa-566	15	658	Femur	6700	ng/g

Table E-1. Lead Analytical Results for NIST 2710a Study Samples

Group	Material	Sample ID	Collection Day	Swine Number	Sample Type	Lead Concentration	Units
2	PbAc	NISTa-533	15	662	Femur	5200	ng/g
2	PbAc	NISTa-562	15	676	Femur	8400	ng/g
2	PbAc	NISTa-571	15	690	Femur	5400	ng/g
3	PbAc	NISTa-525	15	665	Femur	9300	ng/g
3	PbAc	NISTa-534	15	666	Femur	12000	ng/g
3	PbAc	NISTa-534	15	666	Femur	130000	ng/g
3	PbAc	NISTa-568	15	667	Femur	8100	ng/g
3	PbAc	NISTa-524	15	681	Femur	12000	ng/g
3	PbAc	NISTa-521	15	691	Femur	8900	ng/g
4	TM1	NISTa-558	15	650	Femur	2800	ng/g
4	TM1	NISTa-545	15	657	Femur	2900	ng/g
4	TM1	NISTa-545	15	657	Femur	124000	ng/g
4	TM1	NISTa-543	15	670	Femur	5800	ng/g
4	TM1	NISTa-530	15	673	Femur	2800	ng/g
4	TM1	NISTa-539	15	687	Femur	2800	ng/g
4	TM1	NISTa-539	15	687	Femur	2800	ng/g
5	TM1	NISTa-561	15	655	Femur	5100	ng/g
5	TM1	NISTa-544	15	674	Femur	5500	ng/g
5	TM1	NISTa-532	15	677	Femur	4300	ng/g
5	TM1	NISTa-556	15	695	Femur	2900	ng/g
5	TM1	NISTa-556	15	695	Femur	2800	ng/g
5	TM1	NISTa-565	15	697	Femur	2200	ng/g
6	TM1	NISTa-537	15	646	Femur	10000	ng/g
6	TM1	NISTa-523	15	652	Femur	8700	ng/g
6	TM1	NISTa-560	15	654	Femur	6000	ng/g
6	TM1	NISTa-560	15	654	Femur	129000	ng/g
6	TM1	NISTa-552	15	656	Femur	5900	ng/g
6	TM1	NISTa-527	15	694	Femur	8400	ng/g
6	TM1	NISTa-527	15	694	Femur	8700	ng/g
10	Control	NISTa-535	15	645	Femur	<300	ng/g
10	Control	NISTa-549	15	684	Femur	<300	ng/g
10	Control	NISTa-538	15	685	Femur	<300	ng/g
1	PbAc	NISTa-484	15	664	Kidney	66	ng/g
1	PbAc	NISTa-484	15	664	Kidney	69	ng/g
1	PbAc	NISTa-519	15	669	Kidney	150	ng/g
1	PbAc	NISTa-505	15	682	Kidney	160	ng/g
1	PbAc	NISTa-517	15	686	Kidney	68	ng/g
1	PbAc	NISTa-512	15	692	Kidney	150	ng/g
2	PbAc	NISTa-474	15	648	Kidney	230	ng/g
2	PbAc	NISTa-510	15	658	Kidney	230	ng/g
2	PbAc	NISTa-492	15	662	Kidney	190	ng/g
2	PbAc	NISTa-492	15	662	Kidney	690	ng/g
2	PbAc	NISTa-491	15	676	Kidney	320	ng/g
2	PbAc	NISTa-520	15	690	Kidney	310	ng/g
3	PbAc	NISTa-515	15	665	Kidney	250	ng/g
3	PbAc	NISTa-515	15	665	Kidney	740	ng/g
3	PbAc	NISTa-507	15	666	Kidney	550	ng/g
3	PbAc	NISTa-509	15	667	Kidney	300	ng/g
3	PbAc	NISTa-495	15	681	Kidney	360	ng/g

Table E-1. Lead Analytical Results for NIST 2710a Study Samples

Group	Material	Sample ID	Collection Day	Swine Number	Sample Type	Lead Concentration	Units
3	PbAc	NISTa-477	15	691	Kidney	230	ng/g
3	PbAc	NISTa-477	15	691	Kidney	670	ng/g
4	TM1	NISTa-493	15	650	Kidney	110	ng/g
4	TM1	NISTa-486	15	657	Kidney	95	ng/g
4	TM1	NISTa-478	15	670	Kidney	190	ng/g
4	TM1	NISTa-472	15	673	Kidney	100	ng/g
4	TM1	NISTa-482	15	687	Kidney	89	ng/g
5	TM1	NISTa-488	15	655	Kidney	190	ng/g
5	TM1	NISTa-513	15	674	Kidney	180	ng/g
5	TM1	NISTa-506	15	677	Kidney	120	ng/g
5	TM1	NISTa-508	15	695	Kidney	630	ng/g
5	TM1	NISTa-508	15	695	Kidney	110	ng/g
5	TM1	NISTa-499	15	697	Kidney	77	ng/g
5	TM1	NISTa-499	15	697	Kidney	78	ng/g
6	TM1	NISTa-516	15	646	Kidney	330	ng/g
6	TM1	NISTa-480	15	652	Kidney	390	ng/g
6	TM1	NISTa-479	15	654	Kidney	220	ng/g
6	TM1	NISTa-518	15	656	Kidney	220	ng/g
6	TM1	NISTa-518	15	656	Kidney	230	ng/g
6	TM1	NISTa-497	15	694	Kidney	380	ng/g
10	Control	NISTa-511	15	645	Kidney	<10	ng/g
10	Control	NISTa-511	15	645	Kidney	<10	ng/g
10	Control	NISTa-489	15	684	Kidney	<10	ng/g
10	Control	NISTa-502	15	685	Kidney	30	ng/g
1	PbAc	NISTa-430	15	664	Liver	150	ng/g
1	PbAc	NISTa-430	15	664	Liver	680	ng/g
1	PbAc	NISTa-463	15	669	Liver	180	ng/g
1	PbAc	NISTa-433	15	682	Liver	<10	ng/g
1	PbAc	NISTa-427	15	686	Liver	200	ng/g
1	PbAc	NISTa-434	15	692	Liver	67	ng/g
2	PbAc	NISTa-426	15	648	Liver	310	ng/g
2	PbAc	NISTa-435	15	658	Liver	310	ng/g
2	PbAc	NISTa-435	15	658	Liver	320	ng/g
2	PbAc	NISTa-443	15	662	Liver	320	ng/g
2	PbAc	NISTa-443	15	662	Liver	830	ng/g
2	PbAc	NISTa-462	15	676	Liver	120	ng/g
2	PbAc	NISTa-453	15	690	Liver	350	ng/g
2	PbAc	NISTa-453	15	690	Liver	360	ng/g
3	PbAc	NISTa-469	15	665	Liver	380	ng/g
3	PbAc	NISTa-469	15	665	Liver	390	ng/g
3	PbAc	NISTa-436	15	666	Liver	670	ng/g
3	PbAc	NISTa-445	15	667	Liver	350	ng/g
3	PbAc	NISTa-455	15	681	Liver	290	ng/g
3	PbAc	NISTa-425	15	691	Liver	270	ng/g
4	TM1	NISTa-428	15	650	Liver	170	ng/g
4	TM1	NISTa-450	15	657	Liver	300	ng/g
4	TM1	NISTa-419	15	670	Liver	280	ng/g
4	TM1	NISTa-454	15	673	Liver	160	ng/g
4	TM1	NISTa-457	15	687	Liver	230	ng/g

Table E-1. Lead Analytical Results for NIST 2710a Study Samples

Group	Material	Sample ID	Collection Day	Swine Number	Sample Type	Lead Concentration	Units
5	TM1	NISTa-424	15	655	Liver	200	ng/g
5	TM1	NISTa-424	15	655	Liver	200	ng/g
5	TM1	NISTa-444	15	674	Liver	80	ng/g
5	TM1	NISTa-421	15	677	Liver	300	ng/g
5	TM1	NISTa-437	15	695	Liver	190	ng/g
5	TM1	NISTa-439	15	697	Liver	280	ng/g
6	TM1	NISTa-461	15	646	Liver	450	ng/g
6	TM1	NISTa-420	15	652	Liver	480	ng/g
6	TM1	NISTa-441	15	654	Liver	300	ng/g
6	TM1	NISTa-423	15	656	Liver	380	ng/g
6	TM1	NISTa-458	15	694	Liver	650	ng/g
6	TM1	NISTa-458	15	694	Liver	3000	ng/g
10	Control	NISTa-449	15	645	Liver	<10	ng/g
10	Control	NISTa-432	15	684	Liver	<10	ng/g
10	Control	NISTa-456	15	685	Liver	220	ng/g

APPENDIX F: ANALYTICAL RESULTS FOR QUALITY CONTROL SAMPLES

Table F-1. Blind Duplicate Samples

Blind Duplicate Sample ID	Sample Type	Swine Number	Collection Days	Original Sample Concentration	Duplicate Sample Concentration	Sample Units	RPD
NISTa-587	Urine	688	6/7	95	98	µg/L	3%
NISTa-584	Urine	651	6/7	170	180	µg/L	6%
NISTa-789	Urine	684	6/7	1	<1	µg/L	67%
NISTa-790	Urine	687	9/10	36	37	µg/L	3%
NISTa-791	Urine	659	9/10	360	360	µg/L	0%
NISTa-868	Urine	672	9/10	110	110	µg/L	0%
NISTa-699	Urine	652	12/13	464	457	µg/L	2%
NISTa-684	Urine	689	12/13	160	160	µg/L	0%
NISTa-792	Urine	679	12/13	97	100	µg/L	3%
NISTa-109	Blood	666	0	10	<10	µg/L	67%
NISTa-187	Blood	676	2	30	31	µg/L	3%
NISTa-194	Blood	673	2	10	10	µg/L	0%
NISTa-201	Blood	684	2	<10	<10	µg/L	0%
NISTa-214	Blood	665	4	20	30	µg/L	40%
NISTa-246	Blood	657	4	<10	30	µg/L	143%
NISTa-288	Blood	652	8	42	48	µg/L	13%
NISTa-289	Blood	669	8	20	20	µg/L	0%
NISTa-293	Blood	695	8	30	30	µg/L	0%
NISTa-354	Blood	655	11	48	51	µg/L	6%
NISTa-406	Blood	656	15	75	77	µg/L	3%
NISTa-408	Blood	685	15	<10	<10	µg/L	0%
NISTa-801	Blood	664	0	10	<10	µg/L	67%
NISTa-802	Blood	691	0	<10	<10	µg/L	0%
NISTa-803	Blood	645	4	<10	<10	µg/L	0%
NISTa-804	Blood	646	11	74	69	µg/L	7%
NISTa-805	Blood	670	11	38	45	µg/L	17%
NISTa-806	Blood	691	15	53	59	µg/L	11%
NISTa-542	Femur	654	15	6000	5600	ng/g	7%
NISTa-814	Femur	676	15	8400	8400	ng/g	0%
NISTa-559	Femur	695	15	2800	3000	ng/g	7%
NISTa-813	Kidney	658	15	230	230	ng/g	0%
NISTa-810	Kidney	667	15	300	290	ng/g	3%
NISTa-470	Kidney	697	15	78	73	ng/g	7%
NISTa-807	Liver	650	15	170	190	ng/g	11%
NISTa-464	Liver	658	15	320	320	ng/g	0%
NISTa-431	Liver	670	15	280	300	ng/g	7%

RPD = relative percent difference
One-half the detection limit was used to calculate RPD in cases where one value was detected and the other was not.

Table F-2. Laboratory Spikes

Spike Sample ID	Sample Type	Original Sample Concentration (ppb)	Added Spike Concentration (ppb)	Measured Sample Concentration (ppb)	Recovered Spike (ppb)	Recovery (%)
NISTa-586	Urine	95	200	290	195	98%
NISTa-600	Urine	130	200	320	190	95%
NISTa-617	Urine	1	200	200	199	100%
NISTa-627	Urine	28	200	220	192	96%
NISTa-641	Urine	96	200	320	224	112%
NISTa-654	Urine	400	200	600	200	100%
NISTa-666	Urine	36	200	260	224	112%
NISTa-698	Urine	1	200	210	209	105%
NISTa-711	Urine	200	200	415	215	108%
NISTa-724	Urine	100	200	320	220	110%
NISTa-737	Urine	2	200	220	218	109%
NISTa-791	Urine	360	200	590	230	115%
NISTa-112	Blood	<10	2500	2400	2395	96%
NISTa-128	Blood	<10	2500	3000	2995	120%
NISTa-144	Blood	<10	2500	2500	2495	100%
NISTa-163	Blood	10	2500	2500	2490	100%
NISTa-175	Blood	<10	2500	2500	2495	100%
NISTa-187	Blood	31	2500	2600	2569	103%
NISTa-201	Blood	<10	2500	2500	2495	100%
NISTa-213	Blood	30	2500	2500	2470	99%
NISTa-229	Blood	20	2500	2400	2380	95%
NISTa-245	Blood	20	2500	2400	2380	95%
NISTa-258	Blood	54	2500	2300	2246	90%
NISTa-268	Blood	<10	2500	2400	2395	96%
NISTa-282	Blood	20	2500	3000	2980	119%
NISTa-295	Blood	34	2500	2400	2366	95%
NISTa-313	Blood	61	2500	2400	2339	94%
NISTa-327	Blood	82	2500	2400	2318	93%
NISTa-343	Blood	63	2500	2630	2567	103%
NISTa-359	Blood	38	2500	2610	2572	103%
NISTa-372	Blood	51	2500	2550	2499	100%
NISTa-386	Blood	42	2500	2560	2518	101%
NISTa-400	Blood	85	2500	2630	2545	102%
NISTa-414	Blood	5	2500	2540	2535	101%
NISTa-806	Blood	59	2500	2540	2481	99%
NISTa-534	Femur	12000	126000	130000	118000	94%
NISTa-545	Femur	2900	123000	124000	121100	98%
NISTa-560	Femur	6000	122000	129000	123000	101%
NISTa-814	Femur	8400	123000	130000	121600	99%
NISTa-477	Kidney	230	490	670	440	90%
NISTa-492	Kidney	190	526	690	500	95%
NISTa-508	Kidney	110	476	630	520	109%
NISTa-515	Kidney	250	481	740	490	102%
NISTa-810	Kidney	290	481	760	470	98%
NISTa-430	Liver	150	505	680	530	105%
NISTa-443	Liver	320	505	830	510	101%
NISTa-458	Liver	650	2500	3000	2350	94%

Table F-3. Laboratory Duplicates

Duplicate Sample ID	Sample Type	Original Sample Concentration (ppb)	Duplicate Concentration (ppb)	RPD	Absolute Difference
NISTa-580	Urine	110	120	9%	10
NISTa-594	Urine	31	32	3%	1
NISTa-608	Urine	34	34	0%	0
NISTa-622	Urine	190	190	0%	0
NISTa-634	Urine	220	230	4%	10
NISTa-649	Urine	471	470	0%	1
NISTa-659	Urine	270	280	4%	10
NISTa-676	Urine	<0.5	1	67%	0.5
NISTa-691	Urine	443	415	7%	28
NISTa-706	Urine	110	99	11%	11
NISTa-719	Urine	79	78	1%	1
NISTa-732	Urine	44	43	2%	1
NISTa-796	Urine	<0.5	<0.5	0%	0
NISTa-793	Urine	0.2	0.1	67%	0.1
NISTa-106	Blood	10	<10	67%	5
NISTa-117	Blood	<10	<10	0%	0
NISTa-135	Blood	<10	<10	0%	0
NISTa-150	Blood	<10	<10	0%	0
NISTa-169	Blood	10	10	0%	0
NISTa-181	Blood	<10	10	67%	5
NISTa-194	Blood	10	20	67%	10
NISTa-207	Blood	20	30	40%	10
NISTa-221	Blood	58	56	4%	2
NISTa-238	Blood	<10	<10	0%	0
NISTa-250	Blood	20	20	0%	0
NISTa-263	Blood	58	58	0%	0
NISTa-275	Blood	53	51	4%	2
NISTa-289	Blood	20	20	0%	0
NISTa-307	Blood	47	46	2%	1
NISTa-319	Blood	30	30	0%	0
NISTa-336	Blood	30	31	3%	1
NISTa-351	Blood	48	46	4%	2
NISTa-365	Blood	47	49	4%	2
NISTa-381	Blood	<10	<10	0%	0
NISTa-393	Blood	75	75	0%	0
NISTa-406	Blood	77	77	0%	0
NISTa-802	Blood	<10	20	120%	15
NISTa-527	Femur	8700	8400	4%	300
NISTa-539	Femur	2800	2800	0%	0
NISTa-556	Femur	2800	2900	4%	100
NISTa-566	Femur	6700	6700	0%	0
NISTa-484	Kidney	66	69	4%	3
NISTa-499	Kidney	78	77	1%	1
NISTa-511	Kidney	<10	<10	0%	0
NISTa-518	Kidney	220	230	4%	10
NISTa-424	Liver	200	200	0%	0
NISTa-435	Liver	320	310	3%	10
NISTa-453	Liver	350	360	3%	10

Table F-3. Laboratory Duplicates

Duplicate Sample ID	Sample Type	Original Sample Concentration (ppb)	Duplicate Concentration (ppb)	RPD	Absolute Difference
NISTa-469	Liver	390	380	3%	10
NISTa-818	Feed	1	1	0%	0
NISTa-821	Water	<40	<50	22%	10

RPD = relative percent difference

Table F-4. Laboratory Quality Control Standards

Sample ID	Associated Sample Type	LET Number	Analyte Measured	Measured Concentration	Units	Detection Limit (ppb)	Reference Material ID	Certified Mean±Standard Deviation	Recovery
QC-1	Urine	L10010126	Arsenic	6	ng/mL	3	NIST 2670a-L	3	200%
QC-2	Urine	L10010150	Arsenic	220	ng/mL	10	NIST 2670a-H	220±10	100%
QC-3	Urine	L10010174	Arsenic	230	ng/mL	10	NIST 2670a-H	220±10	105%
QC-4	Urine	L10010198	Arsenic	230	ng/mL	10	NIST 2670a-H	220±10	105%
QC-5	Urine	L10010222	Arsenic	230	ng/mL	10	NIST 2670a-H	220±10	105%
QC-6	Urine	L10010246	Arsenic	230	ng/mL	10	NIST 2670a-H	220±10	105%
QC-7	Urine	L10010258	Arsenic	55	ng/mL	1	NIST 1643e	58.98±0.7	93%
QC-8	Urine	L10010264	Arsenic	7.7	µg/g	0.2	NIST 1566b	7.65±0.65	101%
QC-1	Blood	V10020022	Lead	28	ng/mL	2	NIST 1640	26.7±0.41	105%
QC-10	Blood	V10020238	Lead	30	ng/mL	2	NIST 1640	26.7±0.41	112%
QC-11	Blood	V10020262	Lead	30	ng/mL	2	NIST 1640	26.7±0.41	112%
QC-12	Blood	V10020274	Lead	29	ng/mL	2	NIST 1640	26.7±0.41	109%
QC-2	Blood	V10020046	Lead	26	ng/mL	2	NIST 1640	26.7±0.41	97%
QC-3	Blood	V10020070	Lead	28	ng/mL	2	NIST 1640	26.7±0.41	105%
QC-4	Blood	V10020094	Lead	28	ng/mL	2	NIST 1640	26.7±0.41	105%
QC-5	Blood	V10020118	Lead	27	ng/mL	2	NIST 1640	26.7±0.41	101%
QC-6	Blood	V10020142	Lead	28	ng/mL	2	NIST 1640	26.7±0.41	105%
QC-7	Blood	V10020166	Lead	28	ng/mL	2	NIST 1640	26.7±0.41	105%
QC-8	Blood	V10020190	Lead	27	ng/mL	2	NIST 1640	26.7±0.41	101%
QC-9	Blood	V10020214	Lead	29	ng/mL	2	NIST 1640	26.7±0.41	109%
QC-2	Feed	V10030011	Lead	0.44	µg/g	1	NRCC Dolt-3	0.319±0.045	138%
QC-1	Femur	V10020386	Lead	8.7	ng/g	30	NIST 1400	9.07±0.12	96%
QC-2	Femur	V10020406	Lead	9.3	ng/g	30	NIST 1400	9.07±0.12	103%
QC-1	Tissue	V10020299	Lead	0.38	ng/g	10	NRCC TORT-2	0.35±0.13	109%
QC-2	Tissue	V10020322	Lead	0.23	ng/g	10	NRCC Dolt-3	0.319±0.045	72%
QC-3	Tissue	V10020346	Lead	0.32	ng/g	10	NRCC TORT-2	0.35±0.13	91%
QC-4	Tissue	V10020362	Lead	0.17	ng/g	10	NRCC Dolt-3	0.319±0.045	53%
QC-1	Water	V10030010	Lead	26	ng/g	1	NIST 1640	9.07±0.12	97%

Table F-5. Arsenic Performance Evaluation Samples

Sample ID	PE ID	PE Standard	PE Concentration	Sample Concentration	Adjusted Concentration	RPD
NISTa-643	as3.100	Sodium arsenite	100	110	108	8%
NISTa-687	as3.20	Sodium arsenite	20	23	21	7%
NISTa-593	as3.400	Sodium arsenite	400	390	388	3%
NISTa-620	as5.100	Sodium arsenate	100	110	108	8%
NISTa-662	as5.20	Sodium arsenate	20	22	20	2%
NISTa-735	as5.400	Sodium arsenate	400	441	439	9%
NISTa-737	ctrl	Control urine	0	2	0	0%
NISTa-625	ctrl	Control urine	0	1	0	0%
NISTa-678	dma100	Disodium methylarsenate	100	100	98	2%
NISTa-626	dma20	Disodium methylarsenate	20	22	20	2%
NISTa-691	dma400	Disodium methylarsenate	400	443	441	10%
NISTa-706	mma100	Dimethyl arsenic acid	100	110	108	8%
NISTa-577	mma20	Dimethyl arsenic acid	20	21	19	3%
NISTa-654	mma400	Dimethyl arsenic acid	400	400	398	0%

PE = performance evaluation. Sample concentration adjusted by subtracting mean of background arsenic (~1.5 µg/L) from sample concentration.
RPD = relative percent difference

Table F-6. Lead CDC Samples

Sample ID	Sample Type	CDC Sample	CDC Concentration (µg/dL)	Sample Concentration	RPD
NISTa-105	Blood	CDC BLLRS sample 294	1.9	1	45%
NISTa-219	Blood	CDC BLLRS sample 294	1.9	1	45%
NISTa-320	Blood	CDC BLLRS sample 294	1.9	1	45%
NISTa-391	Blood	CDC BLLRS sample 294	1.9	1	45%
NISTa-101	Blood	CDC BLLRS sample 199	5.5	3.6	95%
NISTa-192	Blood	CDC BLLRS sample 199	5.5	3.4	105%
NISTa-295	Blood	CDC BLLRS sample 199	5.5	3.4	105%
NISTa-341	Blood	CDC BLLRS sample 199	5.5	3.8	85%
NISTa-165	Blood	CDC BLLRS sample 592	13.9	12	95%
NISTa-210	Blood	CDC BLLRS sample 592	13.9	12	95%
NISTa-292	Blood	CDC BLLRS sample 592	13.9	12	95%
NISTa-373	Blood	CDC BLLRS sample 592	13.9	13	45%

RPD = relative percent difference

Table F-7. Blanks

Sample ID	Associated Sample Type	Analyte Measured	Measured Concentration	Detection Limit	Units
Blank-8	Feed	Arsenic	<0.1	0.1	µg/g
Blank-1	Urine	Arsenic	<1	1	ng/mL
Blank-2	Urine	Arsenic	<1	1	ng/mL
Blank-3	Urine	Arsenic	<1	1	ng/mL
Blank-4	Urine	Arsenic	<1	1	ng/mL
Blank-5	Urine	Arsenic	<1	1	ng/mL
Blank-6	Urine	Arsenic	<1	1	ng/mL
Blank-7	Urine	Arsenic	<1	1	ng/mL
Blank-1	Water	Arsenic	1	1	ng/mL
Blank-1	Blood	Lead	<10	10	ng/mL
Blank-2	Blood	Lead	<10	10	ng/mL
Blank-3	Blood	Lead	<10	10	ng/mL
Blank-4	Blood	Lead	<10	10	ng/mL
Blank-5	Blood	Lead	<10	10	ng/mL
Blank-6	Blood	Lead	<10	10	ng/mL
Blank-7	Blood	Lead	<10	10	ng/mL
Blank-8	Blood	Lead	<10	10	ng/mL
Blank-9	Blood	Lead	<10	10	ng/mL
Blank-10	Blood	Lead	<10	10	ng/mL
Blank-11	Blood	Lead	<10	10	ng/mL
Blank-12	Blood	Lead	<10	10	ng/mL
Blank-1	Femur	Lead	<300	300	ng/g
Blank-2	Femur	Lead	<300	300	ng/g
Blank-1	Tissue	Lead	<10	10	ng/g
Blank-2	Tissue	Lead	<10	10	ng/g
Blank-3	Tissue	Lead	<10	10	ng/g
Blank-4	Tissue	Lead	<10	10	ng/g

Figure F-1. Urinary Arsenic Blind Duplicates

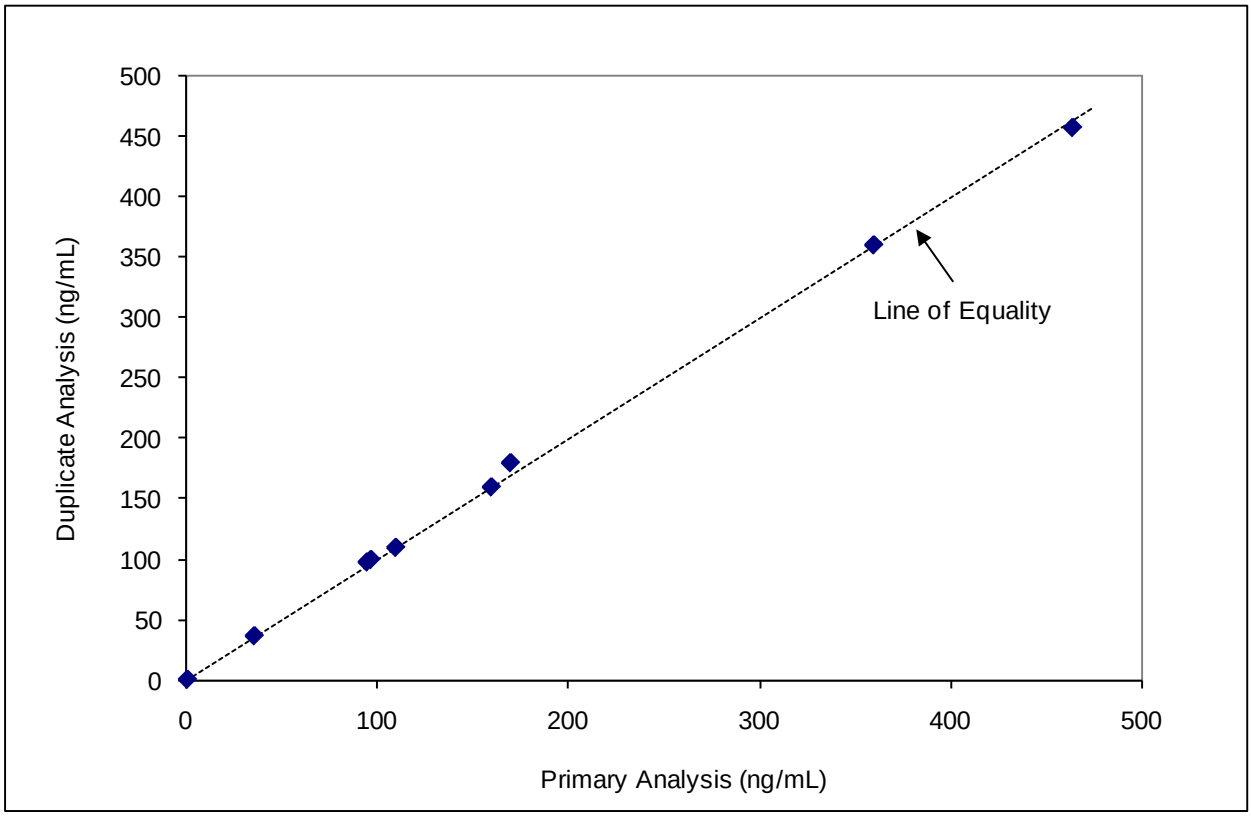
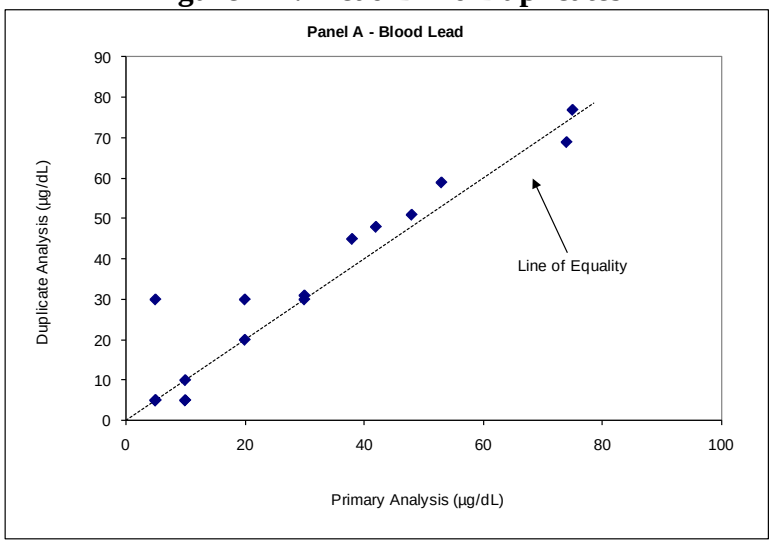
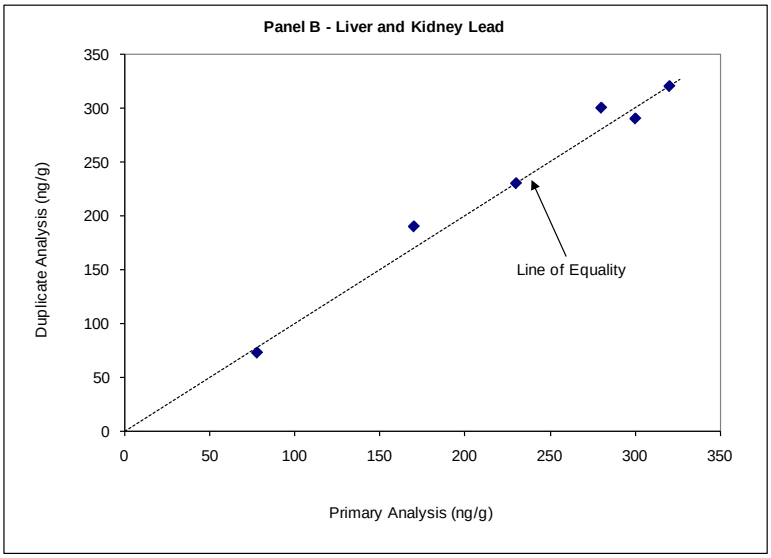


Figure F-2. Lead Blind Duplicates





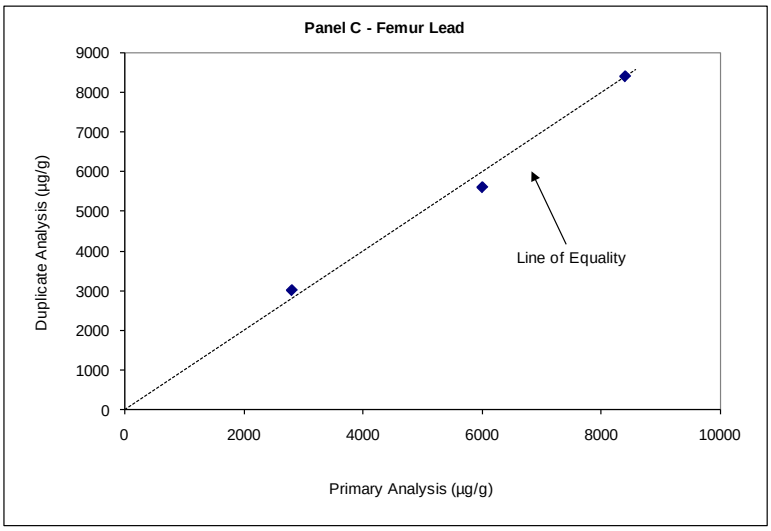


Figure F-3. Performance Evaluation Samples

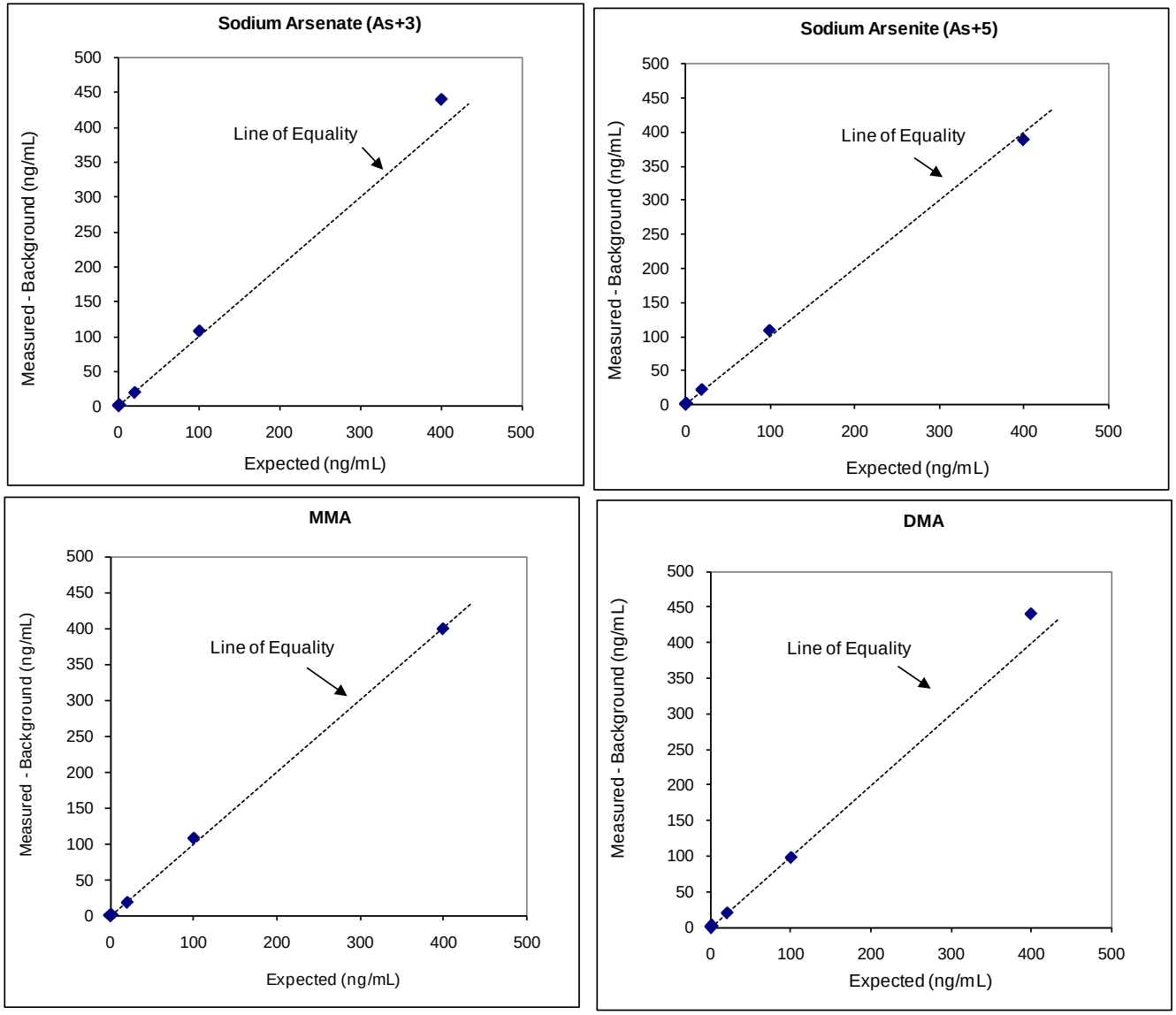
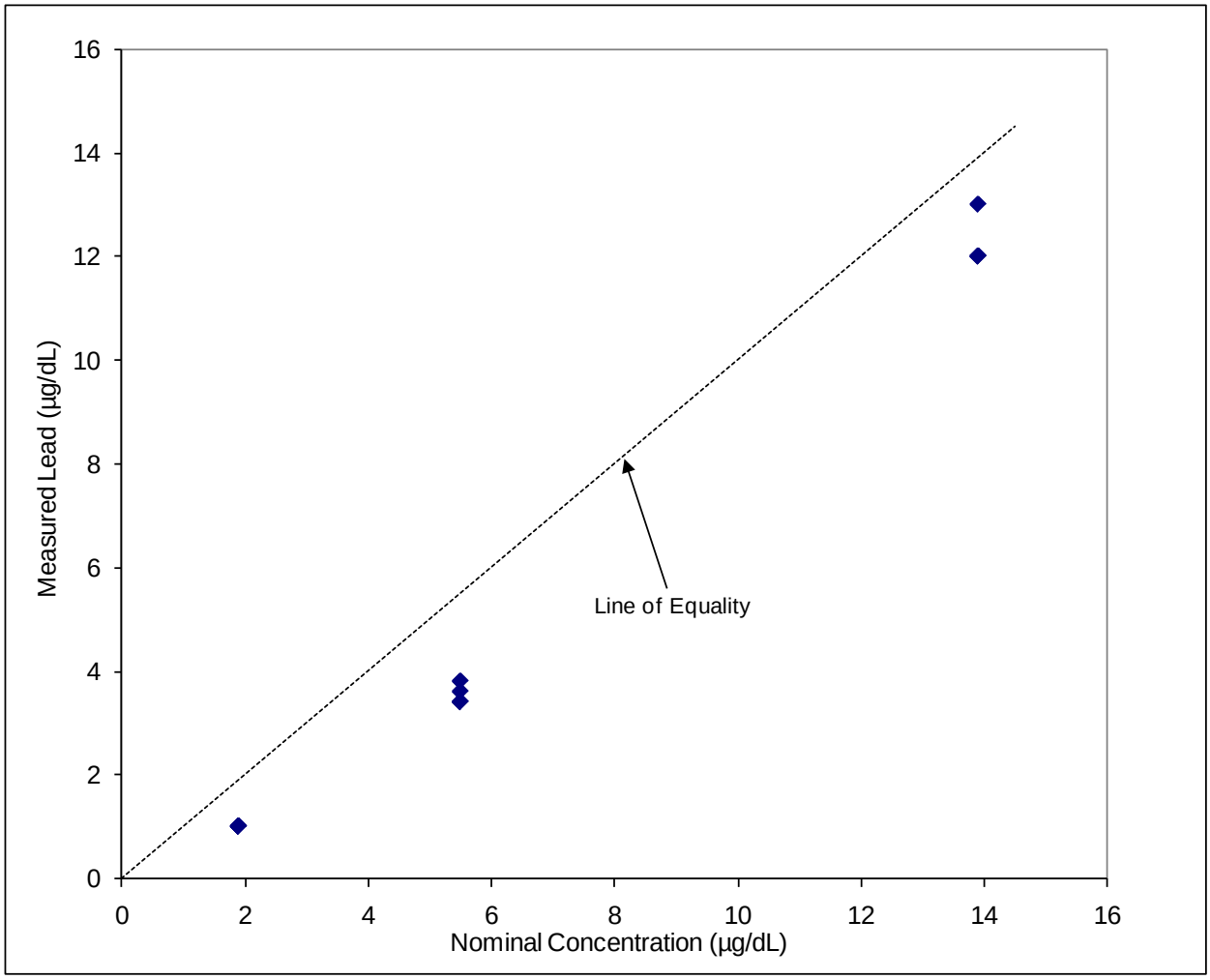


Figure F-4. CDC Blood Lead Check Sample



RELATIVE BIOAVAILABILITY OF ARSENIC AND VANADIUM IN SOIL FROM A SUPERFUND SITE IN PALESTINE, TEXAS

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- Dr. Edward Hinderberger of L.E.T., Inc., Columbia, Missouri, who provided prompt and reliable chemical analysis of all of the samples for total arsenic and vanadium concentrations.

EXECUTIVE SUMMARY

A study using juvenile swine as test animals was performed to measure the gastrointestinal absorption of arsenic and vanadium from soil collected from a Superfund site in Palestine, Texas. The relative bioavailability of arsenic and vanadium was assessed by comparing the absorption of arsenic or vanadium from the test soil to that of a reference material (sodium arsenate or vanadyl sulfate). Groups of five swine were given oral doses of sodium arsenate, vanadyl sulfate, or the test soil twice a day for 15 days; a group of three non-treated swine served as a control. The arsenic concentration in the test soil was 47 µg/g and the vanadium concentration was 121 µg/g.[1]

Arsenic

The amount of arsenic absorbed by each animal was evaluated by measuring the amount of arsenic excreted in the urine (collected over 48-hour periods beginning on days 6, 9, and 12). The urinary excretion fraction (UEF) (the ratio of the amount excreted per 48 hours divided by the dose given per 48 hours) was calculated for both the test soil and sodium arsenate using linear regression analysis. The relative bioavailability (RBA) of arsenic in the test soil compared to that in sodium arsenate was calculated as follows:

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

The results are summarized below:

Measurement Endpoint	Estimated Soil RBA (90% Confidence Interval)
Days 6/7	0.19 (0.17 - 0.21)
Days 9/10	0.16 (0.14 - 0.19)
Days 12/13	0.13 (0.11 - 0.15)
All Days	0.15 (0.14 - 0.16)

Using sodium arsenate as a relative frame of reference, the RBA estimate for the test soil is approximately 15%. This value is markedly lower than the default value range of 80%-100% for arsenic in soil that is usually employed when reliable site-specific data are lacking. This indicates that the arsenic in this soil is not as well absorbed as soluble arsenic.

[1] Due to an insufficient quantity of soil provided at the start of the study, the primary soil sample was used for dosing on days 0-11 only. For the final dose preparation (administered on days 12-14), the remaining soil was mixed with additional soil obtained from the supplier. The arsenic concentration of this combined soil sample was 62 µg/g and the vanadium concentration was 147 µg/g.

Vanadium

The amount of vanadium absorbed by each animal was evaluated by measuring the concentration of vanadium in liver, kidney, and bone (measured on day 15 at study termination). The dose-response data for vanadium in each tissue were modeled using a linear equation. RBA for each tissue was calculated as the ratio of the slope term from the test soil equation to the slope term from the vanadyl sulfate equation. The suggested point estimate is calculated as the simple mean of the three endpoint-specific estimates. The results are summarized below:

Measurement Endpoint	Estimated Soil RBA (90% Confidence Interval)
Liver Vanadium	0.08 (0.06 - 0.10)
Kidney Vanadium	0.06 (0.05 - 0.08)
Bone Vanadium	0.08 (0.06 - 0.10)
Point Estimate	0.08 (0.06 - 0.10)

Using vanadyl sulfate as a relative frame of reference the RBA point estimate for the test soil is approximately 8%. This value indicates that the vanadium in the test soil is not as well absorbed as soluble vanadium.

These relative bioavailability estimates may be used to improve accuracy and decrease uncertainty in estimating human health risks from exposure to this test soil.

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ACRONYMS AND ABBREVIATIONS

ABA	Absolute bioavailability
AF_o	Oral absorption fraction
As+3	Trivalent inorganic arsenic
As+5	Pentavalent inorganic arsenic
DMA	Dimethyl arsenic
D	Ingested dose
g	Gram
GLP	Good Laboratory Practices
ICP-AES	Inductively coupled plasma atomic emission spectroscopy
kg	Kilogram
K_u	Fraction of absorbed arsenic which is excreted in urine
mL	Milliliter
MMA	Monomethyl arsenic
N	Number of data points
QA	Quality assurance
RBA	Relative bioavailability
ref	Reference material
RfD	Reference dose
SD	Standard deviation
SF	Slope factor
test	Test material
UEF	Urinary excretion fraction
USEPA	United States Environmental Protection Agency
µg	Microgram
µm	Micrometer
°C	Degrees Celsius

1.0 INTRODUCTION

1.1 Overview of Bioavailability

Analysis of the potential hazard to humans from ingestion of a chemical depends upon accurate information on a number of key parameters, including the concentration of the chemical in environmental media (e.g., soil, dust, water, food, air, paint), intake rates of each medium, and the rate and extent of absorption ("bioavailability") of the chemical by the body from each ingested medium. Bioavailability is a measure of the amount of chemical that is absorbed by the body from an ingested medium. The amount of bioavailable chemical depends on the physical-chemical properties of the chemical and of the medium. For example, some metals in soil may exist, at least in part, as poorly water-soluble minerals, and may also exist inside particles of inert matrix such as rock or slag of variable size, shape, and association. These chemical and physical properties may influence (usually decrease) the bioavailability of the metals when ingested. Thus, equal ingested doses of different forms of a chemical in different media may not be of equal health concern.

Bioavailability of a chemical in a particular medium may be expressed either in absolute terms (absolute bioavailability) or in relative terms (relative bioavailability):

> Absolute bioavailability (ABA) is the ratio of the amount of the chemical absorbed to the amount ingested:
>
> $$ABA = \frac{Absorbed\ Dose}{Ingested\ Dose}$$
>
> This ratio is also referred to as the oral absorption fraction (AF_o).
>
> Relative bioavailability (RBA) is the ratio of the AF_o of the chemical present in some test material (*test*) to the AF_o of the chemical in some appropriate reference material (e.g., either the chemical dissolved in water or a solid form that is expected to fully dissolve in the stomach) (*ref*):
>
> $$RBA(test\ vs\ ref) = \frac{AF_o(test)}{AF_o(ref)}$$

For example, if 100 micrograms (µg) of a chemical (e.g., arsenic) dissolved in drinking water were ingested and a total of 50 µg were absorbed into the body, the AF_o would be 50/100, or 0.50 (50%). Likewise, if 100 µg of a chemical contained in soil were ingested and 30 µg were absorbed into the body, the AF_o for this chemical in soil would be 30/100, or 0.30 (30%). If the chemical dissolved in water were used as the frame of reference for describing the relative amount of the same chemical absorbed from soil, the RBA would be 0.30/0.50, or 0.60 (60%).

For additional discussion about the concept and application of bioavailability, see Gibaldi and Perrier (1982), Goodman et al. (1990), and/or Klaassen et al. (1996).

1.2 Using RBA Data to Improve Risk Calculations

When reliable data are available on the relative bioavailability (RBA) of a chemical in a site medium (e.g., soil), this information can be used to improve the accuracy of exposure and risk calculations at that site. Available RBA data can be used to adjust default oral toxicity values (reference dose and slope factor) to account for differences in absorption between the chemical ingested in water and the chemical ingested in site media, assuming the toxicity factors are based on a readily soluble form of the chemical. For non-cancer effects, the default reference dose ($RfD_{default}$) can be adjusted ($RfD_{adjusted}$) as follows:

$$RfD_{adjusted} = \frac{RfD_{default}}{RBA}$$

For potential carcinogenic effects, the default slope factor ($SF_{default}$) can be adjusted ($SF_{adjusted}$) as follows:

$$SF_{adjusted} = SF_{default} \cdot RBA$$

Alternatively, it is also acceptable to adjust the dose (rather than the toxicity factors) as follows:

$$Dose_{adjusted} = Dose_{default} \cdot RBA$$

This dose adjustment is mathematically equivalent to adjusting the toxicity factors as described above.

1.3 Purpose of this Study

The objective of this study was to use juvenile swine as a test system to determine the RBA of arsenic and vanadium in soil collected from a Superfund site in Palestine, Texas compared to a soluble form of arsenic (sodium arsenate) and vanadium (vanadyl sulfate).

2.0 STUDY DESIGN

This investigation of arsenic and vanadium RBA was performed according to the basic design presented in Table 2-1. The study investigated arsenic and vanadium absorption from sodium arsenate ($NaHAsO_4$), vanadyl sulfate ($VOSO_4$), and a test material (TM1). Each material was administered to groups of five animals at three different dose levels for 15 days (a detailed schedule is presented in Appendix A, Table A-1). Additionally, the study included a non-treated group of three animals to serve as a control for determining background arsenic and vanadium levels. All doses were administered orally.

The study design was based on the standardized study protocol for measuring lead relative bioavailability (USEPA 2007) using the juvenile swine model. The basic model for estimating arsenic RBA differed from lead in that the urinary excretion fraction (UEF) of arsenic administered in test material and in reference material (sodium arsenate) was measured, and the ratio of the two UEF values then calculated:

RBA(test material) = UEF(test material) / UEF(sodium arsenate)

The UEF for each material (test soil, sodium arsenate) was estimated by plotting the mass of arsenic excreted by each animal as a function of the dose administered, and then fitting a linear regression line to the combined data. The process of deriving the best fit linear regression were fit using simultaneous weighted linear regression.

The study was performed as nearly as possible within the spirit and guidelines of Good Laboratory Practices (GLP: 40 CFR 792).

2.1 Test Material

2.1.1 Sample Description

The test material used in this investigation was a soil sample collected from a Superfund site in Palestine, Texas. Due to an insufficient quantity of soil provided at the start of the study, the initial soil sample was only used for dosing on days 0-11. The final dose (administered on days 12-14) used the remaining soil mixed with new, additional soil obtained from the supplier.

2.1.2 Sample Preparation

The soil sample was sieved through a 250 micrometer (μm) sieve prior to test substance analysis and characterization. Only material that passed through the sieve (corresponding to particles smaller than about 250 μm) were used in the bioavailability study. The study was limited to this fine-grained soil fraction because it is believed that soil particles less than about 250 μm are most likely to adhere to the hands and be ingested by hand-to-mouth contact, especially in young children.

2.1.3 Arsenic and Vanadium Concentrations

The dried and sieved soil samples were analyzed for arsenic and vanadium by L. E. T., Inc., (Columbia, Missouri). Arsenic and vanadium concentrations were measured in duplicate by inductively coupled plasma atomic emission spectroscopy (ICP-AES). The resulting mean arsenic values were 47 μg/g for the initial soil sample and 62 μg/g in the supplementary combined soil sample. The resulting mean vanadium values were 121 μg/g in the initial soil sample and 147 μg/g in the supplementary combined soil sample.

2.2 Experimental Animals

Juvenile swine were selected for use in this study because they are considered to be a good physiological model for gastrointestinal absorption in children (Weis and LaVelle, 1991; Casteel et al., 1996). The animals were intact males of the Pig Improvement Corporation genetically defined Line 26, and were purchased from Chinn Farms, Clarence, Missouri.

The number of animals purchased for the study was several more than required by the protocol. These animals were purchased at an age of about 5-6 weeks (weaning occurs at age 3 weeks) and housed in individual stainless steel cages. The animals were then held under quarantine for one week to observe their health before beginning exposure to dosing materials. Each animal was examined by a certified veterinary clinician (swine specialist) and any animals that appeared to be in poor health during this quarantine period were excluded from the study. To minimize weight variations among animals and groups, extra animals most different in body weight (either heavier or lighter) six days prior to exposure (day -6) were also excluded from the study. The remaining animals were assigned to dose groups at random (group assignments are presented in Appendix A, Table A-2).

When exposure began (day zero), the animals were about 6-7 weeks old and weighed an average of about 10.5 kilograms (kg). The animals were weighed every three days during the course of the study. On average, animals gained about 0.37 kg/day and the rate of weight gain was comparable in all dosing groups, ranging from 0.32 to 0.44 kg/day. These body weight data are presented in Appendix A, Table A-3, and summarized in Figure 2-1.

All animals were examined daily by an attending veterinarian while on study. Most animals (N = 41) exhibited no problems throughout the study. Several animals (N = 7) exhibited elevated temperatures, diarrhea, and/or anorexia and were treated with Naxcel for a duration of 3 days (see Appendix A, Table A-4).

2.3 Diet

Animals were weaned onto standard pig chow (purchased from MFA Inc., Columbia, Missouri) by the supplier. The animals were gradually transitioned from the MFA feed to a special feed originally developed for lead RBA studies (purchased from Zeigler Brothers, Inc., Gardners, Pennsylvania), and this feed was maintained 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 2-2. Each day every animal was given an amount of feed equal to 4% of the mean body weight of all animals on study, except for animals dosed with soil (groups 4-6), which received an amount of feed equal to 3.7% of the mean body weight of all animals (to compensate for the extra feed required when dosing with soil). Feed amounts were adjusted every three days, when pigs were weighed. Feed was administered in two equal portions at 11:00 AM and 5:00 PM daily. Analysis of random feed samples indicated that the arsenic levels did not exceed 0.2 μg/g; vanadium concentrations did not exceed 1.0 ug/g.

Drinking water was provided *ad libitum* (i.e., free feeding) via self-activated watering nozzles within each cage. Analysis of samples from randomly selected drinking water nozzles indicated the arsenic and vanadium concentrations were below a level of detection.

2.4 Dosing

The protocol for exposing animals to arsenic and vanadium is shown in Table 2-1. Animals were exposed to dosing materials (sodium arsenate, vanadyl sulfate, test soil) for 15 days, with the dose for each day being administered in two equal portions beginning at 9:00 AM and 3:00 PM (two hours before feeding), with two minute intervals allowed for individual pig dosing. To facilitate dose administration, dosing materials were placed in a small depression in a ball of dough consisting of moistened feed (typically about 5g) and the dough was pinched shut. The doughballs were administered to the animals by hand.

Occasionally, some animals did not consume their entire dose and there were some difficulties with doughball preparation. In these instances, the missed doses were estimated and recorded and the time-weighted average dose calculation for each animal was adjusted downward accordingly (see Appendix A, Table A-3).

Due to an insufficient quantity of soil provided at the start of the study, the initial soil sample was only used for dosing on days 0-11. For the final dose preparation (administered on days 12-14), the remaining soil was mixed with additional soil obtained from the supplier. However, there was still insufficient soil to prepare the second half of the day 14 dosing, so no animals received the 3:00 PM dose on day 14.

Administered amounts of dose materials were based on the arsenic or vanadium concentration in the dosing materials and the measured group mean body weights. Specifically, the amount of dosing material to be administered for the three days following each weighing was based on the group mean body weight adjusted by the addition of 1 kg to account for the expected weight gain over each time interval. After completion of the study, body weights were estimated by interpolation for those days when measurements were not collected and the actual administered doses were calculated for each day and then averaged across all days. The actual mean doses for each dosing group are included in Table 2-1; the actual daily doses administered to each pig are presented in Appendix A, Tables A-6 (arsenic) and A-7 (vanadium).

2.5 Collection of Biological Samples

Urine

Samples of urine were collected from each animal for 48-hour periods on days 6 to 7 (U1), 9 to 10 (U2), and 12 to 13 (U3) of the study. Collection began at 9:00 AM and ended 48 hours later. The urine was collected in a stainless steel pan placed beneath each cage, which drained into a plastic storage bottle. Each collection pan was fitted with a nylon screen to minimize contamination with feces, spilled food, or other debris. Due to the length of the collection period, collection containers were emptied periodically (typically twice daily) into a separate holding container to ensure that there was no loss of sample due to overflow.

At the end of each collection period, the total urine volume for each animal was measured (see Appendix A, Table A-8) and three 60-milliliter (mL) portions were removed and acidified with 0.6 mL concentrated nitric acid. Two of the aliquots were archived in the refrigerator and one aliquot was sent for arsenic analysis. All samples were refrigerated until arsenic analysis.

Liver, Kidney, and Bone

On day 15, all animals were humanely euthanized and samples of liver, kidney, and bone (the right femur, defleshed) were removed and stored at -80 degrees Celsius (°C) in plastic bags for vanadium analysis.

Subsamples of all biological samples collected were archived in order to allow for reanalysis and verification of arsenic or vanadium levels, if needed. All animals were also subjected to detailed examination at necropsy by a certified veterinary pathologist in order to assess overall animal health. All samples were assigned random chain-of-custody tag numbers and submitted to the analytical laboratory for analysis in a blind fashion.

2.6 Analysis of Biological Samples

Urine

Urine samples were assigned random chain-of-custody tag numbers and submitted to the analytical laboratory for analysis in a blind fashion. The samples were analyzed for arsenic by L. E. T., Inc., (Columbia, Missouri). In brief, 25 mL samples of urine were digested by refluxing and then heating to dryness in the presence of magnesium nitrate and concentrated nitric acid. Following magnesium nitrate digestion, samples were transferred to a muffle furnace and ashed at 500°C. The digested and ashed residue was dissolved in hydrochloric acid and analyzed by the hydride generation technique using a PerkinElmer 3100 atomic absorption spectrometer. Preliminary tests of this method established that each of the different forms of arsenic that may occur in urine, including trivalent inorganic arsenic (As+3), pentavalent inorganic arsenic (As+5), monomethyl arsenic (MMA), and dimethyl arsenic (DMA), are all recovered with high efficiency.

Urine analytical results are presented in Appendix A, Table A-9.

Liver and Kidney

Five grams of liver were placed in a screw-cap Teflon container with 5 mL of concentrated (70%) nitric acid and heated in an oven to 90°C overnight. After cooling, the digestate was transferred to a clean 50 mL volumetric flask and diluted to volume with deionized distilled water. The same procedure was followed for kidney, except quantities were halved due to less tissue available.

Bone

The right femur of each animal was defleshed, broken, and dried at 100°C overnight. The dried bones were then placed in a muffle furnace and dry-ashed at 450°C for 48 hours. Following dry ashing, the bone was ground to a fine powder using a mortar and pestle, and 200 mg was removed and dissolved in 10.0 mL of 1:1 (volume:volume) concentrated nitric acid/water. After the powdered bone was dissolved and mixed, 5.0 mL of the acid solution was removed and diluted to 25.0 mL in deionized distilled water.

Liver, kidney, and bone samples and other materials (e.g., food, water, reagents, solutions) were analyzed for vanadium by ICP-AES. Vanadium analytical results for study samples are presented in Appendix A, Table A-10. All responses below the quantitation limit were evaluated at one-half the quantitation limit. Quality assurance samples are described in the following section.

2.7 Quality Assurance

A number of quality assurance (QA) steps were taken during this project to evaluate the accuracy of the analytical procedures. The results for quality assurance samples are presented in Appendix A, Table A-11, and are summarized below.

Spike Recovery

Randomly selected samples were spiked with known amounts of arsenic (sodium arsenate) or vanadium (vanadyl sulfate) and the recovery of the added analyte was measured. Arsenic recovery for individual samples ranged from 101% to 113%, with an average of 106 ± 4.1% (N = 9). Vanadium recovery for individual samples ranged from 113% to 134%, with an average of 119 ± 8.3% (N = 6).

Laboratory Duplicates

Periodically during arsenic analysis, urine samples were randomly selected by the analyst for duplicate analysis (i.e., the same prepared sample was analyzed twice). Urinary arsenic duplicates had a percent deviation of 0% to 9.5%, with an average of 2.1% ± 3.3% (N = 11).

In addition, a random selection of about 20% of all tissue samples (liver, kidney, and femur) generated during the study were prepared for vanadium analysis in duplicate (i.e., two separate subsamples of tissue were prepared for analysis); the identity of these samples was known by the

analytical laboratory. Tissue vanadium duplicates had a percent deviation of 0% to 17%, with an average of 8.3% ± 5.2% (N = 9).

Blind Duplicates (Sample Preparation Replicates)

A random selection of about 20% of all urine samples generated during the study were prepared for laboratory analysis in duplicate (i.e., two separate subsamples of urine were prepared for analysis) and submitted to the laboratory in a blind fashion. The results for the blind duplicates are shown in Figure 2-2. There was good agreement between results for the duplicate pairs.

No blind duplicates of liver, kidney, or femur samples were submitted to the analytical laboratory for vanadium analysis.

Laboratory Control Standards

Laboratory control standards (samples of reference materials for which a certified concentration of specific analytes has been established) were tested periodically during sample analysis. Results for the standards are summarized below:

Analyte	Standard	Certified Mean ± SD	Mean	SD	Mean % Recovery	N
Arsenic	NIST 1566b	7.65 ± 0.65	7.9	0.07	102.6%	2
	NIST 1640	.0267 ± 0.0004	0.030	0.001	110.5%	2
	NRCC TORT-2	21.6 ± 1.8	21.0	0.0	97.2%	2
Vanadium	NIST 1640	.01299 ± 0.0004	0.013	0.0	100.1%	6
	NRCC TORT-2	1.64 ± 0.19	1.70	0.0	103.7%	4

SD = Standard deviation
N = Number of data points used in curve fitting

As seen, recovery of arsenic and vanadium from these standards was generally good and within the acceptable range.

Blanks

Blank samples run along with each batch of samples never yielded a measurable level of arsenic or vanadium (N = 16).

Summary of QA Results

Based on the results of all of the quality assurance samples and steps described above, it is concluded that the analytical results are of sufficient quality for derivation of reliable estimates of arsenic and vanadium absorption from the test material.

3.0 DATA ANALYSIS FOR ARSENIC

3.1 Overview

Figure 3-1 shows a conceptual model for the toxicokinetic fate of ingested arsenic. Key points of this model are as follows:

- In most animals (including humans), absorbed arsenic is excreted mainly in the urine over the course of several days. Thus, the urinary excretion fraction (UEF), defined as the amount excreted in the urine divided by the amount given, is usually a reasonable approximation of the AF_o or ABA. However, this ratio will underestimate total absorption, because some absorbed arsenic is excreted in the feces via the bile, and some absorbed arsenic enters tissue compartments (e.g., skin, hair) from which it is cleared very slowly or not at all. Thus, the urinary excretion fraction should not be equated with the absolute absorption fraction.

- The RBA of two orally administered materials (i.e., a test material and reference material) can be calculated from the ratio of the urinary excretion fraction of the two materials. This calculation is independent of the extent of tissue binding and of biliary excretion:

$$RBA(test\ vs\ ref) = \frac{AF_o(test)}{AF_o(ref)} = \frac{D \cdot AF_o(test) \cdot K_u}{D \cdot AF_o(ref) \cdot K_u} = \frac{UEF(test)}{UEF(ref)}$$

  where:

  D = Ingested dose (μg)

  K_u = Fraction of absorbed arsenic that is excreted in the urine

Based on the conceptual model above, the basic method used to estimate the RBA of arsenic in a particular test material compared to arsenic in a reference material (sodium arsenate) is as follows:

1. Plot the amount of arsenic excreted in the urine (μg/day) as a function of the administered amount of arsenic (μg/day), both for reference material (sodium arsenate) and for test material.

2. Find the best fit linear regression line through each data set. The slope of each line (μg/day excreted per μg/day ingested) is the best estimate of the urinary excretion fraction (UEF) for each material.

3. Calculate RBA for each test material as the ratio of the UEF for test material compared to UEF for reference material:

$$RBA(test\ vs\ ref) = \frac{UEF(test)}{UEF(ref)}$$

A detailed description of the curve-fitting methods and rationale and the methods used to quantify uncertainty in the arsenic RBA estimates for a test material are summarized below. All model fitting was performed in Microsoft Excel® using matrix functions.

3.2 Dose-Response Model

Simultaneous Regression

The techniques used to derive linear regression fits to the dose-response data are based on the methods recommended by Finney (1978). According to Finney (1978), when the data to be analyzed consist of two dose-response curves (the reference material and the test material), both curves must have the same intercept because there is no difference between the curves when the dose is zero. This requirement is achieved by combining the two dose response equations into one and solving for the parameters simultaneously, as follows:

Separate Models:

$$\mu_r(i) = a + b_r \cdot x_r(i)$$

$$\mu_t(i) = a + b_t \cdot x_t(i)$$

Combined Model

$$\mu(i) = a + b_r \cdot x_r(i) + b_t \cdot x_t(i)$$

where $\mu(i)$ indicates the expected mean response of animals exposed at dose $x(i)$, and the subscripts r and t refer to reference and test material, respectively. The coefficients of this combined model are derived using multivariate regression, with the understanding that the combined data set is restricted to cases in which one (or both) of x_r and x_t are zero (Finney, 1978).

Weighted Regression

Regression analysis based on ordinary least squares assumes that the variance of the responses is independent of the dose and/or the response (Draper and Smith, 1998). This assumption is generally not satisfied in swine-based RBA studies, where there is a tendency toward increasing variance in response as a function of increasing dose (heteroscedasticity). One method for dealing with heteroscedasticity is through the use of weighted least squares regression (Draper and Smith, 1998). In this approach, each observation in a group of animals is assigned a weight that is inversely proportional to the variance of the response in that group:

$$w_i = \frac{1}{\sigma_i^2}$$

where:

w_i = weight assigned to all data points in dose group i

σ_i^2 = variance of responses in animals in dose group i

When the distributions of responses at each dose level are normal, weighted regression is equivalent to the maximum likelihood method.

There are several alternative strategies for assigning weights. The method used in this study estimates the value of σ_i^2 using an "external" variance model based on an analysis of the relationship between variance and mean response using data consolidated across many different swine-based arsenic RBA studies. Log-variance increases as an approximately linear function of log-mean response:

$$\ln(s_i^2) = k1 + k2 \cdot \ln(\bar{y}_i)$$

where:

s_i^2 = observed variance of responses of animals in dose group i
$\bar{y}_i$ = mean observed response of animals in dose group i

Goodness of Fit

The goodness-of-fit of each dose-response model was assessed using the F test statistic and the adjusted coefficient of multiple determination (Adj R^2) as described by Draper and Smith (1998). A fit is considered acceptable if the p-value is less than 0.05.

Assessment of Outliers

In biological assays, it is not uncommon to note the occurrence of individual measured responses that appear atypical compared to the responses from other animals in the same dose group. In this study, an analysis was made by looking at responses that yielded standardized weighted residuals greater than 3.5 or less than -3.5 (Canavos,1984). When such data points were encountered in a data set, the UEF and RBA values were calculated both with and without the potential outlier(s) excluded, and the result with the outlier(s) excluded was used as the preferred estimate.

3.3 Calculation of Arsenic RBA Estimates

The arsenic RBA values were calculated as the ratio of the slope term for the test material data set (b_t) and the reference material data set (b_r):

$$RBA = \frac{b_t}{b_r}$$

The uncertainly range about the RBA ratio was calculated using Fieller's Theorem as described by Finney (1978).

4.0 DATA ANALYSIS FOR VANADIUM

4.1 Overview

The basic approach for measuring vanadium absorption *in vivo* is to administer an oral dose of vanadium to test animals and measure the increase in vanadium levels in one or more body compartments (e.g., soft tissue, bone). In order to calculate the RBA value of a test material, the increase in vanadium in a body compartment is measured both for that test material and a reference material (vanadyl sulfate). Because equal absorbed doses of vanadium will produce equal responses (i.e., equal increases in concentration in tissues) regardless of the source or nature of the ingested vanadium, the RBA of a test material is calculated as the ratio of doses (test material and reference material) that produce equal increases in vanadium concentration in the body compartment. Thus, the basic data reduction task required to calculate an RBA for a test material is to fit mathematical equations to the dose-response data for both the test material and the reference material, and then solve the equations to find the ratio of doses that would be expected to yield equal responses.

The curve-fitting methods and rationale, along with the methods used to quantify uncertainty in the RBA estimates, are summarized below.

4.2 Measurement Endpoints

Three independent measurement endpoints were evaluated based on the concentration of vanadium observed in liver, kidney, and bone (femur). The measurement endpoint was the concentration in the tissue at the time of sacrifice (day 15).

4.3 Dose-Response Model

Basic Equation

Selection of an appropriate dose-response model and weighting factors requires data from multiple studies and, in contrast to arsenic for which multiple studies support the use of a linear dose-response model, data are only available for a single vanadium study. Therefore, the vanadium data set was evaluated using weighted linear regression, which was selected for most endpoints investigated by USEPA, including liver, kidney, and bone lead (USEPA, 2007). Indeed, inspection of the data (see Figures 5-10, 5-11, and 5-12) suggested that they could be well-fit using a linear equation.

Simultaneous Regression

Similar to arsenic analysis, data analysis consists of two dose-response curves for each endpoint (the reference material and test material) and because there is no difference between the curves when the dose is zero, both curves for a given endpoint must have the same intercept. This

requirement is achieved by combining the two dose response equations into one and solving for the parameters simultaneously, resulting in the following equation:

$$y = a + b_r \cdot x_r + b_t \cdot x_t$$

where:

y = response
x = dose
a, b = empirical coefficients for reference material (r) and test material (t)

All model fitting was performed using JMP® version 3.2.2, a commercial software package developed by SAS®.

Weighted Regression

Regression analysis based on ordinary least squares assumes that the variance of the responses is independent of the dose and/or the response (Draper and Smith, 1998). This assumption is generally not satisfied in swine-based RBA studies, where there is a tendency toward increasing variance in response as a function of increasing dose (heteroscedasticity). One method for dealing with heteroscedasticity is through the use of weighted least squares regression (Draper and Smith, 1998). In this approach, each observation in a group of animals is assigned a weight that is inversely proportional to the variance of the response in that group:

$$w_i = \frac{1}{\sigma_i^2}$$

where:

w_i = weight assigned to all data points in dose group i

σ_i^2 = variance of responses in animals in dose group i

As discussed previously for arsenic (Section 3.2), the preferred method for estimating the value of σ_i^2 uses an "external" variance model based on an analysis of the relationship between variance and mean response using data consolidated across many different swine-based arsenic RBA studies. However, because vanadium data are only available from a single study, it was not possible to develop an external variance model. Instead, the observed variance (s_i^2) in the responses of animals in dose group i was used to estimate the value of σ_i^2.

Goodness of Fit

The goodness-of-fit of each dose-response model was assessed using the F test statistic and the adjusted coefficient of multiple determination (Adj R^2) as described by Draper and Smith (1998). A fit is considered acceptable if the p-value is less than 0.05.

Assessment of Outliers

In biological assays, it is not uncommon to note the occurrence of individual measured responses that appear atypical compared to the responses from other animals in the same dose group. In this study, an analysis was made by looking at responses that yielded standardized weighted residuals greater than 3.5 or less than -3.5 (Canavos,1984). When such data points were encountered in a data set, the UEF and RBA values were calculated both with and without the potential outlier(s) excluded, and the result with the outlier(s) excluded was used as the preferred estimate.

4.4 Calculation of Vanadium RBA Estimate

Endpoint-specific RBA Estimates

Vanadium RBA values were estimated using the basic statistical techniques recommended by Finney (1978). Each endpoint-specific RBA value was calculated as the ratio of the slope term for the test material data set (b_t) to the reference material data set (b_r):

$$RBA = \frac{b_t}{b_r}$$

The uncertainly range about the RBA ratio was calculated using Fieller's Theorem as described by Finney (1978).

RBA Point Estimate

Because there are three independent estimates of RBA for the test material (one from each measurement endpoint), the final RBA estimate involves combining the three endpoint-specific RBA values into a single value (point estimate) and estimating the uncertainty around that point estimate. As reflected in the coefficient of variation for endpoint-specific RBA estimate, the three endpoint-specific RBA values are all approximately equally reliable. Therefore, the RBA point estimate for each test material was calculated as the simple mean of all three endpoint-specific RBA values.

The uncertainty bounds around the point estimate were estimated using Monte Carlo simulation. Values for RBA were drawn from the uncertainty distributions for each endpoint with equal frequency. Each endpoint-specific uncertainty distribution was assumed to be normal, with the mean equal to the best estimate of RBA and the standard deviation estimated from Fieller's Theorem (Finney, 1978). The uncertainty in the point estimate was characterized as the range from the 5^{th} to the 95^{th} percentile of the mean across endpoints.

5.0 RESULTS

5.1 Clinical Signs

The doses of arsenic and vanadium administered in this study are below a level that is expected to cause toxicological responses in swine. No clinical signs of arsenic- or vanadium-induced toxicity were noted in any of the animals used in the study.

5.2 Data Exclusions

Occasionally, the dilution of urine by spilled water is so large that the concentration of arsenic in the urine cannot be quantified. These instances are defined by having a urine arsenic concentration at or below the quantitation limit (2 μg/liter) and a total urine volume greater than 10,000 mL. When both of these conditions are met, the data are deemed unreliable and excluded from further calculations. In this study, one result (pig #709 from group 10 on days 12/13) was deemed unreliable for this reason and excluded from all analyses.

In addition, pig #713 (group 5, middle dose of test soil) spilled a large portion of its dose in its urine bucket on day 6. Therefore, the urine collected from this animal on days 6/7 was excluded *a priori*.

5.3 Dose-Response Patterns

Urinary Arsenic Variance

Discussed in Section 3.2, the urinary arsenic dose-response data are analyzed using weighted least squares regression and the weights are assigned using an "external" variance model. The data used to derive the variance model are shown in Figure 5-1. This data was gathered from previous RBA studies on swine. Based on these data, values of $k1$ and $k2$ were derived using ordinary least squares minimization. The resulting values were -1.10 for $k1$ and 1.64 for $k2$.

Superimposed on Figure 5-1 is the variance data from this study (as indicated by the solid symbols) on top of the historic data set (open symbols). As seen, the variance of the urinary arsenic data from this study is consistent with the data used to generate the variance model.

Urinary Arsenic

The dose-response data for arsenic in urine were modeled using a linear equation (see Section 3.2). All data were used in the initial fittings. The results of the initial fittings are shown in Figures 5-2 (days 6/7), 5-3 (days 9/10), 5-4 (days 12/13), and 5-5 (all days). Two outliers were identified based on the identification process described earlier. Outliers are identified in Figures 5-2 through 5-5. These outliers were subsequently excluded from the final evaluation for arsenic (Figures 5-6 through 5-9).

Tissue Vanadium

The dose-response data for vanadium in liver, kidney, and bone (measured at sacrifice on day 15) were modeled using a linear equation (see Section 4.3). All data were included in the initial fittings. The results of these fittings are shown in Figures 5-10 (liver), 5-11 (kidney), and 5-12 (femur). No outliers were identified in the vanadium data sets.

5.4 Calculated RBA Values

Arsenic

The dose-response curves are approximately linear (Figures 5-6 through 5-9), with the slope of the best-fit straight line being equal to the best estimate of the UEF.

As discussed previously (Section 3.1), the relative bioavailability of arsenic in a specific test material is calculated as follows:

$$RBA(test\ vs\ ref) = \frac{UEF(test)}{UEF(ref)}$$

The following table summarizes the estimated RBA values:

Measurement Endpoint	Estimated Soil RBA (90% Confidence Interval)
Days 6/7	0.19 (0.17 - 0.21)
Days 9/10	0.16 (0.14 - 0.19)
Days 12/13	0.13 (0.11 - 0.15)
All Days	0.15 (0.14 - 0.16)

As shown, using sodium arsenate as a relative frame of reference, the RBA estimate for the test soil is approximately 15%.

Vanadium

Vanadium RBA values were calculated for each measurement endpoint (liver, kidney, and bone) using the method described in Section 4.4; the suggested point estimate is calculated as the simple mean of the three endpoint-specific estimates. The results are shown below:

Measurement Endpoint	Estimated Soil RBA (90% Confidence Interval)
Liver Vanadium	0.08 (0.06 - 0.10)
Kidney Vanadium	0.06 (0.05 - 0.08)
Bone Vanadium	0.08 (0.06 - 0.10)
Point Estimate	0.08 (0.06 - 0.10)

As shown, using vanadyl sulfate as a relative frame of reference, the RBA estimate for the test soil is approximately 8%.

5.5 Uncertainty

The bioavailability estimates above are subject to uncertainty that arises from several different sources. One source of uncertainty is the inherent biological variability between different animals in a dose group, which in turn causes variability in the amount of arsenic or vanadium absorbed by the exposed animals. This between-animal variability in response results in statistical uncertainty in the best-fit dose-response curves and, hence, uncertainty in the calculated values of RBA. Such statistical uncertainty is accounted for by the statistical models used above and is characterized by the uncertainty range around the endpoint-specific and the point estimate values of RBA.

However, there is also uncertainty in the extrapolation of RBA values measured in juvenile swine to young children or adults, and this uncertainty is not included in the statistical confidence bounds above. Even though the immature swine is believed to be a useful and meaningful animal model for gastrointestinal absorption in children, it is possible that there are differences in physiological parameters that may influence RBA and that RBA values in swine are not identical to values in children. In addition, RBA may depend on the amount and type of food in the stomach, since the presence of food can influence stomach pH, holding time, and possibly other factors that may influence solubilization of arsenic or vanadium. In this regard, it is important to recall that RBA values measured in this study are based on animals that have little or no food in their stomach at the time of exposure and, hence, are likely to yield high-end values of RBA. Thus, these RBA values may be somewhat conservative for humans who ingest the site soil along with food. The magnitude of this bias is not known.

Dosing Anomalies

There were a few instances where some animals did not consume their entire dose (see Appendix A, Tables A-6 and A-7). During the study, however, the dosing technician observed each animal and attempted to estimate the fraction of dose not consumed; these estimates of missed doses were then used to adjust the time-weighted average dose calculation for each animal downward. Because these estimates of missed doses are subjective, they introduce some uncertainty; however, the magnitude of this uncertainty is thought to be small. All calculations are based on actual administered doses (not target doses) to compensate for dosing errors.

There was insufficient soil to prepare the second half of final dosing (day 14) dosing, so dosing for all animals was terminated after the day 14 morning dosing (i.e., no animals in any group received the 3:00 PM dose on day 14). This could result in a decrease in the magnitude of the measured vanadium concentrations in the endpoint tissues. However, because the animals were dosed for 15 days, the magnitude of this decrease is likely to be small. In addition, because the lack of dosing was applied to all groups, it is expected that any observable effect will be cancelled and it is not expected to introduce a significant error. Urine collections ended on day 13, so arsenic concentrations are unaffected by this dosing anomaly.

6.0 CONCLUSIONS AND RECOMMENDATIONS

Arsenic

When reliable site-specific data are lacking, a default RBA value in the range of 80%-100% is usually employed for arsenic in soil. The RBA estimate of 15% for the test soil used in this study is markedly lower than the default range, indicating that the arsenic in this soil is not as well absorbed as soluble arsenic. It is appropriate to take this into account when evaluating potential risks to humans from incidental ingestion of this soil.

Vanadium

Due to a general lack of data, the RBA typically employed for vanadium in soil is 100%. The RBA estimate of 8% obtained for the test soil used in this study is markedly lower than that default assumption, indicating that the vanadium in this soil is not as well absorbed as soluble vanadium. It is appropriate to take this into account when evaluating potential risks to humans from incidental ingestion of this soil.

Recommendations

These site-specific RBA estimates for arsenic and vanadium are an improvement over the default values and should be considered for use in site-specific risk assessments. However, it important to consider that the values are specific to the soil tested in this study. Use of the RBA estimates may improve accuracy and decrease uncertainty in estimating human health risks from exposure to this test soil, as well as increase confidence in computations of site-specific risk-based cleanup levels.

7.0 REFERENCES

Canavos, C. G. 1984. Applied Probability and Statistical Methods. Little, Brown and Co., Boston.

Casteel, S. W., R. P. Cowart, C. P. Weis, G. M. Henningsen, E. Hoffman, W. J. Brattin, M. F. Starost, J. T. Payne, S. L. Stockham, S. V. Becker, and J. R. Turk. 1996. A swine model for determining the bioavailability of lead from contaminated media. In: Advances in Swine in Biomedical Research. Tumbleson and Schook, eds. Vol 2, Plenum Press, New York. Pp. 637-46.

Draper, N. R., and H. Smith. 1998. Applied Regression Analysis (3rd Edition). John Wiley & Sons, New York.

Finney, D. J. 1978. Statistical Method in Biological Assay (3rd Edition). Charles Griffin and Co., London.

Gibaldi, M., and Perrier, D. 1982. Pharmacokinetics (2nd edition), pp 294-297. Marcel Dekker, Inc, NY, NY.

Goodman, A.G., Rall, T.W., Nies, A.S., and Taylor, P. 1990. The Pharmacological Basis of Therapeutics (8th ed.), pp. 5-21. Pergamon Press, Inc. Elmsford, NY.

Klaassen, C.D., Amdur, M.O., and Doull, J. (eds). 1996. Cassarett and Doull's Toxicology: The Basic Science of Poisons, pp. 190. McGraw-Hill, Inc. NY, NY.

USEPA. 2007. Estimation of Relative Bioavailability of Lead in Soil and Soil-Like Materials by *In Vivo* and *In Vitro* Methods OSWER9285.7-77. Office of Solid Waste and Emergency Response, Washington DC, USA.

Weis, C.P., and LaVelle, J.M. 1991. Characteristics to consider when choosing an animal model for the study of lead bioavailability. In: The proceedings of the international symposium on the bioavailability and dietary uptake of lead. Science and Technology Letters 3:113-119.

TABLES AND FIGURES

TABLE 2-1 DOSING PROTOCOL

Group	Number of Animals	Dose Material Administered	Arsenic Dose (µg/kg-day)		Vanadium Dose (µg/kg-day)	
			Target	Actual [a]	Target	Actual [a]
1	5	$NaHAsO_4$	30	30.4	0	0.0
2	5	$NaHAsO_4$	60	60.3	0	0.0
3	5	$NaHAsO_4$	120	121.1	0	0.0
4	5	Soil	40	42.6	103	107.8
5	5	Soil	80	84.8	206	214.3
6	5	Soil	160	165.8	412	418.9
7	5	$VOSO_4$	0	0.0	80	88.3
8	5	$VOSO_4$	0	0.0	160	162.3
9	5	$VOSO_4$	0	0.0	320	322.5
10	3	Control	0	0	0	0

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

Doses were administered in two equal portions given at 9:00 AM and 3:00 PM each day. Doses were based on the mean weight of the animals in each group, and were adjusted every three days to account for weight gain.

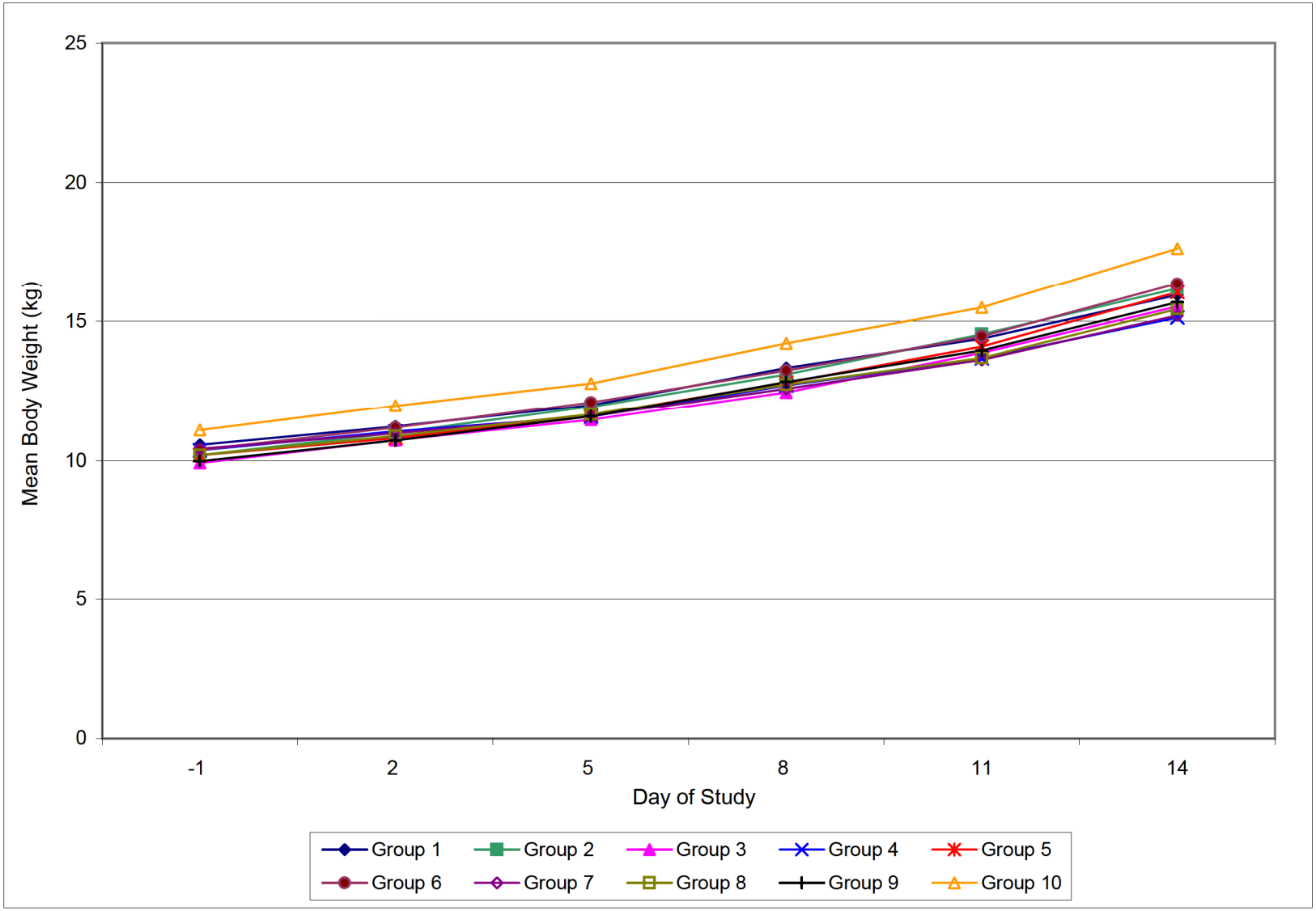
TABLE 2-2 TYPICAL FEED COMPOSITION

Nutrient Name	Amount
Protein	20.1021%
Arginine	1.2070%
Lysine	1.4690%
Methionine	0.8370%
Met+Cys	0.5876%
Tryptophan	0.2770%
Histidine	0.5580%
Leucine	1.8160%
Isoleucine	1.1310%
Phenylalanine	1.1050%
Phe+Tyr	2.0500%
Threonine	0.8200%
Valine	1.1910%
Fat	4.4440%
Saturated Fat	0.5590%
Unsaturated Fat	3.7410%
Linoleic 18:2:6	1.9350%
Linoleic 18:3:3	0.0430%
Crude Fiber	3.8035%
Ash	4.3347%
Calcium	0.8675%
Phos Total	0.7736%
Available Phosphorous	0.7005%
Sodium	0.2448%
Potassium	0.3733%

Nutrient Name	Amount
Chlorine	0.1911%
Magnesium	0.0533%
Sulfur	0.0339%
Manganese	20.4719 ppm
Zinc	118.0608 ppm
Iron	135.3710 ppm
Copper	8.1062 ppm
Cobalt	0.0110 ppm
Iodine	0.2075 ppm
Selenium	0.3196 ppm
Nitrogen Free Extract	60.2340%
Vitamin A	5.1892 kIU/kg
Vitamin D3	0.6486 kIU/kg
Vitamin E	87.2080 IU/kg
Vitamin K	0.9089 ppm
Thiamine	9.1681 ppm
Riboflavin	10.2290 ppm
Niacin	30.1147 ppm
Pantothenic Acid	19.1250 ppm
Choline	1019.8600 ppm
Pyridoxine	8.2302 ppm
Folacin	2.0476 ppm
Biotin	0.2038 ppm
Vitamin B12	23.4416 ppm

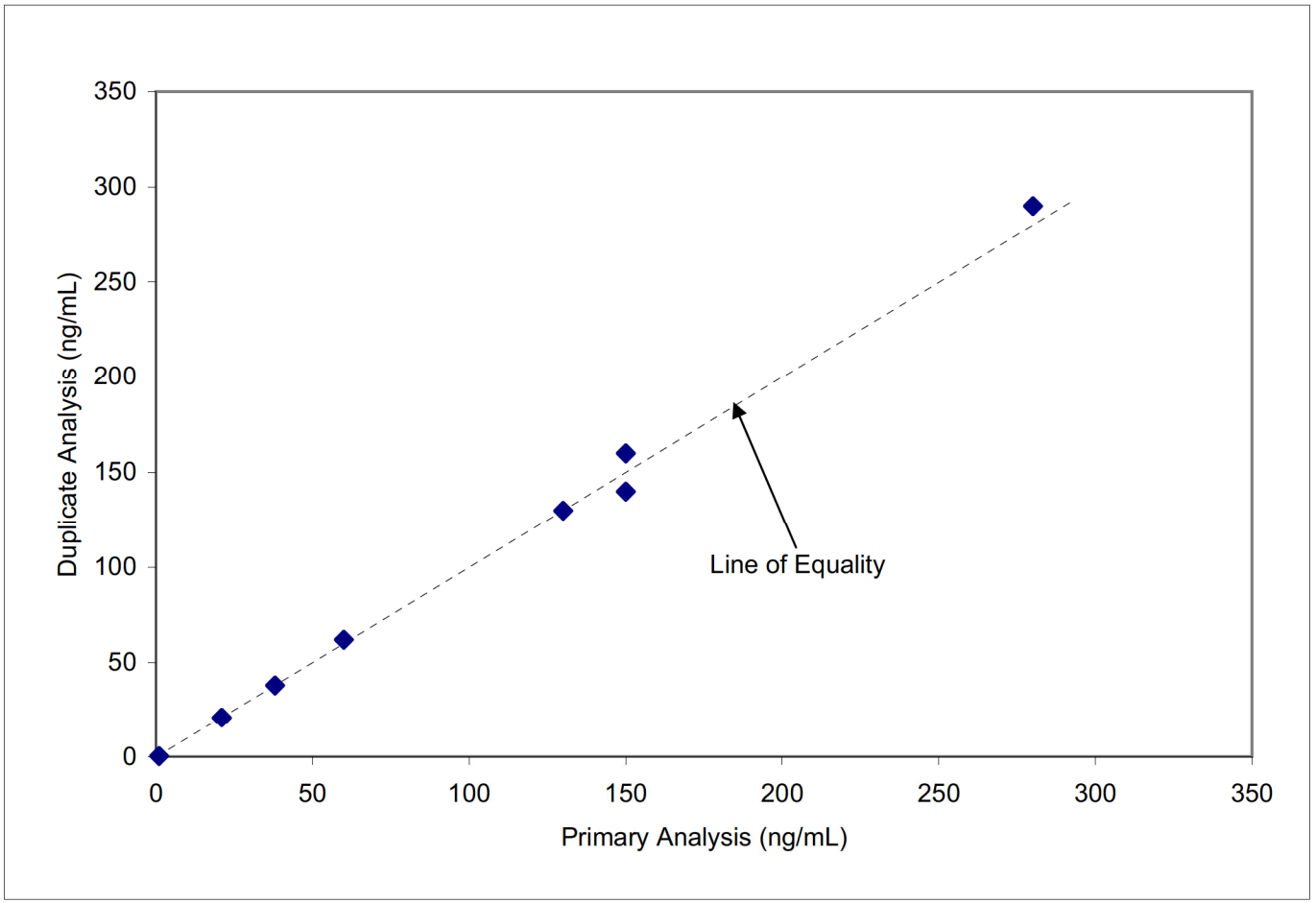
Feed obtained from and nutritional values provided by Zeigler Bros., Inc

FIGURE 2-1 BODY WEIGHT GAIN



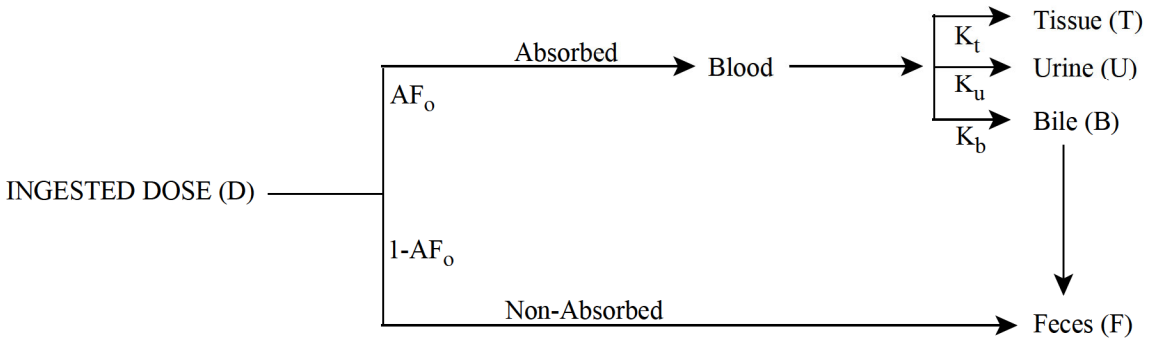
1_PTX Weights.xls (Fig2-1_group weights)

FIGURE 2-2 URINARY ARSENIC BLIND DUPLICATES (SAMPLE PREPARATION



5_PTX Lab QC analysis.xls (Fig2-2_BlindDupes)

Figure 3-1. Conceptual Model for Arsenic Toxicokinetics



where:

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

BASIC EQUATIONS:

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

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

= $D \bullet\bullet AF_o \bullet\bullet K_u$

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

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

= $AF_o \bullet\bullet K_u$

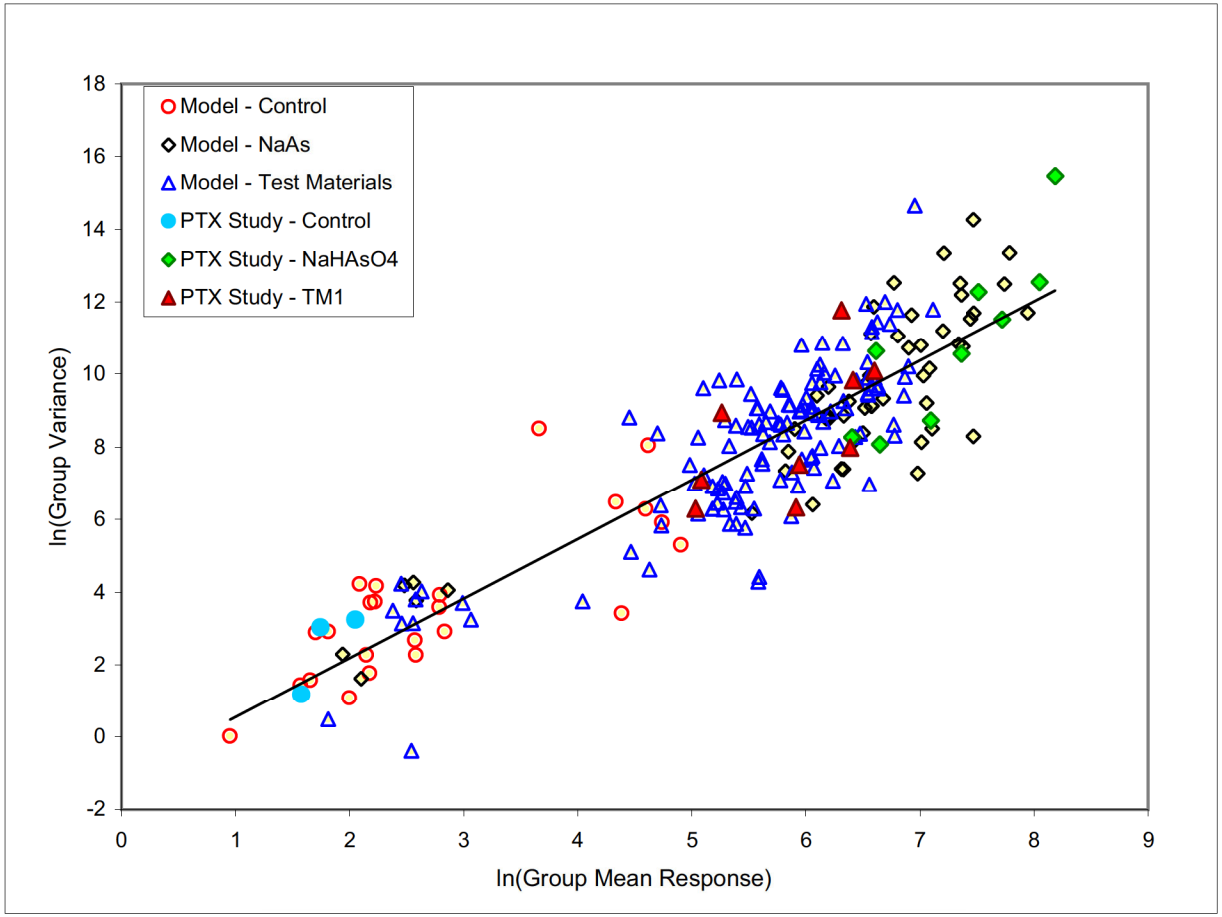
Relative Bioavailability (x vs. y) = UEF(x) / UEF(y)

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

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

Fig 3-1_Toxicokinetics.wpd

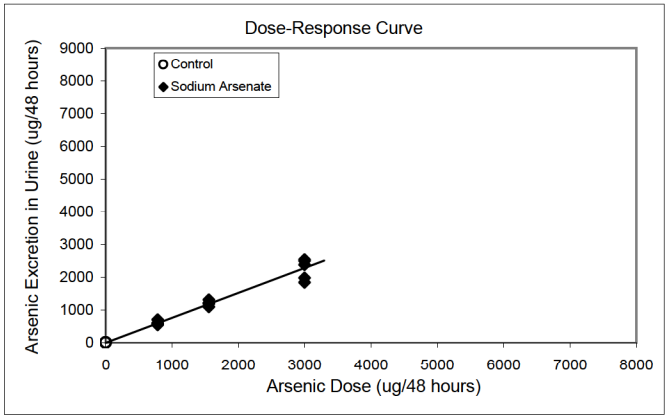
FIGURE 5-1 URINARY ARSENIC VARIANCE



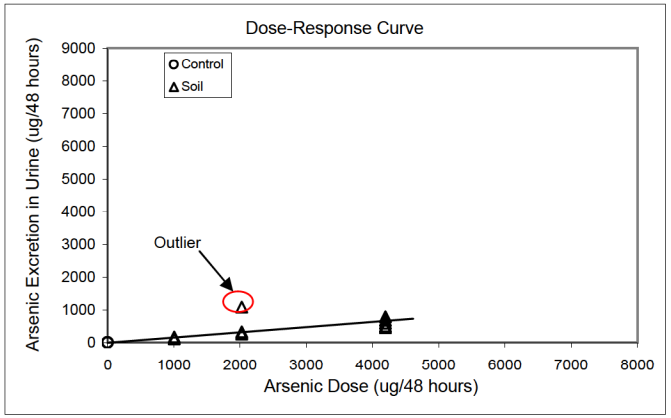
4_PTX Variance.xls (Fig A-5_PhIII Oral)

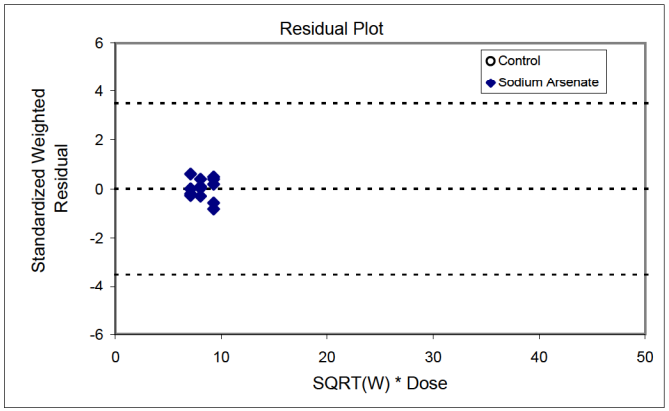
FIGURE 5-2 URINARY EXCRETION OF ARSENIC: Days 6/7 (All Data)

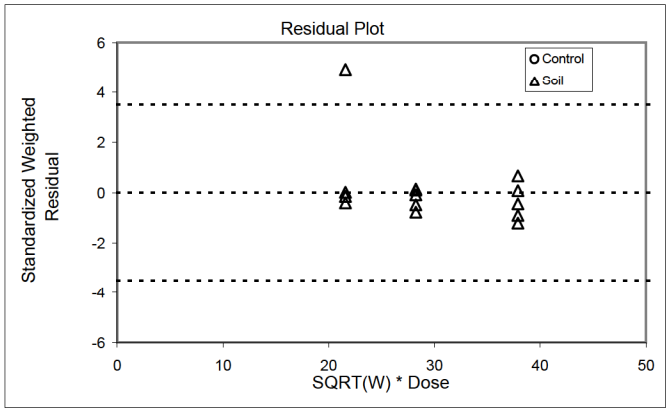
Reference Material (Sodium Arsenate)



Test Material (Soil)







Summary of Fitting[a]

Parameter	Estimate	SE
a	4.9	2.1
b1	0.76	0.05
b2	0.16	0.01
Covariance (b1,b2)	0.0018	--
Degrees of Freedom	30	--

[a] y = a + b1*x1 + b2*x2

ANOVA

Source	SSE	DF	MSE
Fit	909.30	2	454.65
Error	83.85	29	2.89
Total	993.15	31	32.04

Statistic	Estimate
F	157.242
p	< 0.001
Adjusted R^2	0.9097

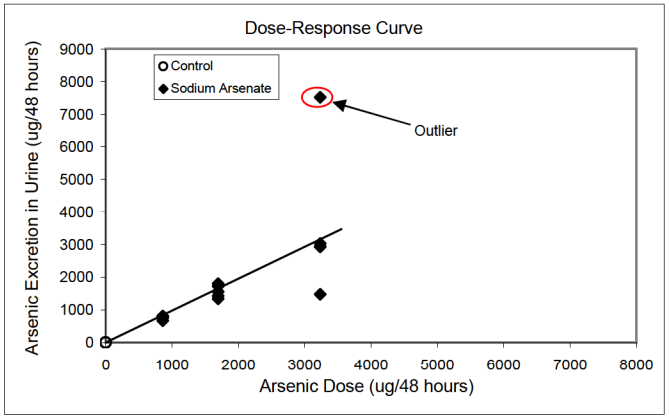
RBA and Uncertainty

Test Material (Soil)	
RBA	0.21
Lower bound[b]	0.17
Upper bound[b]	0.25
Standard Error[b]	0.025

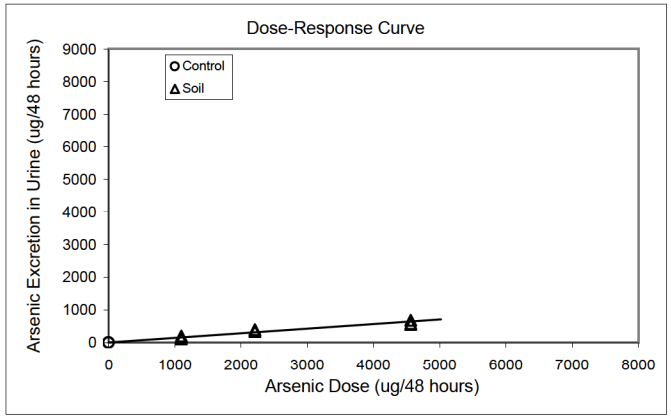
[b] Calculated using Fieller's theorem

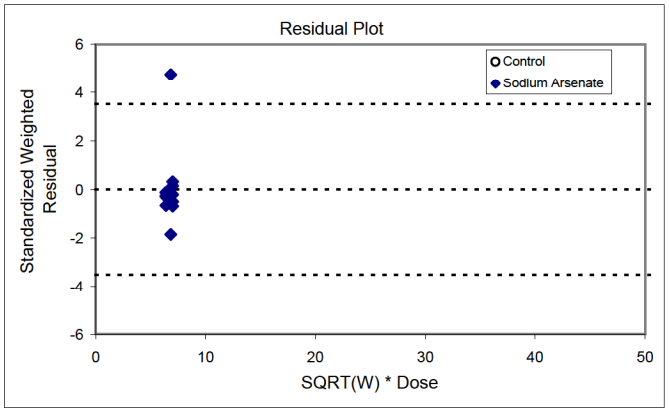
FIGURE 5-3 URINARY EXCRETION OF ARSENIC: Days 9/10 (All Data)

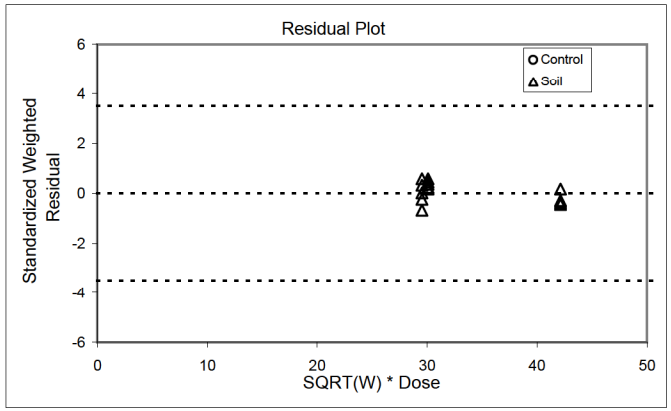
Reference Material (Sodium Arsenate)



Test Material (Soil)







Summary of Fitting[a]

Parameter	Estimate	SE
a	5.7	2.8
b1	0.98	0.08
b2	0.14	0.02
Covariance (b1,b2)	0.0020	--
Degrees of Freedom	31	--

[a] y = a + b1*x1 + b2*x2

ANOVA

Source	SSE	DF	MSE
Fit	1003.04	2	501.52
Error	119.23	30	3.97
Total	1122.27	32	35.07

Statistic	Estimate
F	126.192
p	< 0.001
Adjusted R^2	0.8867

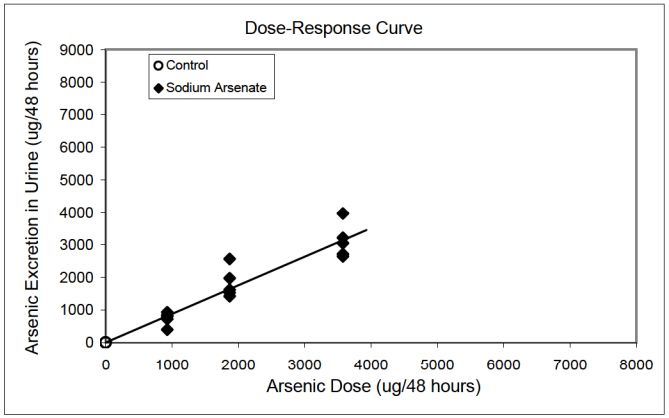
RBA and Uncertainty

Test Material (Soil)	
RBA	0.14
Lower bound[b]	0.11
Upper bound[b]	0.18
Standard Error[b]	0.019

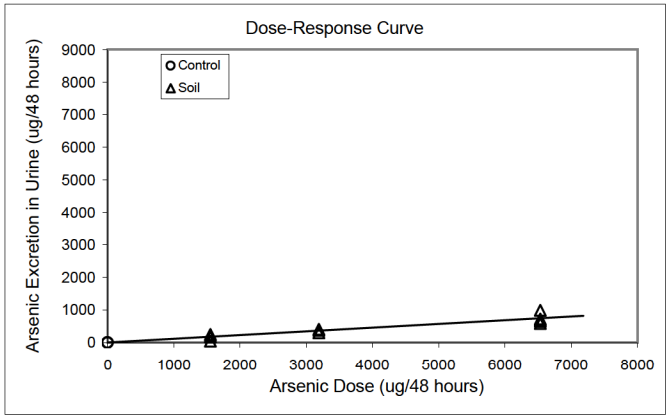
[b] Calculated using Fieller's theorem

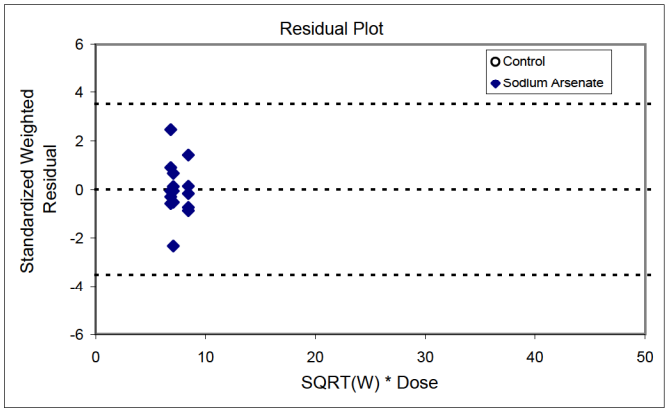
FIGURE 5-4 URINARY EXCRETION OF ARSENIC: Days 12/13 (All Data)

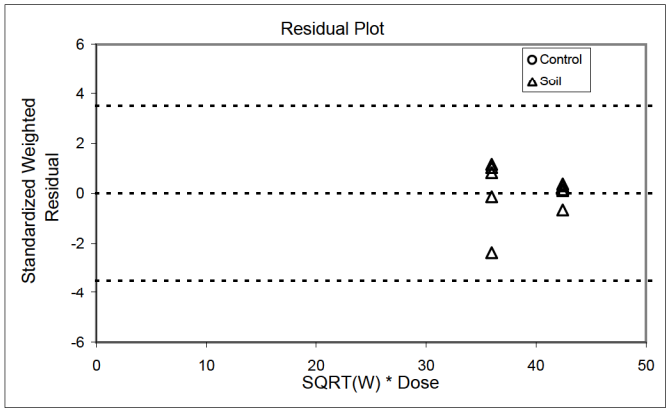
Reference Material (Sodium Arsenate)



Test Material (Soil)







Summary of Fitting[a]

Parameter	Estimate	SE
a	9.2	3.5
b1	0.88	0.05
b2	0.11	0.01
Covariance (b1,b2)	0.0056	--
Degrees of Freedom	30	--

[a] y = a + b1*x1 + b2*x2

ANOVA

Source	SSE	DF	MSE
Fit	1006.66	2	503.33
Error	58.60	29	2.02
Total	1065.26	31	34.36

Statistic	Estimate
F	249.093
p	< 0.001
Adjusted R^2	0.9412

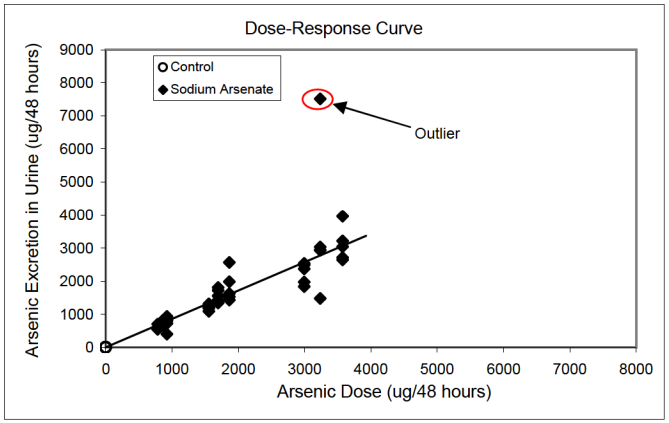
RBA and Uncertainty

Test Material (Soil)	
RBA	0.13
Lower bound[b]	0.11
Upper bound[b]	0.15
Standard Error[b]	0.012

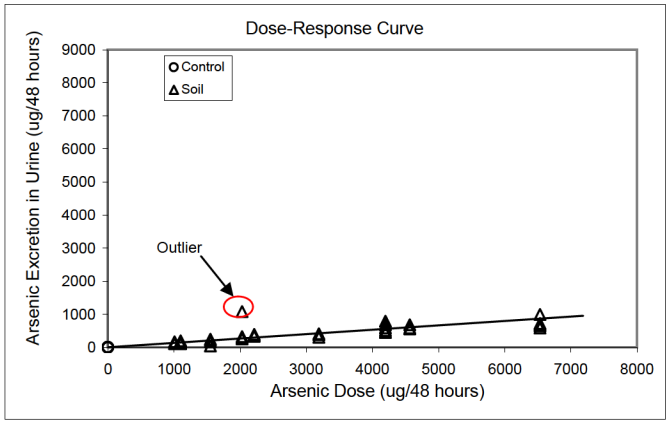
[b] Calculated using Fieller's theorem

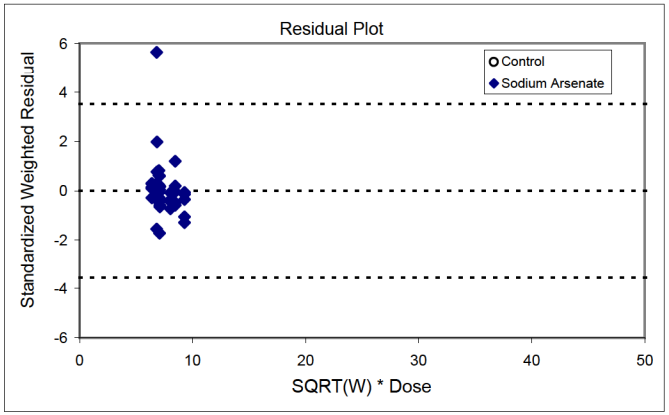
FIGURE 5-5 URINARY EXCRETION OF ARSENIC: All Days (All Data)

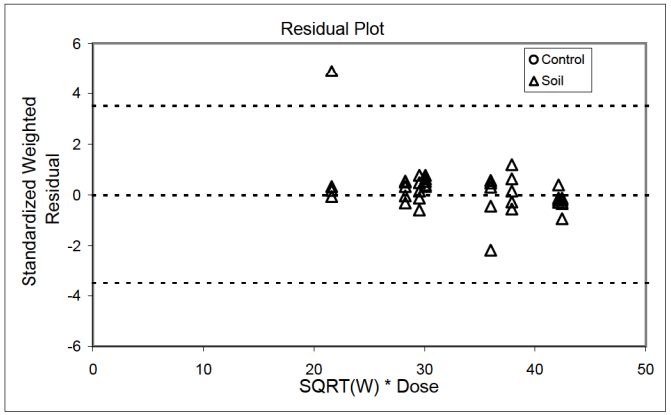
Reference Material (Sodium Arsenate)



Test Material (Soil)







Summary of Fitting[a]

Parameter	Estimate	SE
a	5.8	1.5
b1	0.86	0.04
b2	0.13	0.01
Covariance (b1,b2)	0.0023	--
Degrees of Freedom	95	--

[a] y = a + b1*x1 + b2*x2

ANOVA

Source	SSE	DF	MSE
Fit	2894.30	2	1447.15
Error	302.37	94	3.22
Total	3196.67	96	33.30

Statistic	Estimate
F	449.890
p	< 0.001
Adjusted R^2	0.9034

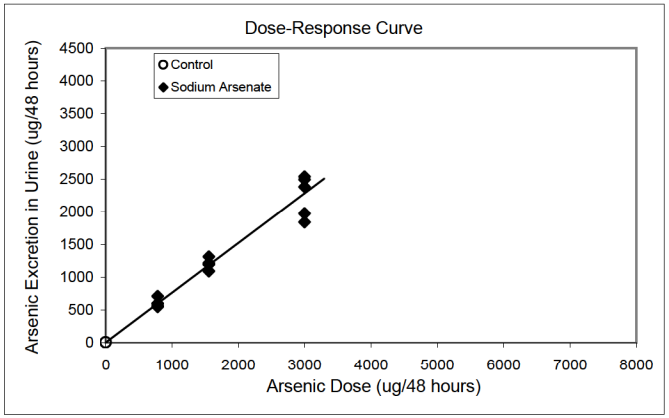
RBA and Uncertainty

Test Material (Soil)	
RBA	0.15
Lower bound[b]	0.14
Upper bound[b]	0.17
Standard Error[b]	0.011

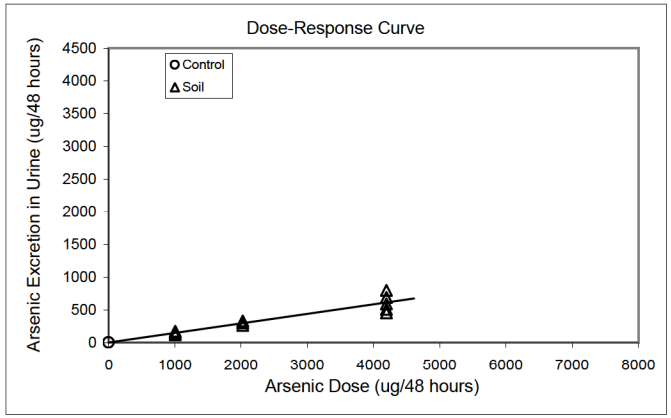
[b] Calculated using Fieller's theorem

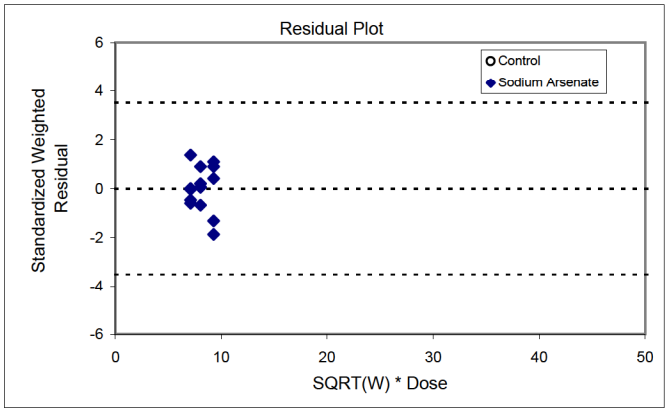
FIGURE 5-6 URINARY EXCRETION OF ARSENIC: Days 6/7 (Outliers Excluded)

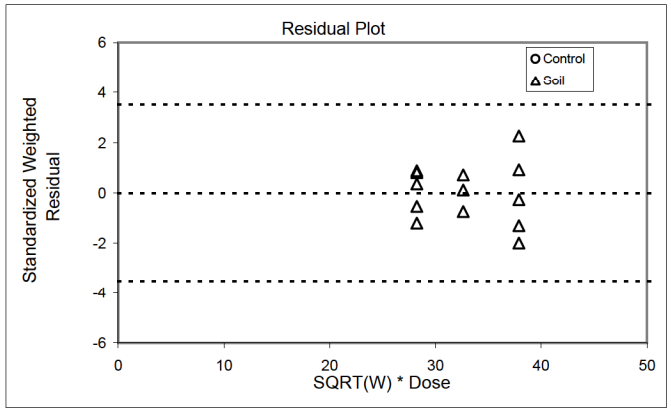
Reference Material (Sodium Arsenate)



Test Material (Soil)







Summary of Fitting[a]

Parameter	Estimate	SE
a	4.9	0.9
b1	0.76	0.02
b2	0.15	0.01
Covariance (b1,b2)	0.0018	--
Degrees of Freedom	29	--

[a] y = a + b1*x1 + b2*x2

ANOVA

Source	SSE	DF	MSE
Fit	882.36	2	441.18
Error	15.82	28	0.57
Total	898.18	30	29.94

Statistic	Estimate
F	780.745
p	< 0.001
Adjusted R^2	0.9811

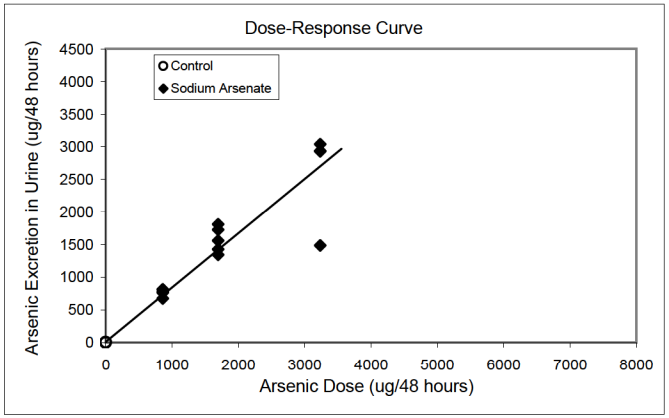
RBA and Uncertainty

Test Material (Soil)	
RBA	0.19
Lower bound[b]	0.17
Upper bound[b]	0.21
Standard Error[b]	0.010

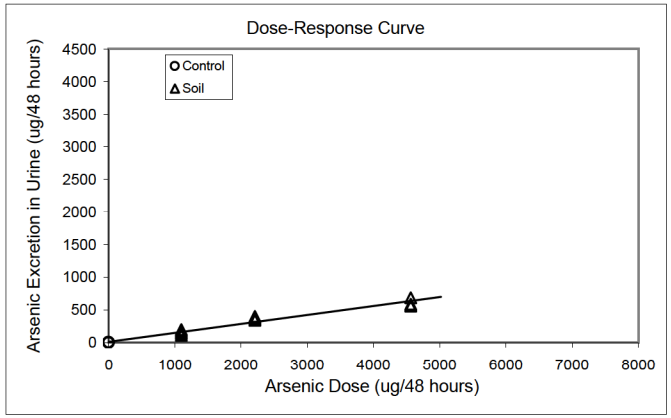
[b] Calculated using Fieller's theorem

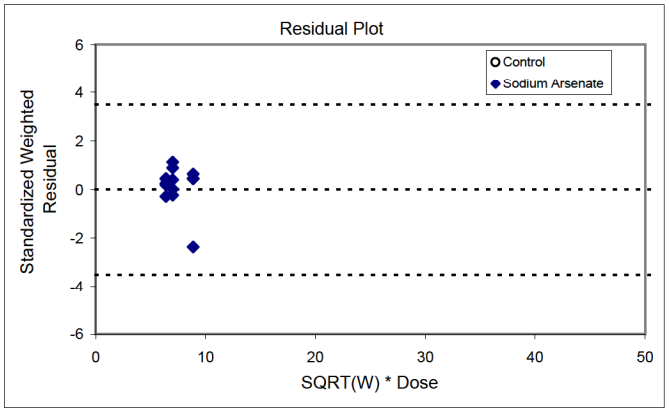
FIGURE 5-7 URINARY EXCRETION OF ARSENIC: Days 9/10 (Outliers Excluded)

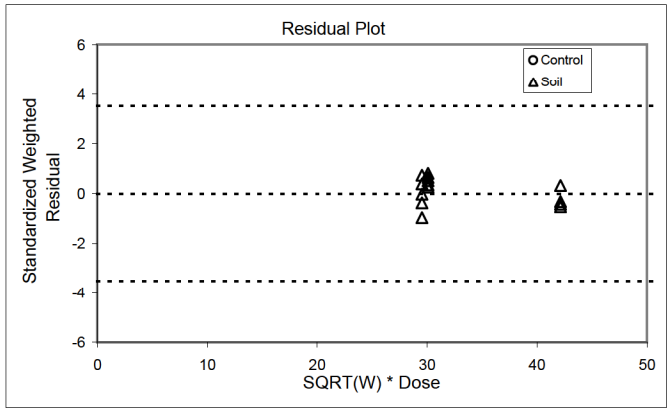
Reference Material (Sodium Arsenate)



Test Material (Soil)







Summary of Fitting[a]

Parameter	Estimate	SE
a	13.2	17.8
b1	0.83	0.04
b2	0.14	0.01
Covariance (b1,b2)	0.1267	--
Degrees of Freedom	30	--

[a] y = a + b1*x1 + b2*x2

ANOVA

Source	SSE	DF	MSE
Fit	887.57	2	443.79
Error	63.32	29	2.18
Total	950.89	31	30.67

Statistic	Estimate
F	203.245
p	< 0.001
Adjusted R^2	0.9288

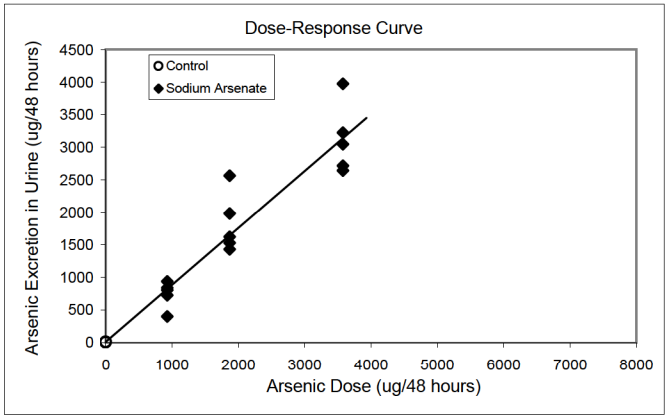
RBA and Uncertainty

Test Material (Soil)	
RBA	0.16
Lower bound[b]	0.14
Upper bound[b]	0.19
Standard Error[b]	0.016

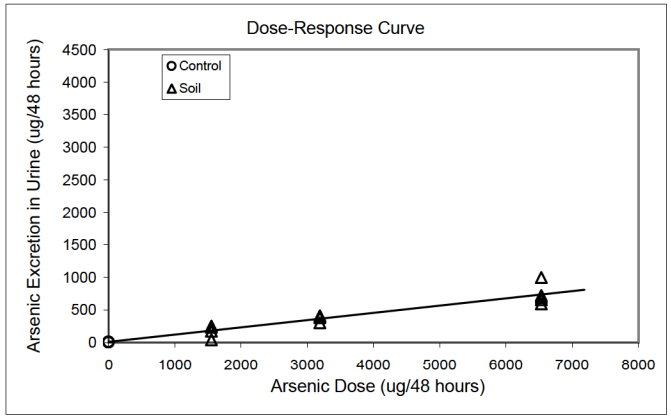
[b] Calculated using Fieller's theorem

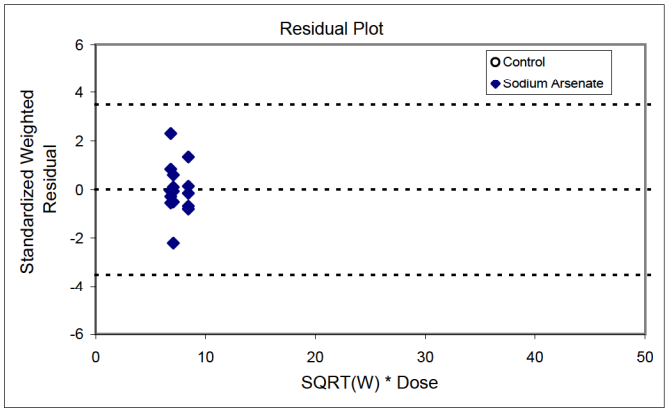
FIGURE 5-8 URINARY EXCRETION OF ARSENIC: Days 12/13 (Outliers Excluded)

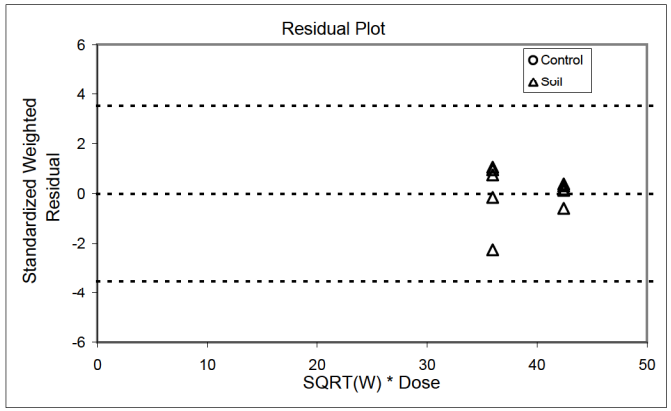
Reference Material (Sodium Arsenate)



Test Material (Soil)







Summary of Fitting[a]

Parameter	Estimate	SE
a	16.3	32.5
b1	0.87	0.04
b2	0.11	0.01
Covariance (b1,b2)	0.2209	--
Degrees of Freedom	30	--

[a] y = a + b1*x1 + b2*x2

ANOVA

Source	SSE	DF	MSE
Fit	987.31	2	493.65
Error	66.72	29	2.30
Total	1054.02	31	34.00

Statistic	Estimate
F	214.578
p	< 0.001
Adjusted R^2	0.9323

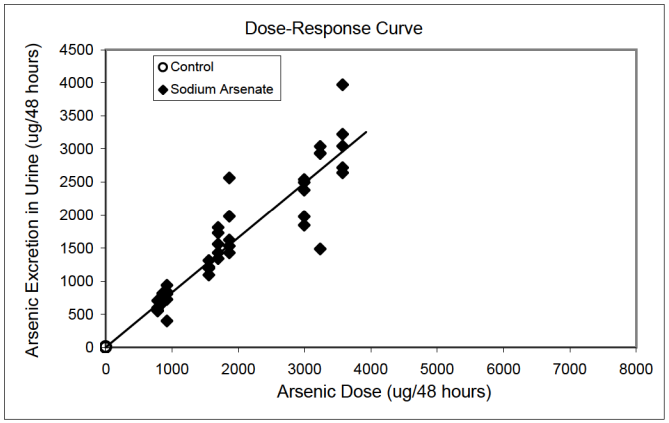
RBA and Uncertainty

Test Material (Soil)	
RBA	0.13
Lower bound[b]	0.11
Upper bound[b]	0.15
Standard Error[b]	0.012

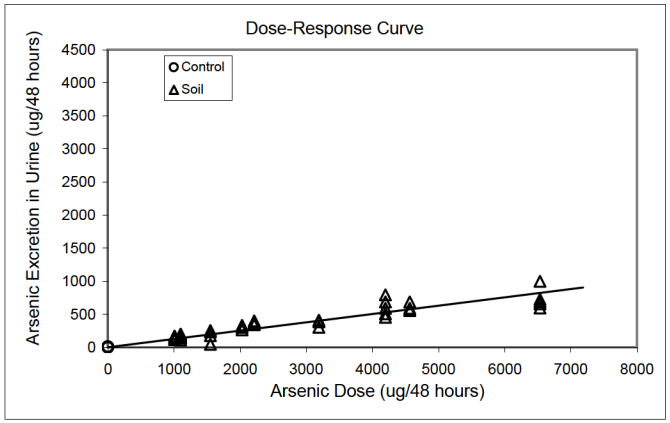
[b] Calculated using Fieller's theorem

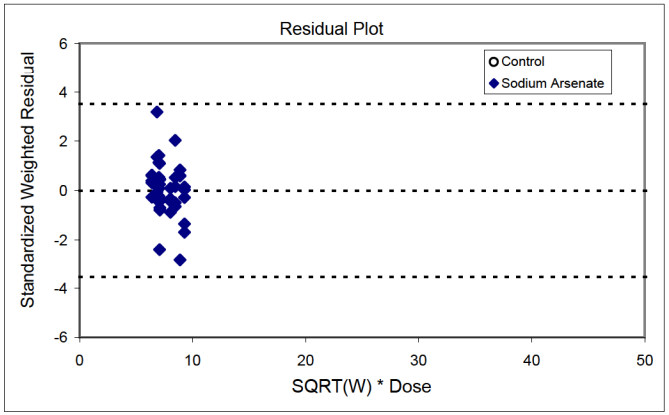
FIGURE 5-9 URINARY EXCRETION OF ARSENIC: All Days (Outliers Excluded)

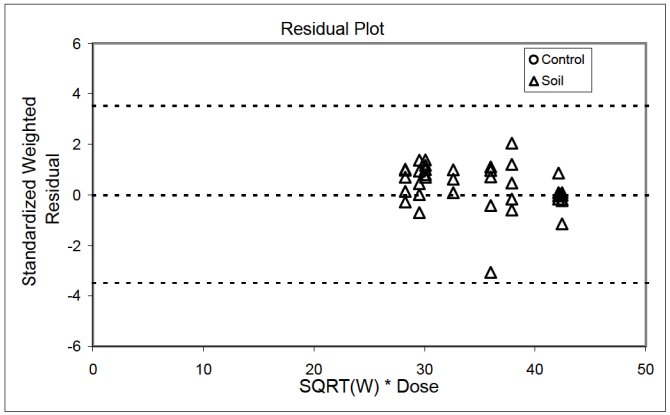
Reference Material (Sodium Arsenate)



Test Material (Soil)







Summary of Fitting[a]

Parameter	Estimate	SE
a	5.1	1.5
b1	0.83	0.02
b2	0.13	0.00
Covariance (b1,b2)	0.0034	--
Degrees of Freedom	93	--

[a] y = a + b1*x1 + b2*x2

ANOVA

Source	SSE	DF	MSE
Fit	2728.52	2	1364.26
Error	132.58	92	1.44
Total	2861.10	94	30.44

Statistic	Estimate
F	946.672
p	< 0.001
Adjusted R^2	0.9527

RBA and Uncertainty

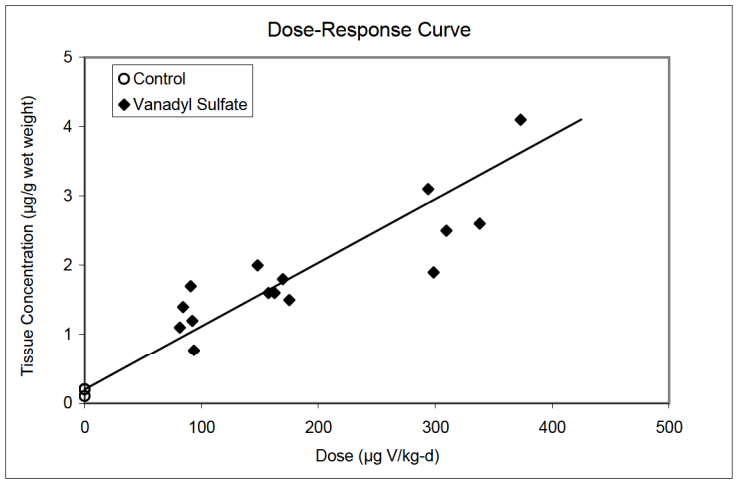
Test Material (Soil)	
RBA	0.15
Lower bound[b]	0.14
Upper bound[b]	0.16
Standard Error[b]	0.007

[b] Calculated using Fieller's theorem

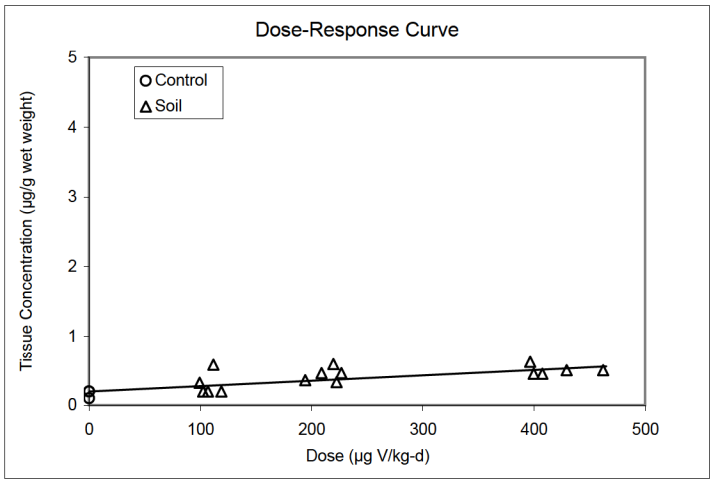
3_PTX RBA Calcs_Arsenic_outliers.xls (Graph_All)

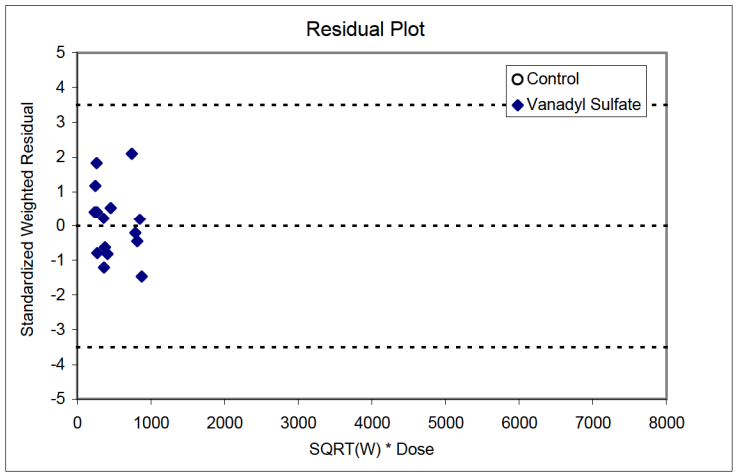
FIGURE 5-10 LIVER VANADIUM DOSE-RESPONSE

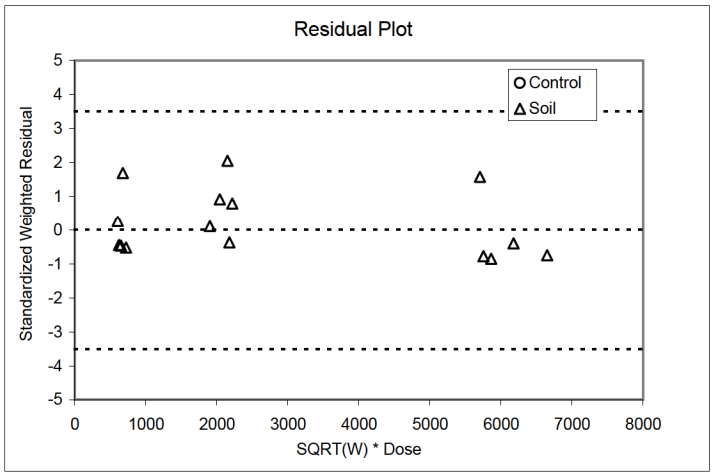
Reference Material (Vanadyl Sulfate)



Test Material (Soil)







Summary of Fitting*

Parameter	Estimate	Standard Error
a	1.99E-01	3.26E-02
b_r	9.20E-03	5.55E-04
b_{tm}	7.69E-04	1.16E-04
Covariance (b_r,b_{tm})	0.2663	--
Degrees of Freedom	30	--

*Data were fit using the linear model: $y = a + b_r \cdot x_r + b_{tm} \cdot x_{tm}$

Goodness of Fit

Statistic	Estimate
F	139.966
p	< 0.001
Adjusted R^2	0.8968

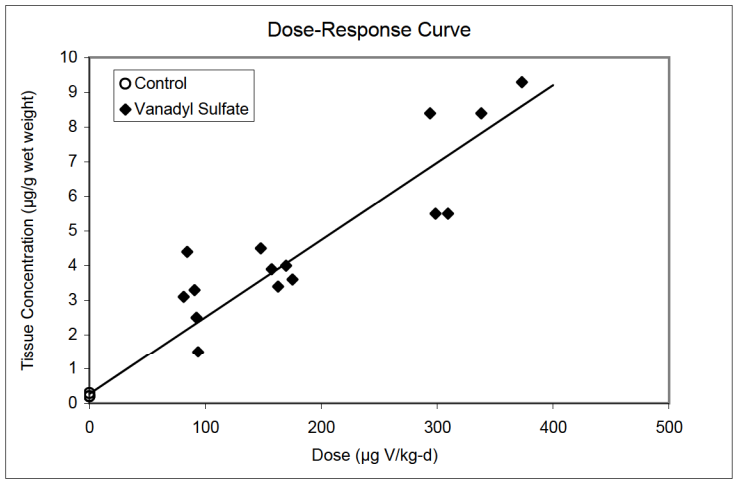
RBA and Uncertainty

Test Material	
RBA	0.08
Lower Bound	0.06
Upper Bound	0.10
Standard Error	0.012

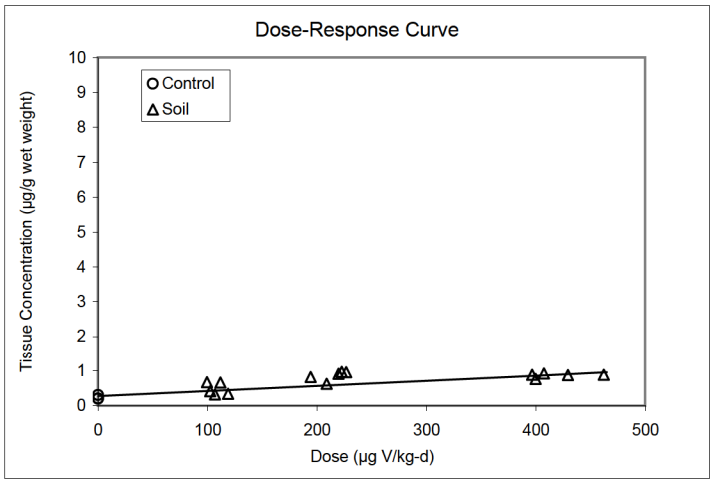
3_PTX RBA Calcs_Vanadium_REV4.xls (Fig5-10_Liver)

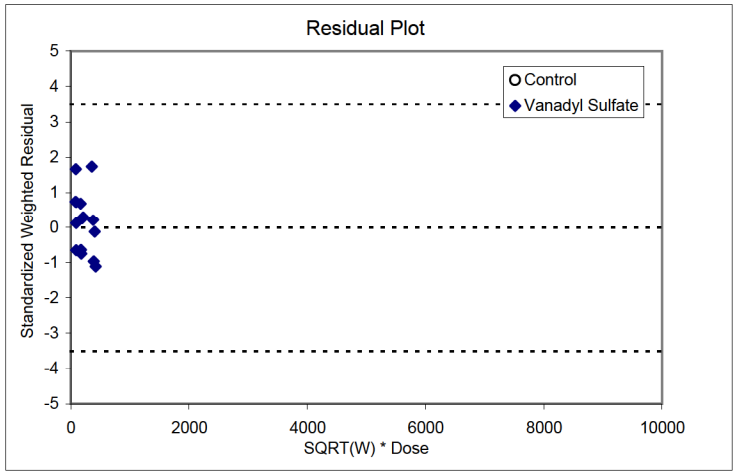
FIGURE 5-11 KIDNEY VANADIUM DOSE-RESPONSE

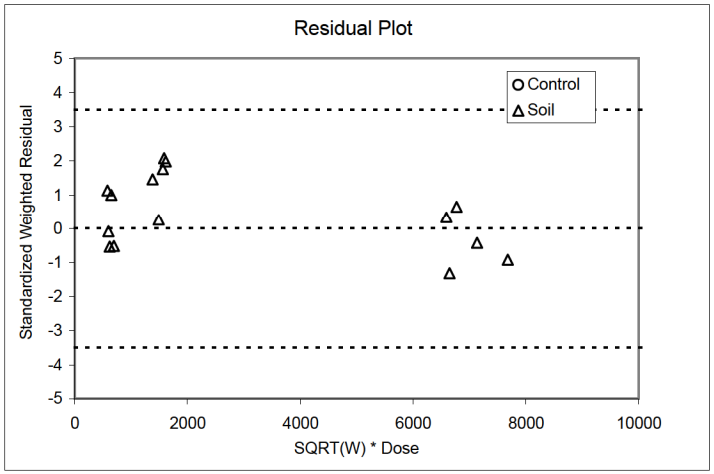
Reference Material (Vanadyl Sulfate)



Test Material (Soil)







Summary of Fitting*

Parameter	Estimate	Standard Error
a	2.80E-01	4.09E-02
b_r	2.23E-02	1.39E-03
b_{tm}	1.45E-03	1.33E-04
Covariance (b_r,b_{tm})	0.1335	--
Degrees of Freedom	30	--

*Data were fit using the linear model: $y = a + b_r \cdot x_r + b_{tm} \cdot x_{tm}$

Goodness of Fit

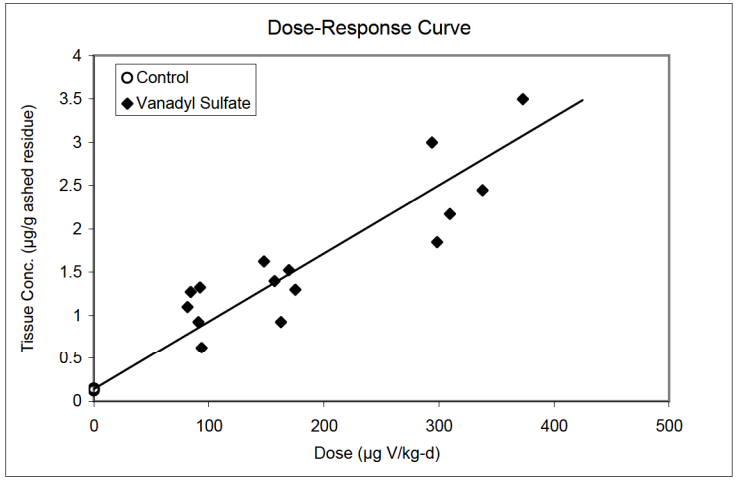
Statistic	Estimate
F	168.998
p	< 0.001
Adjusted R^2	0.9130

RBA and Uncertainty

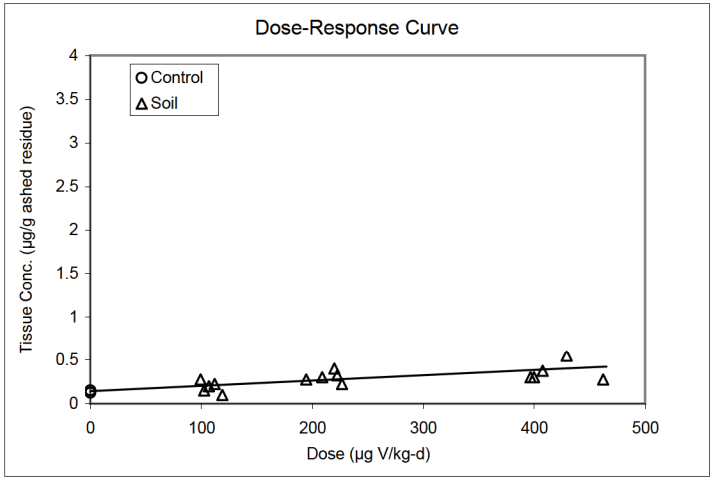
Test Material	
RBA	0.06
Lower Bound	0.05
Upper Bound	0.08
Standard Error	0.007

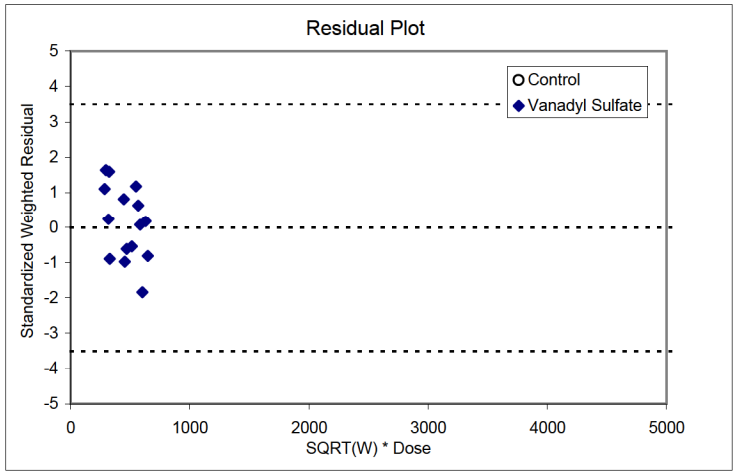
FIGURE 5-12 FEMUR VANADIUM DOSE-RESPONSE

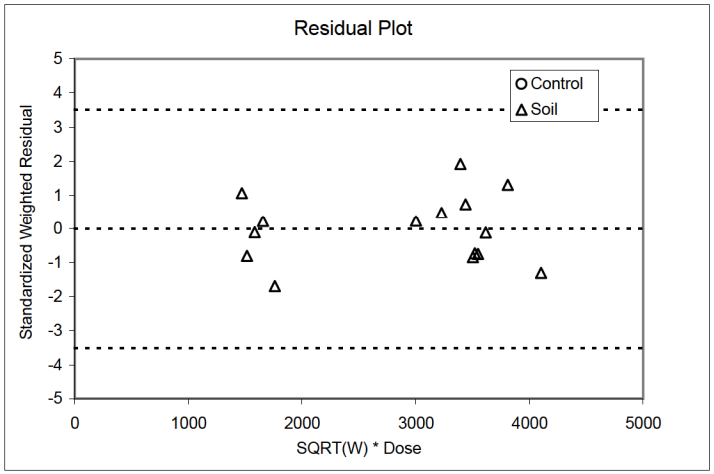
Reference Material (Vanadyl Sulfate)



Test Material (Soil)







Summary of Fitting*

Parameter	Estimate	Standard Error
a	1.43E-01	8.45E-03
b_r	7.87E-03	5.55E-04
b_{tm}	6.04E-04	9.40E-05
Covariance (b_r,b_{tm})	0.0310	--
Degrees of Freedom	30	--

*Data were fit using the linear model: $y = a + b_r \cdot x_r + b_{tm} \cdot x_{tm}$

Goodness of Fit

Statistic	Estimate
F	118.484
p	< 0.001
Adjusted R^2	0.8801

RBA and Uncertainty

Test Material	
RBA	0.08
Lower Bound	0.06
Upper Bound	0.10
Standard Error	0.013

APPENDIX A

DETAILED RESULTS

TABLE A-1 SCHEDULE

Study Day	Day	Date	Feed Special Diet	Cull Pigs/ Assign Dose Group	Weigh	Dose Preparation	Dose Administration	48-hour Urine Collection	Sacrifice/ Necropsy
-6	Tuesday	02/08/05	transition	X	X				
-5	Wednesday	02/09/05	transition						
-4	Thursday	02/10/05	transition						
-3	Friday	02/11/05	X						
-2	Saturday	02/12/05	X						
-1	Sunday	02/13/05	X		X	X			
0	Monday	02/14/05	X				X		
1	Tuesday	02/15/05	X				X		
2	Wednesday	02/16/05	X		X	X	X		
3	Thursday	02/17/05	X				X		
4	Friday	02/18/05	X				X		
5	Saturday	02/19/05	X		X	X	X		
6	Sunday	02/20/05	X				X	U-1	
7	Monday	02/21/05	X				X		
8	Tuesday	02/22/05	X		X	X	X		
9	Wednesday	02/23/05	X				X	U-2	
10	Thursday	02/24/05	X				X		
11	Friday	02/25/05	X		X	X	X		
12	Saturday	02/26/05	X				X	U-3	
13	Sunday	02/27/05	X				X		
14	Monday	02/28/05	X		X		X		
15	Tuesday	03/01/05							X

TABLE A-2 GROUP ASSIGNMENTS

Pig Number	Dose Group	Material Administered	Target Dose of Arsenic (µg/kg-day)	Target Dose of Vanadium (µg/kg-day)
705 727 732 742 749	1	$NaHAsO_4$	30	0
718 721 722 726 751	2	$NaHAsO_4$	60	0
701 707 724 734 748	3	$NaHAsO_4$	120	0
704 708 712 719 735	4	Soil	40	103
713 714 715 731 750	5	Soil	80	206
723 738 739 747 752	6	Soil	160	412
703 710 717 740 746	7	$VOSO_4$	0	80
716 720 736 737 743	8	$VOSO_4$	0	160
702 728 733 744 745	9	$VOSO_4$	0	320
709 711 730	10	Control	0	0

TABLE A-3 BODY WEIGHTS BY DAY

Body weights were measured on days -1, 2, 5, 8, 11, and 14. Weights for other days are estimated, based on linear interpolation between measured values.
All weights shown in kilograms (kg).

Group	Pig #	Day -1	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
1	705	10.1	10.1	10.1	10.2	10.3	10.4	10.6	11.0	11.4	11.9	12.1	12.4	12.7	12.9	13.2	13.4
1	727	9.9	10.2	10.5	10.8	11.1	11.5	11.8	12.3	12.7	13.2	13.5	13.9	14.3	14.9	15.5	16.1
1	732	11.1	11.3	11.5	11.8	12.0	12.3	12.6	13.0	13.4	13.8	14.2	14.6	15.0	15.5	16.0	16.6
1	742	10.4	10.7	11.0	11.3	11.5	11.7	12.0	12.4	12.9	13.3	13.7	14.2	14.6	15.2	15.9	16.5
1	749	11.5	11.7	12.0	12.2	12.5	12.8	13.2	13.7	14.2	14.7	14.9	15.2	15.4	16.0	16.6	17.2
2	718	11.3	11.5	11.7	11.9	12.2	12.5	12.8	13.2	13.6	14.0	14.6	15.2	15.8	16.3	16.8	17.3
2	721	10.0	10.2	10.5	10.8	11.2	11.5	11.9	12.2	12.6	12.9	13.4	13.8	14.3	14.8	15.3	15.9
2	722	10.5	10.8	11.1	11.5	11.7	11.9	12.2	12.7	13.2	13.7	14.1	14.6	15.0	15.5	16.0	16.6
2	726	9.7	9.9	10.2	10.4	10.7	11.1	11.4	11.8	12.2	12.6	13.1	13.6	14.1	14.6	15.1	15.7
2	751	9.6	9.9	10.2	10.5	10.8	11.2	11.5	11.8	12.2	12.5	12.9	13.2	13.6	14.2	14.9	15.6
3	701	9.6	9.7	9.9	10.1	10.5	10.9	11.3	11.5	11.7	11.9	12.4	12.9	13.4	14.1	14.7	15.4
3	707	9.6	9.9	10.3	10.6	10.7	10.9	11.0	11.4	11.7	12.1	12.5	13.0	13.5	13.9	14.3	14.7
3	724	9.9	10.1	10.4	10.6	10.9	11.2	11.5	11.9	12.2	12.6	13.1	13.6	14.1	14.6	15.1	15.6
3	734	10.5	10.8	11.2	11.5	11.7	12.0	12.2	12.6	13.0	13.4	13.8	14.1	14.5	15.2	15.8	16.5
3	748	10.1	10.4	10.7	11.0	11.1	11.3	11.4	11.8	12.1	12.5	13.0	13.5	14.0	14.5	15.1	15.6
4	704	9.8	10.0	10.3	10.6	10.8	10.9	11.1	11.6	12.0	12.5	12.8	13.1	13.5	14.0	14.6	15.1
4	708	11.2	11.4	11.7	12.0	12.2	12.4	12.7	13.0	13.4	13.8	14.2	14.6	15.1	15.7	16.4	17.0
4	712	10.9	11.1	11.3	11.6	11.8	12.1	12.4	12.7	13.1	13.5	13.8	14.2	14.6	15.2	15.8	16.5
4	719	10.5	10.7	11.0	11.2	11.4	11.5	11.7	12.0	12.4	12.8	13.2	13.6	14.1	14.6	15.2	15.8
4	735	9.6	9.7	9.8	9.9	10.0	10.1	10.2	10.5	10.8	11.1	11.2	11.3	11.4	11.4	11.4	11.4
5	713	9.7	10.0	10.3	10.6	10.9	11.2	11.5	12.0	12.5	13.0	13.4	13.9	14.3	15.0	15.7	16.4
5	714	11.7	11.9	12.1	12.3	12.5	12.8	13.0	13.4	13.8	14.2	14.6	14.9	15.3	15.9	16.6	17.3
5	715	10.2	10.3	10.5	10.6	10.9	11.2	11.5	11.7	11.9	12.1	12.5	13.0	13.4	14.1	14.8	15.5
5	731	9.6	9.8	9.9	10.1	10.5	10.8	11.2	11.5	11.9	12.2	12.6	13.0	13.5	14.1	14.7	15.3
5	750	9.8	10.1	10.3	10.6	10.8	11.0	11.2	11.6	12.1	12.6	13.1	13.6	14.2	14.7	15.3	15.9
6	723	10.3	10.6	10.9	11.2	11.5	11.8	12.1	12.5	12.9	13.3	13.7	14.1	14.5	15.2	15.9	16.6
6	738	10.3	10.5	10.8	11.1	11.4	11.8	12.1	12.5	12.9	13.4	13.7	14.1	14.5	15.2	15.9	16.7
6	739	10.2	10.3	10.5	10.6	10.8	11.1	11.3	11.6	11.9	12.3	12.6	12.9	13.3	13.7	14.2	14.7
6	747	10.5	10.8	11.1	11.4	11.8	12.2	12.6	12.9	13.3	13.7	14.1	14.5	14.9	15.5	16.1	16.8
6	752	10.7	11.0	11.4	11.7	12.0	12.2	12.5	12.9	13.3	13.8	14.2	14.7	15.2	15.8	16.5	17.1
7	703	9.7	10.0	10.3	10.6	10.8	11.0	11.2	11.6	12.0	12.4	12.8	13.2	13.6	14.1	14.6	15.1
7	710	10.0	10.1	10.2	10.4	10.5	10.7	10.9	11.3	11.6	12.0	12.5	13.0	13.5	14.2	14.9	15.6
7	717	11.3	11.6	11.9	12.3	12.6	12.9	13.2	13.3	13.5	13.6	14.2	14.7	15.3	15.6	16.0	16.4
7	740	11.3	11.3	11.4	11.5	11.6	11.6	11.7	12.0	12.3	12.6	12.6	12.5	12.5	13.0	13.5	14.0
7	746	10.0	10.1	10.2	10.3	10.6	10.8	11.1	11.6	12.0	12.5	12.8	13.1	13.4	13.9	14.5	15.0
8	716	9.5	9.7	9.8	10.0	10.2	10.4	10.7	11.1	11.5	11.9	12.2	12.5	12.8	13.4	13.9	14.5
8	720	10.1	10.3	10.6	10.8	11.0	11.3	11.5	11.9	12.3	12.7	13.1	13.5	13.9	14.4	14.9	15.4
8	736	10.9	11.2	11.5	11.8	12.1	12.5	12.8	13.3	13.7	14.2	14.5	14.8	15.1	15.7	16.3	16.9
8	737	10.7	10.9	11.2	11.4	11.7	12.0	12.4	12.5	12.6	12.7	13.1	13.5	13.9	14.6	15.3	16.0
8	743	9.9	10.1	10.3	10.5	10.7	10.9	11.1	11.5	11.9	12.4	12.5	12.7	12.9	13.5	14.0	14.6
9	702	10.9	11.1	11.4	11.7	12.0	12.3	12.6	13.1	13.7	14.2	14.6	15.1	15.5	16.1	16.6	17.2
9	728	10.4	10.7	11.1	11.4	11.7	11.9	12.2	12.5	12.8	13.1	13.6	14.1	14.6	15.3	15.9	16.6
9	733	10.7	10.9	11.2	11.4	11.7	11.9	12.2	12.5	12.9	13.3	13.7	14.0	14.4	14.8	15.3	15.8
9	744	8.5	8.7	8.9	9.1	9.4	9.7	10.0	10.4	10.8	11.3	11.6	11.9	12.2	12.6	13.1	13.6
9	745	9.5	9.7	9.9	10.1	10.4	10.7	11.0	11.5	11.9	12.4	12.7	13.0	13.3	13.9	14.6	15.3
10	709	11.9	12.3	12.7	13.1	13.4	13.7	14.0	14.5	15.1	15.6	16.1	16.6	17.1	17.9	18.8	19.7
10	711	10.6	10.8	11.0	11.2	11.5	11.7	12.0	12.4	12.8	13.2	13.7	14.1	14.6	15.1	15.7	16.3
10	730	10.9	11.1	11.4	11.7	11.9	12.2	12.4	12.9	13.4	13.9	14.2	14.6	14.9	15.6	16.2	16.9

TABLE A-4 ANIMAL HEALTH

Naxcel Treatment

First Day of Treatment*	Pig	Group	Indications
Day -5 (2/09/05)	749	1	Elevated temperature, diarrhea
	710	7	
	750	5	
Day -1 (2/13/05)	719	4	Elevated temperature, anorexia
Day 1 (2/15/05)	735	4	Elevated temperature, diarrhea
	705	1	
Day 5 (2/19/05)	737	8	Elevated temperature, anorexia
Day 13 (2/27/05)	735	4	Diarrhea
	705	1	

*Treatment duration: 3 days

Necropsy

Pig 737 (group 8) had one testicle retained in abdomen.
Kidneys appeared small in $VOSO_4$ groups; however, organs were not weighed so this observation could not be verified statistically.

TABLE A-5 DOSE PREPARATION AND ADMINISTRATION

Quantifiable missed doses are noted at the bottom of Tables A-6 and A-7.

There were two major difficulties in dose preparation: 1) this batch of special feed became very sticky when mixed with water and 2) a large amount of soil was necessary for the soil groups. Details are provided below.

Day -1 (2/13/05): **Dose preparation:** All doses were made by adding the dose material to doughballs, which consisted of special feed mixed with water. Reference material doses were made by pipeting the stock solution into a small hole in the doughball made with a flask stopper, allowed to soak in, and then squeezed shut. Soil doses were made by first mixing soil with an equal amount of special feed, wetting this mixture and rolling it into small logs, and allowing it to dry for a few hours; these logs were then broken into pieces and placed in the center of doughballs in an attempt to reduce the number of soil doughballs and still prevent the soil from falling out. Upon storing, all doughballs became very wet and sticky in the storage bags and were difficult to get out; the soil stayed as a hard lump in the center and the dough did not cling to them well.

Day 2 (2/16/05): **Dose preparation:** Doughballs were made from a mixture of 3/4 cup vegetable shortening, 1 cup powdered sugar, 1 pound cornstarch, an equal amount of special feed, and enough water to make the mixture malleable. This dough was non-sticky and did not become wet over time. Reference material doses were prepared the same way as on Day -1. Soil doses were prepared as follows: 1) a log of dough about 3 inches long was flattened on cornstarch-dusted bench paper to approximately 3" by 4"; 2) this was brushed with a mixture of equal amounts of powdered sugar and water to dampen the surface; 3) the weighed soil was sprinkled over the dough, staying back from the edge; 4) the soil-covered dough was rolled up cinnamon-roll style and placed in a dosing bag. Soil for groups 4 and 5 were able to be placed in just one doughball, while Group 6 required 2, and then 3, later on. The soil wetted into the doughball, so they were easily broken into bite-size pieces at dosing without the soil falling out. Group 4 doughballs had some flour in them instead of cornstarch.

Day 5 (2/19/05): **Dose preparation:** Doughballs were made from a mixture of 3/4 cup vegetable shortening, 1 pound flour, an equal amount of special feed, and enough water to make the mixture malleable. It became apparent that there was insufficient soil to last through the end of the study. In order to extend the soil supply, doses for the soil groups (groups 4-6) consisted of the archived soil doughballs from the previous two dose preparations (Day -1 and Day 2), which had been stored in the freezer, in addition to a new doughball made with an amount of soil calculated to supplement the amount in the archived sample to make the dose necessary for this preparation. No archives were made at this dose preparation or in further dose preparations.

Day 6 (2/20/05): **Dosing:** Pig 713 (Group 5) drinks excessively; lots of soil in urine bucket (morning and afternoon doses). Loss of dose not quantified, so actual dose not adjusted.

Day 8 (2/22/05): **Dosing:** At the afternoon dosing, there was uncertainty regarding the prepared doughballs for Group 4, so new doughballs were made; animals were dosed 20 minutes late.
Dose preparation: Doughballs were made using the same recipe as Day 5. Only 200g of soil remained after this preparation.

Day 11 (2/25/05): **Dose preparation:** Doughballs were made using the same recipe as Day 5. The supplier sent more soil, which was mixed with the remaing 200 g, rolled, and used. A sample of the new mixed soil was taken for analysis. There still was insufficient soil to make the afternoon dose for all three soil groups on Day 14 (the last dosing day), so no doughballs for any groups were prepared for the Day 14 afternoon dose.

Day 14 (2/28/05): **Dosing:** No animals received the afternoon dose; dosing ended with the morning dose of Day 14.

TABLE A-6 ACTUAL ADMINISTERED ARSENIC DOSES

Doses shown have been adjusted for individual body weights (see Table A-3); units are µg/kg-d.

Group	Pig #	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	Mean Dose (Days 0-14)
1	705	34.4	34.3	34.2	35.7	35.2	34.8	35.5	34.2	32.9	35.5	34.7	34.0	35.8	35.1	25.8	34.1
1	727	34.1	33.1	32.1	33.0	32.0	31.1	31.8	30.7	29.7	31.8	30.9	30.1	31.0	29.8	21.5	30.8
1	732	30.7	30.1	29.5	30.5	29.9	29.2	30.1	29.2	28.4	30.4	29.5	28.7	29.8	28.8	20.9	29.0
1	742	32.5	31.7	30.9	32.0	31.3	30.7	31.5	30.4	29.3	31.3	30.4	29.5	30.3	29.1	21.0	30.1
1	749	29.7	29.0	28.5	29.3	28.6	27.9	28.6	27.6	26.6	28.9	28.4	27.9	28.9	27.8	20.1	27.9
2	718	58.4	57.4	56.5	59.0	57.6	56.3	58.9	57.3	55.7	58.1	55.8	53.6	57.3	55.6	40.5	55.9
2	721	65.7	63.9	62.2	64.5	62.4	60.5	63.5	61.8	60.2	63.3	61.2	59.2	62.9	60.8	44.1	61.1
2	722	62.1	60.4	58.7	61.6	60.4	59.3	61.4	59.1	56.9	60.0	58.2	56.4	60.1	58.2	42.3	58.3
2	726	67.7	66.1	64.6	67.1	65.1	63.2	65.9	63.9	61.9	64.9	62.5	60.3	63.9	61.7	44.7	62.9
2	751	68.2	66.2	64.3	66.7	64.6	62.6	65.7	63.9	62.2	65.9	64.1	62.5	65.5	62.5	44.8	63.3
3	701	134.9	132.6	130.4	134.8	129.8	125.2	130.7	128.4	126.3	130.7	125.5	120.6	126.9	121.2	87.0	125.7
3	707	131.9	127.6	123.6	131.3	129.6	128.1	131.8	127.9	124.2	129.1	124.5	120.2	128.8	125.0	91.1	125.0
3	724	129.3	126.4	123.6	129.2	125.8	122.5	126.1	122.3	118.8	123.5	119.1	115.0	122.7	118.6	86.1	120.6
3	734	121.0	117.3	113.9	120.1	117.7	115.5	118.8	115.1	111.7	117.4	114.4	111.5	117.9	113.0	81.4	113.8
3	748	126.6	123.0	119.7	126.9	125.2	123.6	127.4	123.7	120.2	124.7	119.9	115.5	122.9	118.5	85.8	120.2
4	704	45.3	44.1	42.9	44.8	44.1	43.4	43.5	41.8	40.2	42.8	41.8	40.8	55.4	53.3	38.5	44.2
4	708	39.8	38.8	37.9	39.5	38.8	38.1	38.6	37.5	36.4	38.6	37.5	36.5	49.4	47.4	34.2	39.3
4	712	40.9	40.1	39.4	40.8	39.9	39.0	39.5	38.4	37.4	39.7	38.7	37.7	51.0	49.0	35.3	40.5
4	719	42.4	41.5	40.6	42.5	41.9	41.4	41.8	40.5	39.3	41.5	40.3	39.1	53.0	50.9	36.8	42.2
4	735	47.1	46.5	26.4	48.3	47.9	47.5	48.1	46.8	45.5	49.2	48.8	48.4	68.3	68.3	17.1	46.9
5	713	89.7	87.3	84.9	87.1	84.7	82.5	42.4	81.3	78.2	82.4	79.7	77.2	106.4	101.8	73.1	82.6
5	714	75.4	74.2	73.1	75.6	74.1	72.7	75.6	73.4	71.3	75.9	74.1	72.4	100.1	96.0	69.1	76.9
5	715	86.7	85.6	84.5	86.7	84.3	82.1	86.5	85.1	83.7	88.1	85.2	82.4	113.2	108.0	77.4	88.0
5	731	91.7	90.2	88.7	90.4	87.5	84.7	88.0	85.4	83.0	87.5	84.7	82.1	113.4	108.6	78.2	89.6
5	750	89.1	87.0	84.9	87.9	86.3	84.7	87.0	83.6	80.3	84.2	81.0	78.1	108.4	104.3	75.5	86.8
6	723	172.6	167.8	163.3	170.3	166.0	161.9	168.4	163.1	158.2	166.9	161.9	157.3	214.8	205.3	147.5	169.7
6	738	64.8	168.3	86.1	170.6	165.8	161.2	167.5	162.1	157.0	166.1	161.5	157.3	214.6	204.9	147.1	157.0
6	739	176.2	174.0	171.8	180.0	176.3	172.6	180.4	175.7	171.1	181.2	176.6	172.1	237.7	229.7	166.6	182.8
6	747	168.6	102.5	159.7	165.5	160.3	155.4	162.3	157.8	153.6	162.1	157.5	153.0	210.4	202.4	146.2	161.2
6	752	165.0	88.1	155.6	163.0	159.4	156.0	162.3	157.2	152.5	160.2	155.0	150.0	206.2	198.3	143.2	158.1
10	709	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	711	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	730	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Dosing Anomalies:

Day 1 - Pig 738 did not eat entire AM or PM dose (ate approximately 50% and 25%, respectively). Daily dose adjusted to 37.5%.
Day 1 - Pig 747 did not eat entire PM dose (ate approximately 25%). Daily dose adjusted to 62.5%.
Day 1 - Pig 752 did not eat entire PM dose (ate approximately 10%). Daily dose adjusted to 55%.
Day 2 - Pig 735 did not eat entire AM dose (ate approximately 15%). Daily dose adjusted to 57.5%.
Day 2 - Pig 738 did not eat entire AM dose (ate approximately 5%). Daily dose adjusted to 52.5%.
Day 6 - Pig 713 was drinking excessively and a large amount of dosing material was found in the urine bucket; however, a reliable estimate of the amount of dose lost could not be made. Therefore, for the purposes of these calculations, a value of 50% was assumed to minimized bias.
Day 14 - Pig 735 did not eat entire AM dose (ate approximately 50%) and did not receive PM dose (see note below). Daily dose adjusted to 25%.
Day 14 - There was insufficient soil to prepare the PM doses for Groups 4, 5, and 6. As a result, no groups received PM doses.

TABLE A-7 ACTUAL ADMINISTERED VANADIUM DOSES

Doses shown have been adjusted for individual body weights (see Table A-3); units are µg/kg-d.

Group	Pig #	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	Mean Dose (Days 0-14)
4	704	116.7	113.5	110.5	115.3	113.5	111.8	111.9	107.6	103.6	110.3	107.6	105.1	131.3	126.3	91.3	111.8
4	708	102.4	100.0	97.6	101.6	99.8	98.1	99.3	96.5	93.8	99.4	96.6	93.9	117.0	112.4	81.1	99.3
4	712	105.4	103.3	101.4	105.1	102.7	100.5	101.8	99.0	96.3	102.3	99.6	97.1	121.0	116.2	83.8	102.4
4	719	109.1	106.8	104.6	109.4	108.0	106.6	107.6	104.3	101.1	106.9	103.7	100.6	125.6	120.8	87.2	106.8
4	735	121.2	119.7	68.0	124.4	123.3	122.3	123.9	120.4	117.2	126.8	125.6	124.5	161.9	161.9	40.5	118.8
5	713	231.0	224.6	218.6	224.2	218.1	212.4	109.1	209.3	201.3	212.2	205.3	198.8	252.3	241.3	173.4	208.8
5	714	194.1	191.1	188.3	194.6	190.8	187.1	194.5	188.9	183.5	195.4	190.8	186.5	237.3	227.5	163.9	194.3
5	715	223.2	220.3	217.6	223.1	217.2	211.5	222.8	219.0	215.4	226.9	219.3	212.2	268.5	256.0	183.5	222.4
5	731	236.1	232.2	228.3	232.7	225.2	218.1	226.6	219.9	213.6	225.4	218.2	211.4	268.8	257.5	185.3	226.6
5	750	229.5	223.9	218.6	226.2	222.1	218.1	224.0	215.1	206.9	216.8	208.6	201.0	256.9	247.4	178.9	219.6
6	723	444.3	432.0	420.4	438.6	427.4	416.7	433.5	420.0	407.3	429.6	416.9	404.9	509.3	486.9	349.7	429.2
6	738	166.9	433.4	221.7	439.2	426.8	415.0	431.2	417.3	404.2	427.5	415.9	404.9	508.7	485.8	348.7	396.5
6	739	453.6	447.9	442.2	463.5	453.8	444.4	464.6	452.2	440.5	466.6	454.5	443.1	563.7	544.5	395.0	462.0
6	747	434.0	263.9	411.2	426.2	412.7	400.1	417.8	406.3	395.4	417.4	405.4	394.0	498.9	479.8	346.6	407.3
6	752	424.9	226.8	400.6	419.6	410.5	401.7	417.8	404.7	392.5	412.5	398.9	386.2	488.9	470.1	339.5	399.7
7	703	91.6	89.1	86.7	89.2	87.6	86.0	87.1	214.2	81.3	85.0	82.4	80.0	83.0	80.2	58.1	92.1
7	710	90.4	89.4	88.3	91.1	89.5	88.0	89.6	221.3	84.4	87.4	84.0	80.9	82.7	78.8	56.4	93.5
7	717	78.9	76.7	74.6	76.4	74.6	72.9	75.8	190.9	74.1	76.9	74.0	71.4	74.9	73.1	53.5	81.3
7	740	80.7	80.1	79.5	82.9	82.5	82.0	84.0	208.7	80.0	86.6	86.8	87.0	90.0	86.7	62.7	90.7
7	746	90.8	89.8	88.8	90.8	88.5	86.4	87.3	84.0	81.0	85.3	83.4	81.5	84.2	81.0	58.5	84.1
8	716	185.7	182.9	180.1	186.6	182.5	178.5	183.5	177.0	171.1	180.8	176.2	171.9	176.2	169.2	122.1	174.9
8	720	174.0	169.9	165.9	172.3	168.7	165.3	170.4	164.8	159.6	168.2	163.4	158.9	163.7	158.0	114.5	162.5
8	736	160.5	156.0	151.9	156.7	152.5	148.5	153.0	148.0	143.3	152.1	148.8	145.7	150.0	144.6	104.7	147.7
8	737	163.9	160.5	157.2	162.2	158.0	153.9	162.6	161.1	159.6	168.2	163.4	158.9	161.7	154.2	110.6	157.1
8	743	177.7	174.5	171.5	178.5	175.2	172.0	176.5	170.1	164.1	175.5	173.0	170.6	174.7	167.6	120.8	169.5
9	702	316.1	308.7	301.6	313.7	305.6	297.9	306.7	294.8	283.7	302.7	293.9	285.7	298.5	288.6	209.5	293.9
9	728	328.4	318.0	308.2	321.8	173.0	307.7	322.3	314.7	307.5	325.7	314.1	303.3	313.8	300.7	216.5	298.4
9	733	321.4	314.7	308.2	322.2	315.5	309.0	321.4	311.9	302.9	324.5	316.3	308.6	323.0	312.8	227.4	309.3
9	744	403.9	394.8	386.1	399.4	387.0	375.4	386.7	371.9	358.1	383.4	373.7	364.5	379.2	365.2	264.2	372.9
9	745	362.3	354.9	347.9	361.0	350.8	341.3	351.8	338.5	326.2	350.1	342.0	334.3	343.8	327.8	234.8	337.8
10	709	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	711	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	730	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Dosing Anomalies:

Day 1 - Pig738 did not eat entire AM or PM dose (ate approximately 50% and 25%, respectively). Daily dose adjusted to 37.5%.
Day 1 - Pig 747 did not eat entire PM dose (ate approximately 25%). Daily dose adjusted to 62.5%.
Day 1 - Pig 752 did not eat entire PM dose (ate approximately 10%). Daily dose adjusted to 55%.
Day 2 - Pig 735 did not eat entire AM dose (ate approximately 15%). Daily dose adjusted to 57.5%.
Day 2 - Pig 738 did not eat entire AM dose (ate approximately 5%). Daily dose adjusted to 52.5%.
Day 4 - Pig 728 did not eat entire AM dose (ate approximately 10%). Daily dose adjusted to 55%.
Day 6 - Pig 713 was drinking excessively and a large amount of dosing material was found in the urine bucket; however, a reliable estimate of the amount of dose lost could not be made. Therefore, for the purposes of these calculations, a value of 50% was assumed to minimized bias.
Day 7 - Pigs 703, 710, 717, and 740 received Group 9's AM dose. Daily dose adjusted upward accordingly, to 255%.
Day 14 - Pig 735 did not eat entire AM dose (ate approximately 50%) and did not receive PM dose (see note below). Daily dose adjusted to 25%.
Day 14 - There was insufficient soil to prepare the PM doses for Groups 4, 5, and 6. As a result, no groups received PM doses.

TABLE A-8 URINE VOLUMES - 48 HOUR COLLECTIONS

Units of Volume: mls

Group	Pig ID	Urine Collection		
		U-1 Days 6-7 2/20-2/21/05	**U-2** Days 9-10 2/23-2/24/05	**U-3** Days 12-13 2/26-2/27/05
1	705	4590	7680	5060
	727	5620	8680	7820
	732	7790	6780	6480
	742	2900	2920	3520
	749	4280	5200	4040
2	718	6075	10220	9580
	721	7980	7100	11020
	722	7480	7880	8420
	726	8220	6400	5580
	751	17900	15720	12500
3	701	7440	5060	4000
	707	18150	15200	24820
	724	7340	9280	8020
	734	6590	4820	8060
	748	2410	4960	3040
4	704	8200	6540	16840
	708	7570	9660	10220
	712	2770	4920	2980
	719	4440	8780	11300
	735	2270	3140	2440
5	713	12600	17460	42520
	714	8380	9240	10280
	715	8600	5440	10400
	731	11740	6520	6220
	750	3020	2020	2300
6	723	5400	3720	5180
	738	11620	8420	6000
	739	4560	5920	3720
	747	13740	8600	12960
	752	14060	9620	10980
7	703	URINE SAMPLES NOT COLLECTED FOR $VOSO_4$ GROUPS		
	710			
	717			
	740			
	746			
8	716			
	720			
	736			
	737			
	743			
9	702			
	728			
	733			
	744			
	745			
10	709	5200	10860	10020
	711	2880	4400	4540
	730	2080	2050	2340
Volume measured by:		AA,JB	AA	AA
Date:		2/22/05	2/24/05	2/28/05

TABLE A-9 URINARY ARSENIC ANALYTICAL RESULTS FOR STUDY SAMPLES

Sample Number	Tag Number	Pig Number	Group	Material Administered	Urine Collection Days	48-hr As Dose (ug/48hr)	Q	Reported Conc (ng/mL)	AdjConc* (ng/mL)	Urine Volume (mL)	Total Excreted (ug/48hrs)
PTX-705-U1	PTX-115	705	1	$NaHAsO_4$	6/7	780		130	130	4590	596.7
PTX-727-U1	PTX-111	727	1	$NaHAsO_4$	6/7	780		100	100	5620	562
PTX-732-U1	PTX-113	732	1	$NaHAsO_4$	6/7	780		91	91	7790	708.89
PTX-742-U1	PTX-117	742	1	$NaHAsO_4$	6/7	780		190	190	2900	551
PTX-749-U1	PTX-122	749	1	$NaHAsO_4$	6/7	780		140	140	4280	599.2
PTX-718-U1	PTX-134	718	2	$NaHAsO_4$	6/7	1554		180	180	6075	1093.5
PTX-721-U1	PTX-135	721	2	$NaHAsO_4$	6/7	1554		150	150	7980	1197
PTX-722-U1	PTX-102	722	2	$NaHAsO_4$	6/7	1554		160	160	7480	1196.8
PTX-726-U1	PTX-104	726	2	$NaHAsO_4$	6/7	1554		160	160	8220	1315.2
PTX-751-U1	PTX-130	751	2	$NaHAsO_4$	6/7	1554		68	68	17900	1217.2
PTX-701-U1	PTX-118	701	3	$NaHAsO_4$	6/7	2992.8		320	320	7440	2380.8
PTX-707-U1	PTX-132	707	3	$NaHAsO_4$	6/7	2992.8		140	140	18150	2541
PTX-724-U1	PTX-106	724	3	$NaHAsO_4$	6/7	2992.8		340	340	7340	2495.6
PTX-734-U1	PTX-129	734	3	$NaHAsO_4$	6/7	2992.8		280	280	6590	1845.2
PTX-748-U1	PTX-114	748	3	$NaHAsO_4$	6/7	2992.8		820	820	2410	1976.2
PTX-704-U1	PTX-119	704	4	Soil	6/7	1005.8		21	21	8200	172.2
PTX-708-U1	PTX-116	708	4	Soil	6/7	1005.8		23	23	7570	174.11
PTX-712-U1	PTX-101	712	4	Soil	6/7	1005.8		58	58	2770	160.66
PTX-719-U1	PTX-128	719	4	Soil	6/7	1005.8		31	31	4440	137.64
PTX-735-U1	PTX-125	735	4	Soil	6/7	1005.8		53	53	2270	120.31
PTX-713-U1	PTX-107	713	5	Soil	6/7	1518.57		60	60	12600	756
PTX-714-U1	PTX-112	714	5	Soil	6/7	2024.76		130	130	8380	1089.4
PTX-715-U1	PTX-131	715	5	Soil	6/7	2024.76		31	31	8600	266.6
PTX-731-U1	PTX-127	731	5	Soil	6/7	2024.76		26	26	11740	305.24
PTX-750-U1	PTX-105	750	5	Soil	6/7	2024.76		110	110	3020	332.2
PTX-723-U1	PTX-108	723	6	Soil	6/7	4192.4		110	110	5400	594
PTX-738-U1	PTX-133	738	6	Soil	6/7	4192.4		44	44	11620	511.28
PTX-739-U1	PTX-123	739	6	Soil	6/7	4192.4		100	100	4560	456
PTX-747-U1	PTX-103	747	6	Soil	6/7	4192.4		58	58	13740	796.92
PTX-752-U1	PTX-110	752	6	Soil	6/7	4192.4		49	49	14060	688.94
PTX-709-U1	PTX-120	709	10	Control	6/7	0		1	1	5200	5.2
PTX-711-U1	PTX-124	711	10	Control	6/7	0		1	1	2880	2.88
PTX-730-U1	PTX-136	730	10	Control	6/7	0		3.1	3.1	2080	6.448
PTX-705-U2	PTX-142	705	1	$NaHAsO_4$	9/10	860.4		100	100	7680	768
PTX-727-U2	PTX-161	727	1	$NaHAsO_4$	9/10	860.4		78	78	8680	677.04
PTX-732-U2	PTX-165	732	1	$NaHAsO_4$	9/10	860.4		120	120	6780	813.6
PTX-742-U2	PTX-143	742	1	$NaHAsO_4$	9/10	860.4		280	280	2920	817.6
PTX-749-U2	PTX-145	749	1	$NaHAsO_4$	9/10	860.4		150	150	5200	780
PTX-718-U2	PTX-172	718	2	$NaHAsO_4$	9/10	1693.2		140	140	10220	1430.8
PTX-721-U2	PTX-153	721	2	$NaHAsO_4$	9/10	1693.2		220	220	7100	1562
PTX-722-U2	PTX-154	722	2	$NaHAsO_4$	9/10	1693.2		230	230	7880	1812.4
PTX-726-U2	PTX-163	726	2	$NaHAsO_4$	9/10	1693.2		210	210	6400	1344
PTX-751-U2	PTX-139	751	2	$NaHAsO_4$	9/10	1693.2		110	110	15720	1729.2
PTX-701-U2	PTX-155	701	3	$NaHAsO_4$	9/10	3232.8		580	580	5060	2934.8
PTX-707-U2	PTX-166	707	3	$NaHAsO_4$	9/10	3232.8		200	200	15200	3040
PTX-724-U2	PTX-170	724	3	$NaHAsO_4$	9/10	3232.8		810	810	9280	7516.8
PTX-734-U2	PTX-158	734	3	$NaHAsO_4$	9/10	3232.8		610	610	4820	2940.2
PTX-748-U2	PTX-171	748	3	$NaHAsO_4$	9/10	3232.8		300	300	4960	1488
PTX-704-U2	PTX-146	704	4	Soil	9/10	1097.92		22	22	6540	143.88
PTX-708-U2	PTX-167	708	4	Soil	9/10	1097.92		21	21	9660	202.86
PTX-712-U2	PTX-168	712	4	Soil	9/10	1097.92		33	33	4920	162.36
PTX-719-U2	PTX-138	719	4	Soil	9/10	1097.92		21	21	8780	184.38
PTX-735-U2	PTX-151	735	4	Soil	9/10	1097.92		36	36	3140	113.04
PTX-713-U2	PTX-162	713	5	Soil	9/10	2209		23	23	17460	401.58
PTX-714-U2	PTX-140	714	5	Soil	9/10	2209		40	40	9240	369.6

TABLE A-9, CONTINUED: URINARY ARSENIC ANALYTICAL RESULTS FOR STUDY SAMPLES

Sample Number	Tag Number	Pig Number	Group	Material Administered	Urine Collection Days	48-hr As Dose (ug/48hr)	Q	Reported Conc (ng/mL)	AdjConc* (ng/mL)	Urine Volume (mL)	Total Excreted (ug/48hrs)
PTX-715-U2	PTX-144	715	5	Soil	9/10	2209		63	63	5440	342.72
PTX-731-U2	PTX-141	731	5	Soil	9/10	2209		54	54	6520	352.08
PTX-750-U2	PTX-157	750	5	Soil	9/10	2209		190	190	2020	383.8
PTX-723-U2	PTX-149	723	6	Soil	9/10	4560.88		150	150	3720	558
PTX-738-U2	PTX-164	738	6	Soil	9/10	4560.88		70	70	8420	589.4
PTX-739-U2	PTX-148	739	6	Soil	9/10	4560.88		97	97	5920	574.24
PTX-747-U2	PTX-160	747	6	Soil	9/10	4560.88		80	80	8600	688
PTX-752-U2	PTX-147	752	6	Soil	9/10	4560.88		58	58	9620	557.96
PTX-709-U2	PTX-169	709	10	Control	9/10	0		1	1	10860	10.86
PTX-711-U2	PTX-159	711	10	Control	9/10	0	<	1	0.5	4400	2.2
PTX-730-U2	PTX-137	730	10	Control	9/10	0		2	2	2050	4.1
PTX-705-U3	PTX-175	705	1	$NaHAsO_4$	12/13	923.6		80	80	5060	404.8
PTX-727-U3	PTX-206	727	1	$NaHAsO_4$	12/13	923.6		120	120	7820	938.4
PTX-732-U3	PTX-195	732	1	$NaHAsO_4$	12/13	923.6		130	130	6480	842.4
PTX-742-U3	PTX-198	742	1	$NaHAsO_4$	12/13	923.6		230	230	3520	809.6
PTX-749-U3	PTX-174	749	1	$NaHAsO_4$	12/13	923.6		180	180	4040	727.2
PTX-718-U3	PTX-194	718	2	$NaHAsO_4$	12/13	1864.8		160	160	9580	1532.8
PTX-721-U3	PTX-197	721	2	$NaHAsO_4$	12/13	1864.8		180	180	11020	1983.6
PTX-722-U3	PTX-187	722	2	$NaHAsO_4$	12/13	1864.8		170	170	8420	1431.4
PTX-726-U3	PTX-173	726	2	$NaHAsO_4$	12/13	1864.8		460	460	5580	2566.8
PTX-751-U3	PTX-183	751	2	$NaHAsO_4$	12/13	1864.8		130	130	12500	1625
PTX-701-U3	PTX-193	701	3	$NaHAsO_4$	12/13	3571.2		680	680	4000	2720
PTX-707-U3	PTX-200	707	3	$NaHAsO_4$	12/13	3571.2		160	160	24820	3971.2
PTX-724-U3	PTX-203	724	3	$NaHAsO_4$	12/13	3571.2		380	380	8020	3047.6
PTX-734-U3	PTX-207	734	3	$NaHAsO_4$	12/13	3571.2		400	400	8060	3224
PTX-748-U3	PTX-191	748	3	$NaHAsO_4$	12/13	3571.2		870	870	3040	2644.8
PTX-704-U3	PTX-185	704	4	Soil	12/13	1550		14	14	16840	235.76
PTX-708-U3	PTX-188	708	4	Soil	12/13	1550		25	25	10220	255.5
PTX-712-U3	PTX-181	712	4	Soil	12/13	1550		60	60	2980	178.8
PTX-719-U3	PTX-199	719	4	Soil	12/13	1550		22	22	11300	248.6
PTX-735-U3	PTX-202	735	4	Soil	12/13	1550		19	19	2440	46.36
PTX-713-U3	PTX-201	713	5	Soil	12/13	3189.28		7.2	7.2	42520	306.144
PTX-714-U3	PTX-196	714	5	Soil	12/13	3189.28		38	38	10280	390.64
PTX-715-U3	PTX-179	715	5	Soil	12/13	3189.28		39	39	10400	405.6
PTX-731-U3	PTX-182	731	5	Soil	12/13	3189.28		62	62	6220	385.64
PTX-750-U3	PTX-190	750	5	Soil	12/13	3189.28		180	180	2300	414
PTX-723-U3	PTX-208	723	6	Soil	12/13	6529.84		140	140	5180	725.2
PTX-738-U3	PTX-192	738	6	Soil	12/13	6529.84		110	110	6000	660
PTX-739-U3	PTX-184	739	6	Soil	12/13	6529.84		160	160	3720	595.2
PTX-747-U3	PTX-178	747	6	Soil	12/13	6529.84		77	77	12960	997.92
PTX-752-U3	PTX-186	752	6	Soil	12/13	6529.84		63	63	10980	691.74
PTX-709-U3	PTX-180	709	10	Control	12/13	0	<	1	0.5	10020	5.01
PTX-711-U3	PTX-189	711	10	Control	12/13	0		3	3	4540	13.62
PTX-730-U3	PTX-205	730	10	Control	12/13	0		2	2	2340	4.68

*Non-detects taken at one-half the detection limit.

TABLE A-10 VANDIUM ANALYTICAL RESULTS FOR STUDY SAMPLES

Sample Number	Tag Number	Pig Number	Group	Material Administered	Event/Day	Actual V BWAdj Dose (ug/kg-d)	Q	Reported Conc (ug/g)	AdjConc* (ug/g)
PTX-704-L	PTX-209	704	4	Soil	15	111.76		0.058	0.058
PTX-708-L	PTX-241	708	4	Soil	15	99.31		0.032	0.032
PTX-712-L	PTX-253	712	4	Soil	15	102.37		0.02	0.02
PTX-719-L	PTX-235	719	4	Soil	15	106.81		0.02	0.02
PTX-735-L	PTX-234	735	4	Soil	15	118.77		0.02	0.02
PTX-713-L	PTX-222	713	5	Soil	15	208.8		0.046	0.046
PTX-714-L	PTX-232	714	5	Soil	15	194.28		0.036	0.036
PTX-715-L	PTX-247	715	5	Soil	15	222.43		0.033	0.033
PTX-731-L	PTX-223	731	5	Soil	15	226.63		0.046	0.046
PTX-750-L	PTX-218	750	5	Soil	15	219.6		0.059	0.059
PTX-723-L	PTX-244	723	6	Soil	15	429.16		0.05	0.05
PTX-738-L	PTX-238	738	6	Soil	15	396.47		0.062	0.062
PTX-739-L	PTX-228	739	6	Soil	15	462		0.05	0.05
PTX-747-L	PTX-243	747	6	Soil	15	407.31		0.045	0.045
PTX-752-L	PTX-233	752	6	Soil	15	399.69		0.045	0.045
PTX-703-L	PTX-215	703	7	$VOSO_4$	15	92.11		0.12	0.12
PTX-710-L	PTX-213	710	7	$VOSO_4$	15	93.48		0.077	0.077
PTX-717-L	PTX-212	717	7	$VOSO_4$	15	81.26		0.11	0.11
PTX-740-L	PTX-239	740	7	$VOSO_4$	15	90.68		0.17	0.17
PTX-746-L	PTX-214	746	7	$VOSO_4$	15	84.09		0.14	0.14
PTX-716-L	PTX-246	716	8	$VOSO_4$	15	174.95		0.15	0.15
PTX-720-L	PTX-219	720	8	$VOSO_4$	15	162.5		0.16	0.16
PTX-736-L	PTX-225	736	8	$VOSO_4$	15	147.74		0.2	0.2
PTX-737-L	PTX-221	737	8	$VOSO_4$	15	157.06		0.16	0.16
PTX-743-L	PTX-259	743	8	$VOSO_4$	15	169.49		0.18	0.18
PTX-702-L	PTX-226	702	9	$VOSO_4$	15	293.85		0.31	0.31
PTX-728-L	PTX-240	728	9	$VOSO_4$	15	298.38		0.19	0.19
PTX-733-L	PTX-255	733	9	$VOSO_4$	15	309.32		0.25	0.25
PTX-744-L	PTX-220	744	9	$VOSO_4$	15	372.91		0.41	0.41
PTX-745-L	PTX-248	745	9	$VOSO_4$	15	337.83		0.26	0.26
PTX-709-L	PTX-250	709	10	Control	15	0		0.02	0.02
PTX-711-L	PTX-254	711	10	Control	15	0		0.01	0.01
PTX-730-L	PTX-256	730	10	Control	15	0		0.02	0.02
PTX-704-K	PTX-283	704	4	Soil	15	111.76		0.066	0.066
PTX-708-K	PTX-295	708	4	Soil	15	99.31		0.067	0.067
PTX-712-K	PTX-301	712	4	Soil	15	102.37		0.041	0.041
PTX-719-K	PTX-261	719	4	Soil	15	106.81		0.032	0.032
PTX-735-K	PTX-275	735	4	Soil	15	118.77		0.034	0.034
PTX-713-K	PTX-271	713	5	Soil	15	208.8		0.063	0.063
PTX-714-K	PTX-276	714	5	Soil	15	194.28		0.082	0.082
PTX-715-K	PTX-269	715	5	Soil	15	222.43		0.097	0.097
PTX-731-K	PTX-278	731	5	Soil	15	226.63		0.096	0.096
PTX-750-K	PTX-284	750	5	Soil	15	219.6		0.091	0.091
PTX-723-K	PTX-298	723	6	Soil	15	429.16		0.087	0.087
PTX-738-K	PTX-300	738	6	Soil	15	396.47		0.088	0.088
PTX-739-K	PTX-306	739	6	Soil	15	462		0.088	0.088
PTX-747-K	PTX-265	747	6	Soil	15	407.31		0.092	0.092
PTX-752-K	PTX-260	752	6	Soil	15	399.69		0.076	0.076
PTX-703-K	PTX-302	703	7	$VOSO_4$	15	92.11		0.25	0.25
PTX-710-K	PTX-277	710	7	$VOSO_4$	15	93.48		0.15	0.15
PTX-717-K	PTX-290	717	7	$VOSO_4$	15	81.26		0.31	0.31
PTX-740-K	PTX-294	740	7	$VOSO_4$	15	90.68		0.33	0.33

TABLE A-10, CONTINUED: VANDIUM ANALYTICAL RESULTS FOR STUDY SAMPLES

Sample Number	Tag Number	Pig Number	Group	Material Administered	Event/Day	Actual V BWAdj Dose (ug/kg-d)	Q	Reported Conc (ug/g)	AdjConc* (ug/g)
PTX-746-K	PTX-279	746	7	$VOSO_4$	15	84.09		0.44	0.44
PTX-716-K	PTX-287	716	8	$VOSO_4$	15	174.95		0.36	0.36
PTX-720-K	PTX-280	720	8	$VOSO_4$	15	162.5		0.34	0.34
PTX-736-K	PTX-291	736	8	$VOSO_4$	15	147.74		0.45	0.45
PTX-737-K	PTX-307	737	8	$VOSO_4$	15	157.06		0.39	0.39
PTX-743-K	PTX-289	743	8	$VOSO_4$	15	169.49		0.4	0.4
PTX-702-K	PTX-281	702	9	$VOSO_4$	15	293.85		0.84	0.84
PTX-728-K	PTX-304	728	9	$VOSO_4$	15	298.38		0.55	0.55
PTX-733-K	PTX-282	733	9	$VOSO_4$	15	309.32		0.55	0.55
PTX-744-K	PTX-272	744	9	$VOSO_4$	15	372.91		0.93	0.93
PTX-745-K	PTX-286	745	9	$VOSO_4$	15	337.83		0.84	0.84
PTX-709-K	PTX-293	709	10	Control	15	0		0.02	0.02
PTX-711-K	PTX-262	711	10	Control	15	0		0.03	0.03
PTX-730-K	PTX-267	730	10	Control	15	0		0.02	0.02
PTX-704-F	PTX-342	704	4	Soil	15	111.76		0.9	0.9
PTX-708-F	PTX-321	708	4	Soil	15	99.31		1.1	1.1
PTX-712-F	PTX-341	712	4	Soil	15	102.37		0.6	0.6
PTX-719-F	PTX-345	719	4	Soil	15	106.81		0.8	0.8
PTX-735-F	PTX-311	735	4	Soil	15	118.77		0.4	0.4
PTX-713-F	PTX-337	713	5	Soil	15	208.8		1.2	1.2
PTX-714-F	PTX-332	714	5	Soil	15	194.28		1.1	1.1
PTX-715-F	PTX-314	715	5	Soil	15	222.43		1.3	1.3
PTX-731-F	PTX-318	731	5	Soil	15	226.63		0.9	0.9
PTX-750-F	PTX-346	750	5	Soil	15	219.6		1.6	1.6
PTX-723-F	PTX-327	723	6	Soil	15	429.16		2.2	2.2
PTX-738-F	PTX-331	738	6	Soil	15	396.47		1.2	1.2
PTX-739-F	PTX-317	739	6	Soil	15	462		1.1	1.1
PTX-747-F	PTX-320	747	6	Soil	15	407.31		1.5	1.5
PTX-752-F	PTX-334	752	6	Soil	15	399.69		1.2	1.2
PTX-703-F	PTX-313	703	7	$VOSO_4$	15	92.11		5.3	5.3
PTX-710-F	PTX-326	710	7	$VOSO_4$	15	93.48		2.5	2.5
PTX-717-F	PTX-344	717	7	$VOSO_4$	15	81.26		4.4	4.4
PTX-740-F	PTX-335	740	7	$VOSO_4$	15	90.68		3.7	3.7
PTX-746-F	PTX-330	746	7	$VOSO_4$	15	84.09		5.1	5.1
PTX-716-F	PTX-343	716	8	$VOSO_4$	15	174.95		5.2	5.2
PTX-720-F	PTX-316	720	8	$VOSO_4$	15	162.5		3.7	3.7
PTX-736-F	PTX-319	736	8	$VOSO_4$	15	147.74		6.5	6.5
PTX-737-F	PTX-323	737	8	$VOSO_4$	15	157.06		5.6	5.6
PTX-743-F	PTX-325	743	8	$VOSO_4$	15	169.49		6.1	6.1
PTX-702-F	PTX-312	702	9	$VOSO_4$	15	293.85		12	12
PTX-728-F	PTX-322	728	9	$VOSO_4$	15	298.38		7.4	7.4
PTX-733-F	PTX-324	733	9	$VOSO_4$	15	309.32		8.7	8.7
PTX-744-F	PTX-328	744	9	$VOSO_4$	15	372.91		14	14
PTX-745-F	PTX-339	745	9	$VOSO_4$	15	337.83		9.8	9.8
PTX-709-F	PTX-333	709	10	Control	15	0		0.6	0.6
PTX-711-F	PTX-340	711	10	Control	15	0		0.6	0.6
PTX-730-F	PTX-336	730	10	Control	15	0		0.5	0.5

*Non-detects taken at one-half the detection limit.

TABLE A-11 ANALYTICAL RESULTS FOR QUALITY CONTROL SAMPLES

Sample Type	Sample Number	Tag Number	Pig Number	Analyte	Matrix	Original Pig #	Group	Material Administered	Urine Collection	Q	Conc (ng/mL)	DL	AdjConc* (ng/mL)	Original Result* (ng/mL)
Blind Dup	PTX-2734-U1	PTX-121	2734	As	urine	734	3	$NaHAsO_4$	U1		290	5	290	280
Blind Dup	PTX-2704-U1	PTX-126	2704	As	urine	704	4	Soil	U1		21	1	21	21
Blind Dup	PTX-2721-U1	PTX-109	2721	As	urine	721	2	$NaHAsO_4$	U1		160	5	160	150
Blind Dup	PTX-2749-U2	PTX-156	2749	As	urine	749	1	$NaHAsO_4$	U2		140	5	140	150
Blind Dup	PTX-2708-U2	PTX-150	2708	As	urine	708	4	Soil	U2		21	1	21	21
Blind Dup	PTX-2709-U2	PTX-152	2709	As	urine	709	10	Control	U2	<	1	1	0.5	1
Blind Dup	PTX-2751-U3	PTX-176	2751	As	urine	751	2	$NaHAsO_4$	U3		130	5	130	130
Blind Dup	PTX-2714-U3	PTX-177	2714	As	urine	714	5	Soil	U3		38	1	38	38
Blind Dup	PTX-2712-U3	PTX-204	2712	As	urine	712	4	Soil	U3		62	1	62	60

*Non-detects taken at one-half the detection limit.

Lab QC Type	Submitter I.D.	Certified Mean	+/- SD	Analyte	DL	Q	Conc	Units	Orig Q	Orig Sample Conc	Lab QC Evaluation	
Lab Dup	PTX-102			As	5		160	ng/mL		160	0	% Deviation
Lab Dup	PTX-114			As	10		810	ng/mL		820	1.2	% Deviation
Lab Dup	PTX-123			As	5		98	ng/mL		100	2	% Deviation
Lab Dup	PTX-132			As	5		130	ng/mL		140	7.4	% Deviation
Lab Dup	PTX-143			As	5		290	ng/mL		280	3.5	% Deviation
Lab Dup	PTX-152			As	1	<	1	ng/mL	<	1	0	% Deviation
Lab Dup	PTX-163			As	5		210	ng/mL		210	0	% Deviation
Lab Dup	PTX-172			As	5		140	ng/mL		140	0	% Deviation
Lab Dup	PTX-183			As	5		130	ng/mL		130	0	% Deviation
Lab Dup	PTX-192			As	5		100	ng/mL		110	9.5	% Deviation
Lab Dup	PTX-202			As	1		19	ng/mL		19	0	% Deviation
Lab Dup	PTX-242			V	0.01		0.12	mcg/g		0.11	8.3	% Deviation
Lab Dup	PTX-258			V	0.01		0.19	mcg/g		0.19	0	% Deviation
Lab Dup	PTX-231			V	0.01		0.034	mcg/g		0.033	2.9	% Deviation
Lab Dup	PTX-309			V	0.01		0.038	mcg/g		0.032	17.1	% Deviation
Lab Dup	PTX-310			V	0.01		1.06	mcg/g		0.93	13	% Deviation
Lab Dup	PTX-308			V	0.01		0.27	mcg/g		0.25	7.7	% Deviation

TABLE A-11, CONTINUED: ANALYTICAL RESULTS FOR QUALITY CONTROL SAMPLES

Lab QC Type	Submitter I.D.	Certified Mean	+/- SD	Analyte	DL	Q	Conc	Units	Orig Q	Orig Sample Conc	Lab QC Evaluation	
Lab Dup	PTX-338			V	0.3		7.8	mcg/g		7.4	5.3	% Deviation
Lab Dup	PTX-315			V	0.3		3.4	mcg/g		3.7	8.3	% Deviation
Lab Dup	PTX-329			V	0.3		1.8	mcg/g		1.6	11.8	% Deviation
Spike	PTX-106			As	5		540	ng/mL		340	**	% Recovery
Spike	PTX-118			As	5		520	ng/mL		320	**	% Recovery
Spike	PTX-127			As	1		230	ng/mL		26	102	% Recovery
Spike	PTX-137			As	1		210	ng/mL		2	104	% Recovery
Spike	PTX-146			As	1		240	ng/mL		22	109	% Recovery
Spike	PTX-157			As	5		400	ng/mL		190	105	% Recovery
Spike	PTX-168			As	1		240	ng/mL		33	104	% Recovery
Spike	PTX-177			As	1		240	ng/mL		38	101	% Recovery
Spike	PTX-188			As	1		250	ng/mL		25	113	% Recovery
Spike	PTX-196			As	1		260	ng/mL		38	111	% Recovery
Spike	PTX-206			As	5		330	ng/mL		120	105	% Recovery
Spike	PTX-226-SPK-M			V	0.01		0.39	Mcg/g		0.31	**	% Recovery
Spike	PTX-254-SPK-H			V	0.01		0.18	Mcg/g		0.01	113	% Recovery
Spike	PTX-256-SPK-L			V	0.01		0.087	Mcg/g		0.02	134	% Recovery
Spike	PTX-277-SPK-H			V	0.01		0.49	Mcg/g		0.15	113	% Recovery
Spike	PTX-281-SPK-L			V	0.01		0.96	Mcg/g		0.84	**	% Recovery
Spike	PTX-304-SPK-M			V	0.01		0.71	Mcg/g		0.55	**	% Recovery
Spike	PTX-317-SPK-L			V	0.3		4.2	Mcg/g		1.1	124	% Recovery
Spike	PTX-326-SPK-M			V	0.3		8.3	Mcg/g		2.5	116	% Recovery
Spike	PTX-345-SPK-H			V	0.3		9.4	Mcg/g		0.8	115	% Recovery
Ref Mat	NIST 1640	0.0267	0.0004	As	0.003		0.03	mcg/mL		0		
Ref Mat	NRCC TORT-2	21.6	1.8	As	0.5		21	mcg/mL		0		
Ref Mat	NIST 1566b	7.65	0.65	As	0.2		7.9	mcg/mL		0		
Ref Mat	NRCC TORT-2	21.6	1.8	As	0.5		21	mcg/mL		0		
Ref Mat	NIST 1566b	7.65	0.65	As	0.2		7.8	mcg/mL		0		
Ref Mat	NIST 1640	0.0267	0.0004	As	0.003		0.029	mcg/mL		0		
Ref Mat	NIST 1640	0.01299	0.0004	V	0.001		0.013	mcg/g		0		
Ref Mat	NRCC TORT-2	1.64	0.19	V	0.1		1.8	mcg/g		0		
Ref Mat	NRCC TORT-2	1.64	0.19	V	0.05		1.7	mcg/g		0		
Ref Mat	NIST 1640	0.01299	0.0004	V	0.001		0.013	mcg/g		0		
Ref Mat	NRCC TORT-2	1.64	0.19	V	0.05		1.7	mcg/g		0		

TABLE A-11, CONTINUED: ANALYTICAL RESULTS FOR QUALITY CONTROL SAMPLES

Lab QC Type	Submitter I.D.	Certified Mean	+/- SD	Analyte	DL	Q	Conc	Units	Orig Q	Orig Sample Conc	Lab QC Evaluation
Ref Mat	NIST 1640	0.01299	0.0004	V	0.001		0.013	mcg/g		0	
Ref Mat	NRCC TORT-2	1.64	0.19	V	0.02		1.6	mcg/g		0	
Ref Mat	NIST 1640	0.01299	0.0004	V	0.001		0.013	mcg/g		0	
Ref Mat	NIST 1640	0.01299	0.0004	V	0.001		0.014	mcg/g		0	
Ref Mat	NIST 1640	0.01299	0.0004	V	0.001		0.012	mcg/g		0	
Blank	Blank-1			As	1	<	1	ng/mL		0	
Blank	Blank-2			As	1	<	1	ng/mL		0	
Blank	Blank-3			As	1	<	1	ng/mL		0	
Blank	Blank-4			As	1	<	1	ng/mL		0	
Blank	Blank-5			As	1	<	1	ng/mL		0	
Blank	Blank-6			As	1	<	1	ng/mL		0	
Blank	Blank-1			V	0.01	<	0.01	mcg/g		0	
Blank	Blank-2			V	0.01	<	0.01	mcg/g		0	
Blank	Blank-3			V	0.01	<	0.01	mcg/g		0	
Blank	Blank-4			V	0.01	<	0.01	mcg/g		0	
Blank	Blank-5			V	0.3	<	0.3	mcg/g		0	
Blank	Blank-6			V	0.3	<	0.3	mcg/g		0	
Blank	Blank-7			V	0.001	<	0.001	mcg/g		0	
Blank	PTX-Blank-Liver			V	0.01	<	0.01	mcg/g		0	
Blank	PTX-BLANK-KIDNEY			V	0.01	<	0.01	mcg/g		0	
Blank	PTX-Blank-Femur			V	0.3	<	0.3	mcg/g		0	

** indicates spike too low

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

DEC 3 1 2012

OFFICE OF
SOLID WASTE AND
EMERGENCY RESPONSE

MEMORANDUM **OSWER Directive 9200.1-113**

SUBJECT: Compilation and Review of Data on Relative Bioavailability of Arsenic in Soil and Recommendations for Default Value for Relative Bioavailability of Arsenic in Soil Documents

FROM: Becki Clark, Director
Assessment and Remediation Division, Office of Superfund Remediation and Technology Innovation

TO: Superfund National Policy Managers, Regions 1 - 10

The purpose of this memorandum is to transmit the OSRTI Technical Report entitled "Compilation and Review of Data on Relative Bioavailability of Arsenic in Soil" and "Recommendations for Default Value for Relative Bioavailability of Arsenic in Soil". This report identifies and evaluates published literature relevant to estimating a relative bioavailability (RBA) value of arsenic in soil.

Based upon the analysis and external independent peer review, the following conclusions have been determined:

1. Currently available research information suggests that an RBA of arsenic in soils can be expected to be less than 100%.

2. Based upon evaluation of current data sets of arsenic RBAs in the US, the upper percentile of the data set results in a default RBA value of 60%.

3. The default RBA for arsenic in soils should only be used if site-specific assessments for arsenic RBA are not feasible.

This report and other efforts related to addressing arsenic in soil can be found on the internet at http://epa.gov/superfund/bioavailability/guidance.htm. Please contact Michele Burgess at (703) 603-9003 if you have questions or concerns.

Attachments

1. "Compilation and Review of Data on Relative Bioavailability of Arsenic in Soil"
2. "Recommendations for Default Value for Relative Bioavailability of Arsenic in Soil"

cc: Mathy Stanislaus, OSWER
Lisa Feldt, OSWER
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OSRTI Managers
Regional Superfund Branch Chiefs, Regions 1 – 10
Lisa Price, Superfund Lead Region Coordinator, Region 6
NARPM Co-Chairs
TRW Committee Members