Department of Toxic Substances Control
Brownfields and Environmental Restoration Program

# FINAL TECHNICAL REPORT

## EPA Brownfields Training, Research and Technical Assistance Grant

## Arsenic Characterization/Bioavailability on Mine-Scarred Lands (Study)

**December 2015**

This is the final technical report for the Brownfields Training, Research and Technical Assistance Grant (Grant) awarded to the Department of Toxic Substances Control's (DTSC's) Brownfields and Environmental Restoration Program (Cleanup Program). The Grant award for Brownfields Research Cooperative Agreement (**TR - 83415101**), dated February 26, 2009, was originally for five years (10/01/2008 – 9/30/2013) in the amount of $900,000. DTSC was granted two separate one year time-only extensions to 9/30/2015 and received a total of $850,000.

## GENERAL INFORMATION

Arsenic (As) is the main chemical of concern at a majority of former gold mines in the California Mother Lode and the Southern California desert areas. The California Department of Conservation has identified more than 47,000 abandoned mines in California which present potential threats to human health and the environment from arsenic, mercury and other heavy metals, acid mine drainage and physical hazards. At a majority of these sites, arsenic has been determined as the primary threat to human health.

At the time the Study was conceived, the only available techniques for estimating the relative bioavailability of arsenic were time consuming and expensive. While animal studies (in vivo bioavailability) can be conducted for a specific site, the associated cost and time requirements are generally prohibitive. Bioavailability is a term used by several branches of scientific study to describe the way chemicals are absorbed by humans and other animals if ingested. In general, most risk assessments assume that the site-specific relative bioavailability of arsenic in soil is 100%. However, DTSC believes that the majority of naturally occurring arsenic sites have significantly reduced arsenic relative bioavailability. Therefore, using the customary default of 100% relative bioavailability leads to an overestimation of risk and excessive cleanup costs. Consequently, many public and private entities avoid the remediation and redevelopment of arsenic contaminated sites in favor of uncontaminated sites.

The objective of the Study was to determine the range of arsenic bioavailability that may exist in contaminated soil at former mine sites, and to develop better methods for

determining the human health effects caused by exposure to arsenic at mine sites, calculating health risk, and developing health based cleanup goals for arsenic.
The Study did not make any provisions for DTSC to make remedial action decisions or conduct remedial action activities. The Grant funding was provided solely for specified investigation and research activities.

To complete the Study, DTSC contracted with Dr. Christopher Kim of Chapman University, Dr. Nick Basta of Ohio State University (OSU), Drs. Charles Alpers and Andrea Foster with the U.S. Geological Survey (USGS), and Dr. Stan Casteel with the University of Missouri (U of M) (investigators, as that term is used in the U.S. EPA grant) to work on various aspects of the Study. Specifics of the investigators' involvement in the Study were set forth in individual contracts/agreements between DTSC and each entity. The table below is a summary of the tasks worked on during the Study that lists individual investigator's participation in each task.

**Summary of tasks for Research on Bioavailability of Arsenic at Mine-Scarred Lands**

| Investigator | Task 1: Sample and Analysis Plan Field Work | Task 2: Develop Database for Predicting Bioavailability | Task 3: Bulk Chemistry, Special Chemistries, and Physical Measurements | Task 4: *In Vitro* Bioaccessibility Testing | Task 5: *In Vivo* Bioavailability Testing | Task 6: Spectroscopy at Synchrotron Energies | Task 7: Public Outreach |
|---|---|---|---|---|---|---|---|
| United States Geological Survey | X | This Task was eliminated due to budgetary restraints | X | | | X | X |
| Ohio State University | X | | X | X | X | X | X |
| University of Missouri | X | | | | X | X | X |
| Chapman University | X | | X | X | | X | X |
| Department of Toxic Substances Control | X | | X | X | X | X | x |

X = Investigator is performed work for this task

Each task, as proposed at the start of the Study, is described in detail below.

### Task 1: Sample and Analysis Plan/Field Work

The purpose of this task was to identify, collect, and analyze samples of soil from the gold mining regions in California. The goal was to collect and analyze samples with varying concentrations of arsenic and other characteristics, which data might be used in combination to predict or explain how arsenic adsorbs to and desorbs from soil. Task 1 proceeded in three phases: Reconnaissance, Sampling Event 1, and Sampling Event 2. The division into three phases constituted the investigators' attempt to maximize the information obtainable from the chosen sampling sites by refining sample requirements according to previously collected data.

## Task 2: Develop Database for Predicting Bioavailability

The purpose of this task was to organize information from the entire research project into a database for predicting bioavailability. The goal was to make the database publicly available at the website of DTSC. Due to budget constraints this task was reduced to compiling data generated during the Study and sharing it with all investigators to aid in decision making.

## Task 3: Conventional Chemical and Physical Measurements

The purpose of this task was to generate data from samples collected for use in predicting *in vitro* bioaccessibility and *in vivo* bioavailability. All of the analyses detailed below were performed during the Study.

**Bulk X-Ray Fluorescence (XRF) and Bulk X-Ray Diffraction (XRD):** Samples collected were subjected to bulk XRD and bulk XRF. This described the elemental composition and crystal structure of the many mineral phases occurring in samples of soil collected.

**Differential XRD:** Residual solid materials from various extractions were analyzed by powder XRD. The XRD data from the residual solids was compared to data from the original bulk samples to quantify any changes in mineralogical composition. The purpose was to identify where arsenic was sorbed or desorbed from the various phases of iron oxide before and after each stage of the extraction procedures.

**Electron Microprobe Analysis (EMPA):** EMPA was used by USGS to characterize the spatial relationships among iron oxides, other primary minerals, and arsenic in the various mineral phases (especially arsenian pyrite) present in the soil. EMPA can identify mineralogical associations, *i.e.* the various mineral phases in a sample and how much of the mass of arsenic in that sample is bound to each phase.

**Scanning Electron Microscopy (SEM):** USGS used SEM to elucidate the mineralogical identification of iron oxides at a spatially resolved scale. SEM can sometimes show the fine structure of particles of soil and provide information on the location of bound arsenic within a particle or which arsenic has been removed by extraction.

**Particle Size Analysis:** The surface area of the soil particles in all samples was analyzed using a BET Surface Area Analyzer (Beckman Coulter, SA-3100). This reference method uses helium to measure the free-space in a sample tube for highly precise information on particle surface area. Data from this method allowed for inferences on mineralogy and crystallinity.

**Extraction Studies:** The goal of wet chemical extractions in this Study was to identify chemical procedures which will remove bioaccessible or bioavailable arsenic from soils. Extraction is a wet chemical technique involving exposing the soil sample to a liquid medium for a period of time, filtering the extracting medium away, leaving residual solid material on the filter. This process can be repeated with different extractants with known effects on various iron oxides. All samples of soil from Sampling Events 1 and 2 were subjected to different extraction procedures: (1) water extraction (ASTM, 2004),: (2) simulated gastric fluid (SGF) extraction (Ruby *et al.*, 1996; Drexler and Brattin, 2007); a sequence of extractions as described by Wenzel *et al.* (2003), and (3) simulated lung fluid (SLF) extraction (Twining *et al.*, 2005), as time and funds permitted.

### Task 4: *In Vitro* Bioaccessibility Testing

The analysis detailed in 4a. and 4b. was performed on all soil samples and the analysis in 4c. as time and funds permitted.
**Extraction in Simulated Gastric Fluid:** The method of Ruby *et al.* (1996), as modified by Drexler and Brattin (2007), was used to produce wet chemical data.

**The OSU *In Vitro* Gastrointestinal Method:** *In vitro* bioaccessibility testing was conducted according to previously published procedures (Rodriguez *et al.,* 1999; 2003; Basta *et al.,* 2007).

**New OSU method:** OSU developed a new/modified version of their existing IVBA method that is better able to predict *in vivo* results. The modified method was run on all samples that underwent the in vivo swine feeding protocol.

### Task 5: *In Vivo* Bioavailability Testing

The purpose of Task 5 was to characterize the bioavailability of arsenic in select soils collected during the Study using juvenile swine as an animal model. The data from these studies were used as the standard by which we compared the validity and accuracy of the *in vitro* results. The University of Missouri performed bioavailability testing on select soil samples as described in SOP 13 in the Quality Assurance Project Plan approved by the U.S. EPA for this Study.

### Task 6: Spectroscopy at Synchrotron Energies

When wet chemical analysis is not adequate to predict *in vitro* bioaccessibility of arsenic in a sample, that sample became a candidate for the various types of measurements in Task 6. Chapman University and USGS used performed the following at the Stanford Synchrotron Research Laboratory (SSRL) Facility in California.
**Synchrotron-Based XRD**: This technique was used for selected solid materials, either soil samples from the field or residua from extractions, to produce rapid, high resolution diffraction patterns for identifying mineralogy and crystallography.

**Bulk X-Ray Absorption (XAS):** Irradiating a sample with X-rays and measuring absorption spectra permits quantifying relative abundance of oxidation states, such as arsenic III and arsenic V. Bulk XAS, using millimeter-sized beams of X-rays, permitted qualitative or quantitative speciation of arsenic resident on mineral phases, including before and after extraction or *in vitro* digestion.

**µ-X-Ray Absorption Spectroscopy (µ-XAS) / µ-X-Ray Fluorescence Spectroscopy (µ-XRF) / µ-X-Ray Diffraction (µ-XRD):** These analyses were performed, as necessary, on select samples.

### Task 7: Public Outreach

See Appendix 1, Publication List, for details on peer-reviewed publications, presentations and abstracts from each of the investigators and DTSC.

Detailed information regarding the work completed and conclusions reached for each investigator who participated in the Study can be found below.

**DTSC**

DTSC staff actively managed the Study throughout the grant period. Contracts were negotiated with the U.S. Geological Survey, University of Missouri, Ohio State University, and Chapman University to complete the work described in the grant application. Periodic meetings with the investigators were used to discuss progress, share data, and make group decisions on how to proceed. Web-based meetings were typically used to facilitate participation of those not located in Sacramento, California. All of the investigators, except for Dr. Stan Casteel with the U of M, met in person while presenting at the Annual Meeting of the National Association of Abandoned Mine Land Programs in Northern California in 2011 and at the Goldshmidt Conference in Sacramento and Nevada City in 2014. DTSC requested quarterly update reports from the investigators and used them to create quarterly reports for EPA to keep them informed of the Study's progress. In all, 28 quarterly reports were submitted to EPA during the life of the grant. Throughout the grant period DTSC completed the administrative duties required by the grant, including requesting amendments to the Cooperative Agreement (four amendments for incremental funding and two for time extensions to complete work not included in the original scope).

After receiving the grant award, DTSC prepared a Quality Assurance Project Plan (QAPP), including a Field Sampling Plan and Standard Operating Procedures (SOPs) for analyses to be performed as part of the Study. The QAPP was put out as a draft for a 30-day public comment period to allow the public to provide input on the project. No comments were received during the comment period and the draft QAPP was approved as final for use on the Study by the Quality Assurance Office of U.S. EPA Region 9 on September 15, 2009. This 400+ page document governed how samples were collected and analyses performed to ensure quality assurance requirements were met and that data generated during the project was reliable enough to support any conclusions reached or guidance documents developed.

California's Empire Mine State Historic Park (EMSHP), owned and operated by the California Department of Parks and Recreation (DPR) was selected as the initial sampling location for the Study due to previous remedial investigation work overseen by DTSC that provided arsenic data for several types of mine waste. Because the EMSHP is on the historical register and cultural artifacts and sensitive biological receptors are potentially present in the sampling areas, an Initial Study and Negative Declaration were prepared to comply with the California Environmental Quality Act (CEQA).

During the summer of 2009, reconnaissance sampling was conducted at the EMSHP to assist in the selection of final sampling locations. Ohio State University ran *in vitro* bioaccessibility testing on all of the reconnaissance samples to provide additional information for the Sampling Event 1 sample location decision process. These results were provided in August 2009 and were used, in part, to select the 14 sampling locations at EMSHP for Sampling Event 1. An agreement with Holdredge and Kull (Hand K) was completed that provided for donated services and equipment. H and K

provided a mini excavator, small backhoe and staff for each of the three days of sampling during Sampling Event 1 at no cost to the project.

Following the approved QAPP, Sampling Event 1 was conducted on September 21, through September 23, 2009. Building on the two reconnaissance sampling events from the previous quarter, 14 separate sampling locations were selected in August 2009 based on the data obtained and field XRF screening. Using a mini excavator and a small backhoe, samples were collected from each of the 14 sampling locations with multiple samples collected at several of the locations if the lithology and/or arsenic concentrations varied with depth. Samples were sieved through a #4 nominal (1/4") screen to reduce volume and collected in multiple 5-gallon containers to ensure sufficient volume to conduct the various analyses detailed in the QAPP. In addition to 21 samples collected at the EMSHP, an additional four samples were collected from the nearby Rattlesnake Gates property with the permission of the property owner. DTSC was providing oversight of the investigation of potential mine waste discovered at this property. Samples collected from the four locations sampled during the Study at this property as part of Sampling Event 1 were collocated with samples that had previously undergone bioaccessibility testing by an entity not associated with this project. Because sampling at the EMSHP was ahead of schedule a decision was made in the field to collect the Rattlesnake Gates samples for possible inclusion in this project. In all, a total of 25 samples in 46 5-gallon containers with a total weight of 2,593 pounds were collected during Sampling Event 1. Resources necessary to complete the sampling included:

DTSC

- 6 staff members each day
- 3 trucks
- Water tender

USGS

- 1 staff member
- 1 graduate student

Holdredge and Kull (H and K)

- Mini excavator
- Small backhoe
- 1 heavy equipment operator
- 1 staff member

DPR

- Cultural monitor
- Biological monitor
- 2 maintenance staff

Samples collected during Sampling Event 1 were shipped to Ohio State University (OSU) for storage and processing per the QAPP. The samples were sieved down to the <250 micron fraction and homogenized before being shipped to the other investigators for various analyses. Extensive sampling in accordance with the QAPP was conducted at OSU to confirm the <250 micron fraction aliquots were properly homogenized before shipment.

Following extensive analysis on the samples collected during Sampling Event 1 it was decided to collect additional samples from throughout the State of California. As part of Sampling Event 2, DTSC collected ten samples from September through December 2013 following the procedures included in the approved QAPP in addition to three samples collected by Dr. Kim from the Randsburg Historic Mining Complex. Dr. Kim collected samples from two sites in Kern and San Bernardino counties while DTSC collected samples from seven sites in Amador, Mono, Sierra, and Shasta counties. All of the samples were shipped to OSU for processing, in accordance with the QAPP, and analysis (US EPA 3051a and OSU modified *in vitro*).

Twelve samples from Sampling Event 1 and six samples from Sampling Event 2 were sent to the University of Missouri for in vivo bioavailability testing using juvenile swine.

DTSC Staff worked with DTSC's Environmental Chemistry Lab and OSU over the summer and fall of 2015 on a laboratory repeatability study of the new in-vitro method developed by OSU as part of the Study. Prima Environmental Incorporated, a commercial laboratory located in Northern California, agreed to perform the new OSU method on a subset of the samples collected during the project and DTSC's lab completed arsenic analysis. Additional details regarding this work may be found in the OSU section of this report.

Dr. Valerie Mitchell Hanley has also provided public outreach in a variety of venues. She presented posters at the Society of Toxicology annual meeting throughout the duration of the grant. In Spring 2015, Dr. Hanley presented on the results of the study at the Interstate Technology and Regulatory Council (ITRC) Spring Meeting to the Bioavailability in Contaminated Soils Team. In Fall 2015, Dr. Hanley was the invited speaker at the Sacramento Professional Environmental Marketers Association (SacPEMA) Luncheon to discuss the outcome of the study. Information regarding the study was also presented to a delegation from the Chinese Sichuan Department of Environmental Protection during a visit to DTSC. It was one of only two DTSC projects highlighted to the delegation. Additional information regarding presentations and abstracts can be found in Attachment 1, Publications List.

## OHIO STATE UNIVERSITY

Ohio State University (OSU) evaluated the bioaccessibility of arsenic in mining soils using both sequential extraction procedure (SEP) and various in vitro gastrointestinal models (OSU IVBA, SBRC, and the modified OSU IVBA). A detailed report presenting their methodologies and results is presented in Attachment 2. Table and figure numbers referenced in this section correspond to those in the attached report. OSU had three main objectives in evaluating the bioaccessibility of arsenic in soils:

**Objective 1: Evaluate the OSU-IVG and SBRC methods for use on arsenic contaminated soils from an abandoned gold mine in CA**
***In vitro and In vivo results***

The results for arsenic extracted by the OSU-IVG and SBRC methods as well as swine RBA are presented in table 2. The two in vitro methods extracted similar amounts of arsenic. In addition, both methods extracted a small percentage of total arsenic (<1 to 14.4%). However, the swine RBA arsenic ranged from 4.00 to 23.7%, two to five times the amount of arsenic indicated by in vitro.

**Table 2: In vitro and Swine RBA results for Empire Mine soils.**

| | OSU- IVG GE | OSU IVG IE | SBRC GE | RBA |
|---|---|---|---|---|
| Soil | ---------- | As (%) | ---------- | |
| EM1 | 9.27 | 10.7 | 4.74 | 23.7 |
| EM3 | 2.97 | 3.14 | 1.29 | 15.3 |
| EM5 | 3.66 | 4.02 | 1.11 | 15.3 |
| EM8 | 2.82 | 3.42 | 1.59 | 19.2 |
| EM13 | 2.22 | 2.60 | 1.10 | 12.5 |
| EM15 | 3.50 | 3.96 | 4.52 | 19.7 |
| EM18 | 1.51 | 1.27 | 2.03 | 4.00 |
| EM19 | 1.77 | 2.24 | 0.361 | 11.7 |
| EM20 | 8.05 | 7.66 | 10.8 | 22.7 |
| EM21 | 7.24 | 7.35 | 14.4 | 23.0 |
| RG1 | 1.92 | 2.97 | 0.987 | 11.8 |
| RG3 | 3.05 | 3.05 | 1.15 | 12.4 |
| min | 1.51 | 1.27 | 0.36 | 4.00 |
| mean | 4.00 | 4.36 | 3.67 | 15.9 |
| max | 9.27 | 10.7 | 14.4 | 23.7 |

**Table 3. Swine RBA predictions for Empire Mine soils using published regression equations compared with measured RBA.**

| | OSU-IVG As | | | | SBRC As | | |
|---|---|---|---|---|---|---|---|
| Soil | % GE eq. 1 | % IE eq. 2 | % GE eq. 4 | % IE eq. 5 | % GE eq. 3 | % GE eq. 6 | RBA As 90% CI |
| EM1 | 17.8 | 22.6 | 22.2 | 25.8 | 6.4[a] | 22.6 | 10.9 - 36.5 |
| EM3 | 12.2 | 15.5 | 16.8 | 17.4 | 2.9[a] | 20.5[b] | 11.7 - 18.8 |
| EM5 | 12.8 | 16.4 | 17.4 | 18.4 | 2.8[a] | 20.4[b] | 15.22 - 15.5 |
| EM8 | 12.1[a] | 15.8[a] | 16.7[a] | 17.7 | 3.2[a] | 20.7[b] | 16.9 - 21.4 |
| EM13 | 11.6 | 15 | 16.2 | 16.8 | 2.8[a] | 20.4[b] | 5.1 - 19.9 |
| EM15 | 12.7[a] | 16.3 | 17.3 | 18.3 | 6.1[a] | 22.5 | 13.1 - 26.2 |
| EM18 | 10.9[b] | 13.8[b] | 15.6[b] | 15.4[b] | 3.7 | 20.9[b] | 3.3 - 4.6 |
| EM19 | 11.2 | 14.7 | 15.8[b] | 16.4[b] | 2.0[a] | 19.9[b] | 8.3 - 15.2 |
| EM20 | 16.7[a] | 19.8[a] | 21.2 | 22.4 | 12.4[a] | 26.4[b] | 21.1 - 24.3 |
| EM21 | 16.0[a] | 19.5 | 20.5 | 22.1 | 15.9[a] | 28.6[b] | 17.6 - 28.5 |
| RG1 | 11.3 | 15.4 | 15.9 | 17.2 | 2.6[a] | 20.3[b] | 6.9 - 16.6 |
| RG3 | 12.3 | 15.5 | 16.9 | 17.3 | 2.8[a] | 20.4[b] | 7.6 - 17.2 |

[a]Under-prediction of RBA (below lower 90% CI)
[b]Over-prediction of RBA (above upper 90% CI)

**Objective 2: Optimize existing in vitro method(s) to measure and/or predict bioavailable arsenic in test soils**

In order to optimize gastrointestinal in vitro extraction of arsenic, key physiological parameters affecting dissolution of arsenic from soil were reviewed and compared to OSU-IVG and SBRC. . Gastric constituents were modified within physiological conditions to optimize for As dissolution in the stomach. Details of the optimization procedures will be published in 2016.

**Objective 3: Validate and provide recommendations for use of modified OSU-IVG to make arsenic bioavailability adjustments for CA soils**

The validation of the modified OSU-IVG was a multistep process. First the potential of the modified OSU-IVG to extract bioaccessible arsenic and predict RBA arsenic in Empire Mine soils was evaluated. Second, IVBA and swine RBA data from Empire Mine was merged with data from an existing Strategic Environmental Research and Development Program (SERDP, Department of Defense) study: *Mechanisms and Permanence of Sequestered Pb and As in Soils: Impact on Human Bioavailability* (Project ER-1742). In addition, six soils were collected under this study from sites outside of Empire Mine for IVBA and RBA determination. Finally, the modified OSU-IVG was tested for reproducibility with a round robin between The Ohio State University (Columbus, OH) and Prima Environmental (El Dorado Hills, CA)

### *Evaluation of modified OSU-IVG to extract bioaccessible arsenic and predict RBA arsenic*

The Empire Mine IVBA arsenic results for the modified OSU-IVG and swine RBA are presented in Figure 4. The results demonstrate that the large under extraction of arsenic by the OSU-IVG and SBRC methods has been corrected with the parameters of the modified OSU-IVG method. However, the modified OSU-IVG extracted more than RBA arsenic in some soils (EM15, EM18, M20, and EM21), thereby negating potential bioavailability adjustments as IVBA arsenic approaches the 60% bioavailability default for site assessment (USEPA, 2012). Of note is that these four soils contain the highest arsenic contents of all the study soils (5,647 – 12,095 mg/kg). As a result, the modified in vitro may not be suitable for accurate estimation of RBA arsenic in soils with high arsenic content. However, the modified OSU-IVG closely brackets RBA arsenic in Empire Mine soils with low to moderate arsenic content.

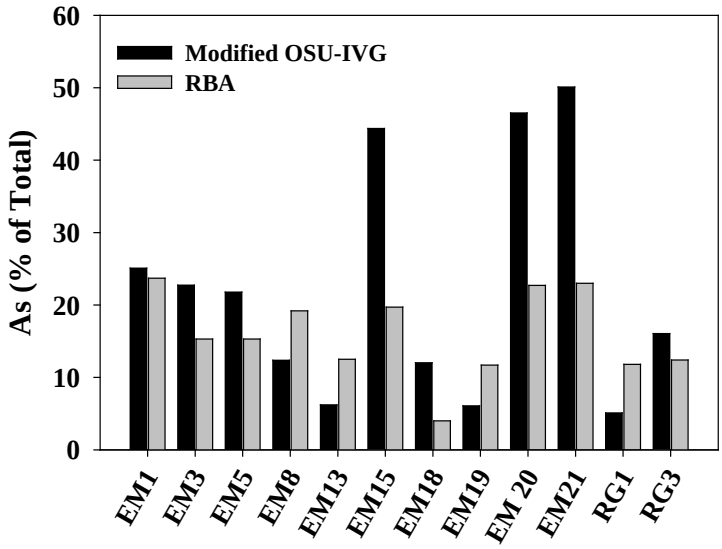



**Figure 3 .** Results of the Modified OSU-IVG compared to swine RBA arsenic in Empire Mine soils.

### *Merged DTSC and SERDP datasets*

The modified OSU-IVG results suggest that accurate extraction and correlation with RBA arsenic may be possible for low to moderately arsenic contaminated soils, but overestimation of RBA is likely in high arsenic soils. As a result, a larger dataset with soils containing low to moderate concentration of arsenic for IVIVC is desirable. This was done by combining the DTSC and SERDP datasets for soils containing less than 1,500 mg As/kg and the addition of data from six soils collected outside of Empire Mine.

The combined dataset resulted in the IVIVC presented in Figure 5. The results of the IVIVC demonstrate that the modified OSU-IVG is highly predictive of RBA arsenic,

meeting the criteria of; an $r^2 > 0.6$, a slope between 0.8 and 1.2 (Denys, Caboche et al. 2012; Wragg et al., 2011) and a y-intercept that does not deviate significantly from zero ((Juhasz et al. 2014). In addition, this regression equation includes soils with widely varying arsenic sources, indicating that the modified OSU-IVG may be applicable to both goldmining and non-gold mining sites.

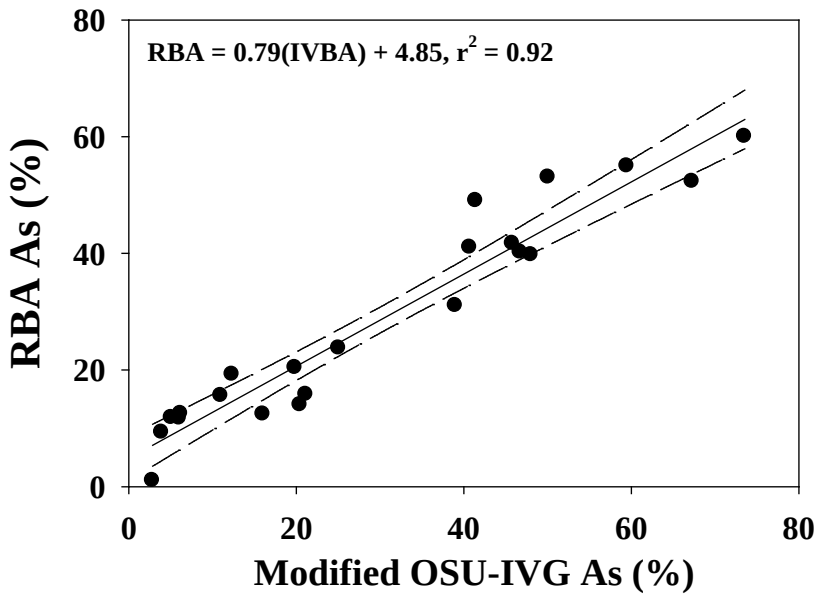



**Figure 4 .** IVIVC (simple linear regression with 95% confidence bands) of modified OSU-IVG vs RBA for DTSC and SERDP soils with 1,500 mg/kg As.

### *Round Robin Validation*

In order to test the reproducibility of the modified OSU-IVG, a round robin was conducted between the data presented in this report by The Ohio State University (Columbus, OH) and Prima Environmental (El Dorado Hills, CA).

The results of the round robin are presented in Table 8. Intra-lab and inter-lab variability was assessed using relative standard deviation (RSD):

$RSD = 100 * (s / |\bar{x}|)$

Where:

s = the sample standard deviation

$\bar{x}$ = sample mean

For intra-lab RSD calculation, the replicate sample extractions were used to calculate RSD. Inter-lab RSD was calculated using the mean IVBA from the respective labs (Table 8). The intra-lab RSDs were below 10% for OSU and Prima, indicating highly reproducible within lab results using the modified OSU-IVG. The inter-lab RSDs ranged from 0.04 to 26% with a mean of 8.5 % and median of 4.9 %. These results demonstrate that when the SOP developed for the round robin is followed, the modified OSU-IVG yields reproducible results.

**Table 6 . Comparison of OSU and Prima Lab results for round robin study.**

| Sample | Lab | n | Mean IVBA | Intra-Lab RSD | Inter-Lab RSD |
|---|---|---|---|---|---|
| | | | % | | |
| | Prima | 5 | 16.1 | 2.8 | |
| Test Soil 1 | OSU | 3 | 11.0 | 6.2 | 26 |
| | Prima | 5 | 33.0 | 4.4 | |
| Test Soil 2 | OSU | 3 | 25.1 | 5.7 | 19 |
| | Prima | 5 | 15.9 | 5.0 | |
| Test Soil 3 | OSU | 3 | 16.1 | 5.2 | 0.7 |
| | Prima | 5 | 51.4 | 3.2 | |
| Test Soil 4 | OSU | 3 | 50.1 | 1.4 | 1.8 |
| | Prima | 5 | 67.8 | 3.4 | |
| Test Soil 5 | OSU | 3 | 67.9 | 1.0 | 0.04 |
| | Prima | 5 | 62.6 | 5.9 | |
| Test Soil 6 | OSU | 3 | 54.6 | 2.6 | 9.7 |
| | Prima | 5 | 92.2 | 5.1 | |
| Test Soil 7 | OSU | 3 | 83.0 | 1.4 | 7.4 |
| | Prima | 5 | 73.1 | 4.4 | |
| NIST 2711A | OSU | 5 | 70.7 | 2.4 | 2.4 |
| | | | Min | 1.0 | 0.04 |
| | | | Mean | 3.8 | 8.5 |
| | | | Median | 3.9 | 4.9 |
| | | | Max | 6.2 | 26 |

## CONCLUSIONS

Two commonly employed in vitro methods (OSU-IVG and SBRC) were evaluated as a surrogate for in vivo swine dosing at Empire Mine State Historic Park. The arsenic fractions solubilized by the OSU-IVG and SBRC (i.e., bioaccessible As) were significantly less than the relative bioavailable arsenic fractions. The results of SEP suggest this may be due to the limited ability of both methods to dissolve amorphous and poorly-crystalline oxides of Fe and Al. In addition, using predictive equations developed from datasets from other studies demonstrated that prediction results vary drastically depending on the study soils used to develop the IVIVC. The SBRC method either drastically either under-predicts RBA arsenic for all but one Empire Mine soil or over predicts for all but two soils depending on which regression equation is used. The regression equations developed for the OSU-IVG are less variable and therefore produce more consistent results. However, the OSU-IVG failed to predict within the RBA 90% confidence interval for every soil regardless of which regression equation was used. In vitro methods that meet IVIVC criteria; an $r^2 > 0.6$, and a slope between 0.8 and 1.2, as well a y-intercept that does not deviate significantly from zero are highly desirable. As a result, modification to the OSU-IVG were made and evaluated. Results show that the modified OSU-IVG meets IVIVC criteria for swine when applied to soils

with less than 1,500 mg As/kg, regardless of arsenic source. A round robin inter-laboratory study was performed to determine the reproducibility of the modified OSU-IVG method. Mean and median intra-laboratory RSDs were 3.8% and 3.9%, respectively. Mean and median inter-laboratory RSD were 8.5% and 4.5%, respectively. The reproducibility meets and exceeds criteria intra-laboratory RSD of < 10% and inter-laboratory RSD of <20% (Wragg et al., 2011). As a result, the SOP (Appendix) developed yields highly reproducible (within and across lab) IVBA results. A robust linear regression of RBA arsenic (%) = 0.79(%IVBA) + 4.85 can be used to predict an accurate and reproducible RBA arsenic from the IVBA measured by the newly developed modified OSU-IVG.

## USGS.

Staff with the USGS participated in most aspects of the Study. Below is a discussion of their activities for each of the major tasks.

### Task 1. Sample and Analysis Plan + Field Work

The USGS Project Director (C. Alpers) communicated frequently with the DTSC Contract Manager (P. Myers) via telephone and e-mail regarding progress on the Scope of Work throughout the project. Staff assisted with collection of soil and rock samples from the EMSHP as part of Sampling Event 1.

### Task 2. Develop Database for Predicting Bioavailability

Data from the study was compiled in spreadsheet form and was shared with other project researchers. The group at Ohio State University took the lead on compiling a final database with all project results for the purpose of statistical analysis.

### Task 3. Bulk Chemistry, Special Chemistries, and Physical Measurements

**Bulk X-ray Fluorescence (XRF) and Bulk X-ray Diffraction (XRD):** A total of 25 samples were analyzed by these methods. Samples sieved to < 250 micrometers were provided by Ohio State University. Results were shared with other investigators.

**Differential XRD**: A total of 12 samples (the same ones analyzed by in vivo methods during rounds 1 and 2) that were leached by in vitro methods were analyzed by XRD using the same methods as unleached samples. Results were compared to determine whether there was any detectable change in mineralogy that could be ascribed to the leach tests. Results were shared with other investigators.

**Electron Microprobe Analysis (EMPA):** Rock samples from trenches and outcrops located near the 25 Sampling Event 1 samples were prepared in polished section and analyzed by EMPA (at the Univ. of California, Davis, [UCD]) for arsenic concentration in sulfide minerals (arsenopyrite, aresnian pyrite, and cobaltite) and oxide minerals (ferrihydrite [HFO], and hydrous ferric arsenate [HFA]). Results were included in Master's thesis by Tamsen Burlak at California State University, Sacramento.

In addition, powdered samples from the 25 Sampling Event 1 samples (sieved to < 250 micrometers by OSU) were also analyzed by EMPA at UCD by T. Burlak, for comparison to the data collected from hand samples. Data were compiled for arsenic content of pyrite and iron oxides for each sampling site and plotted on maps so that spatial trends could be assessed. Statistics were derived for arsenic content of pyrite and iron oxides for the entire EMSHP site. Overall, the median arsenic content of pyrite was about 1 weight percent and the median arsenic content of iron oxides was about 2 weight percent. The higher arsenic content of iron oxides is attributed to contributions from weathering of arsenopyrite.

**SEM analyses (including QEMSCAN):** Qualitative data were collected for mineral abundance in polished sections, and then quantitative data were collected for the 12 sieved samples that were analyzed by in vivo methods. A method was developed to distinguish high-arsenic (> 6 weight percent) iron oxide from low-arsenic (< 6%) iron oxide. Mineral maps indicate that the high-arsenic iron oxides are typically associated with weathered arsenopyrite and the low-arsenic iron oxides are typically associated with weathered (arsenian) pyrite. Data were shared with other investigators.

### Task 4. In Vitro Bioaccessibility Testing

This task was carried out by the group at Ohio State University. The USGS group assisted the OSU group with discussion of method development and data interpretation in light of results of mineralogical and geochemical investigations done by USGS.

### Task 5. In Vivo Bioavailability Testing

This task was carried out the group at the University of Missouri. The USGS group assisted the U of M group with discussion of method development and data interpretation in light of results of mineralogical and geochemical investigations done by USGS.

### Task 6. Spectroscopy at Synchrotron Energies

<u>Summary of Work Completed</u>

Collected synchrotron micro-XRF maps at ca. 50 micron resolution on 11 whole thin sections from variably-weathered hand specimen rock samples from tailings/waste piles at the EMSHP. Both arsenic (As) redox and Fe redox maps have been collected.

Purpose: to track the mineralogical fate of arsenic in tailings/waste piles, and to understand the range of different types of As speciation that exists in the rocks of the EMSHP.

General Conclusions to date:
At the grain scale, the amount of arsenic in secondary ferrihydrite could be related to the As concentration of the adjacent sulfides (mainly pyrite vs. arsenopyrite)
Secondary arsenate minerals such as arseniosiderite and scorodite were identified by EPMA and QEMSCAN, but do not appear to be abundant

Collected bulk As and Fe XAFS spectra from 19 (or more) < 250-micron-sieved contaminated soil samples that were used in bioaccessibility/bioavailability measurements (Sampling Event 1 samples); collected As and Fe XAFS spectra from unsieved or hand sieved samples from the same sites (reconnaissance samples).

Purpose: to identify and quantify the relative abundance of the predominant As and Fe species in samples in order to identify correlations between the occurrence or abundance of a given species and geochemical parameters (XRD, EPMA, QEMSCAN, sequential extraction), and/or bioaccessibility/bioavailability data. To identify sources of uncertainty in linear combination, least-squares data analysis and to attempt to quantify these.

General Conclusions to date
Multivariate analysis identified approximately five unique As species and between 5-10 unique Fe species
First example (to our knowledge) of cluster analysis applied to model and sample As and Fe XAFS spectra. It provides a model-independent way of quantifying spectral similarity
Ubiquitous, predominant As-bearing minerals: Fe oxyhydroxides, Fe sulfides (pyrite, arsenopyrite). Accessory As-bearing minerals (presence is sample dependent): arseniosiderite, scorodite, jarosite, As sorbed on Al-hydroxide or aluminosilicate clay, orpiment
Apparent identification of Ca arsenate mineral in several samples is still equivocal. Could be arsenic in apatite, or could be a stand-in for other, as-yet unidentified As mineral. Cluster analysis shows the spectrum of Ca arsenate to be more similar to As(V) on aluminosilicate minerals than to arseniosiderite, which also contains Ca.

Collected synchrotron micro-XRF maps at ca. 2-10 micron resolution of regions of interest from Sampling Event 1 soil samples. Both As redox and Fe redox maps have been collected.

Purpose: to validate the bulk As and Fe data in terms of the ID and relative abundance of major As species, to identify minor As species that might not be detected in the bulk XAFS analysis, and to compare microscale As/Fe speciation in soils to that analyzed in weathered rock samples (#1)

General Conclusions to date:
Bulk XAFS analysis is generally validated by the microscale samples
Microscale measurement has not proved very valuable in helping to reveal the exact nature of the Ca-arsenate and As-Al hydroxide or As-aluminosilicate species quantified in bulk samples.

**Task 7. Public Outreach**

Presentations:

See Attachment 1, Publication List

Field trips

**2011:** annual meeting of National Association of Abandoned Mine Lands Programs (NAAMLP), held in Squaw Valley, CA (Oct. 2011), co-led by Alpers, Myers, Foster, Kim, Basta, and Mitchell
**2012:** Reclaiming the Sierra conference, held in Nevada City, CA (May, 2012), led by Alpers
**2014:** Goldschmidt Conference, held in Sacramento, CA (June, 2014), co-led by Alpers, Myers, Foster, Kim, Basta, and Mitchell

Peer-reviewed publications:

See Attachment 1, Publication List

Manuscripts in Preparation

Note: the following manuscripts (to be submitted to peer-reviewed journals) are expected to be completed during 2015-16.

**Microscale repartitioning of arsenic and iron during weathering of mine tailings and waste rock from the Empire Mine State Historic Park, a historically-mined lode gold complex**
Foster: Burlak, Brown: collection/analysis of As and Fe EXAFS spectra
Foster: Raman spectroscopy
Petersen, Burlak, Alpers: QEMSCAN data
Burlak, Alpers: Electron microprobe data

**Arsenic and iron speciation in soils and mine wastes from the Empire Mine State Historic Park, a historically-mined lode gold complex**
Foster, Brown: collection/analysis of As and Fe EXAFS spectra
Foster: Raman spectroscopy
Petersen, Burlak, Alpers: QEMSCAN data
Burlak, Alpers: Electron microprobe data

**Relationships among geochemical and in vitro/in vivo datasets from the Empire Mine State Historic Park, a historically-mined lode gold complex**
Foster, Brown-collection/analysis of bulk As and Fe EXAFS spectra
Blum, Alpers: quantitative XRD/XRF data
Basta, Whitacre: OSU-IVG dataset (old or improved), sequential extraction results
Casteel+ co-authors: bioavailability data

## CHAPMAN UNIVERSITY

Major tasks conducted:

Size separation analysis of 20 samples from Empire Mine, Rattlesnake Gate, Chemung Mine, and Eureka Mine area into 11 discrete particle size fractions (see table at right)
Digestion and ICP-MS analysis of all samples' size fractions for concentrations of 49 elements, including arsenic.
Production of mass distribution, elemental concentration, and elemental mass distribution plots as a function of particle size for all samples analyzed.
BET surface area analysis on all size fractions to determine reactive surface area as a function of particle size in $m^2/g$.
Simulated gastric fluid (SGF) extractions of all size fractions
Statistical and graphing analysis of arsenic bioaccessibility (expressed as $[As]_{released}$ and $\%As_{released}$, correlating with:
Initial arsenic concentration (ppm)
Particle size range/average
Reactive surface area ($m^2/g$)
Extended X-ray absorption fine structure (EXAFS) and micro-X-ray fluorescence (µXRF) spectroscopic analysis of As speciation, microspatial distribution, and chemical association (e.g. with other elements such as Fe) in size-fractionated mine wastes [in collaboration with A. Foster, USGS]

| Size Ranges | |
|---|---|
| Split | Particle Diameter |
| S1 | > 2830 |
| S2 | 2830 - 1700 |
| S3 | 1700 - 1000 |
| S4 | 1000 - 500 |
| S5 | 500 - 250 |
| S6 | 250 - 125 |
| S7 | 125 - 75 |
| S8 | 75 - 45 |
| S9 | 45 - 32 |
| S10 | 32 - 20 |
| S11 | <20 um |

Primary expenses associated with work:

ICP-MS analyses (conducted at an external lab)
Materials and supplies associated with surface area measurements (sample holders, liquid nitrogen)
Materials and supplies associated with SGF extractions (chemicals, sample vessels) and analyses (conducted externally)
Travel costs for spectroscopic work conducted at Stanford Synchrotron Radiation Lightsource (SSRL)
Compensation for co-investigator, research assistants

Key conclusions:

Most trace metal(loid) concentrations in mine wastes are inversely related to particle size and, in most fine-grained (≤250 µm) size fractions, are elevated above the bulk concentrations of these metals when all size ranges are considered. This has implications for the proper assessment of risk based on bulk grab sampling, as is commonly done by governmental agencies.
Mine tailings produced through stamp milling and leaching processes were found to have both a narrower and finer particle size distribution than background samples, with significant fractions of particles available in a size range (≤250 µm) that could be incidentally ingested.

Arsenic is strongly correlated with iron in most tailings and background samples, with X-ray absorption spectroscopy identifying phases including arseniosiderite, As(V) sorbed to ferrihydrite, and (minor) arsenopyrite which confirm such a correlation.
Processed mine tailings release a much higher proportion of arsenic than unprocessed waste rock when exposed to both water and simulated gastric fluid; in addition to the finer size fractions present, the secondary arsenic phases likely produced during ore crushing and leaching (to remove gold) appear to be more soluble and mobile.
Initial arsenic concentration present in a mine waste sample is the most significant predictor of the degree to which arsenic will be mobilized in either water or gastric fluid (over surface area, size fraction, and waste type).
Simulated gastric fluid releases on average an order of magnitude more arsenic from a given mine waste material than does water, largely thought to be due to the significant pH difference between the two media (1.5 vs. 5.5) which facilitates particle dissolution in the SGF.
Differences in As speciation between mine tailings and background samples suggest that weathering of crystalline As-bearing phases in tailings leads to sorption of dissolved arsenic to iron hydroxides in non-tailings background material.

## University of Missouri

Dr. Stan Casteel (University of Missouri) completed the swine dosing trials for a total of 18 materials selected for in-vivo testing. Testing was conducted in three phases (rounds 1, 2, and 3) over the course of the Study. The relative oral bioavailability of arsenic was assessed by comparing the absorption of arsenic from the soil samples ("test materials") to that of sodium arsenate. Groups of five 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 negative 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 arsenic 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 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:

| Test Material | Total As (mg/kg) | 90% Confidence Interval | | | |
|---|---|---|---|---|---|
| | | **RBA Day 6/7** | **RBA Day 9/10** | **RBA day 12/13** | **All Days** |
| EM01 | 302 | 26.8 (20.3-33.4) | 29.2 (24.1-34.3) | 15.0 (7.8-22.3) | 23.7 (10.9-36.5) |
| EM03 | 2541 | 17.0 (13.4-20.6) | 15.9 (13.0-18.8) | 12.9 (11.2-14.6) | 15.3 (11.7-18.8) |
| EM08 | 633 | 20.3 (18.4-22.2) | 19.5 (14.2-24.8) | 17.7 (12.3-23.2) | 19.2 (16.9-21.4) |
| EM18 | 10482 | 6.8 (5.8-7.7) | 4.4 (2.2-6.5) | 3.8 (1.3-6.2) | 4.0 (3.3-4.6) |
| EM19 | 370 | 13.8 (11.1-16.4) | 11.7 (9.5-13.9) | 9.8 (7.2-12.5) | 11.7 (8.3-15.2) |
| EM21 | 12041 | 23.5 (19.1-28.0) | 26.0 (22.3-29.8) | 19.6 (14.2-25.1) | 23.0 (17.6-28.5) |
| EM05 | 1906 | 15.3 (13.1-17.4) | 15.4 (12.6-18.2) | 15.3 (6.0-24.6) | 15.3 (15.22-15.5) |
| EM13 | 1237 | 13.7 (7.1-20.3) | 14.7 (12.5-16.9) | 9.1 (4.9-13.4) | 12.5 (5.1-19.9) |
| EM15 | 12095 | 19.8 (11.9-27.7) | 22.2 (17.2-27.2) | 17.0 (12.4-21.5) | 19.7 (13.1-26.2) |
| EM20 | 5647 | 22.5 (18.9-26.1) | 22.1 (24.4-19.8) | 23.5 (17.1-29.8) | 22.7 (21.1-24.3) |
| RG01 | 200 | 12.1 (8.3-15.9) | 13.5 (10.5-16.6) | 9.7 (2.3-17.0) | 11.8 (6.9-16.6) |
| RG03 | 610 | 12.8 (8.2-17.4) | 14.06 (11.3-16.8) | 10.3 (6.0-14.6) | 12.4 (7.6-17.2) |
| MC2 | 603 | 0.9 (-0.6-2.6) | 1.9 (0.7-3.2) | -0.3 (-2.4-1.6) | 1.3 (0.5-2.1) |
| MC3 | 641 | 10.8 (4.1-21.0) | 12.8 (6.9=21.1) | 5.3 (1.0-10.6) | 9.2 (6.-12.7) |
| CE1 | 753 | 36.7 (21.8-59.4) | 41.6 (27.2- 62.1) | 34.2 (23.1-49.3) | 37.6 (301.- 46.6) |
| WR33 | 6681 | 21.3 (11.7- 35.6) | 20.6 (11.1-34.6) | 8.2 (3.1- 14.8) | 14.2 (10.1- 19.2) |
| T81 | 205 | 6.5 (0.9-15.3) | 12.7 (6.4- 21.8) | 6.4 (1.6-12.7) | 8.2 (5.3- 11.7) |
| IM01 | 731 | 4.2 (1.6-7.3) | 6.7 (1.6- 13.4) | 6.7 (1.6-13.4) | 5.8 (3.8-8.0) |

All dose-response models were assessed with the regression function in Excel. Goodness of fit was considered acceptable if the p-value was less than 0.05.

Individual reports for each round of in vivo testing can be found in Attachment 3.

Department of Toxic Substances Control
Brownfields and Environmental Restoration Program

**U.S. EPA Brownfields Training, Research and Technical Assistance Grant**
**Arsenic Characterization/Bioavailability on Mine-Scarred Lands**

# FINAL BUDGET REPORT

**December 2015**

This is the final budget report for the Brownfields Training, Research and Technical Assistance Grant (Grant) awarded to the Department of Toxic Substances Control's (DTSC's) Brownfields and Environmental Restoration Program (Cleanup Program). The Grant award for Brownfields Research Cooperative Agreement (**TR - 83415101**), dated February 26, 2009, was originally for five years (10/01/2008 – 9/30/2013). DTSC was granted two separate one year, time-only extensions to 9/30/2015.

The total Approved Assistance Amount for the Grant was $900,000, of which DTSC received $850,000. Initial funding of $300,000 was provided with the Grant award followed by incremental funding increases of $150,000 in September 2010, $150,000 in July 2011, $100,000 in May 2012, and $150,000 in June 2013. The table below provides budget and expenditure details. Due to travel restrictions imposed by the Governor of California, travel costs during the budget period were less than anticipated and unused Travel funds were shifted to Personnel and Fringe Benefits in 2015. Unexpended Supply funds were also shifted to Personnel and Fringe Benefits in 2015. Total fund shifts represented less than 10% of the budget and were discussed with the U.S. EPA Grant Manager prior to the shifts being made.

| Table A Object Class Category | Total Approved Allowable Budget Period Cost | Fund Shifts Budget Period Cost | Total Expended Budget Period Cost |
|---|---|---|---|
| Personnel | $188,200 | + $12,455.79 | $125,385.96 |
| Fringe Benefits | $58,342 | | $83,611.83 |
| Travel | $8,500 | -$6,328.52 | $2,171.48 |
| Equipment | $0 | | $0 |
| Supplies | $7,000 | + $10,000<br>-$15,810.09 | $1,189.91 |
| Contractual | $637,958 | - $317.18 | $637,640.82 |
| Construction | | | $0 |
| Other | $0 | | $0 |
| **Totals** | **$900,000** | | **$850,000** |
| Total EPA Amount Awarded | $850,000 | | |
| Total Direct Charges Allowed | $900,000 | | |

All of the investigators and DTSC provided in-kind services to keep the project on track at various times over the course of the Grant. Namely: 80 and 475 staff hours from Chapman University and DTSC, respectively, $165,000 in-kind services and staff hours from the United States Geological Survey, and staff time and analytical services beyond the contracted scope of work from the Ohio State University and the University of Missouri that is too difficult to quantify.

For Federal Grants (U.S. EPA, DoD, DOE), DTSC will be delayed in its ability to provide expenditure information. The State of California switched (in tiers/phases) to a new Accounting System, Financial Information System of California (FI$Cal), on July 1, 2015. DTSC's Accounting Office is working with the State's FI$Cal staff to determine how to extract and provide the data that our Federal agencies require. DTSC staff working on the Grant will receive expenditure reports as soon as possible, but this will not be before the end of the calendar year. Because of this issue, Personnel and Fringe Benefit costs from July 1 through September 30, 2015 were calculated from the number of hours logged by each staff person and their effective hourly rate (adjusted to only include charges allowed by the Grant, i.e., no indirect cost/overhead) instead of relying on accounting reports.

ATTACHMENT 1

PUBLICATION LIST

# Arsenic Relative Bioavailability Study – Publication List

## Peer-reviewed publications

Alpers, C.N., Myers, P., Millsap, D., and Regnier, T.B., 2014, Arsenic associated with historical gold mining in the Sierra Nevada: Case study and field trip guide for Empire Mine State Historic Park, California. *In*: Bowell, R., Alpers, C.N., Nordstrom, D.K., Jamieson, H.E., and Majzlan, J. (eds), Arsenic – Environmental Geochemistry, Mineralogy, and Microbiology, Reviews in Mineralogy and Geochemistry v. 79, p. 553-587. http://www.minsocam.org/msa/RIM/index2.html

Basta, N.T., and Juhasz, A., 2014, Using in vivo bioavailability and/or in vitro gastrointestinal bioaccessibility testing to adjust human exposure to arsenic from soil ingestion. *In:* Bowell, R., Alpers, C.N., Nordstrom, D.K., Jamieson, H.E., and Majzlan, J. (eds), Arsenic – Environmental Geochemistry, Mineralogy, and Microbiology, Reviews in Mineralogy and Geochemistry v. 79, p. 451-472. http://www.minsocam.org/msa/RIM/index2.html

Bowell, R., Alpers, C.N., Nordstrom, D.K., Jamieson, H.E., and Majzlan, J., 2014, Arsenic -- Environmental Geochemistry, Mineralogy, and Microbiology, Reviews in Mineralogy and Geochemistry v. 79, 627 p. http://www.minsocam.org/msa/RIM/index2.html

Bowell, R., Alpers, C.N., Nordstrom, D.K., Jamieson, H.E., and Majzlan, J., 2014, The Environmental Geochemistry of Arsenic – An Overview, *In*: Bowell, R., Alpers, C.N., Nordstrom, D.K., Jamieson, H.E., and Majzlan, J. (eds.), Arsenic -- Environmental Geochemistry, Mineralogy, and Microbiology, Reviews in Mineralogy and Geochemistry v. 79, p. 1-16. http://www.minsocam.org/msa/RIM/index2.html

Foster, A.L, and Kim, C.S., 2014, Arsenic speciation in solids using X-ray absorption spectroscopy. *In:* Bowell, R., Alpers, C.N., Nordstrom, D.K., Jamieson, H.E., and Majzlan, J. (eds), Arsenic – Environmental Geochemistry, Mineralogy, and Microbiology, Reviews in Mineralogy and Geochemistry v. 79, p. 257-369.

Mitchell, V.L., 2014 Health risks associated with chronic exposures to arsenic in the environment. *In:* Bowell, R., Alpers, C.N., Nordstrom, D.K., Jamieson, H.E., and Majzlan, J. (eds), Arsenic – Environmental Geochemistry, Mineralogy, and Microbiology, Reviews in Mineralogy and Geochemistry v. 79, p. 435-449.

## Master's Thesis

Burlak, T., 2012, Geochemistry of iron- and arsenic-bearing minerals in soil and bedrock associated with gold-quartz vein mineralization at Empire Mine State Historic Park, Nevada County, California. M.Sc. thesis, Department of Geology, California State University, Sacramento, CA, 142 p. http://csus-dspace.calstate.edu/handle/10211.9/1885

**Abstracts and Presentations**

**2010**

Burlak, T., Alpers, C.N., Foster, A.L., Brown, A., Hammersley, L., and Petersen, E., 2010, Tracking the mineralogical fate of arsenic in weathered sulfides from the Empire Mine gold-quartz vein deposit using X-ray analytical techniques: Potential implications for arsenic bioavailability in mine waste. 2010 Fall Meeting, American Geophysical Union, December 17–20, San Francisco, CA. (POSTER, presented by Burlak) http://abstractsearch.agu.org/meetings/2010/FM/sections/V/sessions/V51C/abstracts/V51C-2220.html

Mitchell, V., Alpers, C., Basta, N., Berry, D., Christopher, J., Eberl, D., Fears, R., Foster, A., Kim, C.S., Myers, P., and Parsons, B., 2010, Identifying predictors for bioavailability of arsenic in arsenic in soil at mining sites. Society of Toxicology, 49th Annual Meeting, Salt Lake City, UT, March 7–11, 2010. *Toxicologist*, v. 114, p. 412. (POSTER, presented by Mitchell)

Brown, A., Foster, A., Alpers, C.N., Dale, J. G., Hansel, C., Lentini, C., Kim, C. S., Stegemeier, J.P., Factors Affecting Principal Component Analysis (PCA) of X-ray Absorption Fine Structure Spectral Datasets of Arsenic and Iron Compounds. Fall Annual Meeting of Geological Society of America, Oct. 2010 (POSTER, presented by Foster)

**2011**

Alpers, C.N., Burlak, T., Foster, A., Hammersley, L., and Petersen, E., 2011, Mineralogy and speciation of arsenic in weathered waste rock from the Empire mine low-sulfide gold-quartz vein deposit, California. Annual Meeting of the National Association of Abandoned Mine Land Programs, Squaw Valley, CA, October 10–12, 2011. (TALK, presented by Alpers)

Foster, A., and Alpers, C.N., 2011, Synchrotron x-ray studies of arsenic species in sediments from the Empire Mine, CA. Annual Meeting of the National Association of Abandoned Mine Land Programs, Squaw Valley, CA, October 10–12, 2011. (TALK, presented by Foster)

Mitchell, V., Alpers, C., Basta, N., Burlak, T., Casteel, S.W., Fears, R.L., Foster, A.L., Kim, C.S., Myers, P.A., and Petersen, E., 2011, The role of iron in the reduced bioavailability of arsenic in soil. Society of Toxicology, 50th Annual Meeting, March, 2011. *Toxicologist*, v. 120 (Supp 2), p. 415 (POSTER, presented by Mitchell)

Myers, P.A., Mitchell, V.L., Alpers, C.N., Basta, N.T., Casteel, S.W., Foster, A.L., and Kim, C.S., 2011, Methods and tools for the evaluation of bioavailability of arsenic at abandoned mine lands: the search for a more cost-effective approach to site clean-up. Annual Meeting of the National Association of Abandoned Mine Land Programs, Squaw Valley, CA, October 10–12, 2011. (TALK, presented by Myers and Mitchell)

**2012**

Alpers, C.N., Burlak, T.L., Foster, A.L., Basta, N.T., and Mitchell, V.L., 2012, Arsenic and old gold mines: mineralogy, speciation, and bioaccessibility. 2012 Goldschmidt Meeting, Montreal, Canada, June 24-29, 2012. (INVITED TALK, KEYNOTE ADDRESS, presented by Alpers) http://www.minersoc.org/files/Goldschmidt2012_Conference_Abstracts_A.pdf

Alpers, C.N., Mitchell, V.L., Basta, N.T., Casteel, S.W., Foster, A.L., Blum, A.E., Kim, C.S., Myers, P., Burlak, T.L., and Hammersley, L., 2012, Evaluating the bioavailability, bioaccessibility, mineralogy, and speciation of arsenic in mine waste and soils: Empire Mine low-sulfide gold-quartz vein deposit, Nevada

County, California. U.S. Environmental Protection Agency Hardrock Mining Conference, Denver, CO, April 3–5, 2012. (TALK, presented by Alpers) http://www.clu-in.org/download/issues/mining/Hard_Rock/ConferenceHandout/HRM_2012_Handout.pdf

Foster, A., 2012, Identification and quantification of arsenic species in gold mine wastes using synchrotron-based x-ray techniques. U.S. Environmental Protection Agency Hardrock Mining Conference, Denver, CO, April 3–5, 2012. (TALK, presented by Foster) http://www.clu-in.org/download/issues/mining/Hard_Rock/ConferenceHandout/HRM_2012_Handout.pdf

Mitchell, V.L., Alpers, C.N., Basta, N.T., Casteel, S.W., Foster, A.L., Kim, C.S., Naught, L., and Myers P.A., 2012, Alternative methods for the prediction of bioavailability of arsenic in mining soils. Society of Toxicology, 51st Annual Meeting, March, 2012. *Toxicologist*, v. 126, p. 321. (POSTER, presented by Mitchell)

Mitchell, V.L., Myers P.A., 2012, Alternative Methods for the Evaluation of Arsenic Bioavailability: Reclaiming Mine-Scarred Lands While Protecting Human Health. Reclaiming the Sierra, Green Solutions to Abandoned Mines Conference, Nevada City, CA, May 3-5, 2012. (TALK, presented by Mitchell) http://reclaimingthesierra.org/wp-content/uploads/2012/06/Mitchell-Myers-Arsenic-Study-RTS-2012.pdf

Mitchell, V.L., 2012, Alternative Methods for the Evaluation of Bioavailability of Arsenic in Mining Soils, Risk Assessment Specialty Section, Society of Toxicology (WEBINAR, presented by Mitchell) http://www.toxicology.org/ISOT/SS/RiskAssess/RASS_Webinar_10_10_2012.pdf

Whitacre, Shane D., Nicholas Basta, Valerie Mitchell and Perry Myers, 2012. Bioavailability Measures for Arsenic in Gold Mine Tailings. Presentation 412-1, ASA, CSSA, and Soil Science Society International Annual Meeting, Cincinnati, OH. Oct. 21 to 24, 2012.

2013

Whitacre, S.D., N.T. Basta, V.L. Mitchell, and P. Myers. 2013. Bioavailability Measures for Arsenic in Gold Mine Tailings Using Agricultural Soil Tests to Estimate Total and Bioaccessible Pb in Urban Soils. Joint MERA/ICOBTE Sponsored Symposium: Trace Element Bioavailability for Human and Ecological Risk Assessment: Concepts and Recent Advances. Organizers: N. Basta, E. Van Genderen, and C. Schlekat. 12th International Conference for Trace Element Biogeochemistry (ICOBTE), Athens, GA, USA. June 16-20, 2013.

**2014**

Basta, N.T., Whitacre, S., Meyers, P., Mitchell, V.L., Alpers, C.N., Foster, A.L., Casteel, S.W., and Kim, C.S., 2014, Using in vitro gastrointestinal and sequential extraction methods to characterize site-specific arsenic bioavailability. Goldschmidt 2014, Sacramento, CA, June 8–13, 2014. (TALK, presented by Basta) http://goldschmidt.info/2014/abstracts/abstractView?abstractId=2172

Buckendorf, L., and Kim, C.S., 2014, Relationships between particle size, arsenic concentration, surface area, and bioaccessibility of mine tailings from the Empire Mine, CA. Goldschmidt 2014, Sacramento, CA, June 8–13, 2014. (POSTER, presented by Buckendorf) http://goldschmidt.info/2014/abstracts/abstractView?abstractId=3209

Foster, A.L., Alpers, C.N., Burlak, T., Blum, A.E., Petersen, E.U., Basta, N.T. , Whitacre, S., Casteel, S.W., Kim, C.S., and Brown, A.L., 2014, Arsenic chemistry, mineralogy, speciation, and bioavailability/bioaccessibilty in soils and mine waste from the Empire Mine, CA, USA. Goldschmidt

2014, Sacramento, CA, June 8–13, 2014. (TALK, presented by Foster)
http://goldschmidt.info/2014/abstracts/abstractView?abstractId=3341

Foster, A.L., and Kim, C.S., 2014, The environmental legacy of California's gold rush: Arsenic and mercury contamination from historic mining. Goldschmidt 2014, Sacramento, CA, June 8–13, 2014. (PLENARY TALK, presented by Foster and Kim, introduced by Alpers)
http://goldschmidt.info/2014/abstracts/abstractView?abstractId=4857
https://www.youtube.com/watch?v=ZvsmiiYL-OU&feature=youtu.be

Kim, C.S, Anthony, T.L. Buckendorf, L., O'Connor, K.P., and Rytuba, J.J.., 2014, Transport, bioaccessibility and risk assessment of fine-grained arsenic-bearing mine tailings. Goldschmidt 2014, Sacramento, CA, June 8–13, 2014. (TALK, presented by Kim)
http://goldschmidt.info/2014/abstracts/abstractView?abstractId=4645

Stevens, B., Basta, N., Whitacre, S., Naber, S., Scheckel, K., Casteel, S., Bradham, K., and Thomas, D., 2014, Evaluation of bioaccessibility methods to predict relative bioavailability of arsenic in contaminated soils. Goldschmidt 2014, Sacramento, CA, June 8–13, 2014. (POSTER, presented by Stevens)
http://goldschmidt.info/2014/abstracts/abstractView?abstractId=2161

Whitacre, S., Basta, N., Casteel, S., Foster, A., Myers, P., and Mitchell, V., 2014, Bioavailability measures for arsenic in California gold mine tailings. Goldschmidt 2014, Sacramento, CA, June 8–13, 2014. (POSTER, presented by Whitacre)
http://goldschmidt.info/2014/abstracts/abstractView?abstractId=2081

Alpers, C.N., 2014, Arsenic Associated with Historical Gold Mining in the Sierra Nevada Foothills. Short Course on "Environmental Geochemistry, Mineralogy and Microbiology of Arsenic," Mineralogical Society of America and the Geochemical Society, Nevada City, CA, June 2014 (ORAL, presented by Alpers)

**2015**

Alpers, C.N., 2015, Arsenic and mercury contamination from historical gold mining in the Sierra Nevada, California. 27th International Applied Geochemistry Symposium (IAGS) hosted by the Association of Applied Geochemists (AAG) in Tucson, Arizona, April 20-24, 2015 (INVITED KEYNOTE TALK, presented by Alpers)

Foster, A. L., 2015, Spectroscopic Methods for Arsenic Characterization. Reclaiming the Sierra Conference, Sacramento, CA, April 2015 (ORAL, presented by Foster)

Mitchell, V.M., Whitacre, S., Casteel S.W., Myers, P.A., Basta, N.T., 2015 (planned) New *In Vitro* Gastrointestinal Model Accurately Predicts Arsenic Bioavailability in Soils, Society of Toxicology, 54$^{th}$ Annual Meeting, March, 2015. (POSTER, presented by Mitchell)

Mitchell, VL, Bioavailability of Arsenic in California Mining Soils: Geochemical Influences and the Development of a Predictive *in vitro* Method, Presentation at the Interstate Technology & Regulatory Council 2015 Spring Meeting, April 2015. (Invited presentation to the Bioavailability in Contaminated Soils Team).

Hanley, VM, Bioavailability of Arsenic in California Mining Soils: Development of a Predictive *in vitro* Method, Presentation to the Sacramento Professional Environmental Marketers Association, November 2015. (Invited Speaker)

ATTACHMENT 2

OSU FINAL REPORT

**OHIO STATE UNIVERSITY**
**FINAL REPORT**

**For the Department of Toxic Substances Control**
**Brownfields and Environmental Restoration Program**
**EPA Brownfields Training, Research and Technical Assistance Grant**
**Arsenic Characterization/Bioavailability on Mine-Scarred Lands**

## INTRODUCTION

Exposure risk associated with soils contaminated with arsenic (As) is assessed by human health risk assessment (HHRA). A critical component of HHRA is exposure assessment by various exposure pathways. In soils, often the most important pathway for As, the risk driver, associated with human exposure is incidental soil ingestion. However, use of total soil As often overestimates exposure because physiochemical properties of the soil matrix can sequester As and reduces its transmission through exposure pathways. A more accurate and site-specific HHRA accounts for bioavailability of As in a soil matrix as part of the exposure assessment.

Appropriate animal models, similar to human gastrointestinal physiology, are often used to determine bioavailability of As in contaminated soil. The most commonly used animal model for determining bioavailable As is the juvenile swine model. (Rodriguez and Basta 1999; Basta, Foster et al. 2007; Rees, Sansom et al. 2009) In addition to juvenile swine, monkeys (Freeman, Schoof et al. 1995; Roberts, Weimar et al. 2002; Roberts, Munson et al. 2007) , adult mouse (Bradham, Scheckel et al. 2011), and rabbit models (Freeman, Johnson et al. 1993) have also been used. Several disadvantages in conducting animal studies include expense, specialized facilities and personnel requirements, and time required to measure contaminant bioavailability.

In order to overcome some of the difficulties and expenses associated with animal dosing trials used to assess bioavailability of contaminants in soil, extensive research efforts have been directed toward development of in vitro gastrointestinal methods, that simulate the gastrointestinal environment, to predict bioavailable As. (Rodriguez and Basta 1999; Rodriguez, Basta et al. 2003; Juhasz, Smith et al. 2006; Basta, Foster et al. 2007; Juhasz, Smith et al. 2007; Juhasz, Smith et al. 2008; Nagar, Sarkar et al. 2009; Bradham, Scheckel et al. 2011, Brattin, Drexler et al. 2013) While there are multiple efforts to advance in vitro methodology, only two in vitro conditions are commonly employed in the United States; a 0.4 M glycine buffered

gastric solution at pH 1.5 (Juhasz, Smith et al. 2006; Juhasz, Smith et al. 2007; Juhasz, Smith et al. 2008; Bradham, Scheckel et al. 2011, Brattin, Drexler et al. 2013), and an unbuffered gastric solution solution at pH 1.8 followed by an unbuffered intestinal solution at pH 6.5 (Basta, Foster et al. 2007; Nagar, Sarkar et al. 2009).

In vitro bioaccessible (IVBA) As is defined as the amount or percent (%) of As potentially dissolved in the GI tract and available for absorption across the epithelium into systemic circulation. An essential requisite of acceptable As in vitro methods is that IVBA be strongly correlated with relative bioavailable (RBA) As determined from animal doing trials. Often, RBA As vs. IVBA As regression equations are used to predict RBA from IVBA. Several studies have reported correlation between IVBA As and RBA As measured from juvenile swine dosing trials. These include the OSU-IVG method of Rodriguez et al. (1999) and Basta et al. (2007), as well as the SBRC method of Juhasz et al. (2007). Basta et al. (2007) reported results from the OSU-IVG method with and without dosing vehicle for a subset of 9 Cu mining soils used in Rodriguez et al. (1999). In this study, the following regression equations (1 and 2) for gastric extractable (GE) and intestinal extractable (IE) used to predict RBA As were determined from the OSU-IVG procedure without dosing vehicle (Basta et al. 2007).

%RBA As = 0.883 (%OSU-IVG GE) + 9.6 (1)

%RBA As = 0.937 (%OSU-IVG IE) + 12.6 (2)

Juhasz et al. (2009) reported results from the Solubility Bioaccessibility Research Consortium assay (SBRC) method (0.4M glycine) and OSU-IVG for 12 soils from former railway corridors, dip sites, mine sites and naturally elevated gossan soils. This study resulted in the following regression equations (3, 4, and 5) to predict RBA As.

%RBA As = 0.992 (%SBRC GE) +1.66 (3)

%RBA As = 0.853 (%OSU-IVG GE) +14.3 (4)

%RBA As = 1.105(%OSU-IVG IE) +13.97 (5)

Brattin al. (2013) reported in vitro results using the same extraction conditions as the SBRC method for 20 soils form US EPA superfund sites as well as As spiked soils. This study resulted in the following regression equation (6) to predict RBA As in swine.

$$\%RBA\ As = 0.62\ (\%SBRC\ GE) + 19.68 \qquad (6)$$

However, it is not known how well the OSU-IVG and SBRC methods will measure and/or predict RBA in soils contaminated with As from sources outside those used in developing the regression equation. The objectives of the study were to:

(1) Evaluate the OSU-IVG and SBRC methods for use on As contaminated soils from an abandoned gold mine in California (CA)

(2) Optimize existing in vitro method(s) to measure and/or predict relative bioavailable As in test soils.

(3) Validate and provide recommendations for use of in vitro to make As relative bioavailability adjustments for CA soils.

## MATERIALS AND METHODS

### Study Soils

Initially, 23 soils were collected from Empire Mine State Historic Park in Grass Valley California for characterization and selection of a subset of 6 soils for swine dosing. Subsequently, 6 additional soils were selected for swine dosing. Soil characterization and objectives 1-3, were conducted on only the Empire Mine soils. Characterization for pH was conducted in a in 1:1 soil:deionized water (Thomas 1996) , texture by pipette method(Kilmer and Alexander 1949), acid ammonium oxalate (Ox) extractable As, Fe, and Al (McKeague and Day 1966), and total As by USEPA method 3051a(USEPA 2007). In addition, soils from Empire mine were fractionated according to the sequential extraction procedure (SEP) of Wenzel et al. (2001) with slight modification. The procedure fractionated As into Five fractions: (F1) non-specifically sorbed; (F2) specifically sorbed; (F3) amorphous and poorly-crystalline oxides of Fe and Al; (F4) well-crystallized oxides of Fe and Al; and (F5) residual As phases. The extraction solution, temperature, and extraction time were: (F1) 0.05M $(NH4)2SO4$, 20ºC/4 h; (F2) 0.05M

NH4H2PO4, 20ºC/16 h; (F3) 0.2M oxalate extraction, pH 3.0, 20ºC/4 h; (F4) 0.2M oxalate + ascorbic acid extraction, pH 3.0, 96ºC/0.5 h: (F5) 3051a As - ∑F1-F4.

Upon completion of objective 2, an additional collection and dosing of 6 soils from sites outside of Empire Mine. Total As in these soils was determined by USEPA method 3051a (USEPA 2007). Bioaccessible As was determined \by three methods (OSU-IVG, SBRC, and modified OSU-IVG) as described below. Bioaccessible As (mg/kg) was converted to a percentage by:

IVBA As (%) = [bioaccessible As (mg/kg)] / [3051a As (mg/kg)] * 100 (7)

**Determination of Bioaccessible As by the OSU-IVG Method**

This method is a 2-step sequential extraction. The first extraction simulates gastric conditions followed by the second extraction which simulates intestinal conditions. In the first step, gastric solution, 150 mL of 0.10 M NaCl and 1% porcine pepsin was heated in an open extraction vessel, in a 37º C hot water bath. When the solution reached 37º C, soil (1 g, < 250 µm) was added. The sample is thoroughly mixed with the solution, using a paddle stirrer to maintain a homogenous suspension, and the pH is adjusted drop wise to 1.8 using 6M trace metal grade HCl. The solution pH is continuously monitored and adjusted 1.8 ± 0.1. After 1 h, 10 mL of gastric solution is removed for analysis. The extract is immediately centrifuged (11,160 g for 15 min) and then filtered (0.45 µm). The pH of the remaining solution is adjusted to 6.1 ± 0.1 using drop wise additions of a saturated $Na_2CO_3$ solution followed by the addition of 0.563 g of porcine bile extract and 0.563 g of porcine pancreatin. After 2 h of mixing, 10 mL of intestinal solution is collected for analysis. The extract is immediately centrifuged (11,160 g for 15 min) and then filtered (0.45 µm). As extracted during the gastric phase is expressed as gastric extractable As (OSU-IVG IVBA GE As) and As extracted during the intestinal phase is expressed as intestinal extractable As (OSU-IVG IE As).

**Determination of Bioaccessible As by the SBRC Method**

This method is a single step extraction in which 100 mL of gastric solution (0.40 M glycine, preheated to 37ºC) and 1.0 g soil (<250 µm) was added to a 175 mL HDPE bottle and placed into a rotator shaker located in a 37ºC incubator. Soil samples were rotated at 30±2 rpm for 1 h, and solution pH was checked and adjusted to 1.5 ± 0.1 using drop wise 50% NaOH and/or 6M trace

metal HCl solution. After 1 h, 10 mL aliquot of suspension was collected with a syringe and filtered (0.45 µm). Contaminant extracted during the gastric phase is expressed as SBRC IVBA gastric extractable (SBRC IVBA GE).

### Determination of Bioaccessible As by a Modified OSU-IVG Method

Details of this method will be published in 2016. Briefly, this method is a single step extraction in which 150mL of gastric solution and 1.0 g soil (< 250 µm) was added to a 175 mL HDPE bottle and placed into a rotator shaker located in a 37ºC incubator. Soil samples were rotated at 30±2 rpm for 2 h. After 2 h, a minimum of 10 mL of gastric solution is removed and filtered (0.45 µm) for analysis. Contaminant extracted during the gastric phase is expressed as Mod IVG IVBA gastric extractable (Mod IVG IVBA GE).

## RESULTS

### Characterization

Characterization of Empire Mine soils is presented in Table 1. Total (3051a) As ranged from 15.3 to 12,095 mg/kg with a mean of 2,980 mg/kg. As extracted in the SEP ranged from (F1) 0 to 21 mg/kg; (F2) 0 to 603mg/kg; (F3) 9.67 to 7,270 mg/kg; (F4) 8.62 to 2,623 mg/kg; and (F5) 0 to 8,658 mg/kg. Oxalate extractable As, Fe and Al ranged from 3.45 to 9,832 mg/kg; 1.15 to 40.5 g/kg; and 0.156 to 131 g/kg respectively. Soil pH ranged from 4.15 to 8.30 with a mean of 5.89. Further SEP As as a percentage of total As is presented in Figure 1. The results of the SEP indicate that a majority of As (>95%) is in the soils from Empire Mine is associated with oxides of Fe and Al (F3-4) and residual phases (F5).

**Table 1. Characterization of Empire Mine sample for 3051a, SEP As, acid ammonium oxalate extractable (As, Fe, Al), texture, and pH.**

| | Sequential Extraction Procedure Fraction | | | | | | Reactive oxides | | | Texture | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3051a As | F1 As | F2 As | F3 As | F4 As | F5 As | Ox As | Ox Fe | Ox Al | Sand | Silt | Clay | |
| Soil | ---------- mg/kg ---------- | | | | | | | g/kg | | -------- % ------- | | | pH |
| EM1 | 302 | 0.53 | 18.3 | 101 | 64.1 | 118 | 164 | 6.64 | 2.40 | 61 | 21 | 19 | 7.81 |
| EM2 | 373 | 0.15 | 19.4 | 113 | 77.5 | 163 | 190 | 7.41 | 2.52 | 40 | 27 | 34 | 7.65 |
| EM3 | 2541 | 0.27 | 86.8 | 1032 | 602 | 819 | 1891 | 31.6 | 4.90 | 55 | 23 | 22 | 4.96 |
| EM4 | 2878 | 0.00 | 103 | 1239 | 458 | 1077 | 1504 | 25.0 | 3.17 | 67 | 17 | 16 | 4.15 |
| EM5 | 1906 | 0.41 | 68.6 | 691 | 531 | 615 | 951 | 17.3 | 2.32 | 64 | 21 | 15 | 4.64 |
| EM6 | 1682 | 0.17 | 73.8 | 655 | 400 | 553 | 896 | 16.5 | 2.00 | 67 | 18 | 14 | 5.49 |
| EM7 | 1687 | 0.00 | 74.9 | 645 | 406 | 561 | 872 | 16.3 | 2.01 | 68 | 20 | 13 | 5.53 |
| EM8 | 633 | 0.00 | 10.6 | 194 | 249 | 179 | 253 | 18.3 | 13.1 | 34 | 48 | 18 | 6.53 |
| EM9 | 15.3 | 0.11 | 0.910 | 15.8 | 0.000 | 0.000 | 12.4 | 1.55 | 0.156 | 97 | 1.6 | 1.3 | 8.30 |
| EM10 | 1617 | 0.51 | 41.6 | 530 | 530 | 515 | 726 | 6.23 | 2.07 | 51 | 36 | 14 | 4.94 |
| EM11 | 23.0 | 0.00 | 0.214 | 11.5 | 12.1 | 0.000 | 3.45 | 2.69 | 6.91 | 22 | 46 | 32 | 5.45 |
| EM12 | 22.0 | 0.00 | 0.000 | 9.67 | 8.62 | 3.73 | 4.33 | 1.64 | 2.92 | 7.6 | 36 | 56 | 5.27 |
| EM13 | 1237 | 1.3 | 23.6 | 74.6 | 542 | 595 | 142 | 1.15 | 1.71 | 42 | 34 | 24 | 4.68 |
| EM14 | 66.0 | 0.00 | 0.000 | 10.7 | 19.5 | 35.7 | 6.20 | 2.71 | 6.83 | 18 | 45 | 37 | 5.96 |
| EM15 | 12095 | 13 | 265 | 7270 | 2579 | 1968 | 9832 | 36.3 | 1.49 | 65 | 23 | 13 | 6.05 |
| EM16 | 8514 | 5.7 | 271 | 4305 | 1195 | 2738 | 6657 | 40.5 | 1.65 | 70 | 18 | 12 | 5.86 |
| EM17 | 9431 | 5.7 | 131 | 3836 | 2623 | 2836 | 6377 | 30.0 | 1.13 | 69 | 20 | 11 | 4.70 |
| EM18 | 10482 | 3.3 | 114 | 1467 | 240 | 8658 | 2066 | 31.6 | 0.734 | 72 | 16 | 12 | 7.63 |
| EM19 | 370 | 0.37 | 5.02 | 27.7 | 60.0 | 277 | 35.8 | 2.19 | 3.90 | 17 | 36 | 47 | 5.88 |
| EM20 | 5647 | 8.3 | 230 | 2504 | 978 | 1927 | 4154 | 25.1 | 5.27 | 41 | 34 | 25 | 6.62 |
| EM21 | 12041 | 21 | 603 | 5592 | 1341 | 4483 | 9038 | 40.1 | 3.35 | 46 | 31 | 23 | 7.29 |
| RG1 | 203 | 0.00 | 3.52 | 17.2 | 47.4 | 135 | 22.1 | 2.11 | 3.22 | 14 | 40 | 47 | 5.30 |
| RG2 | 88.0 | 0.39 | 1.50 | 18.4 | 21.9 | 45.8 | 12.0 | 1.61 | 3.59 | 15 | 31 | 54 | 4.99 |
| RG3 | 610 | 0.00 | 15.7 | 79.5 | 155 | 360 | 123 | 4.36 | 3.10 | 56 | 21 | 23 | 6.02 |
| RG4 | 28.3 | 0.027 | 0.304 | 12.2 | 12.5 | 3.32 | 7.68 | 3.30 | 7.89 | 19 | 48 | 33 | 5.43 |
| Min | 15.3 | 0.00 | 0.000 | 9.67 | 8.62 | 0.000 | 3.45 | 1.15 | 0.156 | 7.6 | 1.6 | 1.3 | 4.15 |
| Mean | 2980 | 2.5 | 86.5 | 1218 | 527 | 1147 | 1838 | 14.9 | 3.53 | 47 | 28 | 25 | 5.89 |
| Max | 12095 | 21 | 603 | 7270 | 2623 | 8658 | 9832 | 40.5 | 13.1 | 97 | 48 | 56 | 8.30 |

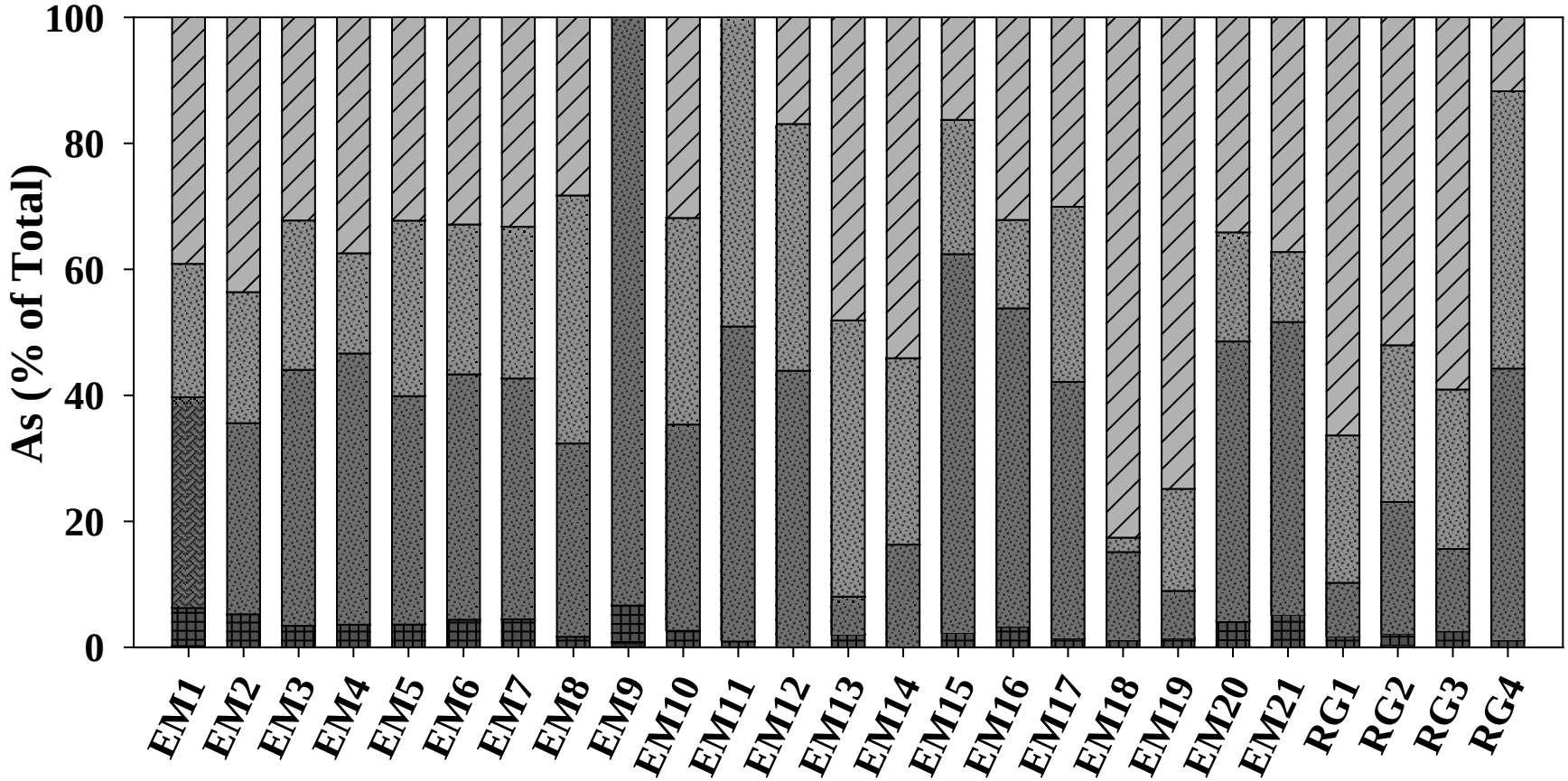



**Figure 1. Percent of total As for each soil in (F1 ) non-specifically sorbed; (F2 ) specifically sorbed; (F3 ) amorphous and poorly-crystalline oxides of Fe and Al; (F4 ) well-crystallized oxides of Fe and Al; and (F5 ) residual As phases.**

**Objective 1: Evaluate the OSU-IVG and SBRC methods for use on As contaminated soils from an abandoned gold mine in CA**

***In vitro and In vivo results***

The results for As extracted by the OSU-IVG and SBRC methods as well as swine RBA are presented in table 2. The two in vitro methods extracted similar amounts of As. In addition, both methods extracted a small percentage of total As (<1 to 14.4%). However, the swine RBA As ranged from 4.00 to 23.7%, two to five times the amount of As indicated by in vitro.

**Table 2. In vitro and Swine RBA results for Empire Mine soils.**

| | OSU- IVG GE | OSU IVG IE | SBRC GE | RBA |
|---|---|---|---|---|
| Soil | ---------- | As (%) | ---------- | |
| EM1 | 9.27 | 10.7 | 4.74 | 23.7 |
| EM3 | 2.97 | 3.14 | 1.29 | 15.3 |
| EM5 | 3.66 | 4.02 | 1.11 | 15.3 |
| EM8 | 2.82 | 3.42 | 1.59 | 19.2 |
| EM13 | 2.22 | 2.60 | 1.10 | 12.5 |
| EM15 | 3.50 | 3.96 | 4.52 | 19.7 |
| EM18 | 1.51 | 1.27 | 2.03 | 4.00 |
| EM19 | 1.77 | 2.24 | 0.361 | 11.7 |
| EM20 | 8.05 | 7.66 | 10.8 | 22.7 |
| EM21 | 7.24 | 7.35 | 14.4 | 23.0 |
| RG1 | 1.92 | 2.97 | 0.987 | 11.8 |
| RG3 | 3.05 | 3.05 | 1.15 | 12.4 |
| min | 1.51 | 1.27 | 0.36 | 4.00 |
| mean | 4.00 | 4.36 | 3.67 | 15.9 |
| max | 9.27 | 10.7 | 14.4 | 23.7 |

***In vitro in vivo correlation***

While both in vitro methods drastically underestimate RBA As, in vitro in vivo correlation (IVIVC) analysis was conducted to determine if a predictive relationship could be established with the Empire Mine soils (Figure 2). The plot demonstrates that the desired linear model does not fit this data set. This is due to large in vitro underestimation (approximately a factor of 5) of RBA As in low (<10%) bioavailability soils, but a smaller underestimation (approximately a factor of two) in higher (10 to 24%) bioavailability soils. As a result, the plot exhibits a nonlinear (plateau) with a steep slope at low RBA and decreases as RBA increases.

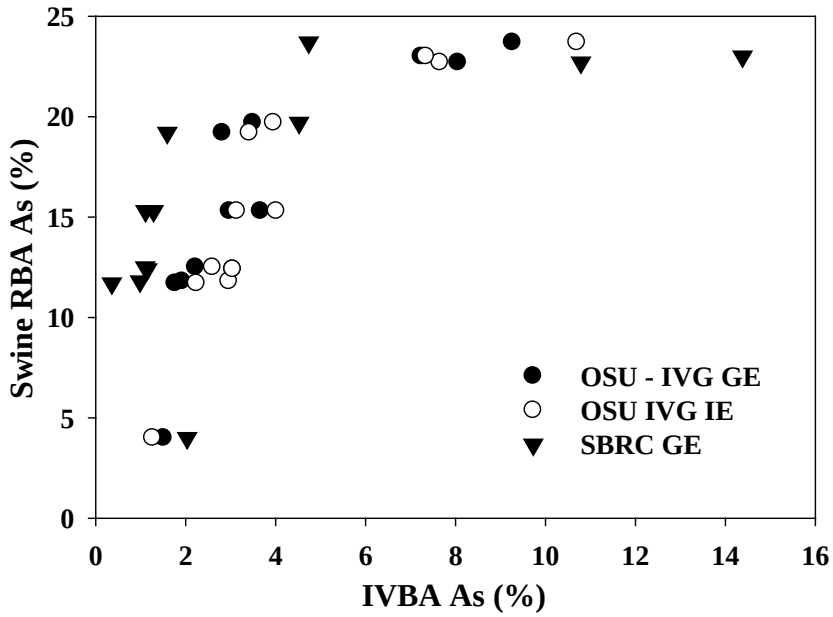



**Figure 2. IVIVC of Empire mine soils using OSU-IVG and SBRC in vitro methods.**

***Prediction from published regression equations***

While both in vitro methods drastically underestimate and failed to exhibit a linear relationship with RBA, another alternative to evaluating the application of the OSU-IVG and SBRC methods on Empire Mine soils is to use previously published regression equations (1-6) and determine if predicted RBA falls within the 90% confidence interval (CI) for RBA. The results of predictive regression equations 1-6 for the twelve Empire Mine soils are presented in Table 3. The SBRC method failed to predict (equation 3) within the RBA 90% CI for all but one soil. The SBRC prediction failed due to drastic underestimation of RBA. Interestingly, the same method parameters of the SBRC produced a drastically different regression equation (6) using a different set of study soils (Brattin et al. 2013). The y-intercept of 19.68 produced RBA predictions ranging from just 19.9% to 28.6%. This resulted in predictions within the RBA 90% CI for only two soils. The OSU-IVG failed to predict within the RBA 90% CI for; five soils using equation 1 (GE), three soils using equation 2 (IE), three soils using equation 4 (GE), and two soils using equation 5 (IE). Due to the inability of previously established regression equations to predict RBA and the large discrepancy in regression equations (3 vs. 6) for the SBRC method, this evaluation also failed to demonstrate the utility of applying in vitro gold mining sites in CA.

**Table 3. Swine RBA predictions for Empire Mine soils using published regression equations compared with measured RBA.**

| | OSU-IVG As | | | | SBRC As | | |
|---|---|---|---|---|---|---|---|
| Soil | % GE eq. 1 | % IE eq. 2 | % GE eq. 4 | % IE eq. 5 | % GE eq. 3 | % GE eq. 6 | RBA As 90% CI |
| EM1 | 17.8 | 22.6 | 22.2 | 25.8 | 6.4[a] | 22.6 | 10.9 - 36.5 |
| EM3 | 12.2 | 15.5 | 16.8 | 17.4 | 2.9[a] | 20.5[b] | 11.7 - 18.8 |
| EM5 | 12.8 | 16.4 | 17.4 | 18.4 | 2.8[a] | 20.4[b] | 15.22 - 15.5 |
| EM8 | 12.1[a] | 15.8[a] | 16.7[a] | 17.7 | 3.2[a] | 20.7[b] | 16.9 - 21.4 |
| EM13 | 11.6 | 15 | 16.2 | 16.8 | 2.8[a] | 20.4[b] | 5.1 - 19.9 |
| EM15 | 12.7[a] | 16.3 | 17.3 | 18.3 | 6.1[a] | 22.5 | 13.1 - 26.2 |
| EM18 | 10.9[b] | 13.8[b] | 15.6[b] | 15.4[b] | 3.7 | 20.9[b] | 3.3 - 4.6 |
| EM19 | 11.2 | 14.7 | 15.8[b] | 16.4[b] | 2.0[a] | 19.9[b] | 8.3 - 15.2 |
| EM20 | 16.7[a] | 19.8[a] | 21.2 | 22.4 | 12.4[a] | 26.4[b] | 21.1 - 24.3 |
| EM21 | 16.0[a] | 19.5 | 20.5 | 22.1 | 15.9[a] | 28.6[b] | 17.6 - 28.5 |
| RG1 | 11.3 | 15.4 | 15.9 | 17.2 | 2.6[a] | 20.3[b] | 6.9 - 16.6 |
| RG3 | 12.3 | 15.5 | 16.9 | 17.3 | 2.8[a] | 20.4[b] | 7.6 - 17.2 |

[a]Under-prediction of RBA (below lower 90% CI)
[b]Over-prediction of RBA (above upper 90% CI)

A high y-intercept in the in vitro in IVIVC is due to IVBA As results significantly less than RBA As as IVBA approaches zero in the data set used to generate the predictive equations (Juhasz et al. 2009, Brattin et al. 2013). Criteria for an acceptable IVIVC has been suggested and includes; an $r^2 > 0.6$, and a slope between 0.8 and 1.2 (Denys et al. 2012). Until recently, no criterion has been formally suggested for y-intercept. However, (Juhasz et al. 2014) suggested a y-intercept that does not deviate significantly from zero is desirable as it would eliminate the possibility of RBA predictions greater than 10% when in vitro results are close to zero.

**Objective 2: Optimize existing in vitro method(s) to measure and/or predict bioavailable As in test soils**

Details of the optimization of the modified OSU-IVG will be published in 2016.

**Objective 3: Validate and provide recommendations for use of modified OSU-IVG to make As bioavailability adjustments for CA soils**

The validation of the modified OSU-IVG was a multistep process. First the potential of the modified OSU-IVG to extract bioaccessible As and predict RBA As in Empire Mine soils was evaluated. Second, IVBA and swine RBA data from Empire Mine was merged with data from an existing Strategic Environmental Research and Development Program (SERDP, Department of Defense) study: *Mechanisms and Permanence of Sequestered Pb and As in Soils: Impact on Human Bioavailability* (Project ER-1742). In addition, six soils were collected under this study from sites outside of Empire Mine for IVBA and RBA determination. Finally, the modified OSU-IVG was tested for reproducibility with a round robin between The Ohio State University (Columbus, OH) and Prima Environmental (El Dorado Hills, CA)

***Evaluation of modified OSU-IVG to extract bioaccessible As and predict RBA As***

The Empire Mine IVBA As results for the modified OSU-IVG and swine RBA are presented in Figure 3. The results demonstrate that the large under extraction of As by the OSU-IVG and SBRC methods has been corrected with the parameters of the modified OSU-IVG method. However, the modified OSU-IVG extracted more than RBA As in some soils (EM15, EM18, M20, and EM21), thereby negating potential bioavailability adjustments as IVBA As approaches the 60% bioavailability default for site assessment (USEPA, 2012). Of note is that these four soils contain the highest As contents of all the study soils (5,647 – 12,095 mg/kg). As a result, the modified in vitro may not be suitable for accurate estimation of RBA As in soils with high As content. However, the modified OSU-IVG closely brackets RBA As in Empire Mine soils with low to moderate As content.

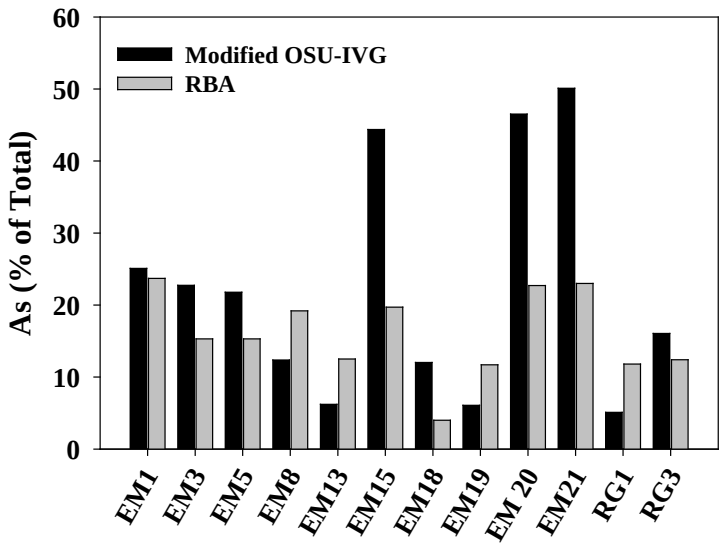



**Figure 3. Results of the Modified OSU-IVG compared to swine RBA As in Empire Mine soils.**

***Merged DTSC and SERDP datasets***

The modified OSU-IVG results suggest that accurate extraction and correlation with RBA As may be possible for low to moderately As contaminated soils, but overestimation of RBA is likely in high As soils. As a result, a larger dataset with soils containing low to moderate concentration of As for IVIVC is desirable. This was done by combining the DTSC and SERDP datasets for soils containing less than 1,500 mg As/kg and the addition of data from six soils collected outside of Empire Mine (Table 4)

**Table 4. Total (3051a), modified OSU-IVG IVBA, and RBA As for combined DTSC and SERDP datasets.**

| Source | ID | Project | 3051a As mg/kg | Modified OSU-IVG ---------- % | RBA ---------- |
|---|---|---|---|---|---|
| Gold Mining | EM1 | DTSC | 302 | 25.1 | 23.7 |
| Gold Mining | EM8 | DTSC | 633 | 12.4 | 19.2 |
| Gold Mining | EM19 | DTSC | 370 | 6.07 | 11.7 |
| Gold Mining | EM13 | DTSC | 1,237 | 6.22 | 12.5 |
| Gold Mining | RG3 | DTSC | 610 | 16.1 | 12.4 |
| Gold Mining | RG1 | DTSC | 203 | 5.10 | 11.8 |
| Silver Mining | T81 | DTSC | 205 | 21.2 | 15.8 |
| Eastern side of the Sierra Nevada | MC2 | DTSC | 603 | 2.86 | 1.00 |
| Eastern side of the Sierra Nevada | MC3 | DTSC | 641 | 11.0 | 15.6 |
| Gold Mining (secondary) | IM01 | DTSC | 731 | 3.95 | 9.28 |
| Gold Mining | CE1 | DTSC | 753 | 46.8 | 40.2 |
| Gold Mining | WR33 | DTSC | 6,681 | 19.9 | 20.4 |
| Unknown Mining | SE8 | SERDP | 162 | 59.5 | 54.9 |
| PbAsO4 orchard pesticide | SE18 | SERDP | 283 | 39.0 | 31.0 |
| PbAsO4 orchard pesticide | SE19 | SERDP | 353 | 40.7 | 41.0 |
| PbAsO4 orchard pesticide | SE21 | SERDP | 375 | 50.1 | 53.0 |
| PbAsO4 orchard pesticide | SE20 | SERDP | 391 | 41.4 | 49.0 |
| PbAsO4 orchard pesticide | SE7 | SERDP | 332 | 67.3 | 52.3 |
| Unknown tailings | SE9 | SERDP | 521 | 20.5 | 14.0 |
| Gold Mining (Australia) | SE6 | SERDP | 839 | 45.8 | 41.7 |
| Pb mining slag | SE12 | SERDP | 1,236 | 48.0 | 39.7 |
| Iron King Mine (Pb, Au, Ag, Zn Cu) | SE11 | SERDP | 249 | 73.6 | 60.0 |

The combined dataset resulted in the IVIVC presented in Figure 4. The results of the IVIVC demonstrate that the modified OSU-IVG is highly predictive of RBA As, meeting the criteria of; an $r^2 > 0.6$, a slope between 0.8 and 1.2 (Denys, Caboche et al. 2012; Wragg et al., 2011) and a y-intercept that does not deviate significantly from zero ((Juhasz et al. 2014). In addition, this regression equation includes soils with widely varying As sources, indicating that the modified OSU-IVG may be applicable to both goldmining and non-goldmining sites.

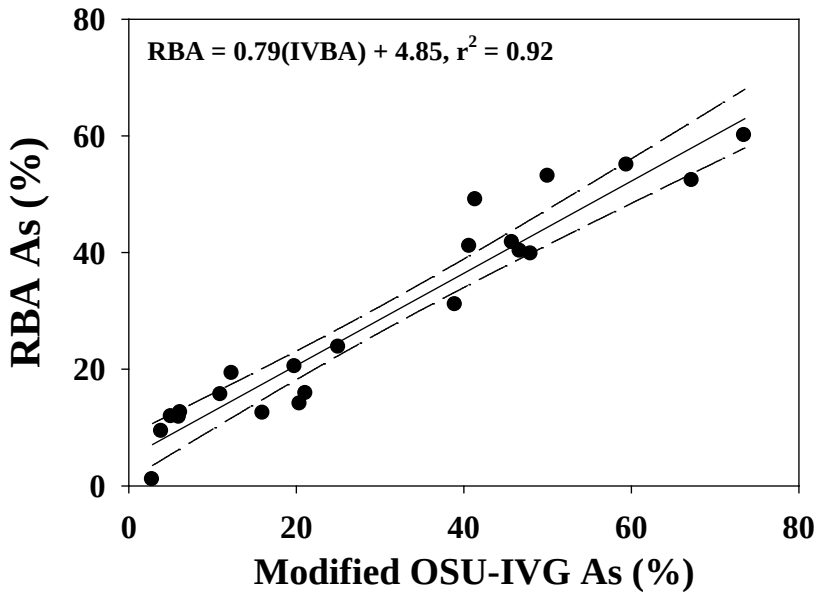



**Figure 4. IVIVC (simple linear regression with 95% confidence bands) of modified OSU-IVG vs RBA for DTSC and SERDP soils with 1,500 mg/kg As.**

***Round Robin Validation***

In order to test the reproducibility of the modified OSU-IVG, a round robin was conducted between the data presented in this report by The Ohio State University (Columbus, OH) and Prima Environmental (El Dorado Hills, CA). The round robin was conducted using the soils in Table 5.

**Table 5. Soils used in round robin study to evaluate reproducibility of the modified OSU-IVG in vitro method.**

| Lab ID | Round Robin ID | 3051a As (mg/kg) |
|---|---|---|
| MC3 | Test Soil 1 | 641 |
| EM1 | Test Soil 2 | 302 |
| RG3 | Test Soil 3 | 610 |
| SE21 | Test Soil 4 | 375 |
| SE7 | Test Soil 5 | 332 |
| BS_39 | Test Soil 6 | 214 |
| 2710A | Test Soil 7 | 1540 |
| 2711A | NIST 2711A | 107 |

The results of the round robin are presented in Table 8. Intra-lab and inter-lab variability was assessed using relative standard deviation (RSD):

$RSD = 100 * (s / |\bar{x}|)$

Where:

s = the sample standard deviation
$\bar{x}$ = sample mean

For intra-lab RSD calculation, the replicate sample extractions were used to calculate RSD. Inter-lab RSD was calculated using the mean IVBA from the respective labs (Table 6). The intra-lab RSDs were below 10% for OSU and Prima, indicating highly reproducible within lab results using the modified OSU-IVG. The inter-lab RSDs ranged from 0.04 to 26% with a mean of 8.5 % and median of 4.9 %. These results demonstrate that when the SOP developed for the round robin is followed, the modified OSU-IVG yields reproducible results.

**Table 6. Comparison of OSU and Prima Lab results for round robin study.**

| | | | Mean IVBA | Intra-Lab RSD | Inter-Lab RSD |
|---|---|---|---|---|---|
| Sample | Lab | n | ---------- | % | ---------- |
| | Prima | 5 | 16.1 | 2.8 | |
| Test Soil 1 | OSU | 3 | 11.0 | 6.2 | 26 |
| | Prima | 5 | 33.0 | 4.4 | |
| Test Soil 2 | OSU | 3 | 25.1 | 5.7 | 19 |
| | Prima | 5 | 15.9 | 5.0 | |
| Test Soil 3 | OSU | 3 | 16.1 | 5.2 | 0.7 |
| | Prima | 5 | 51.4 | 3.2 | |
| Test Soil 4 | OSU | 3 | 50.1 | 1.4 | 1.8 |
| | Prima | 5 | 67.8 | 3.4 | |
| Test Soil 5 | OSU | 3 | 67.9 | 1.0 | 0.04 |
| | Prima | 5 | 62.6 | 5.9 | |
| Test Soil 6 | OSU | 3 | 54.6 | 2.6 | 9.7 |
| | Prima | 5 | 92.2 | 5.1 | |
| Test Soil 7 | OSU | 3 | 83.0 | 1.4 | 7.4 |
| | Prima | 5 | 73.1 | 4.4 | |
| NIST 2711A | OSU | 5 | 70.7 | 2.4 | 2.4 |
| | | | Min | 1.0 | 0.04 |
| | | | Mean | 3.8 | 8.5 |
| | | | Median | 3.9 | 4.9 |
| | | | Max | 6.2 | 26 |

## CONCLUSIONS

Two commonly employed in vitro methods (OSU-IVG and SBRC) were evaluated as a surrogate for in vivo swine dosing at Empire Mine State Historic Park. The the As fractions solubilized by the OSU-IVG and SBRC (i.e., bioaccessible As) were significantly less than the relative bioavailable As fractions. The results of SEP suggest this may be due to the limited ability of both methods to dissolve amorphous and poorly-crystalline oxides of Fe and Al. In addition, using predictive equations developed from datasets from other studies demonstrated that prediction results vary drastically depending on the study soils used to develop the IVIVC. The SBRC method either drastically either under-predicts RBA As for all but one Empire Mine soil or over predicts for all but two soils depending on which regression equation is used. The regression equations developed for the OSU-IVG are less variable and therefore produce more consistent results. However, the OSU-IVG failed to predict within the RBA 90% confidence interval for every soil regardless of which regression equation was used. In vitro methods that meet IVIVC criteria; an $r^2 > 0.6$, and a slope between 0.8 and 1.2, as well a y-intercept that does not deviate significantly from zero are highly desirable. As a result, modification to the OSU-IVG were made and evaluated. Results show that the modified OSU-IVG meets IVIVC criteria for swine when applied to soils with less than 1,500 mg As/kg, regardless of As source. A round robin inter-laboratory study was performed to determine the reproducibility of the modified OSU-IVG method. Mean and median intra-laboratory RSDs were 3.8% and 3.9%, respectively. Mean and median inter-laboratory RSD were 8.5% and 4.5%, respectively. The reproducibility meets and exceeds criteria intra-laboratory RSD of < 10% and inter-laboratory RSD of <20% (Wragg et al., 2011). As a result, the SOP (Appendix) developed yields highly reproducible (within and across lab) IVBA results. A robust linear regression of RBA As (%) = 0.79(%IVBA) + 4.85 can be used to predict an accurate and reproducible RBA As from the IVBA measured by the newly developed modified OSU-IVG.

**References**

Basta, N. T., J. N. Foster, et al. (2007). "The effect of dosing vehicle on arsenic bioaccessibility in smelter-contaminated soils." Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering 42(9): 1275-1281.

Rodriguez RR, Basta NT). (1999. “An in vitro gastrointestinal method to estimate bioavailable arsenic in contaminated soils and solid media”. Environ Sci Technol 33(4): 642-649.

Bradham, K. D., K. G. Scheckel, et al. (2011). "Relative Bioavailability and Bioaccessibility and Speciation of Arsenic in Contaminated Soils." Environ Health Perspect.

Brattin, W., J. Drexler, et al. (2013). "An in vitro method for estimation of arsenic relative bioavailability in soil." J Toxicol Environ Health A 76(7): 458-478.

Denys, S., J. Caboche, et al. (2012). "In vivo validation of the unified BARGE method to assess the bioaccessibility of arsenic, antimony, cadmium, and lead in soils." Environ Sci Technol 46(11): 6252-6260.

Freeman, G. B., J. D. Johnson, et al. (1993). "BIOAVAILABILITY OF ARSENIC IN SOIL IMPACTED BY SMELTER ACTIVITIES FOLLOWING ORAL-ADMINISTRATION IN RABBITS." Fundamental and Applied Toxicology 21(1): 83-88.

Freeman, G. B., R. A. Schoof, et al. (1995). "Bioavailability of arsenic in soil and house dust impacted by smelter activities following oral administration in cynomolgus monkeys." Fundamental and Applied Toxicology 28(2): 215-222.

Juhasz, A. L., E. Smith, et al. (2008). "Effect of soil ageing on in vivo arsenic bioavailability in two dissimilar soils." Chemosphere 71(11): 2180-2186.

Juhasz, A. L., E. Smith, et al. (2006). "In vivo assessment of arsenic bioavailability in rice and its significance for human health risk assessment." Environ Health Perspect 114(12): 1826-1831.

Juhasz, A. L., E. Smith, et al. (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., E. Smith, et al. (2007). "In vitro assessment of arsenic bioaccessibility in contaminated (anthropogenic and geogenic) soils." Chemosphere 69(1): 69-78.

Juhasz, A. L., J. Weber, et al. (2009). "Assessment of four commonly employed in vitro arsenic bioaccessibility assays for predicting in vivo relative arsenic bioavailability in contaminated soils." Environ Sci Technol 43(24): 9487-9494.

Juhasz, A. L., E. Smith, et al. (2014). "Variability associated with as in vivo-in vitro correlations when using different bioaccessibility methodologies." Environ Sci Technol 48(19): 11646-11653.

Kilmer, V. J. and L. T. Alexander (1949). "Methods of making mechanical analysis of soils." Soil Sci. 68: 15-24.

McKeague, J. A. and J. H. Day (1966). "Dithionite- and oxalate-extractable Fe and Al as aids in differentiating various classes of soils." Canadian Journal of Soil Science 46: 13-22.

Meunier, L., J. Wragg, et al. (2010). "Method variables affecting the bioaccessibility of arsenic in soil." J Environ Sci Health A Tox Hazard Subst Environ Eng 45(5): 517-526.

Nagar, R., D. Sarkar, et al. (2009). "Bioavailability and Bioaccessibility of Arsenic in a Soil Amended with Drinking-Water Treatment Residuals." Archives of Environmental Contamination and Toxicology 57(4): 755-766.

Rees, M., L. Sansom, et al. (2009). "Principles and application of an in vivo swine assay for the determination of arsenic bioavailability in contaminated matrices." Environ Geochem Health 31 Suppl 1: 167-177.

Roberts, S. M., J. W. Munson, et al. (2007). "Relative oral bioavailability of arsenic from contaminated soils measured in the cynomolgus monkey." Toxicol Sci 95(1): 281-288.

Roberts, S. M., W. R. Weimar, et al. (2002). "Measurement of arsenic bioavailability in soil using a primate model." Toxicological Sciences 67(2): 303-310.

Rodriguez, R. R. and N. T. Basta (1999). "An in vitro gastrointestinal method to estimate bioavailable arsenic in contaminated soils and solid media." Environmental Science & Technology 33(4): 642-649.

Rodriguez, R. R., N. T. Basta, et al. (2003). "Chemical extraction methods to assess bioavailable arsenic in soil and solid media." Journal of Environmental Quality 32(3): 876-884.

Rodriguez, R. R., N. T. Basta, et al. (2003). "Chemical extraction methods to assess bioavailable arsenic in soil and solid media." J Environ Qual 32(3): 876-884.

Smith, E., R. Naidu, et al. (2008). "The impact of sequestration on the bioaccessibility of arsenic in long-term contaminated soils." Chemosphere 71(4): 773-780.

Tang, X. Y., Y. G. Zhu, et al. (2007). "The ageing effect on the bioaccessibility and fractionation of arsenic in soils from China." Chemosphere 66(7): 1183-1190.

Thomas, G. W. (1996). "Soil pH and soil acidity. In Sparks, D.L. Methods of soil analysis. Part 3- Chemical Methods. SSSA Book Series 5." Soil Science Society of America, Madison, WI: 475-490.

USEPA "Contract Laboratory Program Statement of work for inorganic analysis, multi-media, multi-concentration. ILM04.0b. USEPA, Washington, DC.".

USEPA (2007). "Method 3051a. Mircowave Assisted Acid Digestion of Sediments, Sludges, Soils, and Oils. SW-846. USEPA, Washington, DC.".

USEPA (2007). "Method 6010C. Inductively Coupled Plasma-Atomic Emission Spectrometry. SW-846. USEPA, Washington, DC.".

Wenzel, W.W.; Kirchbaumer, N.; Prohaska, T.; Stingeder, G.; Lombi, E.; Adriano, D.C. Arsenic fractionation in soils using an improved sequential extraction procedure. Analytica Chimica Acta. 2001, 436(2), 309-323.

Whitacre, S. D., N. T. Basta, et al. (2013). "Bioaccessible and non-bioaccessible fractions of soil arsenic." J Environ Sci Health A Tox Hazard Subst Environ Eng 48(6): 620-628.

Wragg J, Cave M, Basta N, Brandon E, Casteel S, Denys S, et al. (2011). “An inter-laboratory trial of the unified BARGE bioaccessibility method for arsenic, cadmium and lead in soil”. Sci Total Environ 409(19): 4016-4030.

ATTACHMENT 3

In Vivo Testing Reports
University of Missouri

# RELATIVE BIOAVAILABILITY OF ARSENIC FOR CALIFORNIA DTSC SOIL STUDY

Prepared for:

California Department of Toxic Substance Control

Prepared by:
Stan. W. Casteel, DVM, PhD, DABVT
Laura Naught, MS

August 25, 2011

## EXECUTIVE SUMMARY

A study using juvenile swine as test animals was performed to measure the gastrointestinal absorption of arsenic from six selected soils for the California DTSC. The arsenic concentrations of the test materials were as follows:

EM01-1-1.3
EM03-0-1.3
EM08-0-0.2
EM18-0-2
EM19-0-1
EM21-1-3

The relative oral bioavailability of arsenic was assessed by comparing the absorption of arsenic from the soil samples ("test materials") to that of sodium arsenate. Groups of five 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 negative 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 arsenic 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 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**

| Test Material | 90% Confidence Interval | | | |
|---|---|---|---|---|
| | **RBA Day 6/7** | **RBA Day 9/10** | **RBA day 12/13** | **All Days** |
| EM01-1-1.3 | 26.8 (20.3-33.4) | 29.2 (24.1-34.3) | 15.0 (7.8-22.3) | 23.7 (10.9-36.5) |
| EM03-0-1.3 | 17.0 (13.4-20.6) | 15.9 (13.0-18.8) | 12.9 (11.2-14.6) | 15.3 (11.7-18.8) |
| EM08-0-0.2 | 20.3 (18.4-22.2) | 19.5 (14.2-24.8) | 17.7 (12.3-23.2) | 19.2 (16.9-21.4) |
| EM18-0-2 | 6.8 (5.8-7.7) | 4.4 (2.2-6.5) | 3.8 (1.3-6.2) | 4.0 (3.3-4.6) |
| EM19-0-1 | 13.8 (11.1-16.4) | 11.7 (9.5-13.9) | 9.8 (7.2-12.5) | 11.7 (8.3-15.2) |
| EM21-1-3 | 23.5 (19.1-28.0) | 26.0 (22.3-29.8) | 19.6 (14.2-25.1) | 23.0 (17.6-28.5) |

All dose-response models were assessed with the regression function in Excel. Goodness of fit was considered acceptable if the p-value was less than 0.05.

# TABLE OF CONTENTS


1.0 INTRODUCTION ..... 1
- 1.1 Overview of Bioavailability ..... 1
- 1.2 Using RBA Data to Improve Risk Calculations ..... 2
- 1.3 Purpose of this Study ..... 2

2.0 STUDY DESIGN ..... 3
- 2.1 Test Materials ..... 3
- 2.2 Experimental Animals ..... 3
- 2.3 Diet ..... 4
- 2.4 Dosing ..... 4
- 2.5 Collection and Preservation of Urine Samples ..... 5
- 2.6 Arsenic Analysis ..... 5
- 2.7 Quality Control ..... 5

3.0 Data Analysis ..... 7
- 3.1 Overview ..... 7
- 3.2 Dose-Response Model ..... 8
- 3.3 Calculation of RBA Estimates ..... 8

4.0 RESULTS ..... 10
- 4.1 Clinical Signs ..... 10
- 4.2 Dosing Deviations ..... 10
- 4.3 Background Arsenic Excretion ..... 10
- 4.4 Dose-Response Modeling ..... 10
- 4.5 Calculated RBA Values ..... 10
- 4.6 Uncertainty ..... 10
- 4.7 Treatment ..... 101
- 4.8 Data Analysis Variations ..... 11

5.0 REFERENCES ..... 12

# LIST OF TABLES

TABLE 2-1 Study Design and Dosing Information

TABLE 2-2 Group Assignments and Weight (kg)

TABLE 2-3 Urinary Arsenic Analytical Results and Urine Volumes for Study Samples

TABLE 2-4 Typical Feed Composition

TABLE 2-5 Laboratory Duplicates

TABLE 2-6 Blanks

TABLE 2-7 Blind Duplicate Samples

TABLE 2-8 Laboratory Quality Control Standards

TABLE 2-9 Laboratory Spikes

TABLE 4-1 Background Urinary Arsenic

TABLE 4-2 Final Results

TABLE 4-3 Day 6/7 Dose Response and Residual Plots

TABLE 4-4 Day 9/10 Dose Response and Residual Plots

TABLE 4-5 Day 11/12 Dose Response and Residual Plots

TABLE 4-6 Estimated RBA for California DTSC Study

LIST OF FIGURES

FIGURE 3-1 Conceptual Model for Arsenic Toxicokinetics

# 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 |
| INAA | Instrumental Neutron Activation Analysis |
| 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 |
| 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 |
| °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 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 six soils ( EM01-1-1.3, EM03-0-1.3, EM08-0-0.2, EM18-0-2, EM19-0-1 and EM21-1-3) 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 five juvenile swine at one dose level 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

| Group Number | Test Material Name | Concentration mg/kg |
|---|---|---|
| 1 | EM01-1-1.3 | 302 |
| 2 | EM03-0-1.3 | 2541 |
| 3 | EM08-0-0.2 | 633 |
| 4 | EM18-0-2 | 10482 |
| 5 | EM19-0-1 | 370 |
| 6 | EM21-1-3 | 12041 |

### 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 purchased from a health-monitored herd owned by 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 represented as part on Table 2-2).

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 Table 2-2.

All animals were examined daily by an attending veterinarian while on study 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 2-4. Arsenic concentration in a randomly selected feed sample measured 0.2 µg/g.

Prior to the start of dosing and throughout the dosing period, each day 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 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 8:00 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. 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 and throughout the study from 0-5, 6-9 and 10-13 days to calculate dose. 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 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 (Table 2-3) 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 Table 2-4.

## 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.

<u>Blind Duplicates (Sample Preparation Replicates)</u>

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 D (see Table D-1 and Table 2-5). There was good agreement between results for the duplicate pairs.

<u>Laboratory Control Standards</u>

National Institute of Technology standard reference materials (NIST SRMs), for which certified concentrations of specific analytes has been established, were tested periodically during sample

analysis. Recovery of arsenic from these standards was good and within the acceptable range (Table 2-6).

Laboratory Duplicates

During analysis, every tenth sample was analyzed in duplicate. Duplicate results for urine samples (Table 2-7) typically agreed within 10% relative percent difference (RPD).

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 2-8.

Spike Recovery

During analysis, one feed and water 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 (Table 2-9) show that mean arsenic concentrations recovered from spiked samples were within 10% of actual concentrations.

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

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:

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

<u>Goodness of Fit</u>

The goodness-of-fit of each dose-response model was assessed by using least squares regression in Excel. Goodness-of-fit was considered p less than 0.05.

### 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 was a half dose eaten (pig #842) on day one PM of the study but did not affect data analysis.

There was a half dose eaten (pig #814) on Day 2 PM dose of the study but did not affect data analysis.

There were two missed doses (Pig #807) on day 3 AM and PM of the study. This was noted during the study. The calculated dose amounts for days 6/7 was affected by this deviation and was removed during data analysis. Day 9/10 and 12/13 were not affected.

There was a missed dose (pig #841) on day 7 PM of the study when it was found that the dose was stuck in the feeder but this did not affect data analysis.

### 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. Mean urinary arsenic concentration was 66.8 +/- 13.9 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 Dose-Response Modeling

The dose-response data for arsenic in urine were modeled using all of the data, and no outliers were identified (using methods discussed in Section 3.2). Modeling results are shown in Figures 4-2 through 4-6.

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 through Table 4-6.

### 4.5 Calculated RBA Values

Estimated RBA values (mean and 90% confidence interval) are shown in Table 4-2 and 4-6.

### 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. 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.

## 4.7 Treatment

Two pigs (#807 and #835) were given 1cc Naxcel for 3 days due to nasal congestion and lack of interest in feed. Injections were give days 4, 5 and 6 of the study.

## 4.8 Data Analysis Variations

For the day 9/10 urine collection group 1 (test soil EM01-1-1.3) only has four data points due to a urine sample accidently not being collected for pig #839 therefore there were only 4 data points used for that group instead of five.

For the day 12/13 Urine Collection group number four (TM EM18-0-2) pig #818 was removed for data analysis. This was because when an individual regression was performed on all data points pig #818 had a negative regression.

## 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 3:113-11

## TABLE 2-1 Study Design and Dosing Information

| Group | Group Name Appreviation | Dose Material Administered | As Conc of the material (ug/g or ug/ul) | No. Pigs in Group | Target (ug/kg BW-day) | Actual[a] (ug/kg BW-day) | Actual[b] (ug-day) Dose Prep 1 (day 0-4) | Actual[b] (ug-day) Dose Prep 2 (day 5-9) | Actual[b] (ug-day) Dose Prep 3 (day 10-13) | Average Actual[b] dose over 0-13 days |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | TM1 | EM01-1-1.3 | 302 | 5 | 60 | 60 | 634.2 | 676.8 | 760.2 | 690.4 |
| 2 | TM2 | EM03-0-1.3 | 2541 | 5 | 60 | 60 | 634.2 | 676.8 | 760.2 | 690.4 |
| 3 | TM3 | EM08-0-0.2 | 633 | 5 | 60 | 60 | 634.2 | 676.8 | 760.2 | 690.4 |
| 4 | TM4 | EM18-0-2 | 10482 | 5 | 60 | 60 | 634.2 | 676.8 | 760.2 | 690.4 |
| 5 | TM5 | EM19-0-1 | 370 | 5 | 60 | 60 | 634.2 | 676.8 | 760.2 | 690.4 |
| 6 | TM6 | EM21-1-3 | 12041 | 5 | 60 | 60 | 634.2 | 676.8 | 760.2 | 690.4 |
| 7 | AsAs | Sodium Arsenate | 10 | 5 | 50 | 50 | 528.5 | 564 | 633.5 | 575.3 |
| 8 | Control | Negative control | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |

[a]Calculated as the administered daily dose divided by the measured or extrapolated daily body weight, averaged over days 0-5, 6-10 and 11-14 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.

Dose 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 each dosing period (day 0-4, 5-9 and 10-13).

## TABLE 2-2 Group Assignments and Weight (kg)

| Group# / Dose | Animal Ear Tag | Day -5 5/4/11 | Group MBW | Day -1 5/8/11 | Group MBW | Day 2 5/11/11 | Group MBW | Day 5 5/14/11 | Group MBW | Day 8 5/17/11 | Group MBW | Day 11 5/20/11 | Group MBW | Day 16 5/25/11 | Group MBW |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 839 | 9 | | 8.8 | | 9.9 | | 10.2 | | 10.8 | | 11.3 | | 12.5 | |
| EM01 | 836 | 11 | | 8.6 | | 10 | | 10.8 | | 11.1 | | 11.7 | | 13.5 | |
| | 826 | 9.2 | | 10.5 | | 11.5 | | 12 | | 12.7 | | 13.4 | | 14.2 | |
| | 815 | 8.7 | | 8 | | 9.1 | | 9.8 | | 10.8 | | 11.5 | | 12.6 | |
| | 842 | 6.9 | 8.96 | 10.2 | 9.22 | 11 | 10.30 | 11.7 | 10.90 | 12.3 | 11.54 | 13.1 | 12.20 | 14.4 | 13.44 |
| 2 | 813 | 8.7 | | 8.1 | | 8.9 | | 9.8 | | 10.8 | | 11.4 | | 12.3 | |
| EM03 | 806 | 8.9 | | 9.7 | | 10.6 | | 11.1 | | 11.8 | | 12.5 | | 13.3 | |
| | 829 | 9.6 | | 9.7 | | 10.8 | | 11.3 | | 12.1 | | 12.9 | | 14.3 | |
| | 833 | 8.9 | | 8.2 | | 9.2 | | 9.9 | | 10.7 | | 11.7 | | 12.6 | |
| | 810 | 10.2 | 9.26 | 10.7 | 9.28 | 11.5 | 10.20 | 12.1 | 10.84 | 12.9 | 11.66 | 13.8 | 12.46 | 15.3 | 13.56 |
| 3 | 841 | 9.3 | | 11.2 | | 11.1 | | 12.9 | | 13.5 | | 14.4 | | 15.3 | |
| EM08 | 817 | 9.5 | | 9.4 | | 8.6 | | 11 | | 11.9 | | 12.6 | | 13.6 | |
| | 814 | 7.3 | | 9.2 | | 9.5 | | 10 | | 11.1 | | 11.9 | | 13.1 | |
| | 822 | 8.4 | | 8.1 | | 10.3 | | 9.2 | | 10.2 | | 11 | | 11.7 | |
| | 801 | 7.9 | 8.48 | 9.8 | 9.54 | 9 | 9.70 | 11 | 10.82 | 11.8 | 11.70 | 12.6 | 12.50 | 13.6 | 13.46 |
| 4 | 827 | 9.7 | | 8.2 | | 10.3 | | 9.7 | | 10.4 | | 11.1 | | 12.3 | |
| EM18 | 811 | 9.2 | | 9.7 | | 8.6 | | 11.2 | | 11.8 | | 12.2 | | 13.6 | |
| | 837 | 9.9 | | 9.3 | | 9.4 | | 9.9 | | 10.6 | | 11.2 | | 12.5 | |
| | 831 | 9.9 | | 8 | | 9.9 | | 9.4 | | 9.9 | | 10.9 | | 12.8 | |
| | 818 | 8.6 | 9.46 | 10.2 | 9.08 | 12.4 | 10.12 | 11.6 | 10.36 | 12.2 | 10.98 | 13.1 | 11.70 | 13.7 | 12.98 |
| 5 | 825 | 10.4 | | 10.4 | | 11.1 | | 11.6 | | 12.6 | | 13.3 | | 14.4 | |
| EM19 | 835 | 7.7 | | 11 | | 10.9 | | 11.2 | | 12.3 | | 12.9 | | 13.7 | |
| | 807 | 10.5 | | 9 | | 9.5 | | 9.8 | | 10.1 | | 1.3 | | 12.6 | |
| | 820 | 9.5 | | 9.2 | | 9.9 | | 10.4 | | 11.4 | | 12.1 | | 13.1 | |
| | 828 | 9.3 | 9.48 | 10.7 | 10.06 | 11.1 | 10.50 | 11.7 | 10.94 | 12.7 | 11.82 | 13.4 | 10.60 | 14.2 | 13.60 |
| 6 | 819 | 10.2 | | 9.8 | | 10.4 | | 11.3 | | 12 | | 12.7 | | 13.9 | |
| EM21 | 802 | 7.5 | | 11.7 | | 12.6 | | 13.2 | | 13.9 | | 14.7 | | 15.8 | |
| | 821 | 10.4 | | 11.2 | | 12.1 | | 12.8 | | 13.7 | | 14.2 | | 15.4 | |
| | 832 | 9.7 | | 8.5 | | 9.4 | | 10 | | 10.8 | | 11.8 | | 12.8 | |
| | 803 | 13.4 | 10.24 | 10.1 | 10.26 | 10.7 | 11.04 | 11.4 | 11.74 | 12 | 12.48 | 12.8 | 13.24 | 13.9 | 14.36 |
| 7 | 804 | 7.6 | | 9.4 | | 10.2 | | 10.8 | | 11.7 | | 12.2 | | 13.5 | |
| NaAs | 824 | 8.3 | | 9.8 | | 10.5 | | 11.2 | | 11.7 | | 12.9 | | 14 | |
| | 812 | 7.9 | | 10.1 | | 10.7 | | 11.7 | | 12.6 | | 13.5 | | 14.7 | |
| | 840 | 10.4 | | 7.6 | | 8.3 | | 9 | | 9.6 | | 10.3 | | 11.5 | |
| | 809 | 10.3 | 8.90 | 8.9 | 9.16 | 9.5 | 9.84 | 10.1 | 10.56 | 10.8 | 11.28 | 11.3 | 12.04 | 12.4 | 13.22 |
| 8 | 808 | 8.7 | | 10.2 | | 10.2 | | 11.1 | | 11.5 | | 12.1 | | 12.9 | |
| Control | 816 | 9.2 | | 10 | | 10.9 | | 11.6 | | 12.4 | | 12.9 | | 13.8 | |
| | 834 | 7.16 | 8.35 | 10.6 | 10.27 | 11.1 | 10.73 | 11.7 | 11.47 | 12.4 | 12.10 | 13.2 | 13.2 | 14 | 13.2 |

**TABLE 2-3 Urinary Arsenic Analytical Results and Urine Volumes for Study Samples**

| | Pig ID | U-1 Days 6-7 | | | U-2 Days 9-10 | | | U-3 Days 12-13 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Vol (mL) | Sample ID | Urine As (ug/L) | Vol (mL) | Sample ID | Urine As (ug/L) | Vol (mL) | Sample ID | Urine As (ug/L) |
| 1 | **839** | 7100 | CAEM1-001 | 32 | NO SAMPLE* | CAEM1-039 | - | 3000 | CAEM1-077 | 52 |
| 1 | **836** | 2600 | CAEM1-002 | 109 | 8580 | CAEM1-040 | 34 | 2660 | CAEM1-078 | 75 |
| 1 | **826** | 3700 | CAEM1-003 | 109 | 10960 | CAEM1-041 | 35 | 3100 | CAEM1-079 | 74 |
| 1 | **815** | 1680 | CAEM1-004 | 180 | 2090 | CAEM1-042 | 160 | 2320 | CAEM1-080 | 160 |
| 1 | **842** | 2540 | CAEM1-005 | 140 | 2840 | CAEM1-043 | 110 | 2320 | CAEM1-081 | 89 |
| **2** | **813** | 900 | CAEM1-006 | 170 | 1060 | CAEM1-044 | 160 | 1100 | CAEM1-082 | 180 |
| **2** | **806** | 840 | CAEM1-007 | 290 | 980 | CAEM1-045 | 230 | 1120 | CAEM1-083 | 210 |
| **2** | **829** | 820 | CAEM1-008 | 290 | 970 | CAEM1-046 | 240 | 1640 | CAEM1-084 | 150 |
| **2** | **833** | 3840 | CAEM1-009 | 59 | 2040 | CAEM1-047 | 92 | 2260 | CAEM1-085 | 95 |
| **2** | **810** | 4720 | CAEM1-010 | 48 | 2280 | CAEM1-048 | 100 | 2120 | CAEM1-086 | 110 |
| 3 | **841** | 6200 | CAEM1-011 | 38 | 3600 | CAEM1-049 | 73 | 3440 | CAEM1-087 | 100 |
| 3 | **817** | 660 | CAEM1-012 | 350 | 840 | CAEM1-050 | 200 | 1360 | CAEM1-088 | 130 |
| 3 | **814** | 1380 | CAEM1-013 | 180 | 1240 | CAEM1-051 | 170 | 1620 | CAEM1-089 | 180 |
| 3 | **822** | 3640 | CAEM1-014 | 77 | 5220 | CAEM1-052 | 54 | 6180 | CAEM1-090 | 45 |
| 3 | **801** | 6860 | CAEM1-015 | 37 | 4600 | CAEM1-053 | 62 | 4360 | CAEM1-091 | 67 |
| 4 | **827** | 1260 | CAEM1-016 | 80 | 1120 | CAEM1-054 | 93 | 1440 | CAEM1-092 | 87 |
| 4 | **811** | 2740 | CAEM1-017 | 42 | 2560 | CAEM1-055 | 45 | 3020 | CAEM1-093 | 43 |
| 4 | **837** | 1520 | CAEM1-018 | 82 | 1220 | CAEM1-056 | 97 | 2400 | CAEM1-094 | 42 |
| 4 | **831** | 2740 | CAEM1-019 | 45 | 2095 | CAEM1-057 | 53 | 2460 | CAEM1-095 | 63 |
| 4 | **818** | 520 | CAEM1-020 | 230 | 480 | CAEM1-058 | 140 | 620 | CAEM1-096 | 120 |
| 5 | **825** | 1060 | CAEM1-021 | 200 | 680 | CAEM1-059 | 240 | 1900 | CAEM1-097 | 110 |
| 5 | **835** | 1540 | CAEM1-022 | 110 | 1680 | CAEM1-060 | 100 | 2440 | CAEM1-098 | 70 |
| 5 | **807** | 260 | CAEM1-023 | 370 | 440 | CAEM1-061 | 360 | 800 | CAEM1-099 | 260 |
| 5 | **820** | 1400 | CAEM1-024 | 140 | 1380 | CAEM1-062 | 150 | 1860 | CAEM1-100 | 120 |
| 5 | **828** | 1700 | CAEM1-025 | 97 | 1200 | CAEM1-063 | 130 | 1260 | CAEM1-101 | 120 |
| 6 | **819** | 3800 | CAEM1-026 | 70 | 3860 | CAEM1-064 | 78 | 3520 | CAEM1-102 | 94 |
| 6 | **802** | 13980 | CAEM1-027 | 22 | 21360 | CAEM1-065 | 17 | 18500 | CAEM1-103 | 20 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 6 | **821** | 12080 | CAEM1-028 | 28 | 9120 | CAEM1-066 | 31 | 10760 | CAEM1-104 | 26 |
| 6 | **832** | 1880 | CAEM1-029 | 150 | 2420 | CAEM1-067 | 120 | 5720 | CAEM1-105 | 52 |
| 6 | **803** | 1800 | CAEM1-030 | 120 | 1800 | CAEM1-068 | 150 | 2090 | CAEM1-106 | 207 |
| **7** | **804** | 1940 | CAEM1-031 | 550 | 2440 | CAEM1-069 | 320 | 2085 | CAEM1-107 | 500 |
| **7** | **824** | 2800 | CAEM1-032 | 370 | 1380 | CAEM1-070 | 770 | 2150 | CAEM1-108 | 540 |
| **7** | **812** | 10135 | CAEM1-033 | 75 | 21440 | CAEM1-071 | 37 | 9860 | CAEM1-109 | 76 |
| **7** | **840** | 6620 | CAEM1-034 | 140 | 6640 | CAEM1-072 | 140 | 5120 | CAEM1-110 | 200 |
| **7** | **809** | 1160 | CAEM1-035 | 490 | 1220 | CAEM1-073 | 460 | 1420 | CAEM1-111 | 640 |
| 8 | **808** | 1020 | CAEM1-036 | 52 | 1020 | CAEM1-074 | 37 | 1620 | CAEM1-112 | 59 |
| 8 | **816** | 780 | CAEM1-037 | 44 | 800 | CAEM1-075 | 94 | 760 | CAEM1-113 | 94 |
| 8 | **834** | 660 | CAEM1-038 | 94 | 640 | CAEM1-076 | 120 | 1140 | CAEM1-114 | 83 |

# TABLE 2-4 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 2-5 Laboratory Duplicates

| Blind Duplicate Sample ID | Sample Type | Pig Number | Collection Days | Original Sample Concentration | Duplicate Sample concentration | Sample Units | RPD |
|---|---|---|---|---|---|---|---|
| CAEM1-005 | Urine | 842 | U-1 | 140 | 140 | ug/L | 0.0 |
| CAEM1-015 | Urine | 801 | U-1 | 37 | 41 | ug/L | 10.3 |
| CAEM1-025 | Urine | 828 | U-1 | 97 | 97 | ug/L | 0.0 |
| CAEM1-035 | Urine | 809 | U-1 | 490 | 500 | ug/L | 2.0 |
| CAEM1-045 | Urine | 806 | U-2 | 230 | 230 | ug/L | 0.0 |
| CAEM1-055 | Urine | 811 | U-2 | 45 | 45 | ug/L | 0.0 |
| CAEM1-065 | Urine | 802 | U-2 | 17 | 17 | ug/L | 0.0 |
| CAEM1-075 | Urine | 816 | U-2 | 94 | 93 | ug/L | 1.1 |
| CAEM1-085 | Urine | 833 | U-3 | 95 | 95 | ug/L | 0.0 |
| CAEM1-095 | Urine | 831 | U-3 | 63 | 62 | ug/L | 1.6 |
| CAEM1-105 | Urine | 832 | U-3 | 52 | 52 | ug/L | 0.0 |
| CAEM1-115 | Water | | | 0.5 | <0.5 | ug/L | * |
| CAEM1-123 | 100 std | | | 514 | 497 | ug/L | 3.4 |
| CAEM1-129 | | 811 dup | U-2 | 46 | 46 | ug/L | 0.0 |

*indicates % Deviation not calculated

## TABLE 2-6 Blanks

| Sample ID | Associated Sample Type | Measured Concentration | Detection Limit | Units |
|---|---|---|---|---|
| Blank-1 | Urine | <1 | 1 | ug/L |
| Blank-2 | Urine | <1 | 1 | ug/L |
| Blank-3 | Urine | <1 | 1 | ug/L |
| Blank-4 | Urine | <1 | 1 | ug/L |
| Blank-5 | Urine | <1 | 1 | ug/L |
| Blank-6 | Urine | <1 | 1 | ug/L |
| Blank-7 | Feed | <1 | 1 | ug/L |

**TABLE 2-9 Laboratory Spikes**

| Spike Sample ID | Sample Type | Original Sample Concentration (ug/L) | Added Spike Concentration (ug/L) | Measured Sample Concentration (ug/L) | Recovered Spike (ug/L) | Recovery |
|---|---|---|---|---|---|---|
| CAEM1-010 | Urine | 48 | 200 | 250 | 202 | 101 |
| CAEM1-020 | Urine | 230 | 200 | 434 | 204 | 102 |
| CAEM1-030 | Urine | 120 | 200 | 320 | 200 | 100 |
| CAEM1-040 | Urine | 34 | 200 | 230 | 196 | 98.0 |
| CAEM1-050 | Urine | 200 | 200 | 390 | 190 | 95.0 |
| CAEM1-060 | Urine | 100 | 200 | 310 | 210 | 105 |
| CAEM1-070 | Urine | 770 | 200 | 1000 | 230 | 115 |
| CAEM1-080 | Urine | 160 | 200 | 350 | 190 | 95.0 |
| CAEM1-090 | Urine | 45 | 200 | 230 | 185 | 92.5 |
| CAEM1-100 | Urine | 120 | 200 | 310 | 190 | 95.0 |
| CAEM1-110 | Urine | 200 | 200 | 413 | 213 | 107 |
| CAEM1-120 | Water | 0.5 | 100 | 110 | 109.5 | 110 |
| CAEM1-126 | Urine | 100 | 200 | 300 | 200 | 100 |
| CAEM1-132 | Urine | 130 | 200 | 340 | 210 | 105 |

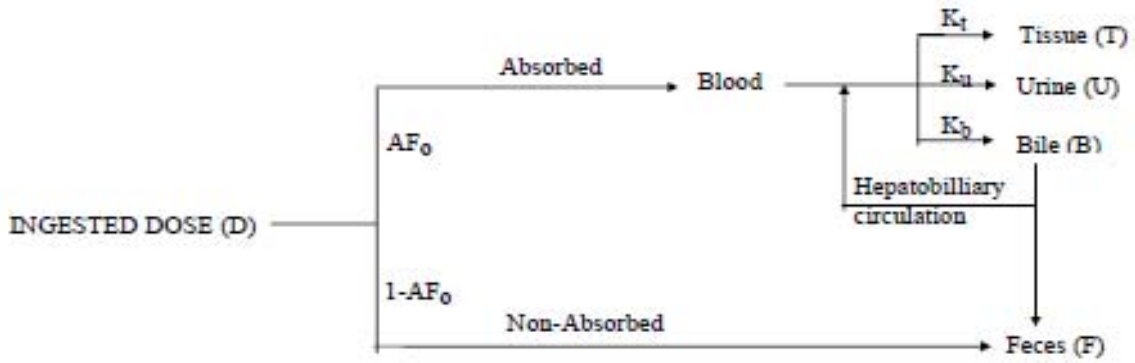
**TABLE 2-7 Blind Duplicate Samples**

| Blind Duplicate Sample ID | Sample Type | Pig Number | Collection Days | Original Sample Concentration | Duplicate Sample concentration | Sample Units | RPD |
|---|---|---|---|---|---|---|---|
| CAEM1-125 | Urine | 829 | U-1 | 290 | 290 | ug/L | 0% |
| CAEM1-126 | Urine | 828 | U-1 | 97 | 100 | ug/L | 3% |
| CAEM1-127 | Urine | 808 | U-1 | 52 | 37 | ug/L | 34% |
| CAEM1-128 | Urine | 810 | U-2 | 100 | 101 | ug/L | 0% |
| CAEM1-129 | Urine | 811 | U-2 | 45 | 46 | ug/L | 2% |
| CAEM1-130 | Urine | 804 | U-2 | 320 | 330 | ug/L | 3% |
| CAEM1-131 | Urine | 826 | U-3 | 74 | 70 | ug/L | 6% |
| CAEM1-132 | Urine | 817 | U-3 | 130 | 130 | ug/L | 0% |
| CAEM1-133 | Urine | 832 | U-3 | 52 | 54 | ug/L | 4% |

**TABLE 2-8 Laboratory Quality Control Standards**

| Sample ID | Associated Sample Type | LET Number | Measured Concentration | Units | Reference Material ID | Certified Mean +/- Std Dev | Recovery |
|---|---|---|---|---|---|---|---|
| QC-1 | Urine | L11060022 | 5 | ng/mL | NIST 2670a-L | 3 | 83% |
| QC-2 | Urine | L11060046 | 230 | ng/mL | NIST2670a-H | 220 +/-10 | 105% |
| QC-3 | Urine | L11060070 | 7 | ng/mL | NIST 2670a-L | 3 | 233% |
| QC-4 | Urine | L11060094 | 210 | ng/mL | NIST2670a-H | 220 +/-10 | 95% |
| QC-5 | Urine | L11060118 | 230 | ng/mL | NIST2670a-H | 220 +/-10 | 105% |
| QC-6 | Urine | L11060142 | 250 | ng/mL | NIST2670a-H | 220 +/-10 | 114% |
| QC-7 | Urine | L11060159 | 230 | ng/mL | NIST2670a-H | 220 +/-10 | 105% |

## 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

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

**TABLE 4-1 Background Urinary Arsenic**

| Pig Number | Urine Control Period (days) | As Dose (ug per collection period) | Urine Volume (mL) | Total As Excreted (ug/48 hours) |
|---|---|---|---|---|
| **808** | 6/7 | **0** | 1020 | 53.04 |
| **816** | 6/7 | **0** | 780 | 34.32 |
| **834** | 6/7 | **0** | 660 | 62.04 |
| **808** | 9/10 | **0** | 1020 | 37.74 |
| **816** | 9/10 | **0** | 800 | 75.2 |
| **834** | 9/10 | **0** | 640 | 76.8 |
| **808** | 12/13 | **0** | 1620 | 95.58 |
| **816** | 12/13 | **0** | 760 | 71.44 |
| **834** | 12/13 | **0** | 1140 | 94.62 |

## Table 4-2 Final Results

Day 6/7 48 hour Urine Collection

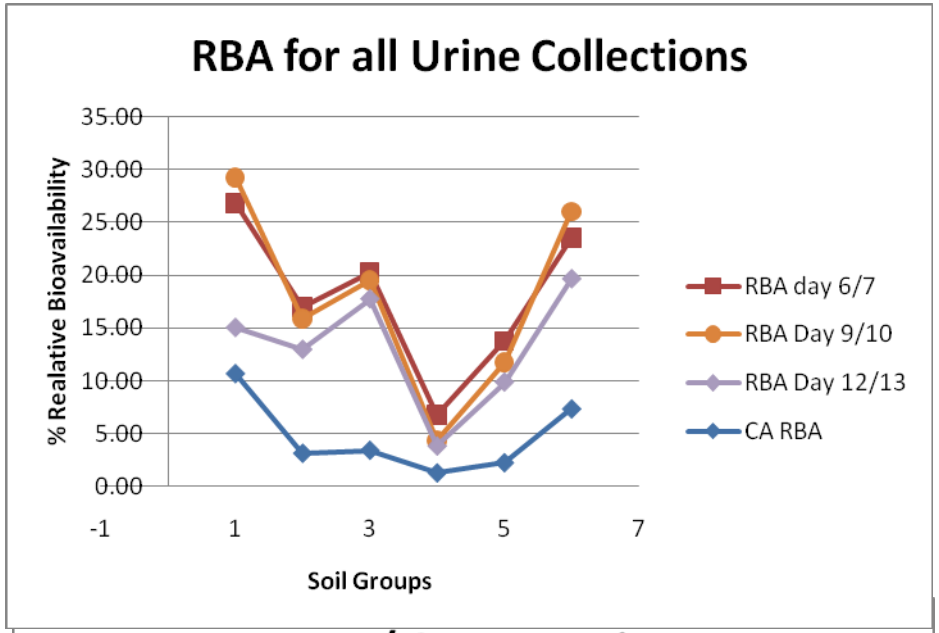
| Test Material | Regression | % RBA | Ca RBA | RBA Ratio |
|---|---|---|---|---|
| EM01-1-1.3 | 0.191613557 | **26.83** | 10.7 | 2.51 |
| EM03-0-1.3 | 0.121454229 | **17.00** | 3.14 | 5.42 |
| EM08-0-0.2 | 0.144858053 | **20.28** | 3.42 | 5.93 |
| EM18-0-2 | 0.048438586 | **6.78** | 1.27 | 5.34 |
| EM19-0-1 | 0.098330678 | **13.77** | 2.24 | 6.15 |
| EM21-1-3 | 0.168134415 | **23.54** | 7.35 | 3.20 |
| Reference | 0.714292543 | | | |

Day 9/10 48 hour Urine Collection

| Test Material | Regression | % RBA | Ca RBA | RBA Ratio |
|---|---|---|---|---|
| EM01-1-1.3 | 0.19357136 | **29.22** | 10.7 | 2.73 |
| EM03-0-1.3 | 0.105337003 | **15.90** | 3.14 | 5.06 |
| EM08-0-0.2 | 0.12926516 | **19.51** | 3.42 | 5.71 |
| EM18-0-2 | 0.028925502 | **4.37** | 1.27 | 3.44 |
| EM19-0-1 | 0.077689262 | **11.73** | 2.24 | 5.24 |
| EM21-1-3 | 0.172521244 | **26.04** | 7.35 | 3.54 |
| Reference | 0.662479866 | | | |

Day 12/13 48 hour Urine Collection

| Test Material | Regression | % RBA | Ca RBA | RBA Ratio |
|---|---|---|---|---|
| EM01-1-1.3 | 0.116094052 | **15.01** | 10.7 | 1.40 |
| EM03-0-1.3 | 0.100091734 | **12.94** | 3.14 | 4.12 |
| EM08-0-0.2 | 0.137102163 | **17.73** | 3.42 | 5.18 |
| EM18-0-2 | 0.021270761 | **3.79** | 1.27 | 2.98 |
| EM19-0-1 | 0.07620703 | **9.85** | 2.24 | 4.40 |
| EM21-1-3 | 0.151797325 | **19.63** | 7.35 | 2.67 |
| Reference | 0.773438786 | | | |



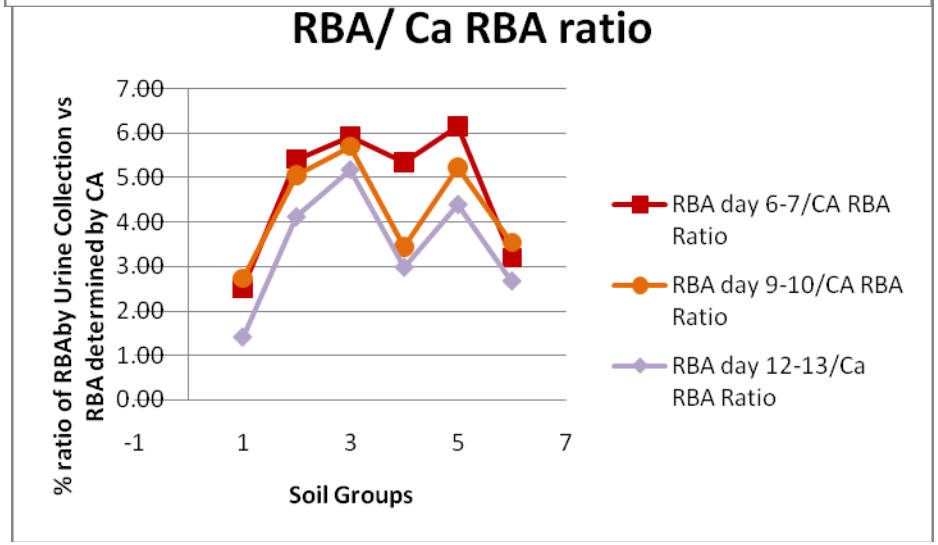
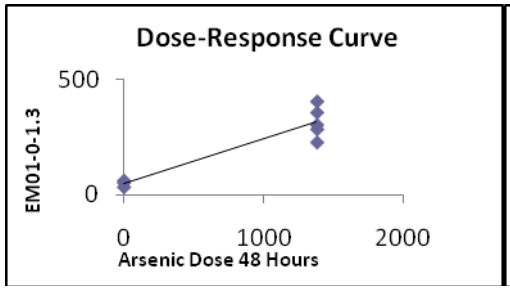
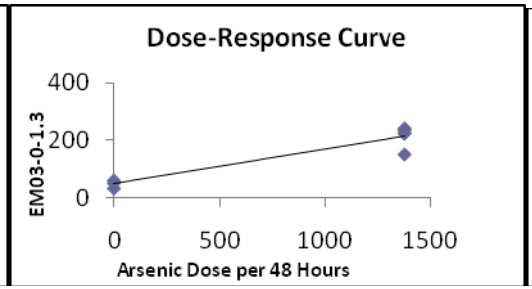
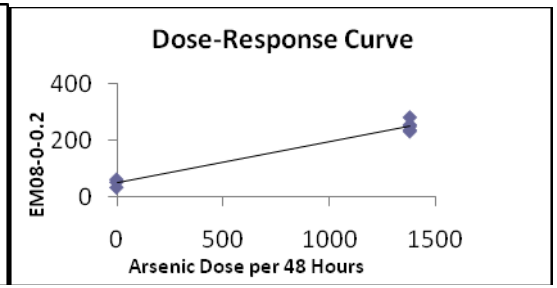
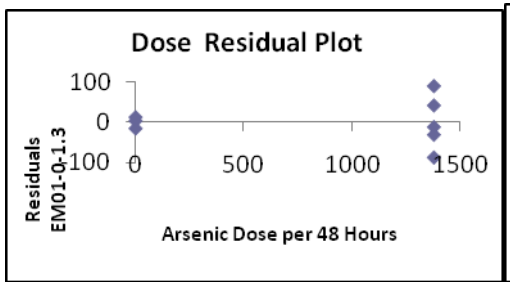



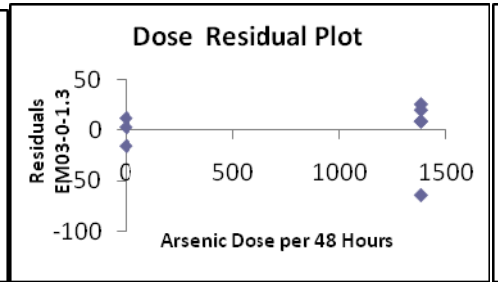
**TABLE 4-3 Day 6/7 Dose Response and Residual Plots**

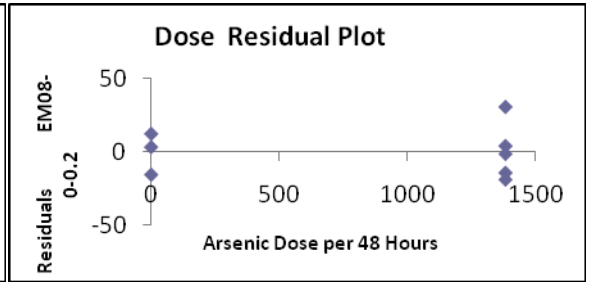


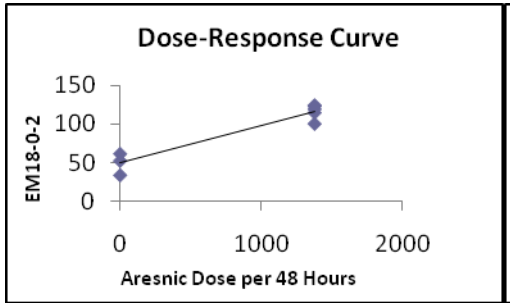



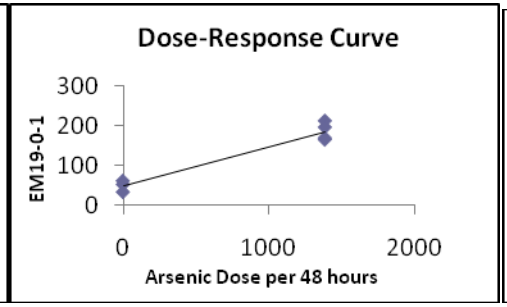



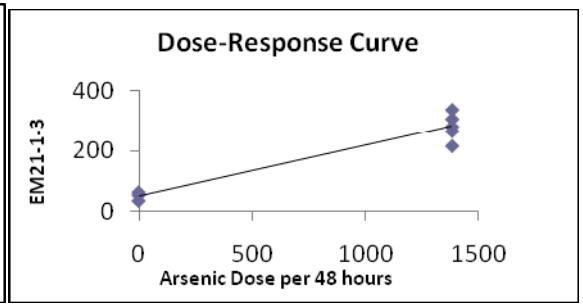



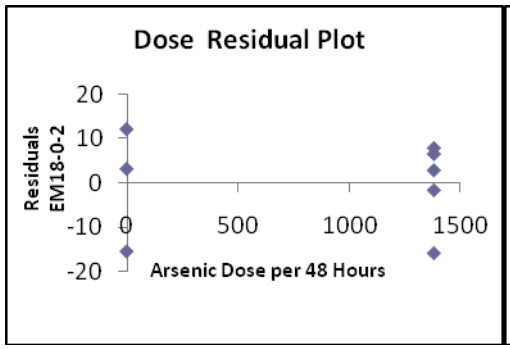



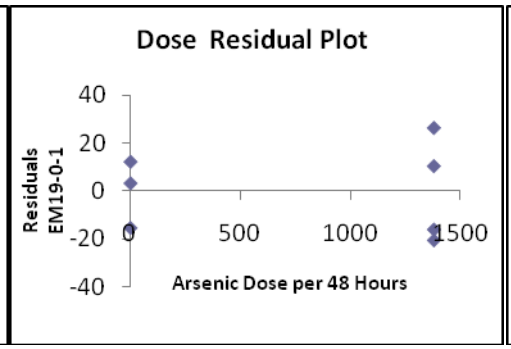



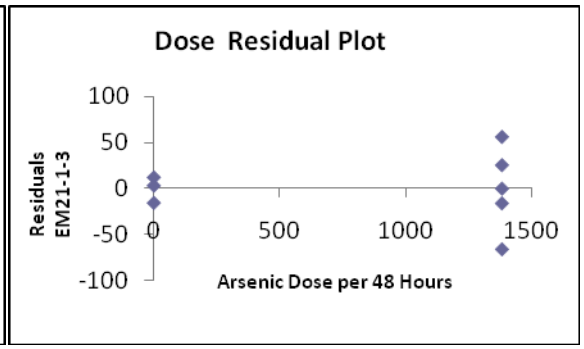
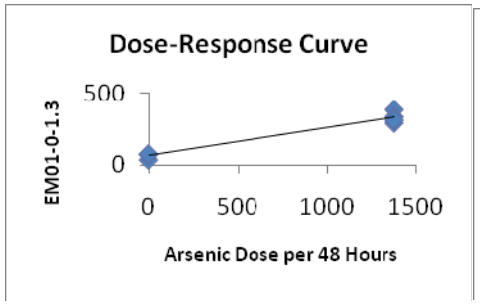
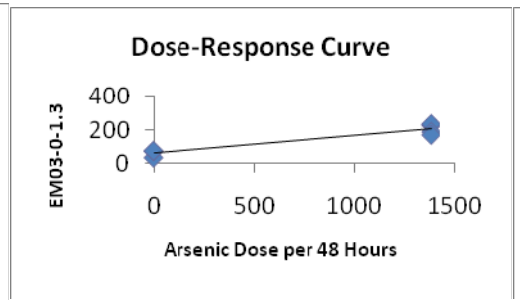
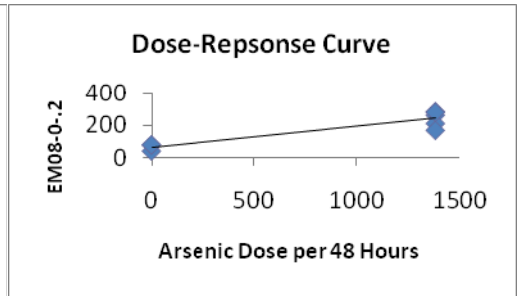
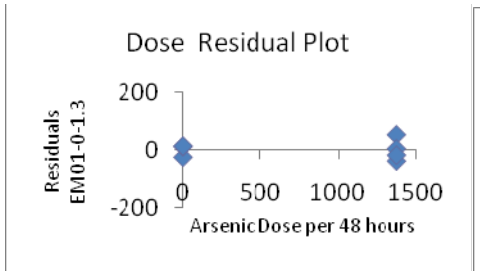



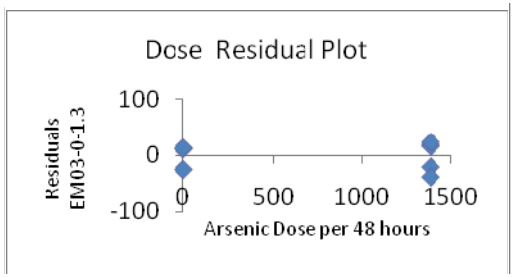
**TABLE 4-4 Day 9/10 Dose Response and Residual Plots**

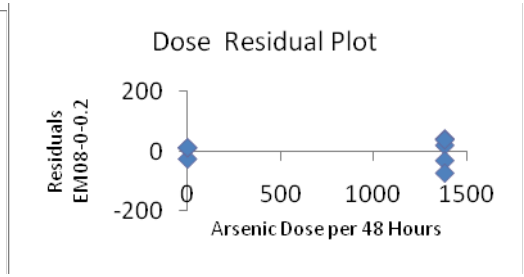


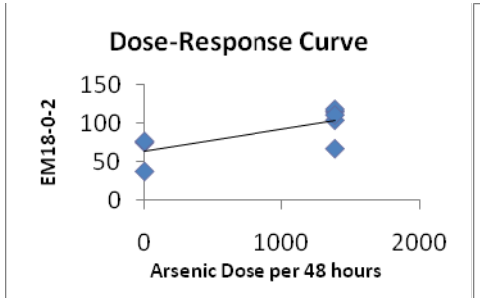



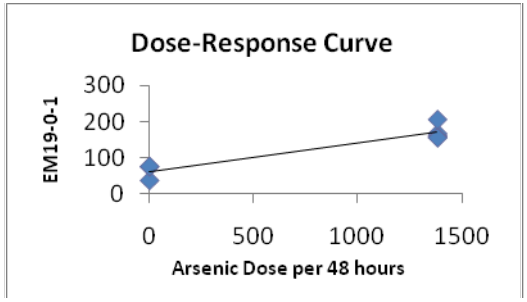



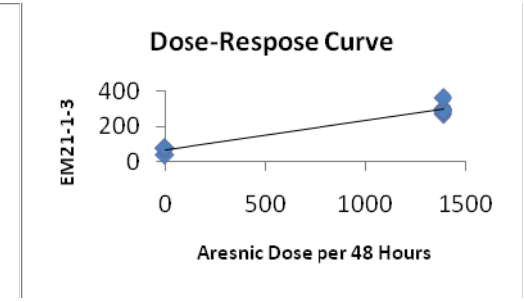



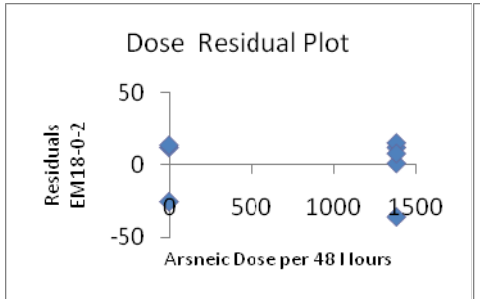



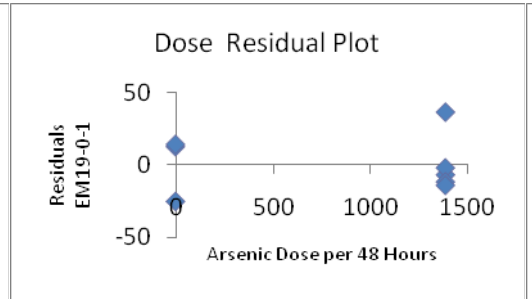



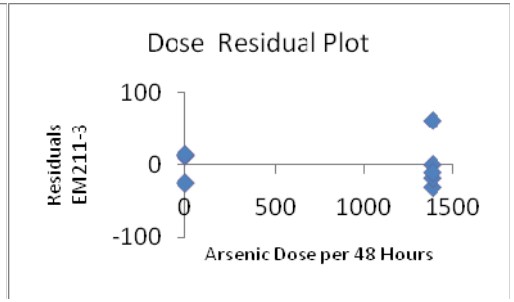
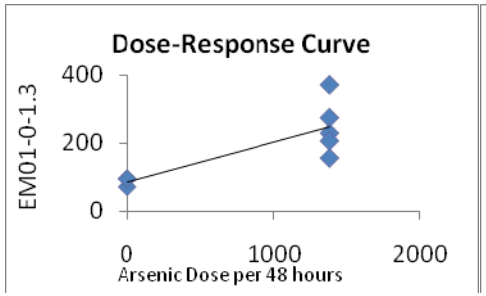
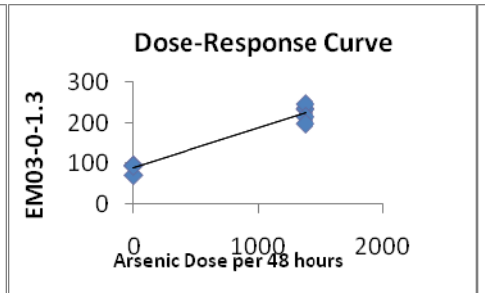
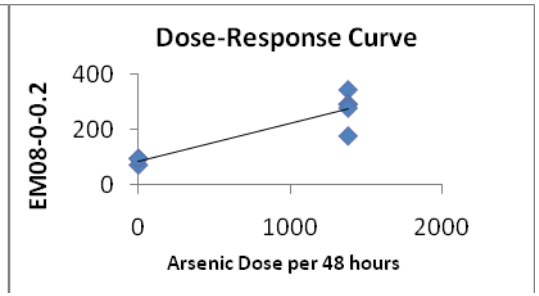
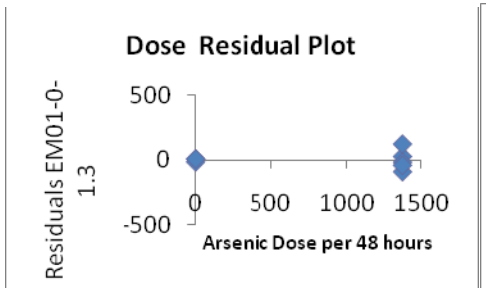



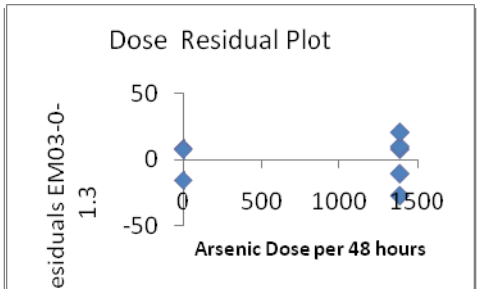
**TABLE 4-5 Day 12/13 Dose Response and Residual Plots**

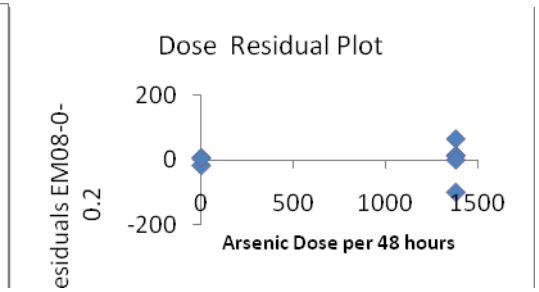


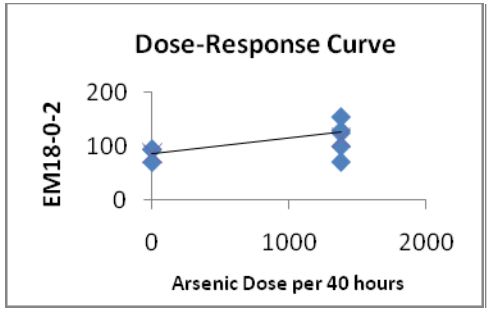



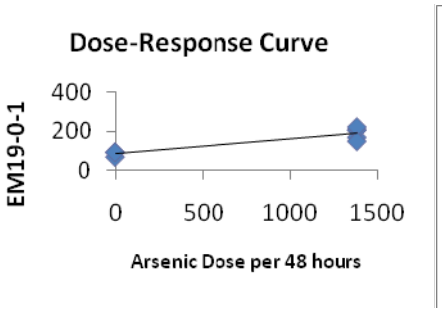



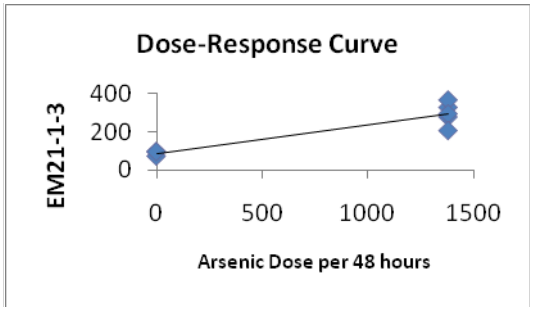



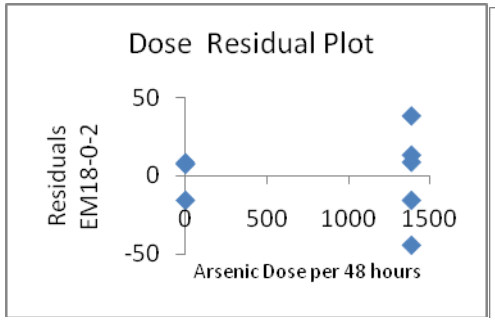



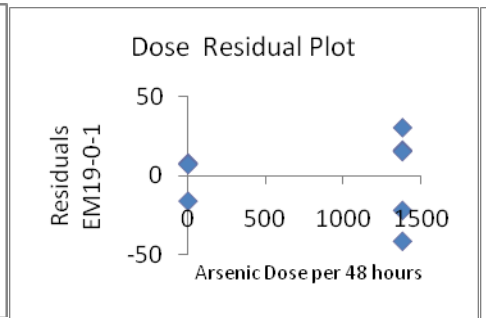



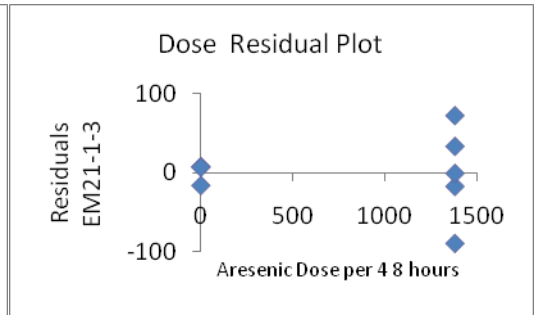



## TABLE 4-6 ESTIMATED RBA FOR CALIFORNIA DTSC

| Test Material | 90% Confidence Interval | | | |
|---|---|---|---|---|
| | **RBA Day 6/7** | **RBA Day 9/10** | **RBA day 12/13** | **All Days** |
| EM01-1-1.3 | 26.8 (20.3-33.4) | 29.2 (24.1-34.3) | 15.0 (7.8-22.3) | 23.7 (10.9-36.5) |
| EM03-0-1.3 | 17.0 (13.4-20.6) | 15.9 (13.0-18.8) | 12.9 (11.2-14.6) | 15.3 (11.7-18.8) |
| EM08-0-0.2 | 20.3 (18.4-22.2) | 19.5 (14.2-24.8) | 17.7 (12.3-23.2) | 19.2 (16.9-21.4) |
| EM18-0-2 | 6.8 (5.8-7.7) | 4.4 (2.2-6.5) | 3.8 (1.3-6.2) | 4.0 (3.3-4.6) |
| EM19-0-1 | 13.8 (11.1-16.4) | 11.7 (9.5-13.9) | 9.8 (7.2-12.5) | 11.7 (8.3-15.2) |
| EM21-1-3 | 23.5 (19.1-28.0) | 26.0 (22.3-29.8) | 19.6 (14.2-25.1) | 23.0 (17.6-28.5) |

# RELATIVE BIOAVAILABILITY OF ARSENIC FOR CALIFORNIA DTSC SOIL STUDY

Prepared for:

California Department of Toxic Substance Control

Prepared by:
Stan. W. Casteel, DVM, PhD, DABVT
Laura Naught, MS

January 4, 2013

## EXECUTIVE SUMMARY

A study using juvenile swine as test animals was performed to measure the gastrointestinal absorption of arsenic from six selected soils for the California DTSC. The arsenic concentrations of the test materials were as follows:

| Test Material | As Conc (ug/g) |
|---|---|
| EM05 | 1906 |
| EM13 | 1237 |
| EM15 | 12095 |
| EM20 | 5647 |
| RG01 | 200 |
| RG03 | 610 |

The relative oral bioavailability of arsenic was assessed by comparing the absorption of arsenic from the soil samples ("test materials") to that of sodium arsenate. Groups of five 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 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 95% confidence interval) are shown below:

**ESTIMATED RBA FOR ASARCO AND HAWAII SOILS**

| | 95% Confidence Interval | | | |
|---|---|---|---|---|
| **Test Material** | **RBA Day 6/7** | **RBA Day 9/10** | **RBA day 12/13** | **All Days** |
| EM05 | 15.3 (13.1-17.4) | 15.4 (12.6-18.2) | 15.3 (6.0-24.6) | 15.3 (15.22-15.5) |
| EM13 | 13.7 (7.1-20.3) | 14.7 (12.5-16.9) | 9.1 (4.9-13.4) | 12.5 (5.1-19.9) |
| EM15 | 19.8 (11.9-27.7) | 22.2 (17.2-27.2) | 17.0 (12.4-21.5) | 19.7 (13.1-26.2) |
| EM20 | 22.5 (18.9-26.1) | 22.1 (24.4-19.8) | 23.5 (17.1-29.8) | 22.7 (21.1-24.3) |
| RG01 | 12.1 (8.3-15.9) | 13.5 (10.5-16.6) | 9.7 (2.3-17.0) | 11.8 (6.9-16.6) |
| RG03 | 12.8 (8.2-17.4) | 14.06 (11.3-16.8) | 10.3 (6.0-14.6) | 12.4 (7.6-17.2) |

All dose-response models were assessed with the regression function in Excel. Goodness of fit was considered acceptable if the p-value was less than 0.05.

# TABLE OF CONTENTS


1.0 INTRODUCTION ..... 1
- 1.1 Overview of Bioavailability ..... 1
- 1.2 Using RBA Data to Improve Risk Calculations ..... 2
- 1.3 Purpose of this Study ..... 2

2.0 STUDY DESIGN ..... 3
- 2.1 Test Materials ..... 3
- 2.2 Experimental Animals ..... 3
- 2.3 Diet ..... 4
- 2.4 Dosing ..... 4
- 2.5 Collection and Preservation of Urine Samples ..... 4
- 2.6 Arsenic Analysis ..... 5
- 2.7 Quality Control ..... 5

3.0 Data Analysis ..... 7
- 3.1 Overview ..... 7
- 3.2 Dose-Response Model ..... 8
- 3.3 Calculation of RBA Estimates ..... 8

4.0 RESULTS ..... 9
- 4.1 Clinical Signs ..... 9
- 4.2 Dosing Deviations ..... 9
- 4.3 Background Arsenic Excretion ..... 9
- 4.4 Dose-Response Modeling ..... 9
- 4.5 Calculated RBA Values ..... 9
- 4.6 Uncertainty ..... 9
- 4.7 Treatment ..... 10
- 4.8 Data Analysis Variations ..... 10

5.0 REFERENCES ..... 11

# LIST OF TABLES

TABLE 2-1 Study Design and Dosing Information

TABLE 2-2 Group Assignments and Weight (kg)

TABLE 2-3 Urinary Arsenic Analytical Results and Urine Volumes for Study Samples

TABLE 2-4 Typical Feed Composition

TABLE 2-5 Laboratory Duplicates

TABLE 2-6 Blanks

TABLE 2-7 Blind Duplicate Samples

TABLE 2-8 Laboratory Quality Control Standards

TABLE 2-9 Laboratory Spikes

TABLE 2-10 Laboratory Control Standards

TABLE 4-1 Background Urinary Arsenic

TABLE 4-2 Final Results

TABLE 4-3 Day 6/7 Dose Response and Residual Plots

TABLE 4-4 Day 9/10 Dose Response and Residual Plots

TABLE 4-5 Day 11/12 Dose Response and Residual Plots

LIST OF FIGURES

FIGURE 3-1 Conceptual Model for Arsenic Toxicokinetics

# 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 |
| INAA | Instrumental Neutron Activation Analysis |
| 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 |
| 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 |
| °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 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 in risk calculations at test 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 six soils (EM05, EM13, EM15, EM20, RG01 and RG03) 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 five juvenile swine at one dose level 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

| Group Number | Test Material Name | Concentration mg/kg |
|---|---|---|
| 1 | EM05 | 1906 |
| 2 | EM13 | 1237 |
| 3 | EM15 | 12095 |
| 4 | EM20 | 5647 |
| 5 | RG01 | 200 |
| 6 | RG03 | 610 |

### 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 represented as part on Table 2-2).

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 Table 2-2.

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 2-4. Arsenic concentration in a randomly selected feed sample measured 3.4 ug/L.

Prior to the start of dosing and throughout the dosing period, each day 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 5 water samples from randomly selected drinking water nozzles were ≤2.5 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). Pigs 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 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 and throughout the study from 0-5, 6-9 and 10-13 days to calculate dose. 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 (Table 2-3) 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 $Ce^2l$ environmental laboratories (Lee's Summit, Missouri) by ICP-MS. In brief, all calibration standards, QC controls and samples were prepared for analysis at 1/10 dilutions. The dilutions were prepared with 2% $HNO_3$ and de-ionized water solution with Gallium as the internal standard at a concentration of 50 ug/L.

Analytical results for the urine samples are presented in Table 2-3.

## 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 summarized below.

<u>Blind Duplicates (Sample Preparation Replicates)</u>

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 Table 2-7. There was good agreement between results for the duplicate pairs.

<u>Laboratory Quality Control and Control Standards</u>

Laboratory low, medium and high controls as well as a laboratory control standard were tested periodically during sample analysis. Recovery of arsenic from these standards were good and within the acceptable range (Table 2-8 and Table 2-10).

<u>Laboratory Duplicates</u>

During analysis, every tenth sample was analyzed in duplicate. Duplicate results for urine samples (Table 2-5) typically agreed within 10% relative percent difference (RPD).

<u>Blanks</u>

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 2-6.

Spike Recovery

During analysis, one feed and water 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 (Table 2-9) show that mean arsenic concentrations recovered from spiked samples were within 10% of actual concentrations.

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

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).

<u>Goodness of Fit</u>

The goodness-of-fit of each dose-response model was assessed by using least squares regression in Excel. Goodness-of-fit was considered p less than 0.05.

### 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 ($b_r$):

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

## 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 no dose eaten (pig #769) on day zero PM of the study but did not affect data analysis.

There was a partially eaten (pig #779) on day one AM of the study but did not affect data analysis.

### 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. Mean urinary arsenic concentration was 135.9 +/- 57.4 µg/L. One control value was omitted from analysis due to being an outlier. Control pig #763 during urine collection day 6/7 appeared to be contaminated since it was analyzed at 658.8 ug total As/48 hours. 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 Dose-Response Modeling

The dose-response data for arsenic in urine were modeled using all of the data, and no outliers were identified (using methods discussed in Section 3.2). Modeling results are shown in Figures 4-3 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 through Table 4-5.

### 4.5 Calculated RBA Values

Estimated RBA values (mean and 95% confidence interval) are shown in Table 4-2.

### 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. 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.

## 4.7 Treatment

No pigs were treated with Naxcel during this study.

## 4.8 Data Analysis Variations

For the day 6/7 urine collection groups the control group only had two points due to a potentially contaminated control sample pig # 763.

## 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 3:113-11

## TABLE 2-1 Study Design and Dosing Information

| Group | Group Name Appreviation | Dose Material Administered | As Conc of the material (ug/g or ug/ul) | No. Pigs in Group | Target (ug/kg BW-day) | Actual[a] (ug/kg BW-day) | Acutal[b] (ug-day) Dose Prep (day 0-13) |
|---|---|---|---|---|---|---|---|
| 1 | TM1 | EM05 | 1906 | 5 | 60 | 60 | 913.2 |
| 2 | TM2 | EM13 | 1237 | 5 | 60 | 60 | 913.2 |
| 3 | TM3 | EM15 | 12095 | 5 | 60 | 60 | 913.2 |
| 4 | TM4 | EM20 | 5647 | 5 | 60 | 60 | 913.2 |
| 5 | TM5 | RG01 | 200 | 5 | 60 | 60 | 913.2 |
| 6 | TM6 | RG03 | 610 | 5 | 60 | 60 | 913.2 |
| 7 | AsAs | Sodium Arsenate | 10 | 5 | 50 | 50 | 761 |
| 8 | Control | 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.

Dose was 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 exposed interval (14 days).

**TABLE 2-2 Group Assignments and Weight (kg)**

| Group# / Dose | Animal Ear Tag | Day -5 | Group MBW | Day -1 | Group MBW | Day 2 | Group MBW | Day 5 | Group MBW | Day 8 | Group MBW | Day 11 | Group MBW | Day 13 | Group MBW |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 753 | 11.5 | | 11.9 | | 12.6 | | 13.3 | | 15 | | 16.7 | | 17.1 | |
| EM05 | 790 | 11 | | 12.2 | | 12.8 | | 13.4 | | 14.5 | | 16.2 | | 16.9 | |
| | 778 | 10.7 | | 10.8 | | 11.9 | | 12 | | 13 | | 14.4 | | 15.2 | |
| | 774 | 11.1 | | 11.7 | | 12 | | 13.6 | | 14.4 | | 16.5 | | 16.8 | |
| | 781 | 11.1 | 11.08 | 11.6 | 11.64 | 12.2 | 12.30 | 13.3 | 13.12 | 14.5 | 14.28 | 16.1 | 15.98 | 16.6 | 16.52 |
| 2 | 775 | 11.5 | | 12 | | 12.8 | | 13.6 | | 14.9 | | 16.8 | | 17.2 | |
| EM13 | 757 | 10.5 | | 11.3 | | 11.9 | | 13 | | 13.8 | | 15.2 | | 16.1 | |
| | 785 | 10.7 | | 10.8 | | 11.8 | | 11.5 | | 13.6 | | 15 | | 15.7 | |
| | 759 | 10.7 | | 11.2 | | 12.1 | | 13.2 | | 14.1 | | 16.1 | | 6.4 | |
| | 773 | 12 | 11.08 | 12.6 | 11.58 | 13.4 | 12.40 | 14.4 | 13.14 | 15 | 14.28 | 16.6 | 15.94 | 17.7 | 14.62 |
| 3 | 780 | 11 | | 11 | | 11.4 | | 12.5 | | 13.3 | | 14.8 | | 15.9 | |
| EM15 | 771 | 10 | | 10.6 | | 11.3 | | 12.3 | | 13 | | 14.9 | | 15.5 | |
| | 752 | 10.6 | | 11 | | 11.6 | | 12.6 | | 13.8 | | 15.3 | | 16.2 | |
| | 769 | 9.9 | | 10.5 | | 11.2 | | 12 | | 12.9 | | 13.6 | | 14.9 | |
| | 765 | 10.5 | 10.40 | 11.1 | 10.84 | 11.9 | 11.48 | 13.2 | 12.52 | 13.9 | 13.38 | 15.9 | 14.90 | 16.8 | 15.86 |
| 4 | 788 | 10.2 | | 10.5 | | 11.5 | | 12.4 | | 13.8 | | 15.3 | | 16 | |
| EM20 | 776 | 11.7 | | 12.5 | | 13.4 | | 13.6 | | 15.2 | | 17 | | 17.6 | |
| | 764 | 11 | | 11.5 | | 12.3 | | 12.9 | | 14.3 | | 15.2 | | 16.3 | |
| | 777 | 10.3 | | 10.5 | | 12.1 | | 13 | | 13.8 | | 15.1 | | 15.7 | |
| | 754 | 11.5 | 10.94 | 12.4 | 11.48 | 13.1 | 12.48 | 14.2 | 13.22 | 15.2 | 14.46 | 17 | 15.92 | 17.7 | 16.66 |
| 5 | 772 | 10.5 | | 12.1 | | 12.9 | | 13.6 | | 14.1 | | 16.2 | | 16.9 | |
| RG01 | 761 | 10.2 | | 11.8 | | 12.3 | | 13.1 | | 14.3 | | 15.8 | | 16.9 | |
| | 787 | 10 | | 11.4 | | 10.7 | | 12.2 | | 14 | | 15.5 | | 16.6 | |
| | 791 | 10.3 | | 11.2 | | 11.9 | | 12.5 | | 13.6 | | 15.2 | | 16 | |
| | 751 | 10.5 | 10.30 | 11.3 | 11.56 | 12.4 | 12.04 | 13 | 12.88 | 14.1 | 14.02 | 15.3 | 15.60 | 17 | 16.68 |
| 6 | 779 | 11.6 | | 12.7 | | 13.1 | | 14.4 | | 15.6 | | 16.8 | | 17.8 | |
| RG03 | 767 | 10.6 | | 11.6 | | 11.9 | | 13 | | 14 | | 16 | | 16.6 | |
| | 768 | 10.3 | | 11.2 | | 11.6 | | 13 | | 13.9 | | 15.5 | | 16.3 | |
| | 760 | 11.3 | | 12.2 | | 12.8 | | 14 | | 14.9 | | 16.3 | | 17.1 | |
| | 755 | 10.7 | 10.90 | 11.2 | 11.78 | 12 | 12.28 | 13.6 | 13.60 | 14.4 | 14.56 | 16.3 | 16.18 | 17 | 16.96 |
| 7 | 789 | 10.1 | | 11.6 | | 12.5 | | 12.2 | | 12.8 | | 14.5 | | 14.8 | |
| NaAs | 782 | 10.2 | | 11.6 | | 12.3 | | 13.3 | | 13.9 | | 15.9 | | 16.8 | |
| | 770 | 10.8 | | 11.3 | | 12.4 | | 13 | | 13.8 | | 16.1 | | 17.1 | |
| | 758 | 10.8 | | 11.1 | | 12.2 | | 13.3 | | 13.9 | | 16 | | 16.5 | |
| | 786 | 9.9 | 10.36 | 10.1 | 11.14 | 11.2 | 12.12 | 12 | 12.76 | 12.7 | 13.42 | 13.2 | 15.14 | 13.8 | 15.80 |
| 8 | 756 | 11.8 | | 12.6 | | 13.9 | | 14.3 | | 14.3 | | 16.2 | | 16.9 | |
| Control | 783 | 11.3 | | 11.8 | | 12.8 | | 13.6 | | 14.5 | | 16.4 | | 17 | |
| | 763 | 10.6 | 11.23 | 10.8 | 11.73 | 11.7 | 12.80 | 12.2 | 13.37 | 13.3 | 14.03 | 14.5 | 15.70 | 15.9 | 16.60 |

**TABLE 2-3 Urinary Arsenic Analytical Results and Urine Volumes for Study Samples**

| | Pig ID | U-1 Days 6-7 | | | U-2 Days 9-10 | | | U-3 Days 12-13 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Vol (ml) | Sample ID | Urine As (ug/L) | Vol (ml) | Sample ID | Urine As (ug/L) | Vol (ml) | Sample ID | Urine As (ug/L) |
| 1 | 753 | 6380 | CAEM2-001 | 55.8 | 4200 | CAEM2-039 | 4.2 | 5100 | CAEM2-077 | 5.1 |
| 1 | 790 | 3540 | CAEM2-002 | 110 | 1760 | CAEM2-040 | 1.76 | 1740 | CAEM2-078 | 1.74 |
| 1 | 778 | 4220 | CAEM2-003 | 79.4 | 7200 | CAEM2-041 | 7.2 | 6940 | CAEM2-079 | 6.94 |
| 1 | 774 | 32200 | CAEM2-004 | 9.46 | 15740 | CAEM2-042 | 15.74 | 17340 | CAEM2-080 | 17.34 |
| 1 | 781 | 3160 | CAEM2-005 | 105 | 5360 | CAEM2-043 | 5.36 | 3960 | CAEM2-081 | 3.96 |
| 2 | 775 | 1560 | CAEM2-006 | 138 | 3940 | CAEM2-044 | 3.94 | 4880 | CAEM2-082 | 4.88 |
| 2 | 757 | 1000 | CAEM2-007 | 220 | 1180 | CAEM2-045 | 1.18 | 7920 | CAEM2-083 | 7.92 |
| 2 | 785 | 2080 | CAEM2-008 | 212 | 2780 | CAEM2-046 | 2.78 | 3020 | CAEM2-084 | 3.02 |
| 2 | 759 | 4620 | CAEM2-009 | 78.6 | 8540 | CAEM2-047 | 8.54 | 7400 | CAEM2-085 | 7.4 |
| 2 | 773 | 4000 | CAEM2-010 | 83.3 | 6380 | CAEM2-048 | 6.38 | 3900 | CAEM2-086 | 3.9 |
| 3 | 780 | 820 | CAEM2-011 | 648 | 800 | CAEM2-049 | 0.8 | 980 | CAEM2-087 | 0.98 |
| 3 | 771 | 5360 | CAEM2-012 | 60.1 | 6200 | CAEM2-050 | 6.2 | 4000 | CAEM2-088 | 4 |
| 3 | 752 | 1440 | CAEM2-013 | 211 | 1120 | CAEM2-051 | 1.12 | 1520 | CAEM2-089 | 1.52 |
| 3 | 769 | 660 | CAEM2-014 | 635 | 540 | CAEM2-052 | 0.54 | 1000 | CAEM2-090 | 1 |
| 3 | 765 | 2800 | CAEM2-015 | 198 | 3940 | CAEM2-053 | 3.94 | 5540 | CAEM2-091 | 5.54 |
| 4 | 788 | 1900 | CAEM2-016 | 275 | 4135 | CAEM2-054 | 4.135 | 7900 | CAEM2-092 | 7.9 |
| 4 | 776 | 2740 | CAEM2-017 | 162 | 2200 | CAEM2-055 | 2.2 | 2072 | CAEM2-093 | 2.072 |
| 4 | 764 | 1780 | CAEM2-018 | 245 | 1900 | CAEM2-056 | 1.9 | 2520 | CAEM2-094 | 2.52 |
| 4 | 777 | 4000 | CAEM2-019 | 108 | 5360 | CAEM2-057 | 5.36 | 7900 | CAEM2-095 | 7.9 |
| 4 | 754 | 1520 | CAEM2-020 | 357 | 1460 | CAEM2-058 | 1.46 | 1640 | CAEM2-096 | 1.64 |
| 5 | 772 | 2148 | CAEM2-021 | 143 | 1300 | CAEM2-059 | 1.3 | 900 | CAEM2-097 | 0.9 |
| 5 | 761 | 2115 | CAEM2-022 | 157 | 3860 | CAEM2-060 | 3.86 | 3180 | CAEM2-098 | 3.18 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 5 | 787 | 2700 | CAEM2-023 | 125 | 3180 | CAEM2-061 | 3.18 | 4150 | CAEM2-099 | 4.15 |
| 5 | 791 | 3080 | CAEM2-024 | 78 | 1220 | CAEM2-062 | 1.22 | 2150 | CAEM2-100 | 2.15 |
| 5 | 751 | 4640 | CAEM2-025 | 46.4 | 3420 | CAEM2-063 | 3.42 | 3880 | CAEM2-101 | 3.88 |
| 6 | 779 | 4720 | CAEM2-026 | 79.3 | 4220 | CAEM2-064 | 4.22 | 5820 | CAEM2-102 | 5.82 |
| 6 | 767 | 30200 | CAEM2-027 | 7.39 | 3680 | CAEM2-065 | 3.68 | 3080 | CAEM2-103 | 3.08 |
| 6 | 768 | 5380 | CAEM2-028 | 65 | 5200 | CAEM2-066 | 5.2 | 3500 | CAEM2-104 | 3.5 |
| 6 | 760 | 5740 | CAEM2-029 | 41.1 | 7760 | CAEM2-067 | 7.76 | 5360 | CAEM2-105 | 5.36 |
| 6 | 755 | 4240 | CAEM2-030 | 73.6 | 5100 | CAEM2-068 | 5.1 | 6000 | CAEM2-106 | 6 |
| **7** | 789 | 1460 | CAEM2-031 | 870 | 2580 | CAEM2-069 | 2.58 | 3100 | CAEM2-107 | 3.1 |
| **7** | 782 | 4800 | CAEM2-032 | 371 | 5620 | CAEM2-070 | 5.62 | 4400 | CAEM2-108 | 4.4 |
| **7** | 770 | 7260 | CAEM2-033 | 251 | 4200 | CAEM2-071 | 4.2 | 2800 | CAEM2-109 | 2.8 |
| **7** | 758 | 5040 | CAEM2-034 | 356 | 2520 | CAEM2-072 | 2.52 | 1100 | CAEM2-110 | 1.1 |
| **7** | 786 | 6360 | CAEM2-035 | 194 | 7280 | CAEM2-073 | 7.28 | 10020 | CAEM2-111 | 10.02 |
| 8 | 756 | 1800 | CAEM2-036 | 61.8 | 1920 | CAEM2-074 | 1.92 | 1560 | CAEM2-112 | 1.56 |
| 8 | 783 | 540 | CAEM2-037 | 38.9 | 6820 | CAEM2-075 | 6.82 | 5920 | CAEM2-113 | 5.92 |
| 8 | 763 | 3660 | CAEM2-038 | 180 | 440 | CAEM2-076 | 0.44 | 500 | CAEM2-114 | 0.5 |

**TABLE 2-4 Typical Feed Composition**

University Feed Mill, Grower Feed
Manufactured: 08/13/2012

| Ingredient | Amount (lbs) |
|---|---|
| Purex Salt | 6 |
| Corn | 1,528 |
| 48% Soybean Meal | 350 |
| Fat | 50 |
| Limestone | 18 |
| L-Lysine | 3 |
| Swine Vit. NB 6104 | 4 |
| Swine Min. NB-8536 | 3 |
| Zinpro-100 | 2 |
| Biotin | 2 |
| Dical | 34 |

## TABLE 2-5 Laboratory Duplicates

| Blind Duplicate Sample ID | QC Batch Sample | Sample Type | Original Sample Concentration | Duplicate Sample concentration | Sample Units | RPD |
|---|---|---|---|---|---|---|
| CAEM2-006 | P211017 | Urine | 138 | 131 | ug/L | 3.4 |
| CAEM2-020 | P211018 | Urine | 357 | 352 | ug/L | 0.9 |
| CAEM2-031 | P211019 | Urine | 870 | 836 | ug/L | 2.6 |
| CAEM2-041 | P211020 | Urine | 42.3 | 43.9 | ug/L | 2.5 |
| CAEM2-051 | P211021 | Urine | 394 | 390 | ug/L | 0.7 |
| CAEM2-061 | P211022 | Urine | 104 | 102 | ug/L | 1.3 |
| CAEM2-071 | P211023 | Urine | 407 | 405 | ug/L | 0.3 |
| CAEM2-081 | P211024 | Urine | 147 | 146 | ug/L | 0.5 |
| CAEM2-091 | P211025 | Urine | 102 | 102 | ug/L | 0 |
| CAEM2-115 | P211026 | Urine | ND | ND | ug/L | * |
| CAEM2-110 | P211027 | Urine | 1560 | 1570 | ug/L | 0.4 |
| CAEM2-121 | P211028 | Urine | 2380 | 2380 | ug/L | 0 |
| CAEM2-130 | P211029 | Urine | 1570 | 1570 | ug/L | 0 |
| CAEM2-002* | P211050[a] | Urine | 110 | 109 | ug/L | 0.6 |

*indicates % Deviation not calculated

[a] Blind Duplicate from an additional analysis of nine samples that were missed during the first analysis

## TABLE 2-6 Blanks

| Sample ID | QC Sample Batch | Associated Sample Type | Measured Concentration | Detection Limit | Units |
|---|---|---|---|---|---|
| Blank-1 | P211017 | Urine | ND | 2.50 | ug/L |
| Blank-2 | P211018 | Urine | 3.44[a] | 2.50 | ug/L |
| Blank-3 | P211019 | Urine | 4.06[a] | 2.50 | ug/L |
| Blank-4 | P211020 | Urine | 3.89[a] | 2.50 | ug/L |
| Blank-5 | P211021 | Urine | 3.71[a] | 2.50 | ug/L |
| Blank-6 | P211022 | Urine | 3.82[a] | 2.50 | ug/L |
| Blank-7 | P211023 | Urine | ND | 2.50 | ug/L |
| Blank-8 | P211024 | Urine | ND | 2.50 | ug/L |
| Blank-9 | P211025 | Urine | ND | 2.50 | ug/L |
| Blank-10 | P211026 | Urine | ND | 2.50 | ug/L |
| Blank-11 | P211027 | Urine | ND | 2.50 | ug/L |
| Blank-12 | P211028 | Urine | ND | 2.50 | ug/L |
| Blank-13 | P211029 | Urine | ND | 2.50 | ug/L |
| Blank-14* | P211050 | Urine | ND | 2.50 | ug/L |

[a]The Method Blank for sample batches 211018. 211019, 211020, 211021 and 211022 displayed arsenic above the detection limit but below the reporting limit. Sample results for these batches should be considered as valid data as arsenic was not detected above the reporting limit in associated method blanks.

*Method blank from an additional analysis of nine samples that were missed during the first analysis.

**TABLE 2-7 Blind Duplicate Samples**

| Blind Duplicate Sample ID | Sample Type | Pig Number | Collection Days | Original Sample Concentration | Duplicate Sample concentration | Sample Units | RPD |
|---|---|---|---|---|---|---|---|
| CAEM2-122 | Urine | 771 | U-1 | 60.1 | 62.7 | ug/L | 4.2 |
| CAEM2-123 | Urine | 755 | U-1 | 73.6 | 74.8 | ug/L | 1.6 |
| CAEM2-124 | Urine | 783 | U-1 | 38.9 | 38.6 | ug/L | 0.8 |
| CAEM2-125 | Urine | 790 | U-2 | 258 | 284 | ug/L | 9.6 |
| CAEM2-126 | Urine | 777 | U-2 | 89.6 | 101 | ug/L | 12.0 |
| CAEM2-127 | Urine | 761 | U-2 | 65.7 | 70.9 | ug/L | 7.6 |
| CAEM2-128 | Urine | 785 | U-3 | 133 | 147 | ug/L | 10.0 |
| CAEM2-129 | Urine | 765 | U-3 | 102 | 100 | ug/L | 2.0 |
| CAEM2-130 | Urine | 758 | U-3 | 1560 | 1570 | ug/L | 0.6 |

**TABLE 2-8 Laboratory Quality Control Standards**

| Sample concentration | Sample ID | Associated Sample Type | Measured Concentration | Units | Recovery |
|---|---|---|---|---|---|
| Low Control- 10ug/L | QC-1 | Urine | 13.361 | ug/L | 133.6 |
| | QC-2 | Urine | 12.389 | ug/L | 123.9 |
| | QC-3 | Urine | 12.727 | ug/L | 127.3 |
| | QC-4 | Urine | 13.123 | ug/L | 131.2 |
| | QC-5 | Urine | 13.804 | ug/L | 138.0 |
| | QC-6[a] | Urine | 14.085 | ug/L | 140.8 |
| | QC-7 | Urine | 10.039 | ug/L | 100.4 |
| | QC-8 | Urine | 11.393 | ug/L | 113.9 |
| | QC-9 | Urine | 12.244 | ug/L | 122.4 |
| | QC-10 | Urine | 12.07 | ug/L | 120.7 |
| | QC-11 | Urine | 10.892 | ug/L | 108.9 |
| | QC-12 | Urine | 12.235 | ug/L | 122.4 |
| | QC-1[*] | Urine | 11.081 | ug/L | 110.8 |
| | QC-2[*] | Urine | 10.65 | ug/L | 106.5 |
| Mid Control- 50ug/L | QC-1 | Urine | 55.56 | ug/L | 111.1 |
| | QC-2 | Urine | 54.569 | ug/L | 109.1 |
| | QC-3 | Urine | 54.795 | ug/L | 109.6 |
| | QC-4 | Urine | 50.947 | ug/L | 101.9 |
| | QC-5 | Urine | 50.591 | ug/L | 101.2 |
| | QC-6 | Urine | 50.935 | ug/L | 101.9 |
| | QC-7 | Urine | 50.147 | ug/L | 100.3 |
| | QC-8 | Urine | 49.893 | ug/L | 99.8 |
| | QC-9 | Urine | 50.207 | ug/L | 100.4 |
| | QC-10 | Urine | 51.855 | ug/L | 103.7 |
| | QC-11 | Urine | 50.634 | ug/L | 101.3 |
| | QC-12 | Urine | 49.591 | ug/L | 99.2 |
| | QC-1[*] | Urine | 48.445 | ug/L | 96.9 |
| | QC-2[*] | Urine | 47.583 | ug/L | 95.2 |
| High Control- 200ug/L | QC-1 | Urine | 207.046 | ug/L | 103.5 |
| | QC-2 | Urine | 204.954 | ug/L | 102.5 |
| | QC-3 | Urine | 206.94 | ug/L | 103.5 |
| | QC-4 | Urine | 210.027 | ug/L | 105.0 |
| | QC-5 | Urine | 206.351 | ug/L | 103.2 |
| | QC-6 | Urine | 209.35 | ug/L | 104.7 |
| | QC-7 | Urine | 203.469 | ug/L | 101.7 |
| | QC-8 | Urine | 206.423 | ug/L | 103.2 |
| | QC-9 | Urine | 208.509 | ug/L | 104.3 |
| | QC-10 | Urine | 209.999 | ug/L | 105.0 |
| | QC-11 | Urine | 202.629 | ug/L | 101.3 |
| | QC-12 | Urine | 205.753 | ug/L | 102.9 |
| | QC-1[*] | Urine | 203.191 | ug/L | 101.6 |
| | QC-2[*] | Urine | 20.114 | ug/L | 101.6 |

[*]Quality Control from an additional analysis of nine samples that were missed during the first analysis.

[a]The sixth low-level quality control check was outside of QA limits of +/- 40%. Recovery was at 140.85%. Mid-level and high-level quality control was within QA limits for the sixth QC check.

**TABLE 2-9 Laboratory Spikes**

| Spike Sample ID | Sample Type | QC Sample Batch | Added Spike Concentration (ug/L) | Measured Sample Concentration (ug/L) | Recovery |
|---|---|---|---|---|---|
| Spike-1 | Urine | P211017 | 200 | 319 | 107 |
| Spike-2 | Urine | P211018 | 200 | 321 | 107 |
| Spike-3 | Urine | P211019 | 200 | 276 | 101 |
| Spike-4 | Urine | P211020 | 200 | 485 | 113 |
| Spike-5 | Urine | P211021 | 200 | 282 | 99 |
| Spike-6 | Urine | P211022 | 200 | 273 | 103 |
| Spike-7 | Urine | P211023 | 200 | 709 | 106 |
| Spike-8 | Urine | P211024 | 200 | 216 | 97 |
| Spike-9 | Urine | P211025 | 200 | 736 | 81 |
| Spike-10 | Urine | P211026 | 200 | 348 | 106 |
| Spike-11 | Urine | P211027 | 200 | 189 | 94 |
| Spike-12 | Urine | P211028 | 200 | 874 | 93 |
| Spike-13 | Urine | P211029 | 200 | 224 | 111 |
| Spike-14* | Urine | P211050 | 200 | 251 | 98 |

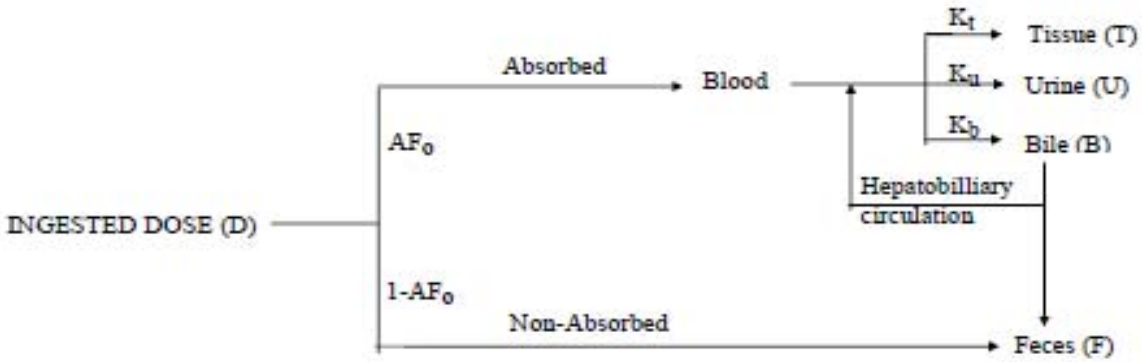
*Quality Control from an additional analysis of nine samples that were missed during the first analysis.

**TABLE 2-10 Laboratory Control Standards (LCS)**

| LCS | QC Batch | Amt. Spiked ug/L | LCS ug/L | % Recovery | LCS Duplicate | % Recovery | RPD |
|---|---|---|---|---|---|---|---|
| LCS-1 | P211017 | 50 | 54.8 | 110 | 54.8 | 110 | 0.04 |
| LCS-2 | P211018 | 50 | 52.6 | 105 | 54.1 | 108 | 3 |
| LCS-3 | P211019 | 50 | 55.5 | 111 | 55.7 | 111 | 0.3 |
| LCS-4 | P211020 | 50 | 54.1 | 108 | 54.1 | 108 | 0.2 |
| LCS-5 | P211021 | 50 | 52.9 | 106 | 56.9 | 114 | 7 |
| LCS-6 | P211022 | 50 | 55.8 | 112 | 55.8 | 112 | 0.06 |
| LCS-7 | P211023 | 50 | 49.7 | 99 | 51.1 | 102 | 3 |
| LCS-8 | P211024 | 50 | 53.9 | 108 | 52.4 | 105 | 3 |
| LCS-9 | P211025 | 50 | 50.9 | 102 | 51.5 | 103 | 1 |
| LCS-10 | P211026 | 50 | 48.9 | 98 | 48.9 | 98 | 0.1 |
| LCS-11 | P211027 | 50 | 53.5 | 107 | 51.5 | 103 | 4 |
| LCS-12 | P211028 | 50 | 50.6 | 101 | 50.4 | 101 | 0.5 |
| LCS-13 | P211029 | 50 | 50.5 | 101 | 50.1 | 100 | 0.8 |
| LCS-14[a] | P211050* | 50 | 49.3 | 99 | 48.9 | 98 | 0.8 |

*Quality Control from an additional analysis of nine samples that were missed during the first analysis.

## 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

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

**TABLE 4-1 Background Urinary Arsenic**

| Pig Number | Urine Control Period (days) | As Dose (ug per collection period) | Urine Volume (ml) | Total As Excreted (ug/48 hours) |
|---|---|---|---|---|
| 756 | 6/7 | **0** | 1800 | 111.24 |
| 783 | 6/7 | **0** | 540 | 21.01 |
| 763 | 6/7 | **0** | 3660 | 658.8* |
| 756 | 9/10 | **0** | 1920 | 97.15 |
| 783 | 9/10 | **0** | 6820 | 127.53 |
| 763 | 9/10 | **0** | 440 | 111.76 |
| 756 | 12/13 | **0** | 1560 | 215.28 |
| 783 | 12/13 | **0** | 5920 | 169.31 |
| 763 | 12/13 | **0** | 500 | 234 |

***This data point was not used in the determination of RBA. Determined to be a contaminated sample.**

## Table 4-2 Final Results

### ESTIMATED RBA FOR ASARCO AND HAWAII SOILS

| Test Material | 95% Confidence Interval | | | |
|---|---|---|---|---|
| | **RBA Day 6/7** | **RBA Day 9/10** | **RBA day 12/13** | **All Days** |
| EM05 | 15.3 (13.1-17.4) | 15.4 (12.6-18.2) | 15.3 (6.0-24.6) | 15.3 (15.22-15.5) |
| EM13 | 13.7 (7.1-20.3) | 14.7 (12.5-16.9) | 9.1 (4.9-13.4) | 12.5 (5.1-19.9) |
| EM15 | 19.8 (11.9-27.7) | 22.2 (17.2-27.2) | 17.0 (12.4-21.5) | 19.7 (13.1-26.2) |
| EM20 | 22.5 (18.9-26.1) | 22.1 (24.4-19.8) | 23.5 (17.1-29.8) | 22.7 (21.1-24.3) |
| RG01 | 12.1 (8.3-15.9) | 13.5 (10.5-16.6) | 9.7 (2.3-17.0) | 11.8 (6.9-16.6) |
| RG03 | 12.8 (8.2-17.4) | 14.06 (11.3-16.8) | 10.3 (6.0-14.6) | 12.4 (7.6-17.2) |

All dose-response models were assessed with the regression function in Excel. Goodness of fit was considered acceptable if the p-value was less than 0.05.

**TABLE 4-3 Day 6/7 Dose Response and Residual Plots**

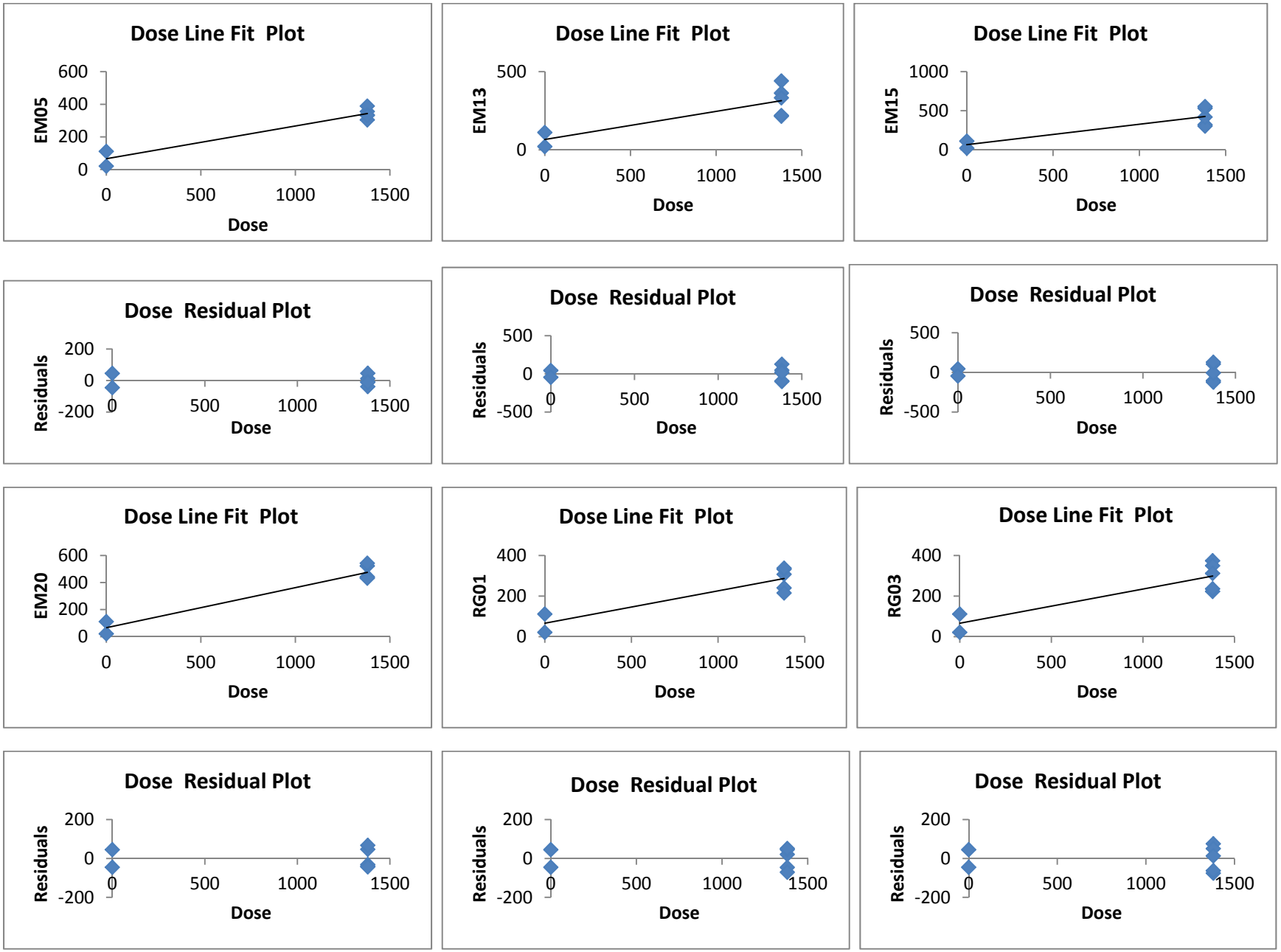


**TABLE 4-4 Day 9/10 Dose Response and Residual Plots**

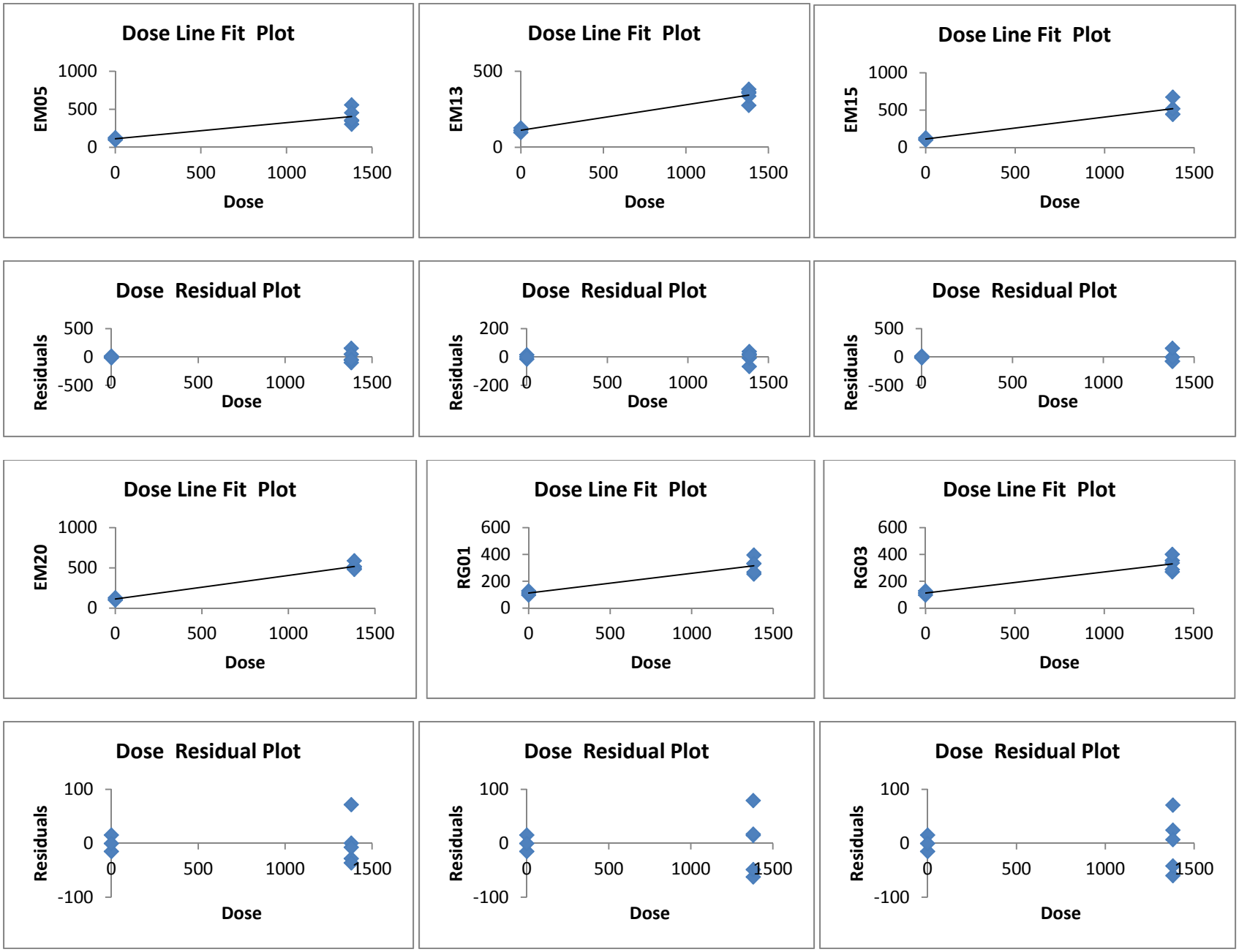
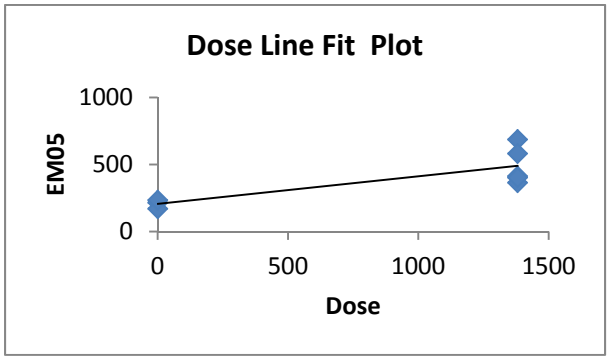
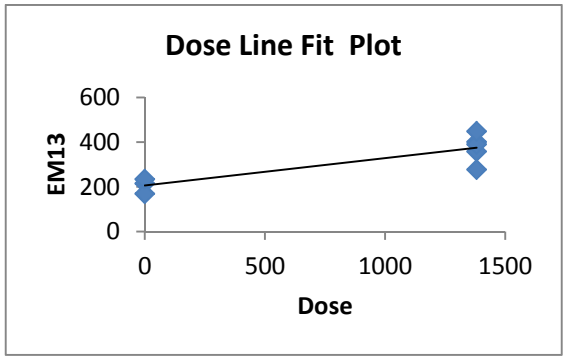
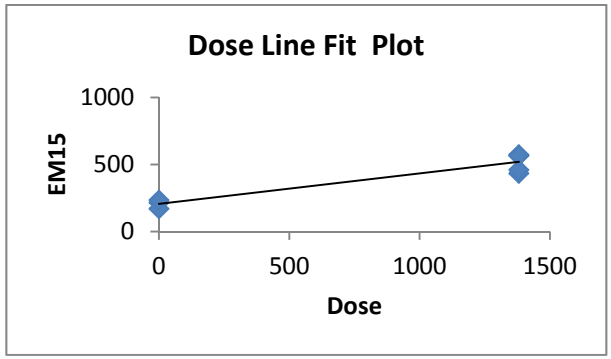
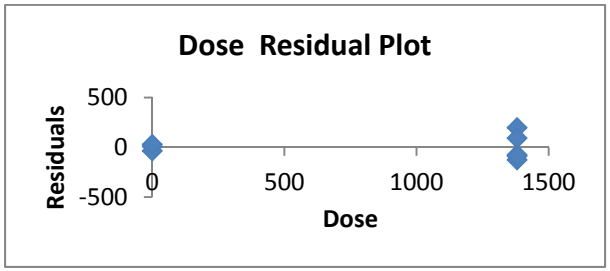


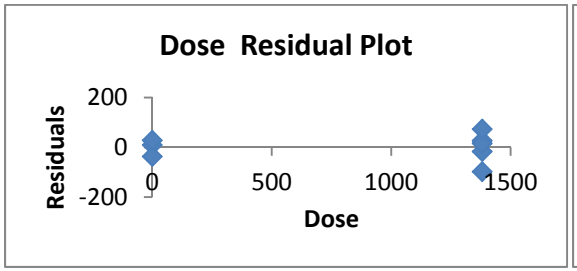
**TABLE 4-5 Day 12/13 Dose Response and Residual Plots**

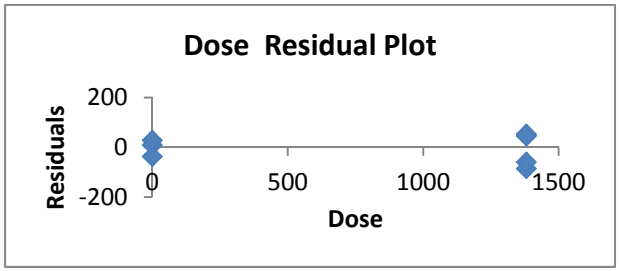


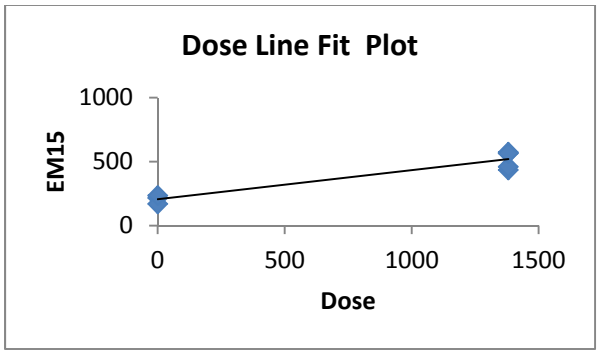



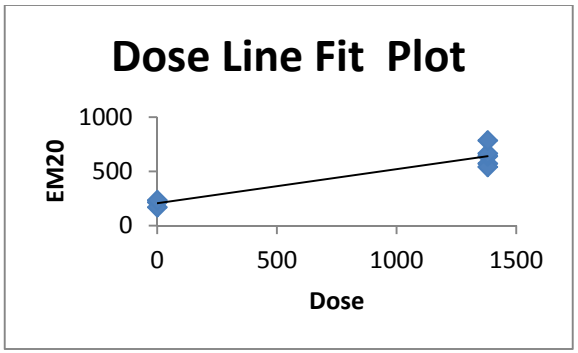



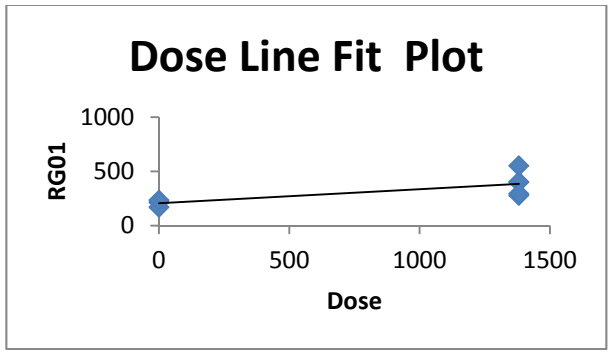



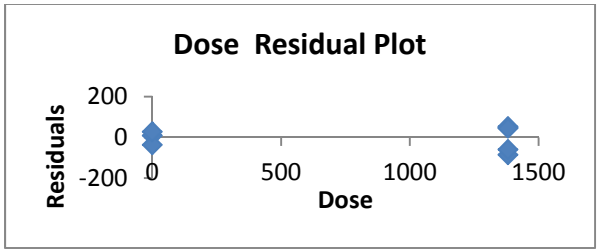



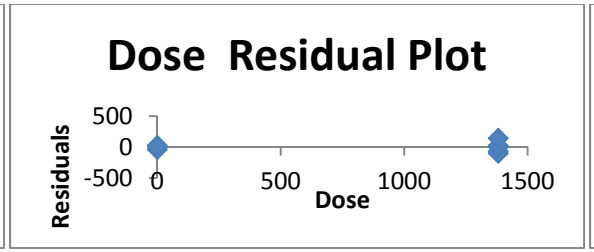



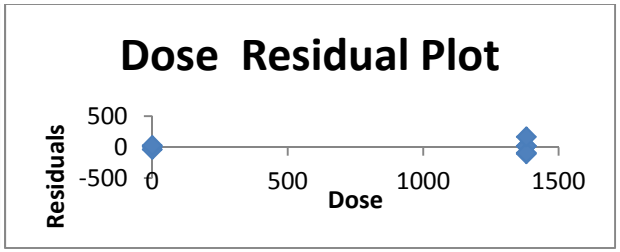



# CAEM3
## RELATIVE BIOAVAILABILITY OF ARSENIC FOR CALIFORNIA DTSC SOIL STUDY

Prepared for:

California Department of Toxic Substance Control

Prepared by:
Stan. W. Casteel, DVM, PhD, DABVT
Trish Parsons, PhD
Margaret Dunsmore, BS

March 30-April 5, 2014

## EXECUTIVE SUMMARY

A study using juvenile swine as test animals was performed to measure the gastrointestinal absorption of arsenic from six selected soils for the California DTSC. The arsenic concentrations of the test materials were as follows:

| Sample Name | Test Material Abbreviation | Study Group ID | As Conc (ug/g) |
|---|---|---|---|
| MC-02-0-1 | MC2 | TM1 | 603 |
| MC-03-0-03 | MC3 | TM2 | 641 |
| CE-01-pile | CE1 | TM3 | 753 |
| 13MGE_WR33 | WR33 | TM4 | 6681 |
| 13CM_T81 | T81 | TM5 | 205 |
| IM-01-0-03 | IM01 | TM6 | 731 |

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

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 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 CALIFORNIA DTSC SOILS**

| **Test Material** | 90% Confidence Interval | | | |
|---|---|---|---|---|
| | **RBA Day 6/7** | **RBA Day 9/10** | **RBA day 12/13** | **All Days** |
| MC2 | 0.01 (-0.01 - 0.03) | 0.02 (0.01 - 0.03) | 0.00 (-0.02 - 0.02) | 0.01 (0.01 - 0.02) |
| MC3 | 0.11 (0.04 - 0.21) | 0.13 (0.07 - 0.21) | 0.05 (0.01 - 0.11) | 0.09 (0.06 - 0.13) |
| CE1 | 0.37 (0.22 - 0.59) | 0.42 (0.27 - 0.62) | 0.34 (0.23 - 0.49) | 0.38 (0.30 - 0.47) |
| WR33 | 0.21 (0.12 - 0.36) | 0.21 (0.11 - 0.35) | 0.08 (0.03 - 0.15) | 0.14 (0.10 - 0.19) |
| T81 | 0.07 (0.01 - 0.15) | 0.13 (0.06 - 0.22) | 0.06 (0.02 - 0.13) | 0.08 (0.05 - 0.12) |
| IM01 | 0.04 (0.02 - 0.07) | 0.07 (0.04 - 0.12) | 0.07 (0.02 - 0.13) | 0.06 (0.04 - 0.08) |

All dose-response models were assessed with the regression function in Excel. Goodness of fit was considered acceptable if the p-value was less than 0.05.

# TABLE OF CONTENTS


1.0 INTRODUCTION ..... 1
- 1.1 Overview of Bioavailability ..... 1
- 1.2 Using RBA Data to Improve Risk Calculations ..... 2
- 1.3 Purpose of this Study ..... 2

2.0 STUDY DESIGN ..... 3
- 2.1 Test Materials ..... 3
- 2.2 Experimental Animals ..... 3
- 2.3 Diet ..... 4
- 2.4 Dosing ..... 4
- 2.5 Collection and Preservation of Urine Samples ..... 5
- 2.6 Arsenic Analysis ..... 5
- 2.7 Analytical Quality Control ..... 5

3.0 Data Analysis ..... 6
- 3.1 Overview ..... 6
- 3.2 Dose-Response Model ..... 7
- 3.3 Calculation of RBA Estimates ..... 7

4.0 RESULTS ..... 8
- 4.1 Clinical Signs ..... 8
- 4.2 Dosing Deviations ..... 8
- 4.3 Background Arsenic Excretion ..... 8
- 4.4 Calculated RBA Values ..... 8
- 4.5 Uncertainty ..... 9
- 4.6 Treatment ..... 9
- 4.7 Data Analysis Variations ..... 9

5.0 REFERENCES ..... 10

# LIST OF TABLES

TABLE 2-1 Study Design and Dosing Information

TABLE 2-2 Group Assignments and Weight (kg)

TABLE 2-3 Mean Body Weight by Groups (Graph)

TABLE 2-4 Urine Collection Volumes

TABLE 2-5 Urinary Arsenic Analytical Results and Urine Volumes for Study Samples

TABLE 2-6 Typical Feed Composition

TABLE 2-7 Doughball Feed Composition

TABLE 4-1 Background Urinary Arsenic

TABLE 4-3 All Days (6/7, 9/10, 11/12) Dose Response and Residual Plots

LIST OF FIGURES

FIGURE 3-1 Conceptual Model for Arsenic Toxicokinetics

# 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 |
| INAA | Instrumental Neutron Activation Analysis |
| 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 |
| 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 |
| °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 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 in risk calculations at test 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 six soils (MC2, MC3, CE1, WR33, T81 and IM01) 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 five juvenile swine at one dose level for 14 days. The study included a non-treated group of three animals to serve as a control for determining background arsenic levels. Study design DTSC 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

| Sample Name | Test Material Abbreviation | Study Group ID | As Conc (ug/g) |
|---|---|---|---|
| MC-02-0-1 | MC2 | TM1 | 603 |
| MC-03-0-03 | MC3 | TM2 | 641 |
| CE-01-pile | CE1 | TM3 | 753 |
| 13MGE_WR33 | WR33 | TM4 | 6681 |
| 13CM_T81 | T81 | TM5 | 205 |
| IM-01-0-03 | IM01 | TM6 | 731 |

### 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 supplied by the University of Missouri-Columbia South Farm swine operations.

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 4-6 weeks (weaning occurs at age 3 weeks) with an approximate receipt weight of 10-12 kilograms. The animals were acclimated to animal room conditions and feed ration for one week before beginning exposure to dosing materials. On Day-5 of the study, animals were randomly assigned to dose groups (group assignments are represented as part of Table 2-2). Animals that appeared to be in poor health at this time (first criteria) or extremely different from the mean body weight of all animals (second criteria) were excluded from the study.

Animals were housed individually in stainless steel metabolism cages with screen and chutes that allowed for collection of urine without fecal contamination. Enrichment for the animals was provided by lengths of plastic chain suspended in the cage. The study protocol was University of Missouri Animal Care and Use Committee approved and in compliance with the provisions of the Animal Welfare Act.

Exposure began on Day 0, at which time the animals were about 5-7 weeks old. The animals were weighed pre-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 Table 2-2.

Animal health and husbandry was monitored daily by study personnel and an ACUC representative. Any animal experiencing illness was examined by a DVM and treated appropriately and in a manner as to not invalidate study results. At study termination animals were euthanized humanely in accordance with AVMA guidelines under supervision of a licensed DVM.

## 2.3 Diet

Animals were weaned onto standard age-appropriate 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 2-4. Arsenic concentration in a randomly selected feed sample measured less than the analytical detection limit of 5µg/L.

Prior to the start of dosing and throughout the dosing period, each animal was given individually 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 provided 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 2 water samples from randomly selected drinking water nozzles was below the analytical detection limit of 5 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). Pigs 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 (about 5grams) and the dough was pinched shut. Doughballs were prepared by mixing a powdered swine diet (Purina Test Diet® Porcine Grower Purified Diet w/Low Lead, Table 2-7) with water to a malleable consistency. This dose was then placed in the feeder at dosing time and the pig was observed to ensure the dose was consumed. Doughballs were sized so the pig could consume them in a single bite.

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 based on the mean weight of the animals in each group and were adjusted every three days to account for weight gain.

Any missed or late doses were recorded and the actual doses adjusted accordingly, if needed.

## 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. 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 (Table 2-3) and two 60-mL portions were removed and acidified with 0.6 ml concentrated nitric acid. All samples were refrigerated. One of the aliquots was archived and one aliquot was sent for arsenic analysis. Refrigeration was maintained until arsenic analysis.

## 2.6 Arsenic Analysis

Urine samples were assigned chain-of-custody tag numbers and submitted to the analytical laboratory for analysis in a blind fashion. The samples were analyzed for arsenic by $Ce^2l$ environmental laboratories (Lee's Summit, Missouri) by ICP-MS. In brief, all calibration standards, QC controls and samples were prepared for analysis at 1/10 dilutions. The dilutions were prepared with 2% $HNO_3$ and de-ionized water solution with Gallium as the internal standard at a concentration of 50 ug/L.

Analytical results for the urine samples are presented in Table 2-3.

## 2.7 Analytical Quality Control

A number of quality control (QC) steps were taken during the sample analysis to ensure the accuracy of the analytical procedures. The results for QC samples are summarized below.

<u>Analytical Replicates</u>

The instrument (ICP-MS) does a triple reading for each sample and averages them. Therefore no technical replicates other than the quality control samples.

<u>Blank and Quality Control</u>

Blanks urine supplied from control pigs was used as an analytical blank and to prepare the standard curve samples and QC samples. Analytical 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).

Arsenic standard material was Ultra purchased from Inorganic Ventures and was used to prepare the standards and QC material. Quality control samples were run as a QC and a QC duplicate every 20 samples. They had to be within 20% agreement (duplicates were completely separate preps) and none of the QC samples or sample duplicates failed.

<u>Summary of QC Results</u>

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

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).

<u>Goodness of Fit</u>

The goodness-of-fit of each dose-response model was assessed by using least squares regression in Excel. Goodness-of-fit was considered p less than 0.05.

### 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 ($b_r$):

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

## 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

In the first few days of dosing, a few (3-5) pigs randomly scattered through the groups did not consume their dose either completely or promptly at an AM or PM dosing. By Study Day 4 all pigs were consuming their doses promptly and completely, so no adjustments to dose versus arsenic excreted were needed for incomplete dose consumption.

### 4.3 Background Arsenic Excretion

Measured values for urinary arsenic excretion for control animals U-1, U-2 and U-3 are shown in Table 4-1. The majority of the control urine samples collected were less than the analytical detection limit of 5µg/L. Only 3 pigs had a detectable level of arsenic in their urine: U-1, 746 was 23.7µg/L; U-3, 846 was 44.8µg/L and 955 was 30.7µg/L. Mean urinary arsenic concentration calculated with a value of 2.5µg/L for non-detects was 17.9µg/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 Calculated RBA Values

The dose-response data for arsenic in urine were modeled using all of the data, and no outliers were identified (using methods discussed in Section 3.2). Results based on the consolidated data from all collection days (days 6/7, 9/10 and 12/13) are shown in Figure 4.3.

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 relative bioavailability of arsenic in a specific test material is calculated as follows:

$$RBA_{(test\ vs\ ref)} = UEF\ (test) / UEF\ (ref)$$

The following table summarizes the estimated RBA values (mean and 90% confidence interval) for the six soils tested.

**ESTIMATED RBA FOR CALIFORNIA DTSC SOILS**

| **Test Material** | 90% Confidence Interval | | | |
|---|---|---|---|---|
| | **RBA Day 6/7** | **RBA Day 9/10** | **RBA day 12/13** | **All Days** |
| MC2 | 0.01 (-0.01 - 0.03) | 0.02 (0.01 - 0.03) | 0.00 (-0.02 - 0.02) | 0.01 (0.01 - 0.02) |
| MC3 | 0.11 (0.04 - 0.21) | 0.13 (0.07 - 0.21) | 0.05 (0.01 - 0.11) | 0.09 (0.06 - 0.13) |

| | | | | |
|---|---|---|---|---|
| CE1 | 0.37 (0.22 - 0.59) | 0.42 (0.27 - 0.62) | 0.34 (0.23 - 0.49) | 0.38 (0.30 - 0.47) |
| WR33 | 0.21 (0.12 - 0.36) | 0.21 (0.11 - 0.35) | 0.08 (0.03 - 0.15) | 0.14 (0.10 - 0.19) |
| T81 | 0.07 (0.01 - 0.15) | 0.13 (0.06 - 0.22) | 0.06 (0.02 - 0.13) | 0.08 (0.05 - 0.12) |
| IM01 | 0.04 (0.02 - 0.07) | 0.07 (0.04 - 0.12) | 0.07 (0.02 - 0.13) | 0.06 (0.04 - 0.08) |

All dose-response models were assessed with the regression function in Excel. Goodness of fit was considered acceptable if the p-value was less than 0.05.

## 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. 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.

## 4.6 Treatment

One pig (966 from Group 5) was treated for illness (diarrhea) with Naxcel® for 3 days from Study Day 0-2. He recovered uneventfully and continued on in the study group.

## 4.7 Data Analysis Variations

Several pigs in various groups had excessive urine volumes leading to an analytical arsenic results of ND (none detected). These were evaluated for calculations at one-half the analytical detection level (5µg/L) as 2.5ug/ L. When this value was multiplied times the urine volume, the results were not statistically out of line with group As values. No outliers in the data were found.

## 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 3:113-11

## TABLE 2-1 Study Design and Dosing Information

| Group | Group Name Abbreviation | Dose Material Administered | As Conc of the material (ug/g or ug/ul) | No. Pigs in Group | Target (ug/kg BW-day) |
|---|---|---|---|---|---|
| 1 | TM1 | MC2 | 603 | 5 | 60 |
| 2 | TM2 | MC3 | 641 | 5 | 60 |
| 3 | TM3 | CE1 | 753 | 5 | 60 |
| 4 | TM4 | WR33 | 6681 | 5 | 60 |
| 5 | TM5 | T81 | 205 | 5 | 60 |
| 6 | TM6 | IM01 | 731 | 5 | 60 |
| 7 | NaAs | Sodium Arsenate | 10 | 5 | 50 |
| 8 | Control | Negative control | 0 | 3 | 0 |

Dose was administered in two equal portions given at 8:00 AM and 3:00 PM each day. Doses were adjusted based on mean body weight per group every three days.

**TABLE 2-2 Group Assignments and Weight (kg)**

| Group Assignment | | Pig ID | Day -5 | Group MBW | Day -1 | Group MBW | Day 3 | Group MBW | Day 6 | Group MBW | Day 9 | Group MBW | Day 12 | Group MBW |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 747 | 10.1 | | 10.6 | | 12.4 | | 12.6 | | 14.3 | | 16 | |
| MC2 | | 749 | 7.5 | | 8.6 | | 10.1 | | 10.5 | | 12.3 | | 13.7 | |
| | | 750 | 8.6 | | 10.2 | | 11.7 | | 11.3 | | 13.5 | | 15.4 | |
| | | 962 | 9.8 | | 10.5 | | 12.1 | | 12.2 | | 13.2 | | 14.3 | |
| | | 967 | 8.5 | 8.9 | 9.8 | 9.94 | 10.8 | 11.42 | 11.6 | 11.66 | 13.2 | 13.3 | 14.9 | 14.86 |
| | 2 | 699 | 9.1 | | 10.6 | | 11.7 | | 12.5 | | 13.8 | | 15.5 | |
| MC3 | | 748 | 8.9 | | 11.1 | | 11.9 | | 12.2 | | 13.6 | | 15.2 | |
| | | 847 | 10.0 | | 11 | | 12.1 | | 12.9 | | 14.4 | | 15.9 | |
| | | 969 | 7.7 | | 10.6 | | 10.4 | | 11.2 | | 12.6 | | 14.3 | |
| | | 972 | 9.1 | 9.0 | 9.6 | 10.58 | 10.9 | 11.4 | 11.7 | 12.1 | 13 | 13.48 | 14.8 | 15.14 |
| | 3 | 700 | 8.8 | | 10.2 | | 12.3 | | 12.3 | | 13.8 | | 15.4 | |
| CE1 | | 848 | 10.0 | | 12.1 | | 13 | | 13.6 | | 14.1 | | 16.8 | |
| | | 960 | 9.2 | | 10.1 | | 11.6 | | 12 | | 13.7 | | 15.2 | |
| | | 965 | 9.8 | | 10.4 | | 11.9 | | 12.5 | | 13.8 | | 15.4 | |
| | | 970 | 9.9 | 9.5 | 11 | 10.76 | 12.3 | 12.22 | 12.8 | 12.64 | 14.4 | 13.96 | 15.4 | 15.64 |
| | 4 | 746 | 9.1 | | 10.3 | | 12 | | 12.4 | | 14 | | 15.3 | |
| WR33 | | 845 | 8.3 | | 9.8 | | 10.9 | | 11.5 | | 13.1 | | 14.7 | |
| | | 849 | 7.9 | | 9.3 | | 10.2 | | 11.8 | | 11.2 | | 13.2 | |
| | | 971 | 9.4 | | 10.5 | | 11.4 | | 12.4 | | 13.8 | | 15.5 | |
| | | 974 | 9.8 | 8.9 | 10.6 | 10.1 | 11.7 | 11.24 | 12.9 | 12.2 | 14.3 | 13.28 | 15.5 | 14.84 |
| | 5 | 600 | 9.4 | | 10.8 | | 12 | | 13 | | 14.6 | | 16 | |
| T81 | | 644 | 10.4 | | 10.3 | | 10.8 | | 12.3 | | 13 | | 14.9 | |
| | | 744 | 9.8 | | 11 | | 12.3 | | 13.1 | | 14.4 | | 15.7 | |
| | | 957 | 9.2 | | 10.3 | | 11.5 | | 12.4 | | 14.1 | | 16.1 | |
| | | 966 | 8.8 | 9.5 | 9.8 | 10.44 | 11.3 | 11.58 | 12.5 | 12.66 | 13.6 | 13.94 | 15.7 | 15.68 |
| | 6 | 844 | 9.1 | | 9.8 | | 11.7 | | 12.2 | | 14.1 | | 15.5 | |
| IM06 | | 953 | 8.7 | | 10.1 | | 11.4 | | 11.9 | | 13.5 | | 15.2 | |
| | | 954 | 7.8 | | 8.7 | | 9.9 | | 10.8 | | 12.2 | | 13.3 | |
| | | 959 | 9.3 | | 10.3 | | 11.6 | | 12.4 | | 13.8 | | 15.6 | |
| | | 975 | 10.3 | 9.0 | 11.5 | 10.08 | 12.9 | 11.5 | 13.6 | 12.18 | 15.2 | 13.76 | 16.7 | 15.26 |
| | 7 | 698 | 9.7 | | 11 | | 12.3 | | 13.1 | | 14.2 | | 15.4 | |
| NaAs | | 843 | 8.3 | | 9.7 | | 11 | | 11.7 | | 12.9 | | 14.3 | |
| | | 964 | 9.4 | | 8.6 | | 9.6 | | 10.1 | | 11.7 | | 12.8 | |
| | | 963 | 9.3 | | 10.3 | | 9.8 | | 12.7 | | 14.2 | | 15.7 | |
| | | 968 | 7.2 | 8.8 | 8.1 | 9.54 | 9 | 10.34 | 10.3 | 11.58 | 12.9 | 13.18 | 12.8 | 14.2 |
| | 8 | 745 | 8.2 | | 9.5 | | 11 | | 11.6 | | 12.6 | | 14.1 | |
| Control | | 846 | 10.2 | | 11.4 | | 12.2 | | 12.8 | | 14.5 | | 16.2 | |
| | | 955 | 9.9 | 9.0 | 10.65 | 9.99 | 11.6 | 10.72 | 12.2 | 11.92 | 13.7 | 13.58 | 15.1 | 15.13333 |

**Table 2-3 Mean Body Weight by Groups**

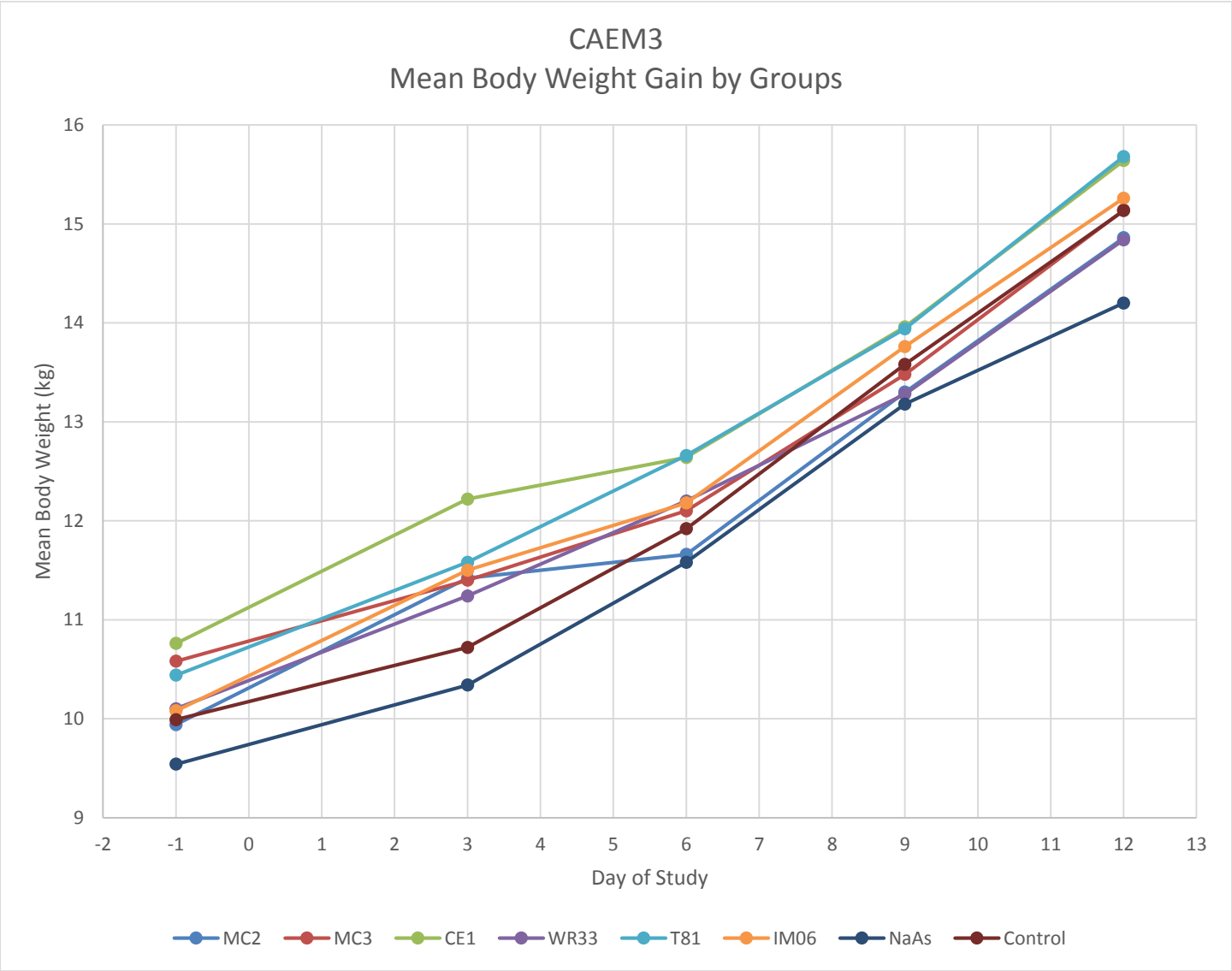


**Table 2-4 Urine Collection Volumes**

| Group | Pig ID | Urine Collection | | |
|---|---|---|---|---|
| | | **U-1** Days 7-8, 04/13-04/14 | **U-2** Days 10-11, 04/16-04/17 | **U-3** Days 13-14, 04/19-04/20 |
| 1 | 749 | 3400 | 3860 | 5300 |
| MC2 | 747 | 7610 | 9500 | 11940 |
| | 967 | 5700 | 7880 | 9600 |
| | 750 | 10126 | 15960 | 16720 |
| | 962 | 11280 | 11560 | 7500 |
| 2 | 699 | 6086 | 6060 | 7380 |
| MC3 | 696 | 2440 | 2460 | 2180 |
| | 972 | 6725 | 5240 | 4720 |
| | 748 | 1540 | 5056 | 8300 |
| | 847 | 4300 | 4480 | 4880 |
| 3 | 960 | 4680 | 6380 | 9620 |
| CE1 | 970 | 5840 | 7400 | 5520 |
| | 700 | 1580 | 2000 | 2420 |
| | 965 | 2131 | 1600 | 3760 |
| | 848 | 1140 | 1450 | 2420 |
| 4 | 746 | 2400 | 2160 | 3740 |
| WR33 | 974 | 2340 | 3200 | 4380 |
| | 971 | 4300 | 7300 | 13520 |
| | 845 | 2129 | 2880 | 3800 |
| | 849 | 5360 | 6500 | 3440 |
| 5 | 644 | 1380 | 1600 | 3200 |
| T81 | 744 | 4600 | 5740 | 14360 |
| | 966 | 4360 | 3900 | 3800 |
| | 600 | 4580 | 2560 | 6420 |
| | 957 | 2400 | 1580 | 3400 |
| 6 | 954 | 10590 | 9820 | 12300 |
| IM03 | 959 | 3580 | 4780 | 2060 |
| | 953 | 6109 | 5120 | 2500 |
| | 975 | 9080 | 9420 | 11460 |
| | 844 | 2760 | 3200 | 3000 |
| 7 | 698 | 6122 | 5700 | 6880 |
| NaAs | 968 | 5400 | 8100 | 6360 |
| | 964 | 1560 | 1930 | 2300 |
| | 963 | 1640 | 2420 | 5780 |
| | 843 | 960 | 1300 | 1500 |
| 8 | 745 | 1600 | 2760 | 4260 |
| Control | 846 | 2800 | 2480 | 2460 |
| | 955 | 2240 | 5960 | 2540 |

**TABLE 2-5 Urinary Arsenic Analytical Results and Urine Volumes for Study Samples**

| SAMPLENAME | Group Number | Pig Number | Collection | Urine volume (mls) | Total ug/L As excreted in Urine |
|---|---|---|---|---|---|
| CAEM3-001 | Group 1 | 749 | U-1 | 3400 | 2.5 |
| CAEM3-002 | Group 1 | 747 | U-1 | 7610 | 2.5 |
| CAEM3-003 | Group 1 | 967 | U-1 | 5700 | 2.5 |
| CAEM3-004 | Group 1 | 750 | U-1 | 10126 | 2.5 |
| CAEM3-005 | Group 1 | 962 | U-1 | 11280 | 2.5 |
| CAEM3-006 | Group 2 | 699 | U-1 | 6086 | 2.5 |
| CAEM3-007 | Group 2 | 696 | U-1 | 2440 | 176.7 |
| CAEM3-008 | Group 2 | 972 | U-1 | 6725 | 2.5 |
| CAEM3-009 | Group 2 | 748 | U-1 | 1540 | 161.7 |
| CAEM3-0011 | Group 2 | 847 | U-1 | 4300 | 87.3 |
| CAEM3-0012 | Group 3 | 960 | U-1 | 4680 | 542.9 |
| CAEM3-0013 | Group 3 | 970 | U-1 | 5840 | 201.5 |
| CAEM3-0014 | Group 3 | 700 | U-1 | 1580 | 312.8 |
| CAEM3-0015 | Group 3 | 965 | U-1 | 2131 | 247.2 |
| CAEM3-0016 | Group 3 | 848 | U-1 | 1140 | 165.3 |
| CAEM3-0017 | Group 4 | 746 | U-1 | 2400 | 173.0 |
| CAEM3-0018 | Group 4 | 974 | U-1 | 2340 | 278.5 |
| CAEM3-0019 | Group 4 | 971 | U-1 | 4300 | 175.0 |
| CAEM3-0020 | Group 4 | 845 | U-1 | 2129 | 212.9 |
| CAEM3-0021 | Group 4 | 849 | U-1 | 5360 | 2.5 |
| CAEM3-0022 | Group 5 | 644 | U-1 | 1380 | 161.5 |
| CAEM3-0023 | Group 5 | 744 | U-1 | 4600 | 2.5 |
| CAEM3-0024 | Group 5 | 966 | U-1 | 4360 | 2.5 |
| CAEM3-0025 | Group 5 | 600 | U-1 | 4580 | 2.5 |
| CAEM3-0026 | Group 5 | 957 | U-1 | 2400 | 115.2 |
| CAEM3-0027 | Group 6 | 954 | U-1 | 10590 | 2.5 |
| CAEM3-0028 | Group 6 | 959 | U-1 | 3580 | 72.0 |
| CAEM3-0029 | Group 6 | 953 | U-1 | 6109 | 51.7 |
| CAEM3-030 | Group 6 | 975 | U-1 | 9080 | 2.5 |
| CAEM3-031 | Group 6 | 844 | U-1 | 2760 | 41.7 |
| CAEM3-032 | Group 7 | 698 | U-1 | 6122 | 512.4 |
| CAEM3-033 | Group 7 | 968 | U-1 | 5400 | 702.0 |
| CAEM3-034 | Group 7 | 964 | U-1 | 1560 | 656.8 |
| CAEM3-0010 | Group 7 | 963 | U-1 | 1640 | 615.0 |
| CAEM3-035 | Group 7 | 843 | U-1 | 960 | 448.3 |
| CAEM3-036 | control | 745 | U-1 | 1600 | 23.7 |
| CAEM3-037 | control | 846 | U-1 | 2800 | 2.5 |

| | | | | | |
|---|---|---|---|---|---|
| CAEM3-038 | control | 955 | U-1 | 2240 | 2.5 |
| CAEM3-039 | Group 1 | 749 | U-2 | 3860 | 2.5 |
| CAEM3-040 | Group 1 | 747 | U-2 | 9500 | 2.5 |
| CAEM3-041 | Group 1 | 967 | U-2 | 7880 | 2.5 |
| CAEM3-042 | Group 1 | 750 | U-2 | 15960 | 2.5 |
| CAEM3-043 | Group 1 | 962 | U-2 | 11560 | 2.5 |
| CAEM3-044 | Group 2 | 699 | U-2 | 6060 | 34.2 |
| CAEM3-045 | Group 2 | 696 | U-2 | 2460 | 236.9 |
| CAEM3-046 | Group 2 | 972 | U-2 | 5240 | 66.5 |
| CAEM3-047 | Group 2 | 748 | U-2 | 5056 | 289.7 |
| CAEM3-048 | Group 2 | 847 | U-2 | 4480 | 112.9 |
| CAEM3-049 | Group 3 | 960 | U-2 | 6380 | 320.3 |
| CAEM3-050 | Group 3 | 970 | U-2 | 7400 | 124.3 |
| CAEM3-051 | Group 3 | 700 | U-2 | 2000 | 428.0 |
| CAEM3-052 | Group 3 | 965 | U-2 | 1600 | 600.0 |
| CAEM3-053 | Group 3 | 848 | U-2 | 1450 | 326.3 |
| CAEM3-054 | Group 4 | 746 | U-2 | 2160 | 257.0 |
| CAEM3-055 | Group 4 | 974 | U-2 | 3200 | 302.1 |
| CAEM3-056 | Group 4 | 971 | U-2 | 7300 | 75.2 |
| CAEM3-057 | Group 4 | 845 | U-2 | 2880 | 225.5 |
| CAEM3-058 | Group 4 | 849 | U-2 | 6500 | 2.5 |
| CAEM3-059 | Group 5 | 644 | U-2 | 1600 | 200.0 |
| CAEM3-060 | Group 5 | 744 | U-2 | 5740 | 2.5 |
| CAEM3-061 | Group 5 | 966 | U-2 | 3900 | 28.5 |
| CAEM3-062 | Group 5 | 600 | U-2 | 2560 | 171.8 |
| CAEM3-063 | Group 5 | 957 | U-2 | 1580 | 165.9 |
| CAEM3-064 | Group 6 | 954 | U-2 | 9820 | 2.5 |
| CAEM3-065 | Group 6 | 959 | U-2 | 4780 | 66.9 |
| CAEM3-066 | Group 6 | 953 | U-2 | 5120 | 126.0 |
| CAEM3-067 | Group 6 | 975 | U-2 | 9420 | 2.5 |
| CAEM3-068 | Group 6 | 844 | U-2 | 3200 | 97.3 |
| CAEM3-069 | Group 7 | 698 | U-2 | 5700 | 815.1 |
| CAEM3-070 | Group 7 | 968 | U-2 | 8100 | 524.1 |
| CAEM3-071 | Group 7 | 964 | U-2 | 1930 | 727.6 |
| CAEM3-072 | Group 7 | 963 | U-2 | 2420 | 788.9 |
| CAEM3-073 | Group 7 | 843 | U-2 | 1300 | 510.9 |
| CAEM3-074 | control | 745 | U-2 | 2760 | 2.5 |
| CAEM3-075 | control | 846 | U-2 | 2480 | 2.5 |
| CAEM3-076 | control | 955 | U-2 | 5960 | 2.5 |
| CAEM3-077 | Group 1 | 749 | U-3 | 5300 | 2.5 |
| CAEM3-078 | Group 1 | 747 | U-3 | 11940 | 2.5 |
| CAEM3-079 | Group 1 | 967 | U-3 | 9600 | 2.5 |

| | | | | | |
|---|---|---|---|---|---|
| CAEM3-080 | Group 1 | 750 | U-3 | 16720 | 2.5 |
| CAEM3-081 | Group 1 | 962 | U-3 | 7500 | 2.5 |
| CAEM3-082 | Group 2 | 699 | U-3 | 7380 | 2.5 |
| CAEM3-083 | Group 2 | 696 | U-3 | 2180 | 120.8 |
| CAEM3-084 | Group 2 | 972 | U-3 | 4720 | 123.2 |
| CAEM3-085 | Group 2 | 748 | U-3 | 8300 | 2.5 |
| CAEM3-086 | Group 2 | 847 | U-3 | 4880 | 113.2 |
| CAEM3-087 | Group 3 | 960 | U-3 | 9620 | 214.5 |
| CAEM3-088 | Group 3 | 970 | U-3 | 5520 | 300.3 |
| CAEM3-089 | Group 3 | 700 | U-3 | 2420 | 447.7 |
| CAEM3-090 | Group 3 | 965 | U-3 | 3760 | 383.5 |
| CAEM3-091 | Group 3 | 848 | U-3 | 2420 | 474.3 |
| CAEM3-092 | Group 4 | 746 | U-3 | 3740 | 144.4 |
| CAEM3-093 | Group 4 | 974 | U-3 | 4380 | 160.3 |
| CAEM3-094 | Group 4 | 971 | U-3 | 13520 | 2.5 |
| CAEM3-095 | Group 4 | 845 | U-3 | 3800 | 154.7 |
| CAEM3-096 | Group 4 | 849 | U-3 | 3440 | 34.3 |
| CAEM3-097 | Group 5 | 644 | U-3 | 3200 | 97.3 |
| CAEM3-098 | Group 5 | 744 | U-3 | 14360 | 2.5 |
| CAEM3-099 | Group 5 | 966 | U-3 | 3800 | 128.1 |
| CAEM3-100 | Group 5 | 600 | U-3 | 6420 | 2.5 |
| CAEM3-101 | Group 5 | 957 | U-3 | 3400 | 181.9 |
| CAEM3-102 | Group 6 | 954 | U-3 | 12300 | 2.5 |
| CAEM3-103 | Group 6 | 959 | U-3 | 2060 | 68.8 |
| CAEM3-104 | Group 6 | 953 | U-3 | 2500 | 87.0 |
| CAEM3-105 | Group 6 | 975 | U-3 | 11460 | 2.5 |
| CAEM3-106 | Group 6 | 844 | U-3 | 3000 | 178.5 |
| CAEM3-107 | Group 7 | 698 | U-3 | 6880 | 694.9 |
| CAEM3-108 | Group 7 | 968 | U-3 | 6360 | 1030.3 |
| CAEM3-109 | Group 7 | 964 | U-3 | 2300 | 706.1 |
| CAEM3-110 | Group 7 | 963 | U-3 | 5780 | 976.8 |
| CAEM3-111 | Group 7 | 843 | U-3 | 1500 | 498.0 |
| CAEM3-112 | control | 745 | U-3 | 4260 | 2.5 |
| CAEM3-113 | control | 846 | U-3 | 2460 | 44.8 |
| CAEM3-114 | control | 955 | U-3 | 2540 | 30.7 |

**TABLE 2-6 Typical Feed Composition**

University Feed Mill, Grower Feed
Manufactured: 08/13/2012

| Ingredient | Amount (lbs) |
|---|---|
| Purex Salt | 6 |
| Corn | 1,528 |
| 48% Soybean Meal | 350 |
| Fat | 50 |
| Limestone | 18 |
| L-Lysine | 3 |
| Swine Vit. NB 6104 | 4 |
| Swine Min. NB-8536 | 3 |
| Zinpro-100 | 2 |
| Biotin | 2 |
| Dical | 34 |

# TABLE 2-7 Doughball 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]

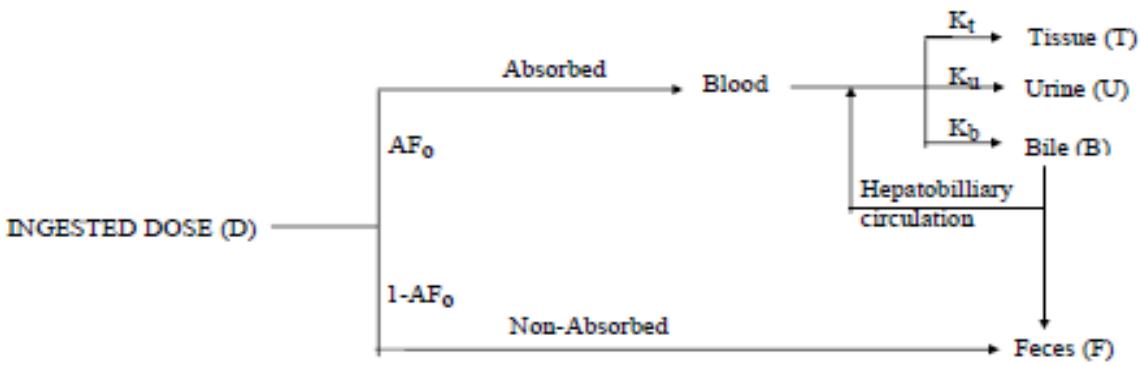
| | | | | |
|---|---|---|---|---|
| **Protein, %** | **21** | **Fat, %** | **3.5** | |
| Arginine, % | 1.42 | Cholesterol, ppm | 0 | |
| Histidine, % | 0.61 | Linoleic Acid, % | 1.95 | |
| Isoleucine, % | 1.14 | Linolenic Acid, % | 0.03 | |
| Leucine, % | 1.95 | Arachidonic Acid, % | 0 | |
| Lysine, % | 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 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

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

## Table 4.1 Background Urinary Arsenic

| SAMPLENAME | Group Number | Pig Number | Collection | Urine volume (mls) | Total ug/L As excreted in Urine |
|---|---|---|---|---|---|
| CAEM3-036 | control | 745 | U-1 | 1600 | 23.7 |
| CAEM3-037 | control | 846 | U-1 | 2800 | 2.5 |
| CAEM3-038 | control | 955 | U-1 | 2240 | 2.5 |
| CAEM3-074 | control | 745 | U-2 | 2760 | 2.5 |
| CAEM3-075 | control | 846 | U-2 | 2480 | 2.5 |
| CAEM3-076 | control | 955 | U-2 | 5960 | 2.5 |
| CAEM3-112 | control | 745 | U-3 | 4260 | 2.5 |
| CAEM3-113 | control | 846 | U-3 | 2460 | 44.8 |
| CAEM3-114 | control | 955 | U-3 | 2540 | 30.7 |
| | | | | **Mean As excreted in Control group** | **12.9 µg/L** |

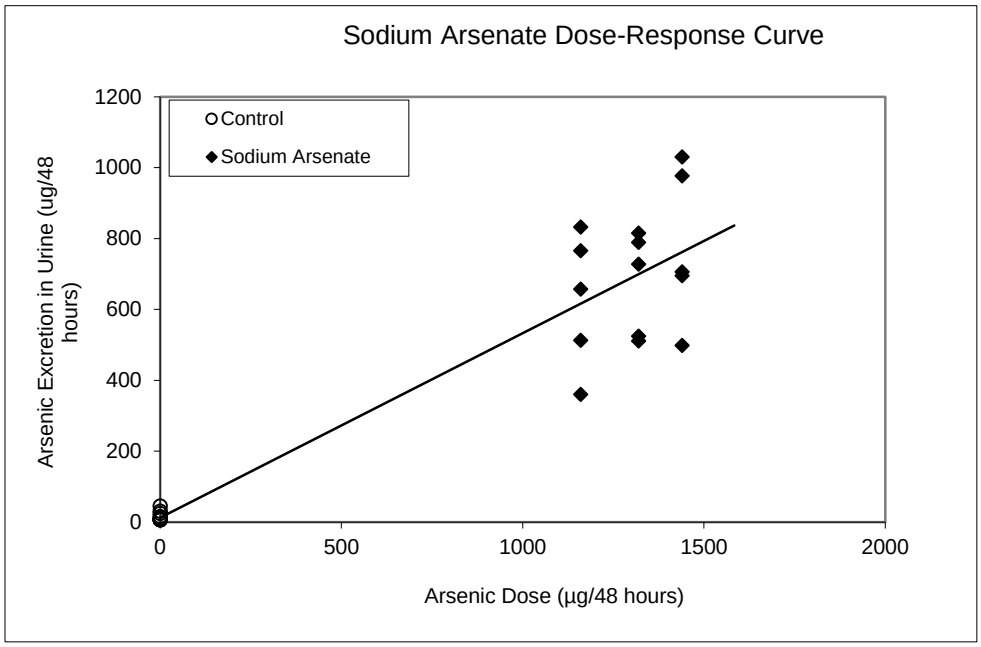
## Table 4-2 Final Results

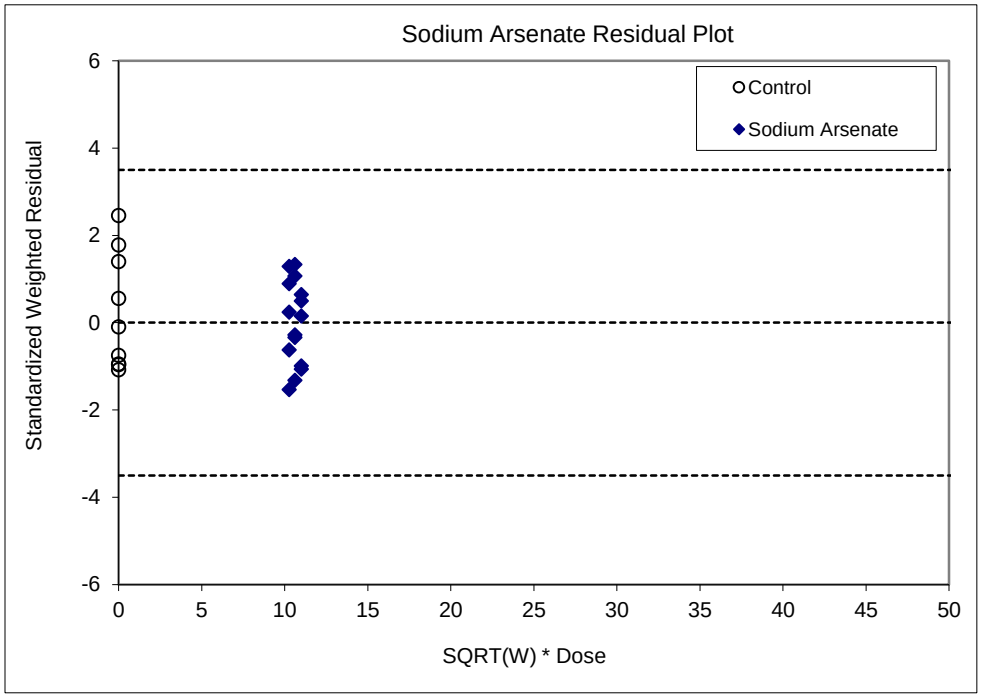
**ESTIMATED RBA FOR CALIFORNIA DTSC SOILS**

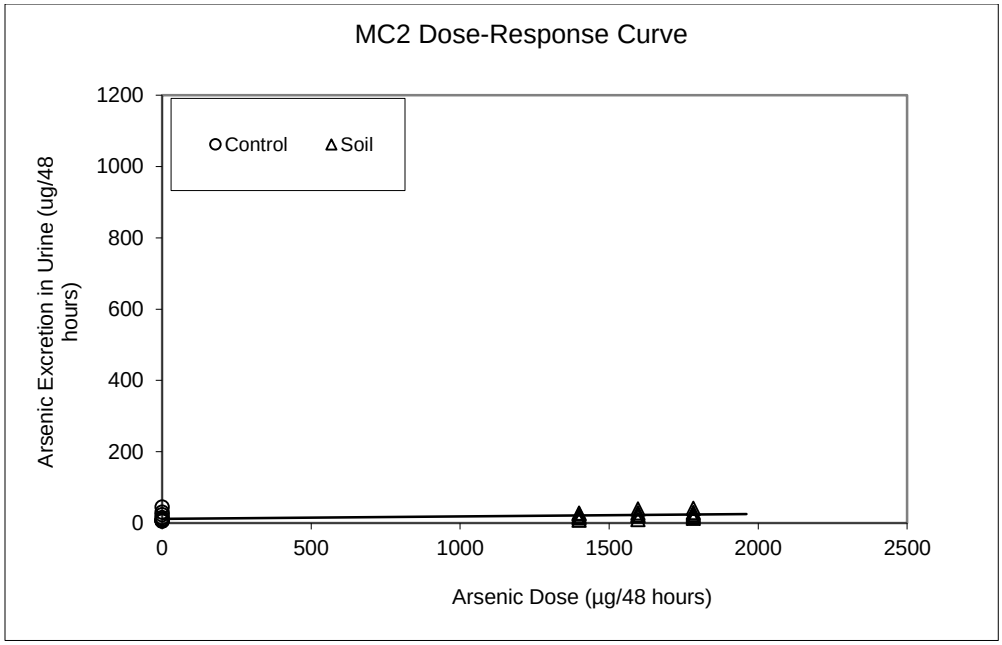
| **Test Material** | 90% Confidence Interval | | | |
|---|---|---|---|---|
| | **RBA Day 6/7** | **RBA Day 9/10** | **RBA day 12/13** | **All Days** |
| MC2 | 0.01 (-0.01 - 0.03) | 0.02 (0.01 - 0.03) | 0.00 (-0.02 - 0.02) | 0.01 (0.01 - 0.02) |
| MC3 | 0.11 (0.04 - 0.21) | 0.13 (0.07 - 0.21) | 0.05 (0.01 - 0.11) | 0.09 (0.06 - 0.13) |
| CE1 | 0.37 (0.22 - 0.59) | 0.42 (0.27 - 0.62) | 0.34 (0.23 - 0.49) | 0.38 (0.30 - 0.47) |
| WR33 | 0.21 (0.12 - 0.36) | 0.21 (0.11 - 0.35) | 0.08 (0.03 - 0.15) | 0.14 (0.10 - 0.19) |
| T81 | 0.07 (0.01 - 0.15) | 0.13 (0.06 - 0.22) | 0.06 (0.02 - 0.13) | 0.08 (0.05 - 0.12) |
| IM01 | 0.04 (0.02 - 0.07) | 0.07 (0.04 - 0.12) | 0.07 (0.02 - 0.13) | 0.06 (0.04 - 0.08) |

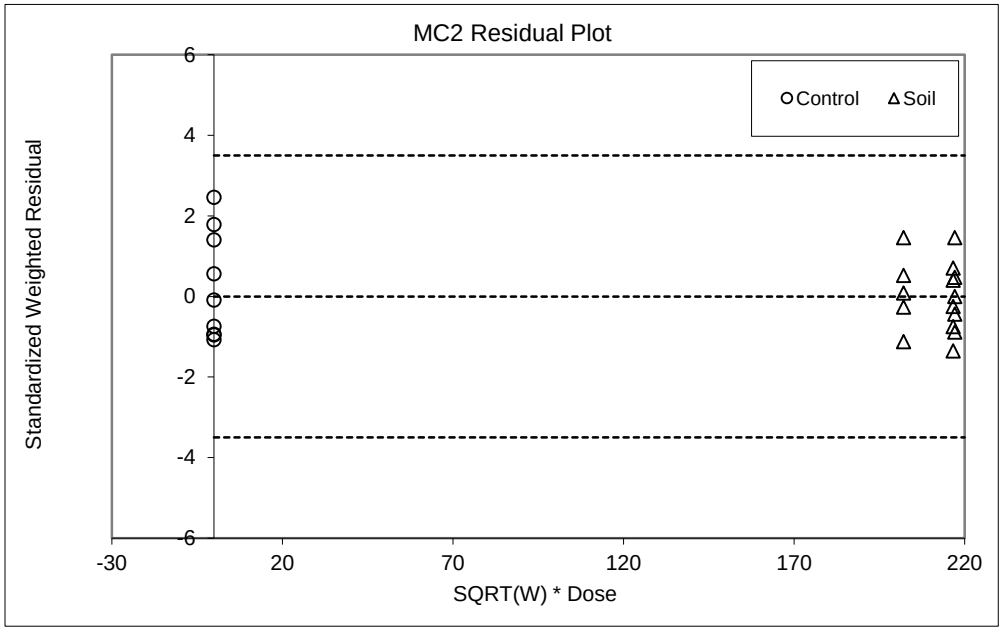
All dose-response models were assessed with the regression function in Excel. Goodness of fit was considered acceptable if the p-value was less than 0.05.

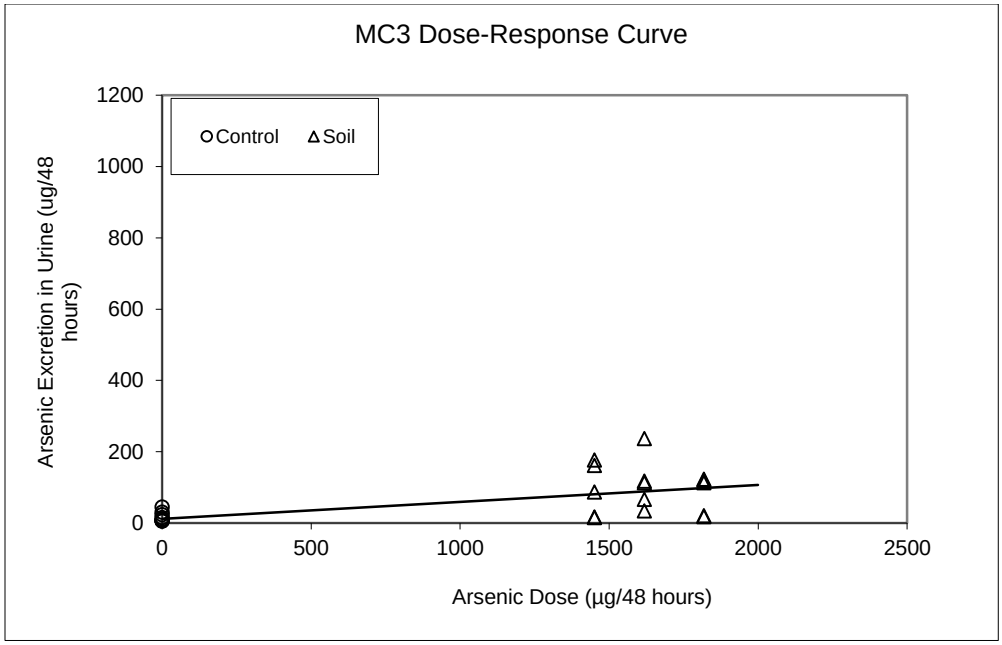
**TABLE 4-3 ALL Days Dose Response and Residual Plots**

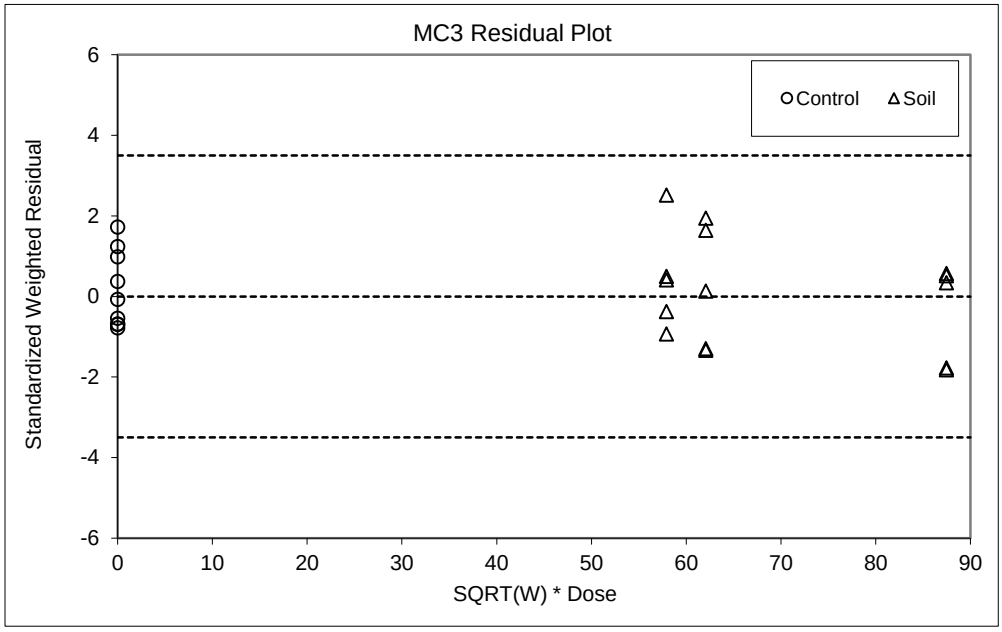


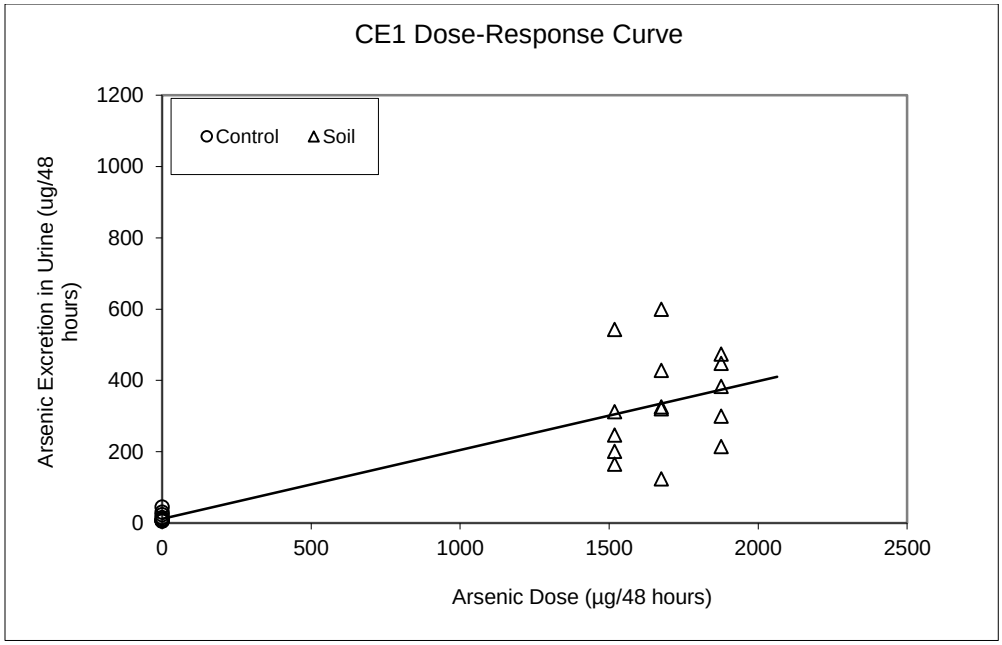



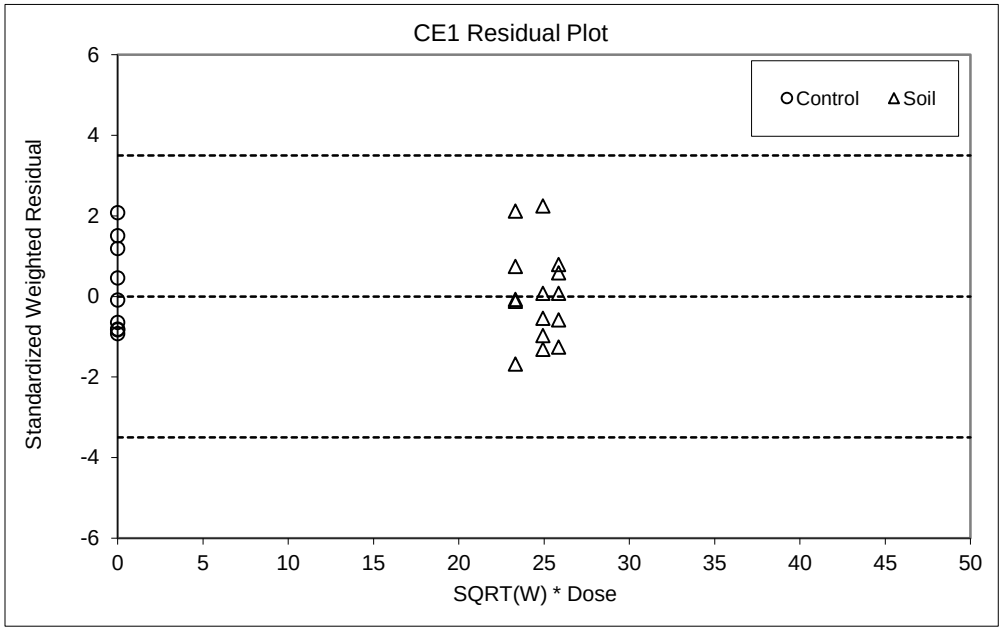

MC2 Dose-Response Curve
Control
Soil
Arsenic Excretion in Urine (ug/48 hours)
1200
1000
800
600
400
200
0
0
500
1000
1500
2000
2500
Arsenic Dose (µg/48 hours)

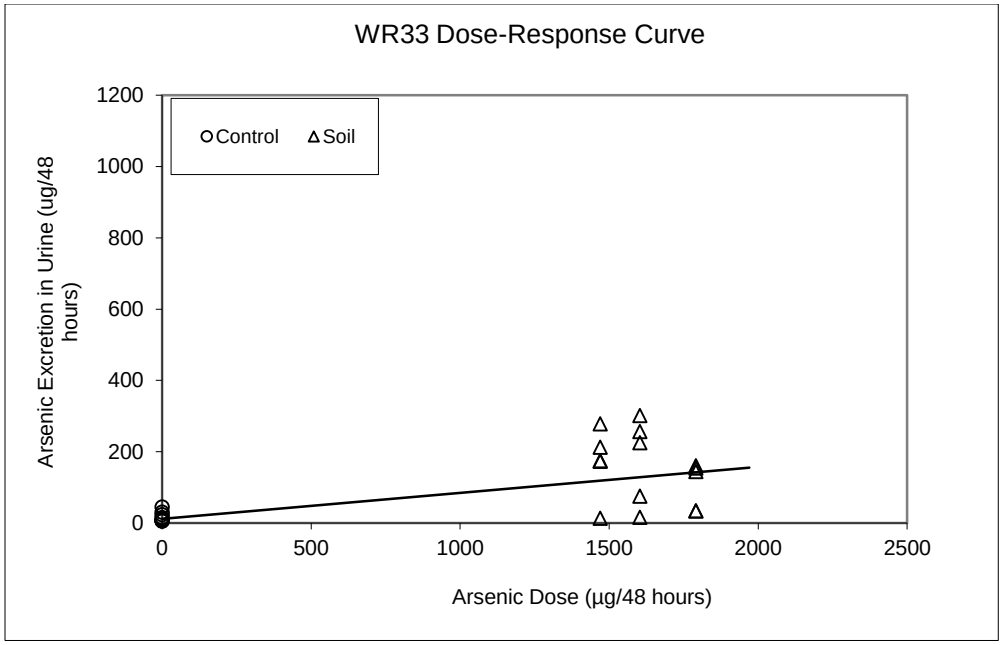

MC2 Residual Plot
Control
Soil
Standardized Weighted Residual
6
4
2
0
-2
-4
-6
-30
20
70
120
170
220
SQRT(W) * Dose

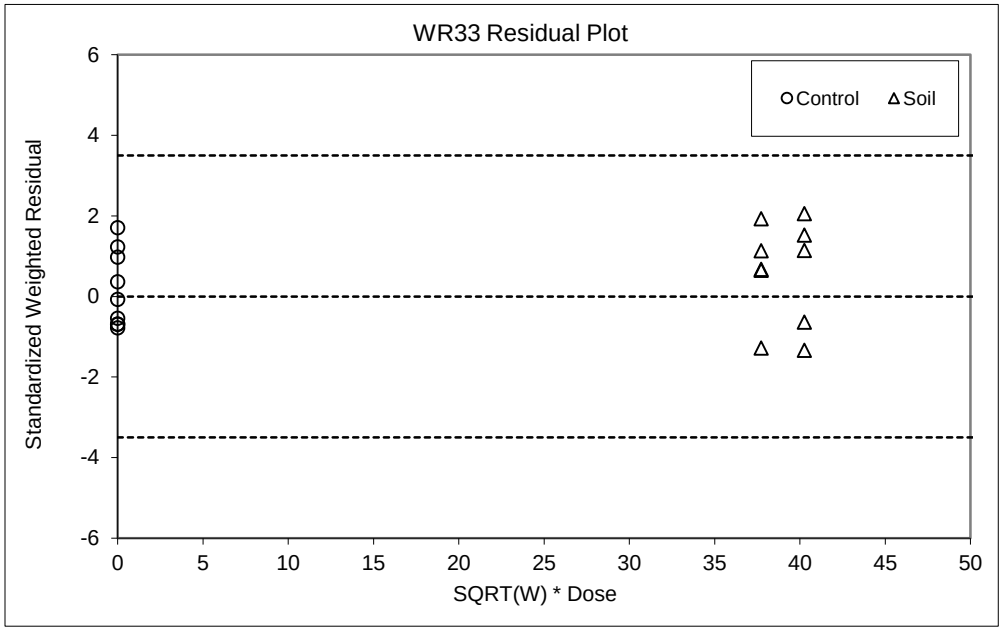

MC3 Dose-Response Curve
Control
Soil
Arsenic Excretion in Urine (ug/48 hours)
1200
1000
800
600
400
200
0
0
500
1000
1500
2000
2500
Arsenic Dose (µg/48 hours)

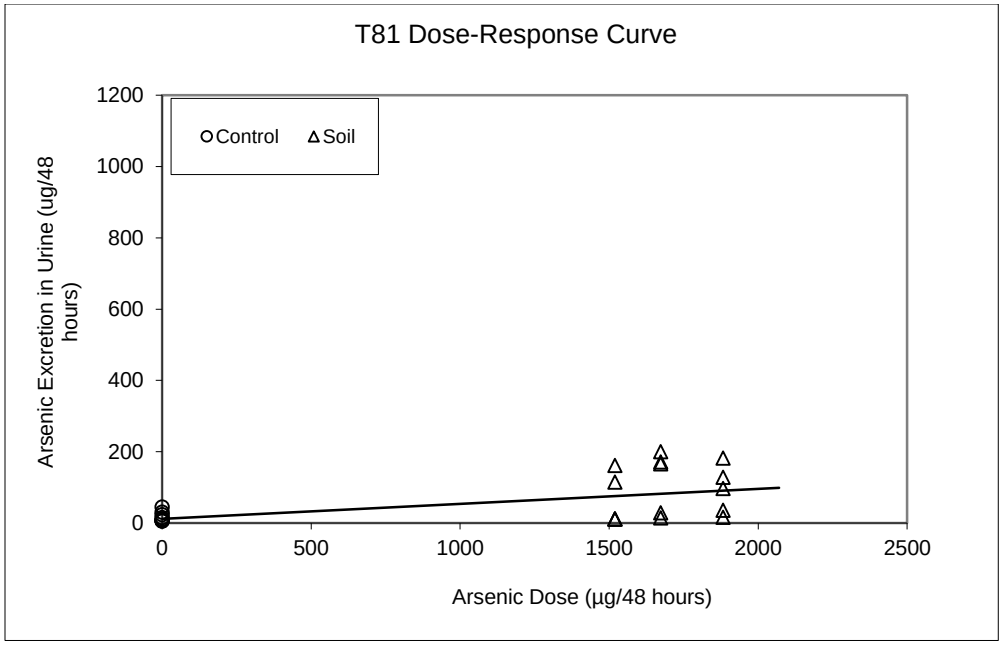

MC3 Residual Plot
Control
Soil
Standardized Weighted Residual
6
4
2
0
-2
-4
-6
0
10
20
30
40
50
60
70
80
90
SQRT(W) * Dose

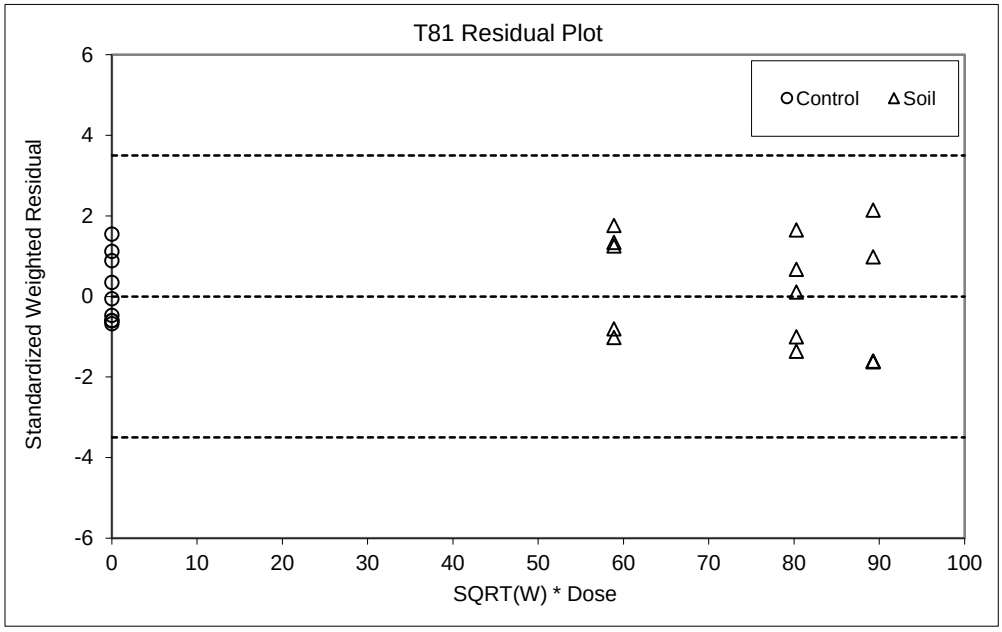

CE1 Dose-Response Curve
Control
Soil
Arsenic Excretion in Urine (ug/48 hours)
1200
1000
800
600
400
200
0
0
500
1000
1500
2000
2500
Arsenic Dose (µg/48 hours)

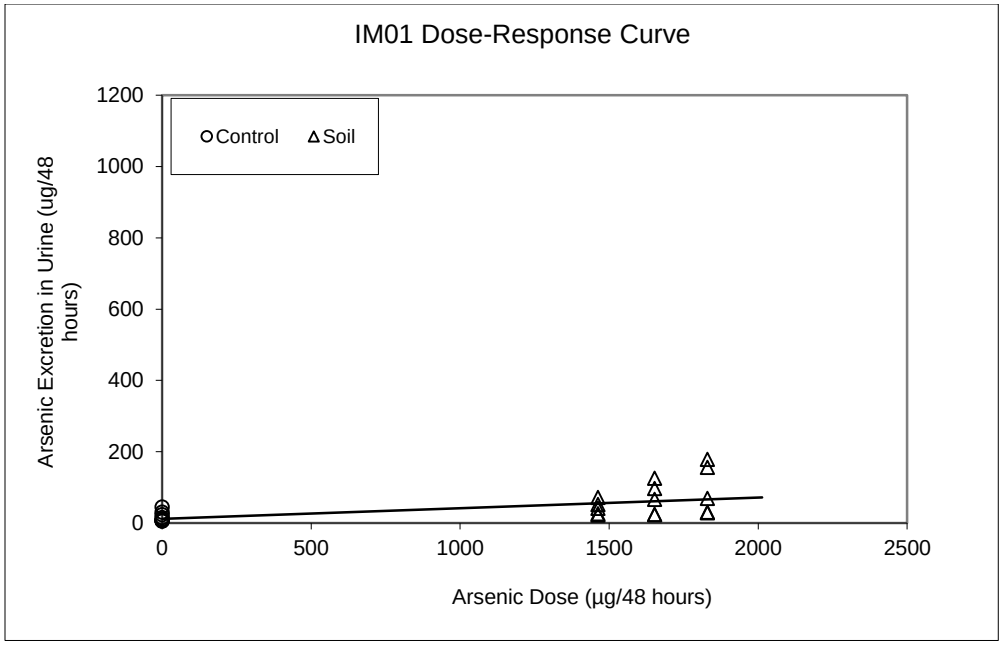

CE1 Residual Plot
Control
Soil
Standardized Weighted Residual
6
4
2
0
-2
-4
-6
0
5
10
15
20
25
30
35
40
45
50
SQRT(W) * Dose

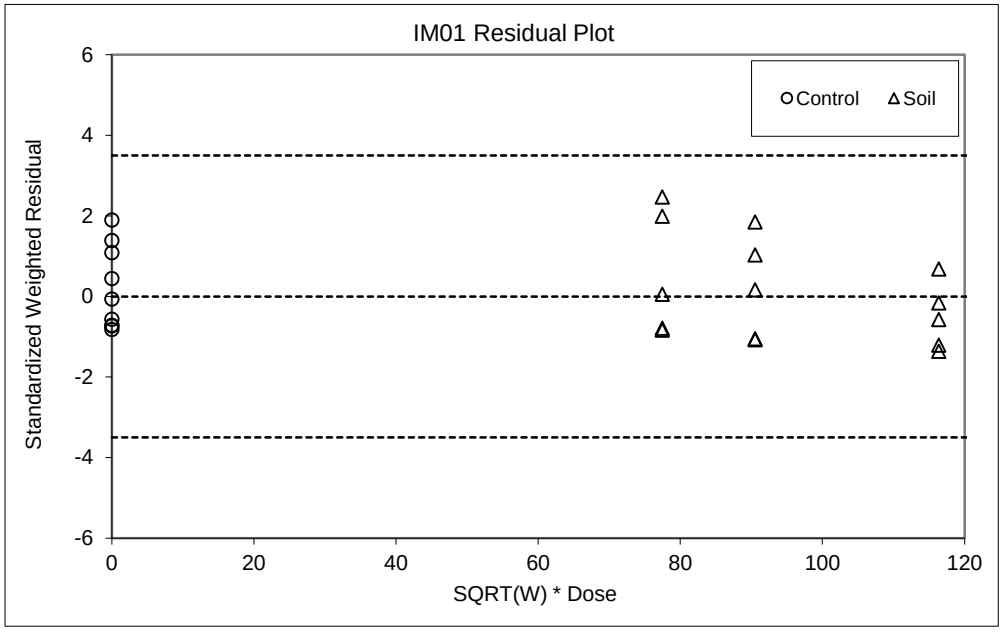

WR33 Dose-Response Curve
Control
Soil
Arsenic Excretion in Urine (ug/48 hours)
1200
1000
800
600
400
200
0
0
500
1000
1500
2000
2500
Arsenic Dose (µg/48 hours)


WR33 Residual Plot
Control
Soil
Standardized Weighted Residual
6
4
2
0
-2
-4
-6
0
5
10
15
20
25
30
35
40
45
50
SQRT(W) * Dose


T81 Dose-Response Curve
Control
Soil
Arsenic Excretion in Urine (ug/48 hours)
1200
1000
800
600
400
200
0
0
500
1000
1500
2000
2500
Arsenic Dose (µg/48 hours)


T81 Residual Plot
Control
Soil
Standardized Weighted Residual
6
4
2
0
-2
-4
-6
0
10
20
30
40
50
60
70
80
90
100
SQRT(W) * Dose


IM01 Dose-Response Curve
Control
Soil
Arsenic Excretion in Urine (ug/48 hours)
1200
1000
800
600
400
200
0
0
500
1000
1500
2000
2500
Arsenic Dose (µg/48 hours)


IM01 Residual Plot
Control
Soil
Standardized Weighted Residual
6
4
2
0
-2
-4
-6
0
20
40
60
80
100
120
SQRT(W) * Dose