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# RELATIVE BIOAVAILABILITY OF ARSENIC IN SOILS AT 11 HAZARDOUS WASTE SITES USING AN *IN VIVO* JUVENILE SWINE METHOD

**Bioavailability Subcommittee of the Technical Review Workgroup**

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# EXECUTIVE SUMMARY

## INTRODUCTION

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. Knowledge of bioavailability is important because the amount of a chemical (e.g., arsenic) that actually enters the body from an ingested medium depends on the physical-chemical properties of the chemical and of the medium. Accurate assessment of the human health risks resulting from oral exposure to arsenic requires knowledge of the amount of arsenic absorbed from the gastrointestinal tract into the body. When reliable data are available on the relative bioavailability (RBA) of a chemical in a site medium (e.g., soil), this information can be used to improve the accuracy of exposure and risk calculations at that site. Available RBA data can be used to adjust default oral toxicity values (reference dose and slope factor) to account for differences in absorption between the chemical ingested in water and the chemical ingested in site media, assuming the toxicity factors are based on a readily soluble form of the chemical.

This document summarizes a number of *in vivo* studies that have been performed in young swine to investigate the RBA of arsenic in different environmental media.

## METHODS

### *Basic In Vivo Experimental Design*

All *in vivo* studies were performed using young swine. Swine were selected for use because available physiological data indicate that young swine are a good model for the human gastrointestinal system. Groups of animals (usually 5 per dose group) were exposed to test material or reference material for 12–15 days. Dosing was usually oral, although some groups were exposed to sodium arsenate by gavage or by intravenous injection.

Samples of urine were collected from each animal on several different days during the study (the exact days varied from study to study). Prior to analysis, samples of urine were digested using one of two alternative methods. Studies that used the first digestion method are referred to as Phase II, and studies that used the second digestion method are referred to as Phase III. After digestion, all samples were analyzed for arsenic using the hydride method.

*Basic Method for Estimating RBA*

Arsenic that is absorbed into the body from the gastrointestinal tract is excreted in the urine within 1–2 days (see Table 2-1). Based on this, the RBA of a test material may be estimated by measuring the urinary excretion fraction (UEF) of arsenic administered in test material and in reference material (sodium arsenate), and calculating the ratio of the two UEF values:

$$RBA(\text{test material}) = UEF(\text{test material}) / UEF(\text{sodium arsenate})$$

The UEF for each material (test soil, sodium arsenate) is estimated by plotting the mass of arsenic excreted by each animal as a function of the dose administered, and then fitting the data for the two test materials to a simultaneous weighted regression model. The slopes estimated for each test material are direct estimates of the UEF. The RBA is estimated as the ratio of the slopes (slope test material/slope sodium arsenate); the regression model also provides estimates of the uncertainty in the slope estimates. A complete description of the regression model is included in Appendix A of the report.

**RESULTS**

In total, 29 test materials were investigated using the *in vivo* swine bioassay (two in duplicate). In three cases, the amount of arsenic administered was too low to allow reliable measurement of RBA, and the results for these samples are not considered to be meaningful. Values for the remaining all 29 test materials are shown below.

**Summary of RBA Estimates for Phase II and Phase III Test Materials**

| Phase | Experiment | Sample | Arsenic Concentration[a] (ppm) | RBA ± SEM |
|---|---|---|---|---|
| Phase II | 2 | Bingham Creek Channel Soil | 149 | 39% ± 8% |
| | 4 | Murray Smelter Slag | 695 | 55% ± 10% |
| | | Jasper County High Lead Mill[b] | 16.4 | 327% ± 105% |
| | 5 | Aspen Berm[b] | 66.9 | 100% ± 46% |
| | | Aspen Residential[b] | 16.7 | 128% ± 52% |
| | 6 | Butte Soil | 234 | 9% ± 3% |
| | | Midvale Slag | 591 | 23% ± 4% |
| | 7 | California Gulch Phase I Residential Soil | 203 | 8% ± 3% |
| | | California Gulch Fe/Mn PbO | 110 | 57% ± 12% |
| | 8 | California Gulch AV Slag | 1050 | 13% ± 4% |
| | 9 | Palmerton Location 2 | 110 | 49% ± 10% |
| | | Palmerton Location 4 | 134 | 61% ± 11% |
| | 10 | California Gulch AV Slag | 1050 | 18% ± 2% |
| | 11 | Murray Smelter Soil | 310 | 33% ± 5% |
| | 15 | Clark Fork Tailings | 181 | 51% ± 6% |

| Summary of RBA Estimates for Phase II and Phase III Test Materials | | | | |
|---|---|---|---|---|
| **Phase** | **Experiment** | **Sample** | **Arsenic Concentration[a] (ppm)** | **RBA ± SEM** |
| Phase III | 1 | VBI70 TM1 | 312 | 40% ± 4% |
| | | VBI70 TM2 | 983 | 42% ± 4% |
| | | VBI70 TM3 | 390 | 37% ± 3% |
| | 2 | VBI70 TM4 | 813 | 24% ± 2% |
| | | VBI70 TM5 | 368 | 21% ± 2% |
| | | VBI70 TM6 | 516 | 24% ± 3% |
| | 3 | Butte TM1 | 234 | 18% ± 3% |
| | | Butte TM2 | 367 | 24% ± 2% |
| | 4 | Aberjona River TM1 | 676.3 | 38% ± 2% |
| | | Aberjona River TM2 | 312.8 | 52% ± 2% |
| | 5 | El Paso TM1 | 74 | 44% ± 3% |
| | | El Paso TM2 | 73 | 37% ± 3% |
| | 6 | ACC Utility Pole Soil | 320 | 47% ± 3% |
| | 7 | ACC Dislodgeable Arsenic | 3500 | 26% ± 1% |

SEM = Standard error of the mean, an indicator of the relative uncertainty around the RBA estimate (see Appendix A)
[a]Same sample as evaluated in Phase II
[b]The amount of arsenic administered was too low to allow reliable measurement of RBA, and the results for these samples are not considered to be meaningful

As seen, using sodium arsenate as a relative frame of reference, estimated RBA values range from less than 10% to more than 60% (excluding the 3 values considered to be unreliable). This wide variability supports the conclusion that there can be important differences in RBA between different types of samples, and that use of a site-specific RBA value is likely to increase the accuracy of risk estimates for arsenic. This conclusion is also consistent with the similarity between the coefficient of variability of the dose-UEF slope for test materials (0.38) and the coefficient of variability of estimated RBAs for the same test materials (0.32).

*Correlation of RBA with Arsenic Geochemistry*

One objective of this project was to obtain preliminary information on which chemical forms or mineral associations of arsenic tend to have high bioavailability and which tend to have low bioavailability. Geochemical speciation data were obtained for 20 different test materials using electron microprobe analysis. A total of 28 different arsenic phases were represented in the test materials; some test materials contained more than one arsenic phase. In order to derive quantitative estimates of phase-specific RBA values, a multivariate linear regression approach was used. Because the total number of phases (28) was larger than the number of RBA measurements (20), the existing data are not sufficient to perform a robust regression analysis based on individual phases. A screening-level analysis was performed by grouping the 28 different phases into broader categories based on professional judgment

regarding the expected degree of similarity between members of a group. Only the arsenic mass in partially or entirely *liberated* particles (arsenic-bearing grains that are partially or entirely exposed on their outer surfaces) was included in this analysis. Based on this analysis, it is possible to assign tentative qualitative estimates of bioavailability, as follows:

| Low Bioavailability | Medium Bioavailability | High Bioavailability |
|---|---|---|
| $As_2O_3$<br>Sulfosalts | As Phosphate<br>FeAs Oxide<br>PbAs Oxide<br>MnAs Oxide<br>Fe and Zn sulfates | FeAsO |

## CONCLUSION

The data from the investigations performed under this program support the following main conclusions:

1. Juvenile swine constitute a useful and stable animal model for measuring the relative bioavailability of arsenic in a variety of soil or soil-like test materials. The Phase III protocol described in this report is the recommended standard operating procedure (SOP) for the juvenile swine RBA assay.
2. There are clear differences in the *in vivo* RBA of arsenic between different types of test materials, ranging from less than 10% to more than 60%. Thus, knowledge of the RBA value for different types of test materials at a site can be important for improving arsenic risk assessments at a site.
3. Available data are not yet sufficient to allow reliable quantitative calculation of the RBA for a test material based only on knowledge of the relative amounts of arsenic mineral phases present. However, tentative qualitative estimates of low, medium, or high bioavailability have been made based on the major phase type of the arsenic containing waste material.
4. Additional extraction steps were identified and necessary to convert urinary organoarsenic metabolites to inorganic arsenic for analysis of total arsenic in urine.
5. Due to limitations in detection limits for measurement of arsenic in urine, a minimum arsenic dose of 25 µg/kg bw-day is recommended for the juvenile swine RBA assay, so that the amount of arsenic excreted in urine reaches a measurable quantity.

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## ACRONYMS AND ABBREVIATIONS

| | |
|---|---|
| AAS | Atomic absorption spectrometer |
| ABA | Absolute bioavailability |
| AFo | Oral absorption fraction |
| bw | Body weight |
| °C | Degrees Celsius |
| CV | Coefficient of variation (SD/mean) |
| DMA | Dimethylarsinic acid |
| EDS | Energy dispersive spectrometer |
| EMPA | Electron Microprobe Analysis |
| ERA | Environmental Resource Associates |
| GLP | Good Laboratory Practices |
| HCl | Hydrochloric acid |
| $HNO_3$ | Nitric acid |
| ICP-AES | Inductively Coupled Plasma-Atomic Emission Spectrometry |
| IRIS | Integrated Risk Information System |
| kg | Kilogram |
| KI | Potassium iodide |
| L | Liter |
| mg | Milligram |
| mL | Milliliter |
| MMA | Monomethylarsonic acid |
| NaAs | Sodium arsenate |
| NIST | National Institute of Standards and Testing |
| NRCC | National Resource Council Canada (Institute for National Measurement Standards) |
| ORD | USEPA Office of Research and Development |
| oRfD | Oral reference dose |
| oSF | Oral slope factor |
| PE | Performance Evaluation |
| ppm | Parts per million |
| QA | Quality assurance |
| RBA | Relative bioavailability |
| RME | Reasonable maximum exposure |
| SD | Standard deviation |
| SEM | Standard error of the mean |
| SOP | Standard operating procedure |
| TAL | Target Analyte List |
| UEF | Urinary excretion fraction |
| µg | Microgram |
| µm | Micrometer |
| U.S. EPA | U.S. Environmental Protection Agency |
| WDS | Wavelength dispersive spectrometers |
| XAS | X-ray absorption spectroscopy |

# 1.0 INTRODUCTION

## 1.1 Overview

Accurate assessment of the human health risks resulting from oral exposure to arsenic requires knowledge of the amount of arsenic absorbed from the gastrointestinal tract into the body. This information on gastrointestinal absorption may be described either in absolute or relative terms:

Absolute Bioavailability (ABA) is the ratio of the amount of arsenic absorbed to the amount ingested:

$$ABA = (Absorbed\ Dose) / (Ingested\ Dose)$$

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

Relative Bioavailability (RBA) is the ratio of the absolute bioavailability of arsenic present in some test material to the absolute bioavailability of arsenic in some appropriate reference material:

$$RBA = ABA(test) / ABA(reference)$$

Usually the form of arsenic used as the reference material is an arsenic compound dissolved in water or a readily soluble form (e.g., sodium arsenate) that is expected to completely dissolve when ingested.

For example, if 100 µg of arsenic dissolved in drinking water were ingested and a total of 90 µg were absorbed into the body, the ABA would be 0.90 (90%). Likewise, if 100 µg of arsenic contained in soil were ingested and 30 µg were absorbed into the body, the ABA for soil would be 0.30 (30%). If the arsenic dissolved in water was used as the frame of reference for describing the relative amount of arsenic absorbed from soil, the RBA would be 0.30/0.90, or 0.33 (33%).

When reliable data are available on the RBA of a chemical (e.g., arsenic) in a site medium (e.g., soil), this information can be used to improve the accuracy of exposure and risk calculations at that site. Available RBA data can be used to adjust default oral toxicity values (reference dose and slope factor) to account for differences in absorption between the chemical ingested in water and the chemical ingested in site media, assuming the toxicity factors are based on a readily soluble form of the chemical.

## 1.2 Using Relative Bioavailability Data to Improve Risk Calculations for Arsenic

The Risk Assessment Guidance for Superfund (RAGS) Part A (U.S. EPA, 1989) and Guidance for Evaluating the Bioavailability of Metals in Soils for Use in Human Health Risk Assessment (U.S. EPA, 2007) discuss making adjustments to exposure estimates in Superfund site-specific risk assessments when the medium of exposure in the exposure assessment differs from the medium of exposure assumed by the toxicity value (cancer slope factor, reference dose value, etc.) based upon site-specific

bioavailability data. When a reliable RBA value is available for a particular site medium (e.g., soil), the RBA can be used to adjust estimate of the daily intake (DI) as follows:

## 2.0 EXPERIMENTAL METHODS FOR ESTIMATING ARSENIC RBA BY *IN VIVO* STUDIES

All *in vivo* studies were performed according to the spirit and guidelines of Good Laboratory Practices (GLP: 40 CFR 792). Standard Operating Procedures (SOPs) that included detailed methods for all of the components of each study were prepared, approved, and distributed to all team members prior to all studies.

### 2.1 Basic Approach for Measuring RBA *In Vivo*

Summary of Arsenic Toxicokinetics

Available data from studies on the absorption and excretion of soluble arsenic compounds in humans and animals are summarized in Table 2-1. Based on the fecal excretion data, absorption of soluble arsenic compounds (sodium arsenate and sodium arsenite) typically appears to be at least 90% in both humans and animals.

Estimates of biliary excretion are available from studies in which soluble arsenic compounds have been given by intravenous injection. Results from studies by Johnson and Farmer (1991) and Freeman et al. (1994) indicate biliary excretion is probably about 4–8% of the absorbed dose. Correction of fecal excretion data by subtraction of 8% to account for biliary excretion suggests that absorption of soluble arsenic is probably close to 100% in most cases.

Figure 2-1 plots the urinary excretion data from Table 2-1. It is apparent that typical urinary recovery of soluble arsenic in humans (top panel) is dose-independent, and averages about 67% (range = 45 to 85%). Urinary recovery of arsenic in rodents (Figure 2-1, lower panel) is similar, with an average value of 70% (range = 36 to 94%). Often the sum of arsenic recovery in urine plus feces is slightly less than 100%. This could be partly due to experimental error, but is more likely due to retention of some arsenic in tissues such as skin and hair.

Conceptual Model

Based on the human and animal data above, it appears that both absorption and excretion are likely to be linear (i.e., dose independent) processes at dose levels well above those expected from

exposure to arsenic in soil (e.g., 1000 ppm × 100 mg/day = 100 µg/day). Figure 2-2 shows a conceptual model for the toxicokinetic fate of ingested arsenic that is based on concept that absorption and excretion are linear. Key points of the model are as follows:

- If 100% of all absorbed arsenic were excreted in the urine, the UEF would be equal to the oral absorption fraction or ABA. However, 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 (e.g., a test soil and sodium arsenate) 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 or biliary excretion, because the fraction of absorbed arsenic that is excreted in urine ($K_u$), which does depend on tissue binding and biliary excretion, cancels in the calculation:

$$RBA(x\ vs.\ y) = \frac{UEF(x)}{UEF(y)} = \frac{AF_o(x) \cdot K_u}{AF_o(y) \cdot K_u} = \frac{AF_o(x)}{AF_o(y)}$$

where:

*RBA(x vs. y)* is the relative bioavailability of As in test material (*x*) vs. sodium arsenate (*y*);

*UEF* is the urinary excretion fraction of the dose excreted in urine;

$AF_O$ is the absorption fraction, which is the fraction of the dose absorbed following oral administration; and

$K_u$ is the fraction of the absorbed dose excreted in urine.

Thus, measurement of the urinary excretion fraction (µg/day excreted in urine per µg/day administered) of test material and reference material (sodium arsenate) is the key experimental goal in these arsenic RBA studies.

Estimation of UEF

The amount of arsenic excreted in urine (µg/day) is calculated as the product of urinary concentration (µg/L) and urinary volume (L/day). The UEF is the rate of As excreted in urine (mL/day) divided by the dose (mg/day). Conceptually, the UEF could be estimated for each animal on each day that data are collected, and the UEF estimates for a particular dose material could then be averaged across different animals, dose levels, and days. However, this approach does not account for baseline intake and excretion of arsenic in the control group (unexposed animals), and tends to overemphasize UEF values at

the low end of the dose range where the estimate of urinary excretion is most uncertain. A more robust approach, used in this evaluation, is to plot the mass excreted by each animal as a function of the dose administered to each animal, and then fit a linear regression line to the combined data. The slope of this line is a direct estimate of the UEF (µg/day excreted per µg/day ingested). This approach automatically accounts for baseline arsenic ingestion and excretion in control (unexposed) animals, and is not disproportionately influenced by measurement error at the low end of the dose curve.

The process of deriving the best fit linear regression lines through the data is complicated by the fact that the equations for each dose material in a study must have the same intercept, and because the variability in the data tend to increase as the dose increases (this is referred to as heteroscedasticity). In order to address these issues, the data from each study were fit using simultaneous weighted linear regression, as detailed in Appendix A.

## 2.2 Experimental Methods

### 2.2.1 Study Designs

Phase II Study Designs

Measurement of arsenic bioavailability in most Phase II studies was performed in parallel with studies designed to estimate lead bioavailability (U.S. EPA, 2007). Groups of animals (typically 4 or 5 per dose group) were given oral doses of a test material (e.g., soil, tailings, slag, sediment) twice daily for 15 days, and 24-hour urine samples were collected several times during the study (typically on days 7 and 14). Because the main focus of these studies was on lead RBA, these early studies did not include groups of animals that were exposed to an arsenic reference material. Thus, these studies, taken alone, were not sufficient to allow for an estimation of the arsenic RBA of the test materials.

In order to address this data gap and provide data on the urinary excretion fraction of a suitable reference material, two "pilot studies" (Phase II, Experiments 10 and 15) were performed to establish the urinary excretion fraction for sodium arsenate administered by three different routes: orally with a small amount of food, orally by gavage (no food), and by intravenous injection.

Appendix B1 provides the detailed study designs for each Phase II study, and Appendix B2 provides the detailed designs for the two pilot studies.

Phase III Study Designs

After the completion of the Phase II studies, a modified study design was developed that was specifically optimized for evaluation of arsenic RBA, rather than lead RBA. In this design, each study includes a set of animals exposed to the reference material (sodium arsenate) and one to three different

test materials, each at two or three different dose levels. In some cases, the doses of arsenic (expressed as µg/day) were held constant over time, rather than being adjusted to account for changing body weight. This is because the basic computational approach used to estimate RBA (described above) compares the mass of arsenic excreted in urine (µg/day) to the mass of arsenic ingested (µg/day), so body weight adjustments are not needed.

Appendix B3 provides the detailed study designs for each Phase III study.

### 2.2.2 Experimental Animals

Juvenile swine were selected for use in these studies because their gastrointestinal physiology is more similar to humans than most other animal models (Weis and LaVelle, 1991). All animals were young males of the Pig Improvement Corporation genetically defined Line 26, and were purchased from Chinn Farms, Clarence, MO. All studies used intact animals, except for one (the second VBI70 study), which used castrated animals. The number of animals purchased for each study was typically 6–8 more than required by the protocol. These animals were usually purchased at age 4–5 weeks (weaning occurs at age 3 weeks), and they were then held under quarantine for one week to observe their health before beginning exposure to test materials. Any animals that appeared to be in poor health during this quarantine period were excluded. To minimize weight variations between animals and groups, extra animals most different in body weight (either heavier or lighter) four days prior to exposure (day-4) were also excluded from the study. The remaining animals were assigned to dose groups at random. When exposure began (day zero), the animals were about 5–6 weeks old and weighed an average of about 7–12 kg.

All animals were housed in individual stainless steel cages. Each animal was examined by a certified veterinary clinician (swine specialist) prior to being placed on study, and all animals were examined daily by an attending veterinarian while on study. There were no instances where animals that became ill could not be promptly restored to good health by appropriate treatment, so no animals were removed from the studies.

### 2.2.3 Diet

Animals provided by the supplier were weaned onto standard pig chow purchased from MFA Inc., Columbia, MO. In order to minimize arsenic exposure from the diet, the animals were gradually transitioned from the MFA feed to a special feed (Zeigler Brothers, Inc., Gardners, PA) over the time interval from day -7 to day -3; this feed was then maintained for the duration of the study. The feed was nutritionally complete and met all requirements of the National Institutes of Health–National Research Council. The typical nutritional components and chemical analysis of the feed is presented in Table 2-2.

Each day every animal was given an amount of feed equal to 5% (4% in the Aberjona River study) of the mean body weight of all animals on study. Feed amounts were adjusted every three days, when pigs were weighed. Feed was administered in two equal portions of 2.5% (2% in the Aberjona River study) of the mean body weight at 11:00 AM and 5:00 PM daily. Periodic analysis of feed samples indicated that the arsenic level was generally below the detection limit (0.1 ppm), which corresponds to a dose contribution from food of less than 5 µg/kg-day (less than 50 µg/day for a 10 kg animal).

Drinking water was provided *ad libitum* via self-activated watering nozzles within each cage. Periodic analysis of samples from randomly selected drinking water nozzles indicated the arsenic concentration was less than the detection limit (about 1 µg/L). Assuming water intake of about 0.1 L/kg-day, this corresponds to a dose contribution from water of less than 0.1 µg/kg-day (1 µg/day for a 10 kg animal).

### 2.2.4 Dosing

Animals were exposed to sodium arsenate (abbreviated in this report as "NaAs") or a test material for 12–15 days, with the dose for each day being administered in two equal portions given at 9:00 AM and 3:00 PM (two hours before feeding). Animals were administered dose material when in a semi-fasted state (i.e., two hours before feeding) to avoid the presence of food in the stomach, which is known to reduce absorption of arsenic. In Phase II, doses were based on measured group mean body weights and were adjusted every three days to account for animal growth. In most Phase III studies, doses were held constant (independent of body weight).

Dose material was placed in the center of a small portion (about 5 grams) of moistened feed (referred to as a "doughball"), which was administered to the animals by hand. In cases where the mass of soil was too large to fit into one doughball, the test material was distributed among two or more doughballs. Occasionally, some animals did not consume some or the entire dose (usually because the dose dropped from their mouth while chewing). All missed doses were recorded and the time-weighted average dose calculation for each animal was adjusted downward accordingly.

### 2.2.5 Collection and Preservation of Urine

Samples of urine were collected from each animal on several different days during the study (the exact days varied from study to study). Collection began at about 8:00 AM and ended 24 hours later in the Phase II studies and 48 hours later in most Phase III studies. The urine was collected in a stainless steel pan placed beneath each cage, which drained into a plastic storage bottle. Each collection pan was fitted with a nylon screen to minimize contamination with feces or spilled food. At the end of each collection period, the urine volume was measured and two 60-mL portions were removed for analysis.

Each 60 mL sample was preserved by addition of 0.6 mL of concentrated nitric acid. These samples were refrigerated until sample analysis.

### 2.2.6 Arsenic Analysis

All samples were assigned random chain-of-custody tag numbers and submitted to the analytical laboratory in a blind fashion. Arsenic concentrations in urine were measured using a hydride generation approach. This method requires that all arsenic exist in the form of inorganic arsenic before hydride generation. Because arsenic in urine can exist in organic forms (monomethylarsonic acid [MMA] and dimethylarsinic acid [DMA]) as well as inorganic forms, digestion of the urine prior to analysis is required.

#### *2.2.6.1 Sample Digestion*

Two different methods of arsenic digestion prior to analysis were employed during this project. The first method was used during Phase II and a revised method was used for Phase III studies. As discussed in greater detail below (see *PE Samples and Blind Duplicates* in Section 2.2.7), this change in digestion method was adopted because recovery of total arsenic from urine and other biological samples using the first method was limited by incomplete conversion of organic metabolites of arsenic (MMA and DMA) to inorganic arsenic. The revised method produced improved recoveries of these metabolites and of total arsenic.

Digestion Method 1

A 25 mL aliquot of acidified urine was removed and placed in a clean 100 mL glass beaker. 20 mL of concentrated nitric acid and 2.5 mL of concentrated perchloric acid were then added. The beaker was covered with a watch glass and placed on a hot plate to reflux for 4–12 hours. After this period, the heat was increased to drive off the nitric acid and to cause the perchloric acid to fume. After about 10 minutes of fuming, the digestate was cooled slightly and diluted with 20 mL of distilled water. This was heated until clear, and then cooled and diluted to 50 mL.

Digestion Method 2

A 25 mL aliquot of acidified urine was removed and placed in a clean 100 mL beaker. 3.0 mL of methanol, 10.0 mL of 40% (w/v) magnesium nitrate hexahydrate, and 10.0 mL of concentrated trace metal grade nitric acid ($HNO_3$) were then added. The beaker was covered with a watch glass and placed on a hot plate to reflux for 8–12 hours at 70–80ºC. After this, the temperature was increased to 200°C, and the watch glass was moved back to allow faster evaporation. The sample was then heated to complete dryness (8–12 hours), covered with a watch glass, and allowed to cool. Dried samples were

transferred to a cool muffle furnace which was heated at a rate of 1 degree/minute to a temperature of 500ºC, and then held at 500ºC for 3 hours before cooling. Ashed samples were dissolved by adding 5 mL distilled water and 5 mL concentrated trace metal grade hydrochloric acid (HCl), and boiling gently until the white residue was completely dissolved. After cooling, the dissolved sample was diluted with distilled water to 50.0 mL and held until analysis.

#### *2.2.6.2 Arsenic Analysis by Hydride Generation*

Arsenic concentrations in urine were measured by hydride generation. Samples were prepared for hydride generation by dilution with a solution of 10% HCl, 10% potassium iodide (KI), and 5% ascorbic acid. The samples were diluted l/10 or 1/5 (v/v), depending on the detection limit desired. Samples were held in the diluting fluid at least 30 minutes before analysis, but overnight was preferred. Analysis was performed on a Perkin-Elmer 3100 atomic absorption spectrometer (AAS) equipped with a FIAS 200 flow injection system. Calibration standards were prepared in dilution fluid (10% HCl, 10% KI, 5% ascorbic acid) at concentrations of 0.0, 0.2, 1.0, 5.0, 10.0, and 15.0 µg/L.

The detection limit of the method was evaluated by performing 10 replicate analyses of a low standard (about 1 µg/L). The detection limit was defined as three times the standard deviation of these 10 analyses. A 1/10 dilution typically gave a detection limit of about 2 µg/L, while a dilution of 1/5 typically yielded a detection limit of about 1 µg/L. All responses below the detection limit were evaluated at one-half the detection limit.

### 2.2.7 Quality Assurance

A number of quality assurance (QA) steps were taken throughout the studies to assess and document the quality of the data that were collected. These steps are summarized below.

Blanks

Blank samples analyzed with each batch of samples never yielded a measurable level of arsenic, with all values being reported as less than 2.0 µg/L of arsenic.

Spike Recovery

Randomly selected samples were spiked with known amounts of inorganic arsenic (5–20 µg) and the recovery of the added arsenic was measured. In Phase II, recovery of arsenic from spiked samples typically ranged from 95 to 105%, with an average across all analyses of 99.8%. In Phase III, recovery of arsenic from spiked samples typically ranged from 83 to 120%, with an average across all analyses of 103%.

Laboratory Duplicates

Random urine samples were selected for duplicate analysis by the analyst. In Phase II, the average absolute difference across all pairs of duplicates samples was 2.4 µg/L (n = 58). In Phase III, the average absolute difference across all samples was 2.3 µg/L (n = 115).

Laboratory Control Standards

Samples of various reference materials were analyzed with each set up test samples. Results for these standards are summarized below:

| Reference Material | Description | Certified Value | Measured Results | | |
|---|---|---|---|---|---|
| | | | Mean (% Certified Value) | Standard Deviation | n |
| **Phase II** | | | | | |
| ERA Potable WatR™ #697 (Trace Metals, Lot 3413) | Plain water spiked with inorganic trace metals | 68.8 µg/L | 23.6 µg/L (34.3%) | 10.2 µg/L | 12 |
| NIST 2670 Elevated | Normal human urine spiked with inorganic trace elements | 480 ± 100 µg/L | 451 µg/L (94%) | 12.8 µg/L | 26 |
| **Phase III** | | | | | |
| ERA Waste WatR™ #500 (Trace Metals, Lot P081) | Plain water spiked with inorganic trace metals | 366 µg/L | 361 µg/L (98.6%) | 7.2 µg/L | 220 |
| ERA Waste WatR #500™ (Trace Metals, Lot 99106) | Plain water spiked with inorganic trace metals | 347 µg/L | 328 µg/L (95%) | 6.7 µg/L | 38 |
| ERA Waste WatR #500™ (Trace Metals, Lot 9978) | Plain water spiked with inorganic trace metals | 92.9 µg/L | 96 µg/L (103%) | 1.7 µg/L | 90 |
| NIST 2670 Elevated | Normal human urine spiked with inorganic trace elements | 480 ± 100 µg/L | 544 µg/L (113%) | 9.6 µg/L | 7 |
| NIST 1640 | Natural water containing trace elements (not spiked) | 0.0267 ± 0.0004 µg/g | 0.027 µg/g (99.4%) | 0.001 µg/g | 2 |
| NRCC Dolt-2 | Dogfish liver (not spiked) | 16.6 ± 1.1 µg/g dry wt | 14.7 µg/g dry wt (88.6%) | 0.8 µg/g dry wt | 10 |
| NRCC Tort-2 | Lobster hepatopancreas (not spiked) | 21.6 ± 1.8 µg/g dry wt | 21.3 µg/g dry wt (98.8%) | 1.2 µg/g dry wt | 12 |
| NIST 1566b | Oyster tissue (not spiked) | 7.65 ± 0.65 µg/g dry wt | 7.6 µg/g dry wt (99.9%) | 0.5 µg/g dry wt | 13 |

ERA: Environmental Resource Associates
NIST: National Institute of Standards and Technology
NRCC: National Resource Council Canada (Institute for National Measurement Standards)

As seen, results were good with the exception of one standard (ERA #697) in Phase II. The low recovery from these samples is not understood.

PE Samples and Blind Duplicates

In addition to these laboratory-based (non-blind) QA procedures, a series of blind Performance Evaluation (PE) samples (known concentrations of sodium arsenate in control urine) and blind duplicates were submitted to the laboratory in a random fashion, commingled with normal test samples.

The combined results for samples evaluated during the Phase II pilot studies are shown in Figure 2-3. As seen in Panel A, there was good accuracy on sodium arsenate PE samples (10, 30, and 1000 µg/L) throughout the duration of each study. As shown in Panel B, there was also good reproducibility between blind duplicate samples.

Initially, these QA results were interpreted to indicate that the analytical procedure was operating correctly. However, the low recovery of arsenic for the ERA standard, as well as the observation that the recovery of arsenic from the urine of animals administered sodium arsenate was lower than expected, suggested that a problem did exist. In order to investigate this, a series of PE samples were prepared by addition of three different concentrations of each of the four major urinary arsenic metabolites to control urine, and each was analyzed in triplicate. The results are summarized below:

| **Urinary Metabolite** | **Average Recovery (Method 1)** |
|---|---|
| Arsenate | 101±2% |
| Arsenite | 93±2% |
| MMA | 73±3% |
| DMA | 15±4% |

As seen, recovery of inorganic forms of arsenic were within reasonable bounds, but recovery of MMA was somewhat decreased and recovery of DMA was very poor. Based on the expectation that this low recovery was based on incomplete conversion of MMA and DMA to inorganic arsenic prior to hydride generation, a more vigorous digestion method was developed (see *Digestion Method 2* in Section 2.2.6). Recovery of each urinary metabolite using this new digestion method is summarized below:

| Urinary Metabolite | Average Recovery (Method 2) |
|---|---|
| Arsenate | 106±2% |
| Arsenite | 106±7% |
| MMA | 107±3% |
| DMA | 113±3% |

As seen, the revised digestion method yielded good recovery of all metabolites, including both MMA and DMA. On this basis, the revised digestion method was used on all arsenic RBA studies following the completion of Phase II.

The results for the Phase III PE samples are shown in Figure 2-4. As seen, the PE samples included several different concentrations each of four different types of arsenic ($As^{+3}$, $As^{+5}$, MMA, and DMA). With the exception of one unexplained outlier, there was good recovery of the arsenic from all four types of PE sample.

The results for the blind duplicates from Phase III are shown in Figure 2-5. As seen, there was good agreement between results for duplicate pairs, with an average absolute difference between pairs of about 6.0 µg/L and an average relative percent difference of about 1.5%.

Inter-laboratory Comparison

In two Phase III studies (Experiments 1 and 2), a series of samples was submitted to a second laboratory for inter-laboratory comparison of results. This included investigative samples (urine samples collected from study animals) as well as several PE samples. The results are shown in Figure 2-6. As seen, there is generally good agreement between the two laboratories, with somewhat better reproducibility for the Phase III studies.

Conclusion

Based on the results of all of the quality assurance samples and steps described above, it is concluded that the analytical results for samples of urine are generally of high quality and are suitable for derivation of reliable estimates of arsenic absorption from test materials. The only potential limitation is that recovery of organic arsenic (especially DMA) is low in Phase II studies, which will tend to result in an underestimate of UEF values. However, since RBA calculations are based on the ratio of two UEFs, if both UEFs are underestimated by the same amount, then the resultant RBA may still be reliable (see Section 2.3.2, below).

### 2.2.8 Test Material Characterization

Table 2-3 describes the test materials for which RBA was measured in this program and provides the analytical results for arsenic. Data on other Target Analyte List (TAL) metals, if available, are provided in Appendix C. As seen, 27 different test materials were investigated (two in duplicate). In all cases, these samples were sieved prior to analysis and dosing, and only materials which passed through a 60-mesh screen (corresponding to particles smaller than about 250 µm) were used. This is because it is believed that soil particles less than about 250 µm are most likely to adhere to the hands and be ingested by hand-to-mouth contact, especially in young children.

Many of the test materials[1] were characterized with regard to arsenic mineral phase, particle size distribution, and matrix association using electron microprobe analysis (EMPA). In this procedure, an electron microprobe with combined energy dispersive spectrometer (EDS) and multiple wavelength dispersive spectrometers (WDS) was used to evaluate the elemental composition of arsenic-bearing particles. A 1 to 2 gram split of dried sample was placed in a 2.5 cm plastic mold and impregnated with epoxy. Once the sample was hardened, it was polished and carbon coated for EMPA. The EMPA was operated at 15 kV accelerating voltage, with a 20 nA current and a 1 micron focused beam. Instrument response was calibrated using certified mineral or pure metal standards and counting times were chosen to provide 3-sigma detection limits of between 100–200 ppm. Elemental concentrations were corrected using ZAF factors and concentration errors were generally less than 5% relative. For a more detailed explanation of the EMPA method of analyses see Birks (1971) or Heinrich (1981).

Although the electron microprobe is capable of determining the precise stoichiometry of the elements in any given particle, this was not attempted in this project. This is mainly because investing time in obtaining precise stoichiometry decreases the number of different particles that can be examined. In addition, many arsenic-bearing particles are not composed of a pure mineral phase with an exact stoichiometry, but are characterized by arsenic that is either adsorbed onto other mineral particles, or is a mixture of phases that are undergoing transition from one phase to another. For this reason, particles were classified into “phases” that may not be purely stoichiometric and may contain a mixture of similar chemical phases. The first step used in the assignment of a phase designation was to determine if the

[1]Arsenic was not speciated in three Phase II samples (Aspen Berm, Aspen Residential, and Jasper County High Lead Mill) because the concentration of arsenic in each material was too low (17 ppm, 67 ppm, and 16 ppm, respectively) to allow reliable evaluation. In addition, speciation data were unavailable for four Phase III samples (El Paso TM1, El Paso TM2, ACC Utility Pole Soil, and ACC Dislodgeable Arsenic).

phase was an oxide, carbonate, sulfide, sulfate, or phosphate. Secondly, with the exception of the "phosphates," the major cation associated with the phase was identified. Therefore, phases such as Fe-sulfate, FeOOH, MnOOH, PbMO, AsMO, or $PbMSO_4$ were identified (where M represents "metal"). Some of these phases could represent a stoichiometric mineral form, but most are likely to be metastable and/or amorphous and have some quantity of arsenic sorbed to their surface.

The "phosphate" group is even more generic in that the only common dominant ion is $PO_4$. Although arsenic and phosphorous are both oxy-anions, a number of particles that contain both arsenic and phosphate have been identified. As above, these might include minerals that contain mixtures of phosphate and arsenate such as walentaite $(Ca,Mn,Fe)Fe_3(AsO_4,PO_4)_4$-$7H_2O$, morelandite $(Ba,Ca,Pb)_5Cl[AsO_4,PO_4]_3$, or turneaureite $Ca_5(Cl)[(AsO_4, PO_4)_3]$, but more likely represent arsenic adsorbed onto other phosphate-containing particles.

Detailed EMPA results are presented in Appendix C and the results, expressed as relative arsenic mass, are summarized in Table 2-4. The relative arsenic mass for a particular phase is the estimated percentage of the total arsenic in a sample that is present in that phase. Of the 28 different phases detected in one or more samples, 14 are relatively minor, with relative arsenic mass values less than 5%. However, the remaining 14 phases occur at concentrations that could contribute significantly to the bioavailability of the sample.

Table 2-5 summarizes data on the size distribution of arsenic-containing particles (measured as the longest dimension) in each sample. As seen, most samples contain a range of particle sizes, with the majority of particles being less than 50 µm in diameter.

Table 2-6 summarizes information on the degree to which arsenic-bearing grains in each sample are partially or entirely exposed on their outer surfaces (*liberated*), or are entirely enclosed within a larger particle of rock or slag (*included*). Data are presented both on a simple particle frequency basis and on the basis of relative arsenic mass. As seen, the majority of arsenic-bearing particles in all samples are partly or entirely *liberated*.

In interpreting the results of the particle speciation studies, it is important to understand that, on a mass basis, only a tiny fraction of the total sample is evaluated by electron microprobe and, hence, there is moderate uncertainty as to whether the results for the grains examined are truly representative of the sample as a whole.

It is also worth noting that other speciation methods are available to determine the chemical forms of metals in soil systems. Each method has distinct advantages and disadvantages; and some methods provide more robust data than others (see D'Amore et al. 2005). One such technique is X-ray

absorption spectroscopy (XAS) for which USEPA Office of Research and Development (ORD) has resident experts to conduct studies and the service is available to support Regional research efforts. XAS probes the sub-atomic structure of elements to distinguish specific bonding mechanisms which leads to precise determination of metal speciation. An example for As is differentiation of As sorbed to an iron oxide versus As present as the mineral scorodite ($FeAsO_4$) for which XAS can easily identify the different phases that have vastly different bioavailability behaviors whereas EMPA will identify both phases as containing As, Fe, and O.

## 2.3 Results

### 2.3.1 RBA Estimates

Detailed raw data for each study are provided in Appendix D. Results of simultaneous weighted linear regression fitting and RBA calculations are presented in Appendix E. The results are summarized below.

The upper portion of Table 2-7 summarizes the RBA results for all Phase II studies, and the lower portion summarizes the results for materials studied during Phase III. As seen, using sodium arsenate as a relative frame of reference, estimated RBA values range from 8% to more than 100%. This wide variability supports the conclusion that there can be important differences in RBA between different types of samples and that use of a site-specific RBA value is likely to increase the accuracy of risk estimates for arsenic. Available data do not include replicate estimates of RBA of the same test materials; therefore, there is no empirical basis for estimating variability in the RBA estimates that might be attributable to within-test material variability as opposed to between-test material variability. Although ABA of As is not estimated in the data reduction procedure for the swine assays, RBA is estimated as the ratio of the slopes of the dose-UEF relationships for sodium arsenate and the test material. Table 2-8 provides summary statistics for the dose-UEF slopes for sodium arsenate and all test materials assayed in the Region 8 Phase III studies. The coefficient of variation (SD/mean) for the sodium arsenate slopes is approximately 0.13 (N=7). This variability reflects an unknown combination of biological variability in As bioavailability and other assay variables that contribute to variability in the measurement of the dose-UEF slope. The coefficient of variability for the dose-UEF slopes for the test materials is 0.38 (N=14), and is greater than that for sodium arsenate by a factor of approximately 3. The difference in the two estimates reflects, at least in part, the additional variability introduced into the dose-UEF slope estimates contributed by differences in bioavailability of the test materials. This outcome suggests that test material characteristics contribute substantially to the observed variability in RBA estimates. This conclusion is also consistent with the similarity between the coefficient of variability of the dose-UEF slope for test materials (0.38) and the estimated RBAs for the same test materials (0.32).

Figure 2-7 shows that the uncertainty in the RBA value for a test material (as reflected by the difference between the upper bound and the lower bound) depends on the dose of arsenic administered in the study. As seen, three of the test materials (Aspen Berm, Aspen Residential, and Jasper County High Lead Mill) were administered only at low dose levels (less than 20 µg/kg bw-day) and have extremely wide uncertainty bounds around the RBA estimates. This is due mainly to the fact that the concentrations of arsenic in the urine were very low and, hence, were difficult to quantify with good accuracy and also difficult to distinguish from baseline. Because of the high uncertainty in these results, the data from these three test materials are not considered further. Thus, based on these results, a minimum daily As dose of 25 µg/kg-bw/day is recommended to ensure the amount if excreted in urine reaches a measurable quantity and, that is to minimize uncertainty in RBA estimates.

### 2.3.2 Effect of Low Analytical Recovery on Phase II RBA Values

As noted above, all of the calculations of arsenic RBA performed during Phase II are based on data obtained using an analytical method that had low recovery of organic metabolites of arsenic, which raises a concern over the accuracy of the results. However, the low recovery of arsenic is not necessarily a basis for complete distrust of the results. This is because the RBA is a ratio of two measured values, and if the degree of error (underestimation) is the same in both the numerator and denominator, then the error will cancel and the resulting ratio will be correct. However, the degree of error in each measurement depends on the relative concentration of the metabolites in the urine: if the level of MMA and DMA is low, the error will be smaller than if the levels of MMA and DMA are high. Thus, the key question is whether or not the ratio of the urinary metabolites tends to be relatively constant as a function of dose and dose material, at least over the range of exposures investigated in the Phase II studies.

The most direct approach for testing this question is to measure the relative concentration of each metabolite ($As^{+3}$, $As^{+5}$, MMA, DMA) in urine from a number of animals exposed to a series of different dose levels and dose materials. This approach was attempted, but the results for quality control samples indicated that the results were not reliable, presumably due to the technical difficulty of performing the separation and quantification of the individual metabolites. Therefore, this approach was not pursued further.

An alternative approach is to measure the UEF and RBA of several test materials using both analytical methods, and to compare the results. This approach was implemented for two different test materials (Butte TM1 and Butte TM2), and the results are shown below:

| Substance Administered | Digestion Method 1 | | Digestion Method 2 | |
|---|---|---|---|---|
| | UEF | RBA | UEF | RBA |
| Sodium Arsenate | 0.238 | [1.00] | 0.890 | [1.00] |
| Butte TM1 | 0.047 | 0.20 | 0.158 | 0.18 |
| Butte TM2 | 0.056 | 0.23 | 0.210 | 0.24 |

As seen, the measured UEF for sodium arsenate based on Digestion Method 1 (24%) is much lower than the UEF based on Digestion Method 2 (89%). However, the UEF of each of two different soil test materials was also lower by approximately the same relative amount when measured by Digestion Method 1 compared to Digestion Method 2, so the ratio (the RBA) was approximately constant when calculated for each method. These results indicate that, even though the low recovery of arsenic in Phase II studies is a basis for uncertainty in the RBA estimates derived during Phase II, the error due to low recovery of organic metabolites of arsenic is likely to approximately cancel, and the final RBA estimates are likely to be approximately correct. For this reason, the Phase II data were included in the overall estimates of As RBA.

### 2.3.3 Effect of Food on Arsenic Absorption

In Phase II Pilot Study 2 (Experiment 15), some animals were dosed with NaAs *via* gavage in order to compare the results with NaAs given in orally in doughballs. These results are shown below:

| Substance Administered | UEF | | |
|---|---|---|---|
| | Slope | SEM | N |
| NaAs – Gavage | 0.189 | 0.014 | 31 |
| NaAs – Doughball | 0.177 | 0.014 | 31 |

As seen, the UEF for sodium arsenate administered orally in a doughball is only slightly lower than the UEF for sodium arsenate administered by gavage, indicating that the amount of feed (about 5 grams) used to administer the arsenic doses does not significantly affect arsenic absorption.

## 2.4 Correlation of RBA with Arsenic Geochemistry

One objective of this project was to obtain preliminary information on which mineral and chemical forms of arsenic tend to have high bioavailability and which tend to have low bioavailability. As noted above, data on chemical form or mineral association were obtained using EMPA. Detailed data are presented in Appendix C and results are summarized in Section 2.2.8 and in Tables 2-4 to 2-6.

In order to derive quantitative estimates of phase-specific RBA values, a multivariate linear regression approach was used, employing the following basic model:

$$RBA = \sum (f_i \cdot RBA_i)$$

where:

*fi* = Fraction of total arsenic present in phase "*i*"

*RBAi* = Inherent RBA of phase "*i*"

However, because a total of 28 different phases were identified and reliable RBA results were obtained for only 20 different samples, it is clear that the existing data are not sufficient to perform a robust regression analysis. Instead, a screening-level analysis was performed, as follows. First, in order to reduce the number of independent variables, the 28 different phases were grouped into 9 categories as described in Table 2-9. These categories were based on professional judgment regarding the expected degree of similarity between members of a group, along with information on the relative abundance of each phase (see Table 2-4). Phases with low relative arsenic mass (maximum relative mass in any test material less than 15%) were grouped together under "Minor Constituents;" these phases included AsMO, $AsMSO_4$, Clays, Paint, Pb Solder, Pb-As Vanidate, PbAsMO, PbAsSbCuO, $PbCrO_4$, PbMO, PbMS, $PbMSO_4$, Pyrite, $TiO_2$, and $ZnSiO_4$. Next, the fraction of arsenic present in each group was calculated by summing the relative arsenic mass for each phase in the group. Based on the expectation that particles that are totally *included* (fully enclosed or encased in mineral or vitreous matrices) are not likely to contribute significantly to the observed RBA value of a sample, only the relative arsenic mass in partially or entirely *liberated* particles (partially or entirely exposed on their outer surfaces) was included in the sum. The results are shown in Table 2-10.

Group-specific RBA values were then estimated by fitting the grouped data to the model using minimization of square errors. Two different options were employed. In the first option, each fitting parameter (group-specific RBA) was fully constrained to be between zero and one, inclusive. In the second option, all parameters were unconstrained. Because the minor constituents do not contribute significantly to the total arsenic mass in any of the tested materials, a reasonable estimate of their specific RBA cannot be obtained. Therefore, an arbitrary coefficient of 0.5 was assumed for this group and the coefficient was not treated as a fitting parameter. The resulting estimates of the group-specific average RBA values for the remaining groups are shown in Table 2-11 (these values apply only to *liberated* particles).

As seen, there is a wide range of group-specific RBA values, with the precise values depending on the method used to constrain the parameters. It is important to stress that these group-specific RBA estimates are derived from a very limited data set, so the group-specific RBA estimates are inherently very uncertain. In addition, both the measured sample RBA values and the relative arsenic mass in each phase are subject to additional uncertainty. Therefore, the group-specific RBA estimates should not be considered to be highly precise, and calculation of a quantitative sample-specific RBA value from these estimates is not appropriate. Rather, it is more appropriate to consider the results of this study as sufficient to support only a qualitative classification of phase-specific RBA values, as follows:

| Low Bioavailability | Medium Bioavailability | High Bioavailability |
|---|---|---|
| $As_2O_3$<br>Sulfosalts | As Phosphate<br>FeAs Oxide<br>PbAs Oxide<br>MnAs Oxide<br>Fe and Zn Sulfates | FeAsO |

## 2.5 Discussion of *In Vivo* Results

The results of this investigation indicate that juvenile swine are a useful model for quantifying gastrointestinal absorption of arsenic from different test materials, using urinary arsenic excretion as the measurement endpoint. In addition, this experimental protocol can be used to estimate lead and arsenic RBA in the same animals. Because of the size of juvenile swine (about 10 kg at the beginning of the study), it is usually possible to administer doses of test soils that are relatively close to the range thought to be of concern to humans. For example, in Pilot Study 1 (Phase II, Experiment 10), the low dose of slag administered averaged about 260 mg/day, only slightly higher than the reasonable maximum exposure (RME) value of 200 mg/day assumed for human children (U.S. EPA, 1991). Thus, most measurements are obtained in a portion of the dose-response curve that is more relevant to humans than is achieved in most other animal models.

Most studies of arsenic absorption employ a single dose protocol and measure urinary excretion for 2–3 days. In contrast, these studies employed a repeated dosing protocol, with repeated 24- or 48-hour urine collections. An advantage of this protocol is that it reflects a more realistic human exposure scenario than does a single dose protocol. Further, multiple measurements can be made from the same animal on different days. In essence, data from different days allow multiple independent estimates of the UEF, and these data can be combined (once steady state has been achieved) to provide a robust estimate of the excretion fraction.

The RBA results for different test materials investigated strongly support the view that absorption of arsenic from soils and mine wastes is highly variable, and generally is not as well absorbed as soluble arsenic. The detailed chemical mechanism accounting for this variable and reduced bioavailability of arsenic in soil-like media is not known, but almost certainly is related to the chemical form of arsenic in the sample.

Because arsenic in most test materials is absorbed less-extensively than soluble forms of arsenic, and because soluble forms of arsenic are the basis of the oral RfD and oral slope factor for arsenic, the use of the unadjusted toxicity factors for assessing human health risk from soil ingestion will usually lead to an overestimate of risk. Consequently, measurement and application of site-specific RBA values to adjust the toxicity factors to account for the lower level of absorption is expected to increase the accuracy and decrease the uncertainty in human health risk assessments for arsenic in soil.

## 3.0 CONCLUSIONS

The data from the investigations performed under this program support the following main conclusions:

1. Juvenile swine constitute a useful and stable animal model for measuring the relative bioavailability of arsenic in a variety of soil or soil-like test materials. The Phase III protocol described in this report is the recommended SOP for the juvenile swine RBA assay.

2. There are clear differences in the *in vivo* RBA of arsenic between different test materials, ranging from less than 10% to more than 60%. Thus, knowledge of the RBA value for different materials at a site can be very important for improving arsenic risk assessments at a site.

3. Available data are not yet sufficient to allow reliable calculation of the RBA for a test material based only on knowledge of the relative amounts of the arsenic mineral phases present. However, tentative qualitative estimates of low, medium, or high bioavailability have been made based on the major phase type of the arsenic containing waste material.

4. For analysis of total arsenic in urine, additional extraction steps were identified and necessary to convert urinary organoarsenic metabolites to inorganic arsenic.

5. Due to limitations in detection limits for measurement of arsenic in urine, a minimum arsenic dose of 25 µg/kg bw-day is recommended for the juvenile swine RBA assay, so that the amount of arsenic excreted in urine reaches a measurable quantity.

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**Table 2-1. Summary of Arsenic Excretion Studies in Humans and Animals Exposed to Soluble Arsenic Compounds in Water**

| Species | Sex | N | Chemical Form | Dose µg/day | Days Exposed | Percent Recovered | | | Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Urine | Feces | Days | |
| Human | M,F | 4 | NS | 8520 | 1 | NA | 4 | 10 | Bettley and O'Shea 1975 |
| Human | M | 3 | $NaAsO_2$ | 500 | 1 | 45 | NA | 4 | Buchet et al. 1981a |
| Human | M | 1 | $NaAsO_2$ | 125 | 5 | 54 | NA | 14 | Buchet et al. 1981b |
| Human | M | 1 | $NaAsO_2$ | 250 | 5 | 73 | NA | 14 | |
| Human | M | 1 | $NaAsO_2$ | 500 | 5 | 74 | NA | 14 | |
| Human | M | 1 | $NaAsO_2$ | 1000 | 5 | 64 | NA | 14 | |
| Human | NS | 2 | $As_2O_3$ | 1000 | 1 | 85 | 1.4 | 5 | Coulson et al. 1935 |
| Human | M | 1 | $As_2O_3$ | 760 | 5 | 70 | NA | 22 | Mappes 1977 |
| Human | M | 1 | Mixture | 63 | 1 | 80 | NA | 3 | Crecelius 1977 |
| Human | M | 1 | $Na_2HAsO_4$ | 200 | 1 | 50 | NA | 3 | |
| Human | M | 6 | $Na_2HAsO_4$ | 0.01 | 1 | 58 | NA | 6 | Tam et al. 1979 |
| Human | M | 2 | $Na_2HAsO_4$ | 220 | 1 | 67 | NA | 7 | Johnson and Farmer 1991 |
| Hamster | NS | 4 | $NaAsO_2$ | 2000 | 1 | 36 | 49 | 3 | Marafante and Vahter 1987 |
| Mouse | M | 5 | $NaAsO_2$ | 400 | 1 | 90 | 7 | 2 | Vahter and Norin 1980 |
| Mouse | M | 5 | $NaAsO_2$ | 4000 | 1 | 65 | 9 | 2 | |
| Mouse | M | 5 | $NaAsO_2$ | 40 | 1 | 88 | NA | 2 | Vahter 1981 |
| Mouse | M | 5 | $NaAsO_2$ | 400 | 1 | 91 | NA | 2 | |
| Mouse | M | 5 | $NaAsO_2$ | 2000 | 1 | 86 | NA | 2 | |
| Mouse | M | 5 | $NaAsO_2$ | 4000 | 1 | 75 | NA | 2 | |
| Monkey | F | 4 | $As_2O_3$ | 1000 | 1 | 73 | NA | 14 | Charbonneau 1978 |
| Monkey | M | 5 | $Na_2HAsO_4$ | 360 | 1 | 49 | 2 | 4 | Roberts et al. 2002 |
| Monkey | M | 7 | $Na_2HAsO_4$ | 50-200 | 1 | 40 | 42 | 4 | Roberts et al. 2007 |
| Hamster | M | 5 | $As_2O_3$ | 4500 | 1 | 49 | 11 | 5 | Yamauchi and Yamamura 1985 |
| Hamster | NS | 4 | $Na_2HAsO_4$ | 2000 | 1 | 74 | 12 | 3 | Marafante and Vahter 1987 |
| Mouse | M | 5 | $Na_2HAsO_4$ | 400 | 1 | 77 | 8 | 2 | Vahter and Norin 1980 |
| Mouse | M | 5 | $Na_2HAsO_4$ | 4000 | 1 | 89 | 6 | 2 | |
| Mouse | M | 5 | $Na_2HAsO_4$ | 40 | 1 | 94 | NA | 2 | Vahter 1981 |
| Mouse | M | 5 | $Na_2HAsO_4$ | 400 | 1 | 93 | NA | 2 | |
| Mouse | M | 5 | $Na_2HAsO_4$ | 2000 | 1 | 92 | NA | 2 | |
| Mouse | M | 5 | $Na_2HAsO_4$ | 4000 | 1 | 85 | NA | 2 | |

## Table 2-2. Typical Swine Feed Composition

| Nutrient Name | Amount |
|---|---|
| Protein | 20.10% |
| Arginine | 1.21% |
| Lysine | 1.47% |
| Methionine | 0.84% |
| Met+Cys | 0.59% |
| Tryptophan | 0.28% |
| Histidine | 0.56% |
| Leucine | 1.82% |
| Isoleucine | 1.13% |
| Phenylalanine | 1.11% |
| Phe+Tyr | 2.05% |
| Threonine | 0.82% |
| Valine | 1.19% |
| Fat | 4.44% |
| Saturated Fat | 0.56% |
| Unsaturated Fat | 3.74% |
| Linoleic 18:2:6 | 1.94% |
| Linoleic 18:3:3 | 0.04% |
| Crude Fiber | 3.80% |
| Ash | 4.33% |
| Calcium | 0.87% |
| Phos Total | 0.77% |
| Available Phosphorous | 0.70% |
| Sodium | 0.24% |
| Potassium | 0.37% |

| Nutrient Name | Amount |
|---|---|
| Chlorine | 0.19% |
| Magnesium | 0.05% |
| Sulfur | 0.03% |
| Manganese | 20.4719 ppm |
| Zinc | 118.0608 ppm |
| Iron | 135.3710 ppm |
| Copper | 8.1062 ppm |
| Cobalt | 0.0110 ppm |
| Iodine | 0.2075 ppm |
| Selenium | 0.3196 ppm |
| Nitrogen Free Extract | 60.23% |
| Vitamin A | 5.1892 kIU/kg |
| Vitamin D3 | 0.6486 kIU/kg |
| Vitamin E | 87.2080 IU/kg |
| Vitamin K | 0.9089 ppm |
| Thiamine | 9.1681 ppm |
| Riboflavin | 10.2290 ppm |
| Niacin | 30.1147 ppm |
| Pantothenic Acid | 19.1250 ppm |
| Choline | 1019.8600 ppm |
| Pyridoxine | 8.2302 ppm |
| Folacin | 2.0476 ppm |
| Biotin | 0.2038 ppm |
| Vitamin B12 | 23.4416 ppm |

Feed obtained from and nutritional values provided by Zeigler Bros., Inc

## Table 2-3. Description of Test Materials

| Phase | Experiment | Sample Designation | Site | Sample Description | Arsenic Concentration[a] (ppm) | Lead Concentration[a] (ppm) |
|---|---|---|---|---|---|---|
| II | 2 | Bingham Creek Channel Soil | Kennecott NPL Site, Salt Lake City, Utah | Soil composite of samples containing 3000 ppm or greater of lead; collected from a residential area (Jordan View Estates) located along Bingham Creek in the community of West Jordan, Utah | 149 | 6330 |
| | 4 | Jasper County High Lead Mill | Jasper County, Missouri Superfund Site | Soil composite collected from an on-site location | 16 | 6940 |
| | | Murray Smelter Slag | Murray Smelter Superfund Site | Composite of samples collected from areas where exposed slag existed on site | 695 | 11,700 |
| | 5 | Aspen Berm | Smuggler Mountain NPL Site, Aspen, Colorado | Composite of samples collected from the Racquet Club property (including a parking lot and a vacant lot) | 67 | 14,200 |
| | | Aspen Residential | Smuggler Mountain NPL Site, Aspen, Colorado | Composite of samples collected from residential properties within the study area | 17 | 3870 |
| | 6 | Butte Soil | Silver Bow Creek/Butte Area NPL Site, Butte, Montana | Soil composite collected from waste rock dumps in Butte Priority Soils Operable Unit (BPSOU) | 234 | 8530 |
| | | Midvale Slag | Midvale Slag NPL Site, Midvale, Utah | Composite of samples collected from a water-quenched slag pile in Midvale Slag Operable Unit 2 | 591 | 8170 |
| | 7 | California Gulch Phase I Residential Soil | California Gulch NPL Site, Leadville, Colorado | Soil composite collected from residential properties within Leadville | 203 | 7510 |
| | | California Gulch Fe/Mn PbO | California Gulch NPL Site, Leadville, Colorado | Soil composite collected from near the Lake Fork Trailer Park located southwest of Leadville near the Arkansas River | 110 | 4320 |
| | 8 and 10 (Pilot 1) | California Gulch AV Slag | California Gulch NPL Site, Leadville, Colorado | Sample collected from a water-quenched slag pile on the property of the former Arkansas Valley (AV) Smelter, located just west of Leadville | 1050 | 10,600 |

**Table 2-3. Description of Test Materials**

| Phase | Experiment | Sample Designation | Site | Sample Description | Arsenic Concentration[a] (ppm) | Lead Concentration[a] (ppm) |
|---|---|---|---|---|---|---|
| | 9 | Palmerton Location 2 | New Jersey Zinc NPL Site, Palmerton, Pennsylvania | Soil composite collected from on-site | 110 | 3230 |
| | | Palmerton Location 4 | New Jersey Zinc NPL Site, Palmerton, Pennsylvania | Soil composite collected from on-site | 134 | 2150 |
| | 11 | Murray Smelter Soil | Murray Smelter Superfund Site | Soil composite collected from on-site | 310 | 3200 |
| | 15 (Pilot 2) | Clark Fork Tailings | Milltown Reservoir Sediments NPL Site, Milltown, Montana | Sample collected from a tailings deposit along the banks of the Clark Fork River on the property of the Grant-Kohrs Ranch near Deer Lodge, Montana | 181 | |
| III | 1 | VBI70 TM1 | Vasquez Boulevard and I-70 NPL Site, Denver, Colorado | Soil composite from impacted residential property (Eastern Swansea/Elyria neighborhood) | 312 | 733 |
| | | VBI70 TM2 | Vasquez Boulevard and I-70 NPL Site, Denver, Colorado | Soil composite from impacted residential property (Western Swansea/Elyria neighborhood) | 983 | 824 |
| | | VBI70 TM3 | Vasquez Boulevard and I-70 NPL Site, Denver, Colorado | Soil composite from impacted residential property (Eastern Cole neighborhood) | 390 | 236 |
| | 2 | VBI70 TM4 | Vasquez Boulevard and I-70 NPL Site, Denver, Colorado | Soil composite from impacted residential property (Western Cole neighborhood) | 813 | 541 |
| | | VBI70 TM5 | Vasquez Boulevard and I-70 NPL Site, Denver, Colorado | Soil composite from impacted residential property (Clayton neighborhood) | 368 | 157 |
| | | VBI70 TM6 | Vasquez Boulevard and I-70 NPL Site, Denver, Colorado | Clean site soil (from the Swansea/Elyria neighborhood) plus added PAX pesticide | 516 | 264 |
| | 3 | Butte TM1 | Silver Bow Creek/Butte Area NPL Site, Butte, Montana | Soil composite collected from waste rock dumps in Butte Priority Soils Operable Unit (BPSOU) | 234 | 7980 |

**Table 2-3. Description of Test Materials**

| Phase | Experiment | Sample Designation | Site | Sample Description | Arsenic Concentration[a] (ppm) | Lead Concentration[a] (ppm) |
|---|---|---|---|---|---|---|
| | | Butte TM2 | Silver Bow Creek/Butte Area NPL Site, Butte, Montana | Soil composite collected from a residential property located adjacent to a railroad grade in Butte, Montana | 367 | 492 |
| | 4 | Aberjona River TM1 | Wells G & H Superfund Site, Woburn, Massachusetts | Composite of sediment samples containing arsenic concentrations greater than 500 ppm, collected along the Aberjona River, Massachusetts | 676 | 410 |
| | | Aberjona River TM2 | Wells G & H Superfund Site, Woburn, Massachusetts | Composite of sediment samples containing arsenic concentrations from 180 to 460 ppm, collected along the Aberjona River, Massachusetts | 313 | 350 |
| | 5 | El Paso TM1 | El Paso/Dona Ana County Metals Survey site, El Paso County, Texas, and Dona Ana County, New Mexico | Soil sample collected approximately 1.5 miles east of the American Canal in El Paso County, Texas | 74 | NM |
| | | El Paso TM2 | El Paso/Dona Ana County Metals Survey site, El Paso County, Texas, and Dona Ana County, New Mexico | Soil sample collected approximately 1.5 miles east of the American Canal in El Paso County, Texas | 73 | NM |
| | 6 | ACC Utility Pole Soil | – (Study sponsored by American Chemistry Council) | Soil affected by chromated copper arsenate (CCA)-treated wood utility poles from a test plot in Conley, Georgia (soil was affected by being adjacent to the poles for over ten years) | 320 | NM |

## Table 2-3. Description of Test Materials

| Phase | Experiment | Sample Designation | Site | Sample Description | Arsenic Concentration[a] (ppm) | Lead Concentration[a] (ppm) |
|---|---|---|---|---|---|---|
| | 7 | ACC Dislodgeable Arsenic | – (Study sponsored by American Chemistry Council) | Dislodgeable material obtained from the surface of chromated copper arsenate (CCA)-treated wood (boards from in-service residential decks, aged outdoors for one to three years) | 3500 | NM |

[a]Values are arithmetic means
All samples were analyzed by ICP/AES in accord with EPA Method 2007.
NM = Not Measured

## Table 2-4. Relative Mass of Arsenic By Mineral Phase in Test Materials

| Phase | Experiment | Sample | Number of Particles Counted | Phase | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | As Phosphate | $As_2O_3$ | AsMO | AsSbO | Pb-As Vanidate | PbAsMO | PbAsSbCuO | $AsMSO_4$ | Barite | Clays | FeAs Oxide | FeAs Sulfate | $ZnSO_4$ | FeAsO | MnAs Oxide | PbAs Oxide | PbMO | PbMS | $PbMSO_4$ | Pyrite | Slag | Sulfosalts | AgAsS | Paint | Pb Solder | $PbCrO_4$ | $TiO_2$ | $ZnSiO_4$ |
| I | 2 | Bingham Creek Channel Soil | 430 | 8% | | | | | | | | <1% | | 11% | 46% | | | <1% | 34% | | | | | | | | | | | | | |
| | 4 | Murray Smelter Slag | 1108 | | | | | | | | | | | 27% | 10% | | | <1% | 49% | <1% | | | | 14% | | | | | | | | |
| | 6 | Butte Soil[a] | 636 | 8% | | | | | | | | <1% | | 20% | 53% | | | 16% | | | | | | | | 2% | | | | | | |
| | | Midvale Slag | 1847 | | | | | | | | | | | <1% | <1% | | | | 87% | | | | | 11% | 1% | | | | | | | |
| | 7 | California Gulch Phase I Residential Soil | 510 | 15% | | | | | | 5% | | <1% | | 29% | 11% | | | 36% | | | | | | 4% | | | | | | | | |
| | | California Gulch Fe/Mn PbO | 380 | 5% | | | | <1% | | | | <1% | | 23% | 5% | | | 66% | | | | | | | | | | | | | | |
| | 8 | California Gulch AV Slag | 1472 | | | 5% | | | <1% | | | | | | <1% | | | | 84% | 2% | 3% | <1% | | 5% | | | | | | | | |
| | 9 | Palmerton Location 2 | 111 | 27% | | | | | | | | 11% | | 21% | <1% | | | 40% | | | | | | | | | | | | | | |
| | | Palmerton Location 4 | 105 | <1% | | | | 4% | | | | <1% | | 5% | | | 38% | 10% | 42% | | | | | | | | | | | | | <1% |
| | 11 | Murray | 355 | | | 2% | | | | | | | | 3% | 6% | | | | 87% | <1% | | | | 2% | | | | | | | | |

## Table 2-4. Relative Mass of Arsenic By Mineral Phase in Test Materials

| Phase | Experiment | Sample | Number of Particles Counted | Phase | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | As Phosphate | $As_2O_3$ | AsMO | AsSbO | Pb-As Vanidate | PbAsMO | PbAsSbCuO | $AsMSO_4$ | Barite | Clays | FeAs Oxide | FeAs Sulfate | $ZnSO_4$ | FeAsO | MnAs Oxide | PbAs Oxide | PbMO | PbMS | $PbMSO_4$ | Pyrite | Slag | Sulfosalts | AgAsS | Paint | Pb Solder | $PbCrO_4$ | $TiO_2$ | $ZnSiO_4$ |
| | | Smelter Soil | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 15 | Clark Fork Tailings | 238 | 16% | | | | | | | | | | 40% | 24% | | 2% | <1% | | | | | <1% | 16% | | | | | | | |
| II | 1 | VBI70 TM1 | 261 | 8% | 54% | | | | | | | | <1% | 3% | <1% | | | 2% | 32% | <1% | | | | | | | | <1% | | <1% | |
| | | VBI70 TM2 | 128 | 4% | 22% | | | | | | | | <1% | 3% | <1% | | | <1% | 70% | <1% | | | | <1% | | | | | | | |
| | | VBI70 TM3 | 97 | 2% | 80% | | | | | | | | <1% | 8% | | | | 5% | 6% | <1% | | | | <1% | | | | <1% | <1% | | |
| | 2 | VBI70 TM4 | 139 | <1% | 86% | | <1% | | | | | | <1% | 2% | <1% | | | <1% | 10% | | | | | <1% | | | | <1% | <1% | | |
| | | VBI70 TM5 | 103 | | 97% | | | | | | | <1% | | 3% | <1% | | | <1% | | <1% | | | | <1% | | | <1% | <1% | | | |
| | | VBI70 TM6 | 124 | <1% | 80% | <1% | 1% | | | | | | <1% | <1% | | | | | 18% | | | | <1% | <1% | | | | <1% | <1% | | |
| | 3 | Butte TM2 | 137 | | | | | | | | <1% | | <1% | 39% | 18% | | | | | | | | <1% | <1% | 42% | | | | | | |
| | 4 | Aberjona River TM1 | 186 | | | | | | | | | | | 69% | 29% | 2% | | | | | | | <1% | | | | | | | | |
| | | Aberjona River TM2 | 123 | | | | | | | | | | | 16% | 27% | 55% | | | | | | | 2% | | | | | | | | |

## Table 2-4. Relative Mass of Arsenic By Mineral Phase in Test Materials

| Phase | Experiment | Sample | Number of Particles Counted | Phase | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | As Phosphate | $As_2O_3$ | AsMO | AsSbO | Pb-As Vanidate | PbAsMO | PbAsSbCuO | $AsMSO_4$ | Barite | Clays | FeAs Oxide | FeAs Sulfate | $ZnSO_4$ | FeAsO | MnAs Oxide | PbAs Oxide | PbMO | PbMS | $PbMSO_4$ | Pyrite | Slag | Sulfosalts | AgAsS | Paint | Pb Solder | $PbCrO_4$ | $TiO_2$ | $ZnSiO_4$ |
| [a] | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

[a] Same sample as evaluated in Phase III Experiment 3 (Butte TM2).

## Table 2-5. Size Distributions of Arsenic Particles

| Phase | Experiment | Sample | Particle Size (µm) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0–5 | 6–10 | 11–20 | 21–50 | 51–100 | 101–150 | 151–200 | 201–250 | >250 |
| II | 2 | Bingham Creek Channel Soil | 71% | 14% | 6% | 6% | 3% | <1% | | | |
| | 4 | Murray Smelter Slag | 14% | 15% | 4% | 15% | 24% | 23% | 2% | 3% | <1% |
| | 6 | Butte Soil[a] | 21% | 9% | 16% | 26% | 17% | 9% | 1% | <1% | <1% |
| | | Midvale Slag | 3% | 1% | 2% | 13% | 19% | 40% | 6% | 14% | <1% |
| | 7 | California Gulch Phase I Residential Soil | 22% | 16% | 14% | 22% | 16% | 6% | 1% | 1% | <1% |
| | | California Gulch Fe/Mn PbO | 35% | 24% | 13% | 17% | 9% | 2% | | | |
| | 8 | California Gulch AV Slag | 21% | 9% | 2% | 11% | 12% | 18% | 14% | 7% | 6% |
| | 9 | Palmerton Location 2 | 40% | 26% | 12% | 15% | 7% | | | | |
| | | Palmerton Location 4 | 21% | 28% | 18% | 19% | 13% | <1% | | | |
| | 11 | Murray Smelter Soil | 18% | 31% | 17% | 10% | 12% | 7% | 3% | 1% | <1% |
| | 15 | Clark Fork Tailings | 34% | 20% | 17% | 21% | 7% | 1% | | | |
| III | 1 | VBI70 TM1 | 81% | 9% | 7% | 3% | | | <1% | | |
| | | VBI70 TM2 | 59% | 20% | 10% | 9% | 2% | | | | |
| | | VBI70 TM3 | 49% | 21% | 18% | 11% | 1% | | | | |
| | 2 | VBI70 TM4 | 45% | 32% | 13% | 9% | 1% | <1% | | | |
| | | VBI70 TM5 | 48% | 18% | 24% | 10% | | | | | |
| | | VBI70 TM6 | 63% | 23% | 6% | 6% | 2% | | | | |
| | 3 | Butte TM2 | 18% | 11% | 20% | 30% | 18% | 4% | | | |
| | 4 | Aberjona River TM1 | 33% | 34% | 6% | 13% | 6% | 4% | <1% | 2% | |
| | | Aberjona River TM2 | 59% | 9% | 15% | 9% | 6% | 2% | | | |

[a] Same sample as evaluated in Phase III Experiment 3 (Butte TM2).

## Table 2-6. Matrix Associations of Arsenic Particles

| Phase | Experiment | Sample | Particle Frequency (Percent) | Relative Arsenic Mass (Percent) | |
|---|---|---|---|---|---|
| | | | *Liberated* | *Liberated* | *Included* |
| II | 2 | Bingham Creek Channel Soil | 100% | 100% | 0% |
| | 4 | Murray Smelter Slag | 99% | 95% | 5% |
| | 6 | Butte Soil[a] | 92% | 87% | 13% |
| | | Midvale Slag | 96% | 78% | 22% |
| | 7 | California Gulch Phase I Residential Soil | 88% | 94% | 6% |
| | | California Gulch Fe/Mn PbO | 98% | 100% | 0% |
| | 8 | California Gulch AV Slag | 85% | 73% | 27% |
| | 9 | Palmerton Location 2 | 100% | 100% | 0% |
| | | Palmerton Location 4 | 84% | 58% | 42% |
| | 11 | Murray Smelter Soil | 92% | 79% | 21% |
| | 15 | Clark Fork Tailings | 99% | 96% | 4% |
| III | 1 | VBI70 TM1 | 100% | 100% | 0% |
| | | VBI70 TM2 | 99% | 95% | 5% |
| | | VBI70 TM3 | 100% | 100% | 0% |
| | 2 | VBI70 TM4 | 100% | 100% | 0% |
| | | VBI70 TM5 | 95% | 100% | 0% |
| | | VBI70 TM6 | 100% | 100% | 0% |
| | 3 | Butte TM2 | 100% | 100% | 0% |
| | 4 | Aberjona River TM1 | 100% | 99% | 1% |
| | | Aberjona River TM2 | 100% | 100% | 0% |

[a] Same sample as evaluated in Phase III Experiment 3 (Butte TM2).

## Table 2-7. RBA Estimates for Arsenic in Test Materials

| Phase | Experiment | Sample | Site | Sample | Arsenic Concentration[a] (ppm) | RBA ± SEM |
|---|---|---|---|---|---|---|
| Phase II | 2 | Bingham Creek Channel Soil | Bingham Creek | Channel Soil | 149 | 39% ± 8% |
| | 4 | Murray Smelter Slag | Murray Smelter | Slag Composite | 695 | 55% ± 10% |
| | | Jasper County High Lead Mill | Region VII Jasper County | High Lead Smelter | 16.4 | 327% ± 105% |
| | 5 | Aspen Berm | Aspen | Berm | 66.9 | 100% ± 46% |
| | | Aspen Residential | Aspen | Residential Soil Composite | 16.7 | 128% ± 52% |
| | 6 | Butte Soil | Butte | Soil 1 | 234 | 9% ± 3% |
| | | Midvale Slag | Midvale | Slag Composite | 591 | 23% ± 4% |
| | 7 | California Gulch Phase I Residential Soil | California Gulch | Phase I Residential Soil Composite | 203 | 8% ± 3% |
| | | California Gulch Fe/Mn PbO | California Gulch | FeMnPb Oxide Soil | 110 | 57% ± 12% |
| | 8 | California Gulch AV Slag | California Gulch | AV Smelter Slag | 1050 | 13% ± 4% |
| | 9 | Palmerton Location 2 | Palmerton | Location 2 | 110 | 49% ± 10% |
| | | Palmerton Location 4 | Palmerton | Location 4 | 134 | 61% ± 11% |
| | 10 | California Gulch AV Slag | California Gulch | AV Smelter Slag (reproducibility) | 1050 | 18% ± 2% |
| | 11 | Murray Smelter Soil | Murray Smelter | Soil Composite | 310 | 33% ± 5% |
| | 15 | Clark Fork Tailings | Clark Fork | Grant Kohrs Tailings | 181 | 51% ± 6% |
| Phase III | 1 | VBI70 TM1 | VBI70 | TM1 | 312 | 40% ± 4% |
| | | VBI70 TM2 | VBI70 | TM2 | 983 | 42% ± 4% |
| | | VBI70 TM3 | VBI70 | TM3 | 390 | 37% ± 3% |
| | 2 | VBI70 TM4 | VBI70 | TM4 | 813 | 24% ± 2% |
| | | VBI70 TM5 | VBI70 | TM5 | 368 | 21% ± 2% |
| | | VBI70 TM6 | VBI70 | TM6 | 516 | 24% ± 3% |
| | 3 | Butte TM1 | Butte Arsenic | Soil 1[b] | 234 | 18% ± 3% |
| | | Butte TM2 | Butte Arsenic | Soil 2 | 367 | 24% ± 2% |
| | 4 | Aberjona River TM1 | Aberjona River | River Sediment – High Arsenic | 676.3 | 38% ± 2% |
| | | Aberjona River TM2 | Aberjona River | River Sediment – Low Arsenic | 312.8 | 52% ± 2% |
| | 5 | El Paso TM1 | El Paso | Soil 1 | 74 | 44% ± 3% |
| | | El Paso TM2 | El Paso | Soil 2 | 73 | 37% ± 3% |
| | 6 | ACC Utility Pole Soil | ACC | Soil Affected by CCA-Treated Wood Utility Poles | 320 | 47% ± 3% |

## Table 2-7. RBA Estimates for Arsenic in Test Materials

| Phase | Experiment | Sample | Site | Sample | Arsenic Concentration[a] (ppm) | RBA ± SEM |
|---|---|---|---|---|---|---|
| | 7 | ACC Dislodgeable Arsenic | ACC | Dislodgeable Arsenic from Weathered CCA-Treated Wood | 3500 | 26% ± 1% |

[a]Values are arithmetic means
[b] Same sample as evaluated in Phase II
SEM = Standard error of the mean, an indicator of the relative uncertainty around the RBA estimate (see Appendix A)

## Table 2-8. Summary Statistics for Dose-UEF Slopes and RBA Estimates for Phase III RBA Assays

| Parameter | Sodium Arsenate Slope | Test Material Slope | Test Material RBA |
|---|---|---|---|
| N | 7 | 14 | 14 |
| Mean | 0.78 | 0.26 | 0.34 |
| SD | 0.099 | 0.098 | 0.118 |
| CV | 0.13 | 0.38 | 0.32 |

CV, coefficient of variation (SD/mean); RBA, relative bioavailability; SD, standard deviation; UEF, urinary excretion fraction

**Table 2-9. Consolidated Arsenic Phases**

| Phase Grouping | Phase | Other Abbreviations Used | Phase Description |
|---|---|---|---|
| As Phosphate | As Phosphate | Phos, Phosphate | Arsenic bearing phosphate: although naturally occurring forms are rare (arsenocrandallite-$CaAl_3AsPO_4$-$OH_6$), these may be metastable forms of phosphate with sorbed arsenic formed by secondary soil processes. |
| $As_2O_3$ | $As_2O_3$ | As | Arsenic trioxide: a common pyrometallurgical-formed phase that is common to arsenic kitchens or copper smelters. It can also be found as a product in old formulas for herbicides, pesticides, and rodenticides. |
| FeAs Oxide | FeAs Oxide | Fe, Fe Oxide, FeSi | Iron oxide (FeOOH) with sorbed arsenic and lead, probably from soil. |
| Fe & Zn Sulfates | FeAs Sulfate | Fe Sulfate, Sulf | Iron-rich sulfates: probably related to jarosite ($KFe_3(OH)_6(SO_4)_2$) or plumbojarosite ($PbFe_3(OH)_6(SO_4)_2$). Can form in oxide zone of hydrothermal deposits, but is also common to baghouse dust associated with copper-lead smelters. |
| | $ZnSO_4$ | – | Zinc sulfates: recognized by an elemental composition dominated by zinc, sulfur, and oxygen with minor quantities of lead, arsenic, and/or cadmium. Generally found as inclusions in slag or in baghouse dust and sometimes used in commercial products. |
| FeAsO | FeAsO | FeAs | Iron oxide (FeOOH) that is highly enriched with arsenic; probably a flue dust. |
| MnAs Oxide | MnAs Oxide | Mn, Mn Oxide | Arsenic sorbed to the surface of manganese oxide-containing particles in soil. Formed by release of arsenic from soluble forms. Recognized by an elemental composition dominated by manganese, arsenic, and oxygen. |
| PbAs Oxide | PbAs Oxide | PbAsO | A product released from smelter flues and sometimes used in commercial products. Recognized by an elemental composition dominated by lead, arsenic, and oxygen. |
| Pyrite | Pyrite | Py | Iron sulfide ($FeS_2$): a gaunge mineral associated with base-metal ore deposits. Pyrite may contain small quantities of arsenic or have arsenic sorbed to its oxidized surface. |
| Sulfosalts | AgAsS | Ags | Silver arsenic sulfides: a mineral form related to mining activity (from a class of minerals referred to as sulfosalts). These ores of silver may be in the chemical form of proustite ($Ag_3AsS_3$), xanthoconite ($Ag_3AsS_3$), pearceite ($(AgCu)_2As_2S_{11}$), or polybasite ($(AgCu)_{16}(Sb,As)_2S_{11}$). |
| | Sulfosalts | – | A group consisting of more than 100 forms of unoxidized minerals composed of metal or semimetals and sulfur, distinct from a sulfide. These include numerous arsenic-bearing phases: tennantite ($Cu_{12}As_4S_{13}$) and enargite ($Cu_3AsS_4$) are perhaps the most common. |
| Minor Constituents | AsMO | – | Arsenic-metal oxides: these are arsenic-rich oxides formed from pyrometallurgical processes. Common associated elements (M) include lead, antimony, copper, zinc, and/or cadmium. |
| | $AsMSO_4$ | – | Arsenic-antimony oxide: this is a common pyrometalurgically formed phase that is common to arsenic kitchens. Its occurrence is significant in “dirty” or “black” arsenic and is still found in trace quantities in “white” arsenic. |

**Table 2-9. Consolidated Arsenic Phases**

| Phase Grouping | Phase | Other Abbreviations Used | Phase Description |
|---|---|---|---|
| | AsSbO | – | Arsenic-antimony oxide: this is a common pyrometalurgically formed phase that is common to arsenic kitchens. Its occurrence is significant in "dirty" or "black" arsenic and is still found in trace quantities in "white" arsenic. |
| | Barite | – | Barium sulfate: common gaunge mineral with base metals. Will adsorb lead and arsenic during smelting. |
| | Clays | AlSi | Arsenic sorbed to the surface of soil-forming clays (hydrated, Al-Mg silicates). |
| | Paint | – | Arsenic may be present in some very old paint pigments or as a trace contaminant in lead, copper, and antimony pigments. |
| | Pb Solder | Pbsold | Lead solder with trace levels of arsenic. Recognized by an elemental composition dominated by lead and tin with minor base metals. |
| | Pb-As Vanidate | $PbAsVo_4$ | A phase probably associated with mining or smelting of copper-rich ores, not used in commercial products. Recognized by an elemental composition dominated by lead, arsenic, vanadium, and oxygen. |
| | PbAsMO | – | Lead-arsenic metal oxides: these are lead-arsenic rich oxides formed from pyrometallurgical processes. Common associated elements (M) include antimony, copper, zinc, and/or cadmium. |
| | PbAsSbCuO | – | Lead-arsenic metal oxides: these are lead-arsenic rich oxides formed from pyrometallurgical processes. |
| | $PbCrO_4$ | – | A common lead pigment in paint and a rare form of lead. |
| | PbMO | – | Lead-metal oxides: these are lead-rich oxides formed from pyrometallurgical processes. Common associated elements (M) include arsenic, antimony, copper, zinc, and/or cadmium. |
| | PbMS | – | Lead-metal sulfides: these are lead-rich oxides formed from pyrometallurgical processes. Common associated elements (M) include arsenic, antimony, copper, zinc, and/or cadmium. |
| | $PbMSO_4$ | – | Lead-metal sulfates: these are lead-rich oxides formed from pyrometallurgical processes. Common associated elements (M) include arsenic, antimony, copper, zinc, and/or cadmium. |
| | Slag | – | A waste by-product of pyrometallurgical activity. Recognized by an elemental composition dominated by silica, calcium, iron, and oxygen with variable quantities of lead, arsenic, copper, and/or zinc. |
| | $TiO_2$ | Ti | Rutile or anatase with surface sorbed arsenic in small quantities. Recognized by an elemental composition dominated by titanium and oxygen. |
| | $ZnSiO_4$ | – | Zinc silicate, recognized by an elemental composition dominated by zinc, silica, and oxygen with minor quantities of lead, arsenic, and/or cadmium. Generally found as inclusions in slag or in baghouse dust and sometimes used in commercial products. |

## Table 2-10. Relative Arsenic Mass for Consolidated Phase Groupings

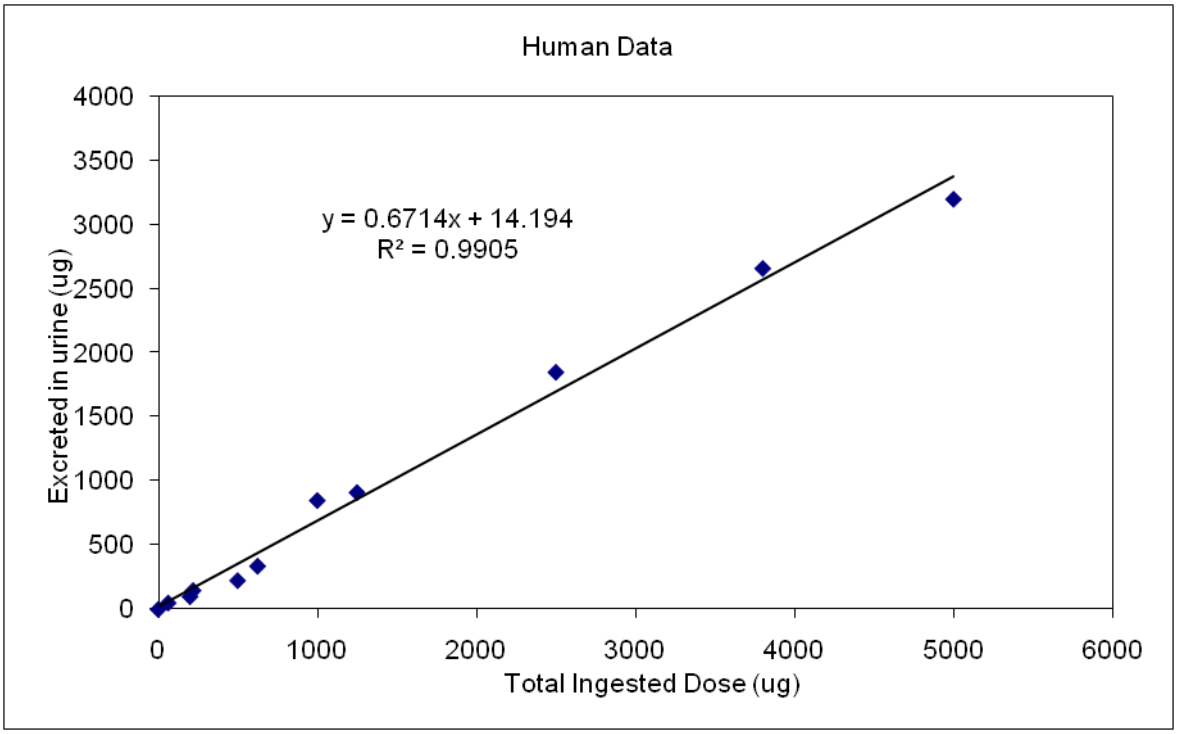
| Phase | Experiment | Sample | RBA | Arsenic Conc. (ppm) | Number of Particles Counted | Phase (Liberated/Included) | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | As Phosphate | | $As_2O_3$ | | FeAs Oxide | | Fe & Zn Sulfates | | FeAsO | | MnAs Oxide | | PbAs Oxide | | Sulfosalts | | Minor Constits. | |
| II | 2 | Bingham Creek Channel Soil | 39.3% | 149 | 430 | 8% | <1% | | | 11% | <1% | 46% | <1% | | | <1% | <1% | 34% | <1% | | | <1% | <1% |
| | 4 | Murray Smelter Slag | 55.1% | 695 | 1108 | | | | | 27% | <1% | 10% | <1% | | | <1% | <1% | 44% | 5% | | | 15% | <1% |
| | 6 | Butte Soil[a] | 17.8% | 234 | 636 | <1% | 7% | | | 18% | 2% | 51% | 3% | | | 16% | <1% | | | 2% | <1% | <1% | <1% |
| | | Midvale Slag | 22.9% | 591 | 1847 | | | | | <1% | <1% | <1% | <1% | | | | | 65% | 22% | 1% | <1% | 11% | <1% |
| | 7 | California Gulch Phase I Residential Soil | 8.4% | 203 | 510 | 14% | <1% | | | 29% | <1% | 11% | <1% | | | 36% | <1% | | | | | 5% | 5% |
| | | California Gulch Fe/Mn PbO | 56.6% | 110 | 380 | 5% | <1% | | | 23% | <1% | 5% | <1% | | | 66% | <1% | | | | | <1% | <1% |
| | 8 | California Gulch AV Slag | 12.9% | 1050 | 1472 | | | | | | | <1% | <1% | | | | | 58% | 26% | | | 16% | <1% |
| | 9 | Palmerton Location 2 | 49.2% | 110 | 111 | 27% | <1% | | | 21% | <1% | <1% | <1% | | | 40% | <1% | | | | | 11% | <1% |
| | | Palmerton Location 4 | 61.0% | 134 | 105 | <1% | <1% | | | 5% | <1% | | | 38% | <1% | 10% | <1% | <1% | 42% | | | 5% | <1% |
| | 11 | Murray Smelter Soil | 33.0% | 310 | 355 | | | | | 3% | <1% | 6% | <1% | | | | | 66% | 21% | | | 4% | <1% |
| | 15 | Clark Fork Tailings | 50.7% | 181 | 238 | 16% | <1% | | | 40% | <1% | 24% | <1% | 2% | <1% | <1% | <1% | | | 13% | 3% | <1% | <1% |
| III | 1 | VBI70 TM1 | 40.3% | 312 | 261 | 8% | <1% | 54% | <1% | 3% | <1% | <1% | <1% | | | 2% | <1% | 32% | <1% | | | <1% | <1% |
| | | VBI70 TM2 | 42.2% | 983 | 128 | 4% | <1% | 17% | 5% | 3% | <1% | <1% | <1% | | | <1% | <1% | 70% | <1% | | | <1% | <1% |
| | | VBI70 TM3 | 36.7% | 390 | 97 | 2% | <1% | 80% | <1% | 8% | <1% | | | | | 5% | <1% | 6% | <1% | | | <1% | <1% |
| | 2 | VBI70 TM4 | 23.8% | 813 | 139 | <1% | <1% | 86% | <1% | 2% | <1% | <1% | <1% | | | <1% | <1% | 10% | <1% | | | <1% | <1% |
| | | VBI70 TM5 | 21.2% | 368 | 103 | | | 97% | <1% | 3% | <1% | <1% | <1% | | | <1% | <1% | | | | | <1% | <1% |
| | | VBI70 TM6 | 23.5% | 516 | 124 | <1% | <1% | 80% | <1% | <1% | <1% | | | | | | | 18% | <1% | | | 1% | <1% |
| | 3 | Butte TM2 | 23.6% | 367 | 137 | | | | | 39% | <1% | 18% | <1% | | | | | | | 42% | <1% | <1% | <1% |
| | 4 | Aberjona River TM1 | 38.1% | 676 | 186 | | | | | 69% | <1% | 30% | 1% | | | | | | | | | <1% | <1% |
| | | Aberjona River TM2 | 52.4% | 313 | 123 | | | | | 16% | <1% | 82% | <1% | | | | | | | | | 2% | <1% |

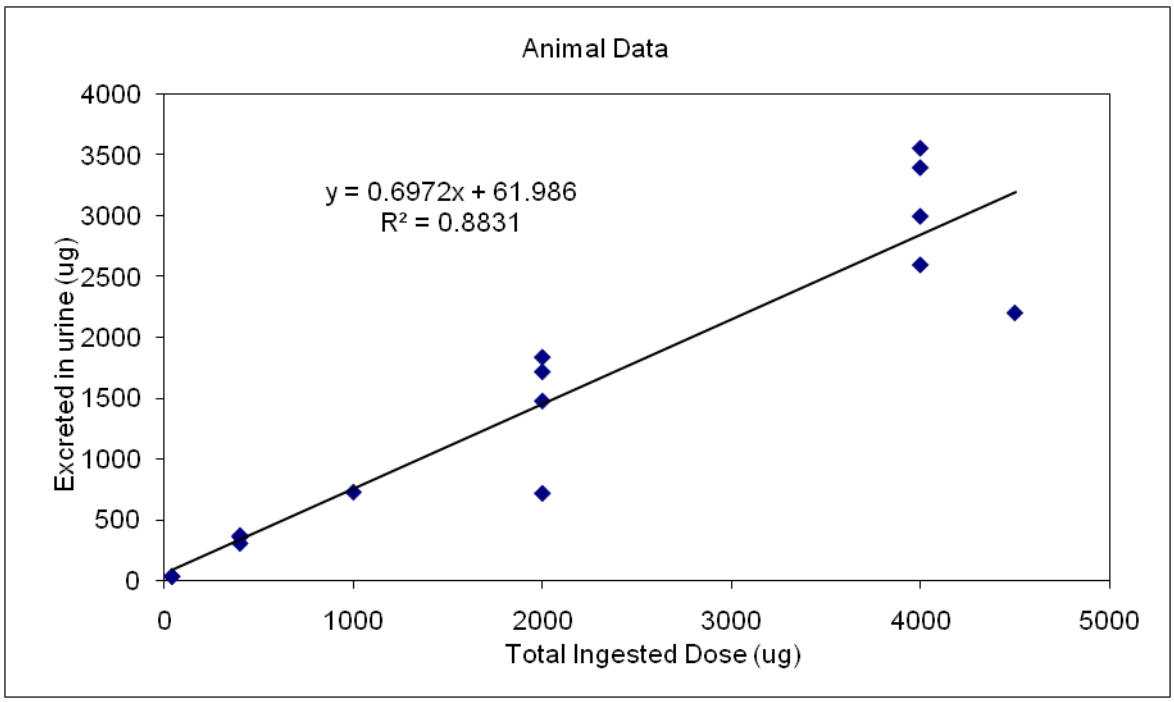
[a] Same sample as evaluated in Phase III Experiment 3 (Butte TM2).

**Table 2-11. Estimated Group-Specific RBA Values for *Liberated* Particles**

| Group Name | Estimated Group-Specific RBA[1] | | RBA Category[2] |
|---|---|---|---|
| | Method 1 | Method 2 | |
| FeAsO | 1.00 | 1.42 | High |
| As Phosphate | 0.55 | 0.59 | Medium |
| FeAs Oxide | 0.45 | 0.44 | Medium |
| Fe & Zn Sulfates | 0.40 | 0.40 | Medium |
| PbAs Oxide | 0.38 | 0.38 | Medium |
| MnAs Oxide | 0.38 | 0.35 | Medium |
| $As_2O_3$ | 0.25 | 0.25 | Low |
| Sulfosalts | 0.02 | 0.01 | Low |

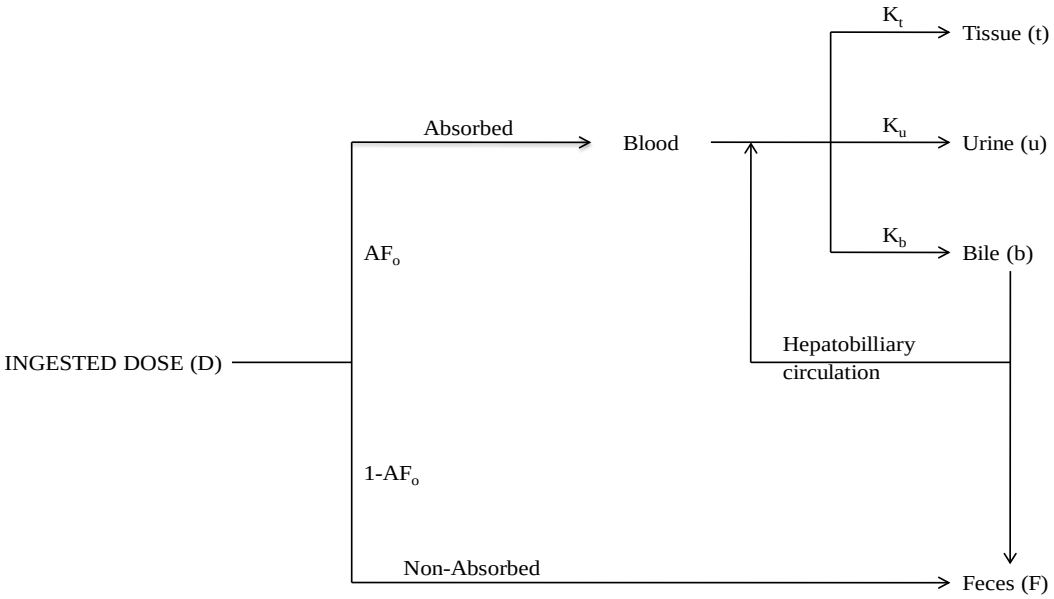
**Figure 2-1. Excretion of Soluble As in Humans and Animals[a]**




[a]See Table 2-1 for literature sources of RBA estimates.

**Figure 2-2. Conceptual Model for Arsenic Absorption and Excretion**




Where:

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

**BASIC EQUATIONS:**

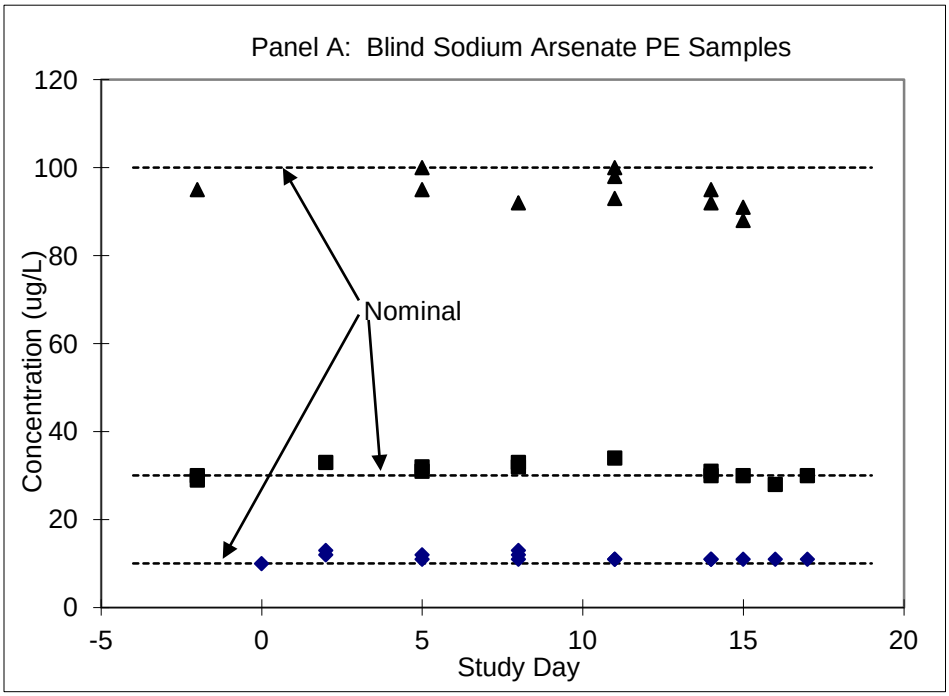
Amount Absorbed (µg) $= D \cdot AF_o$

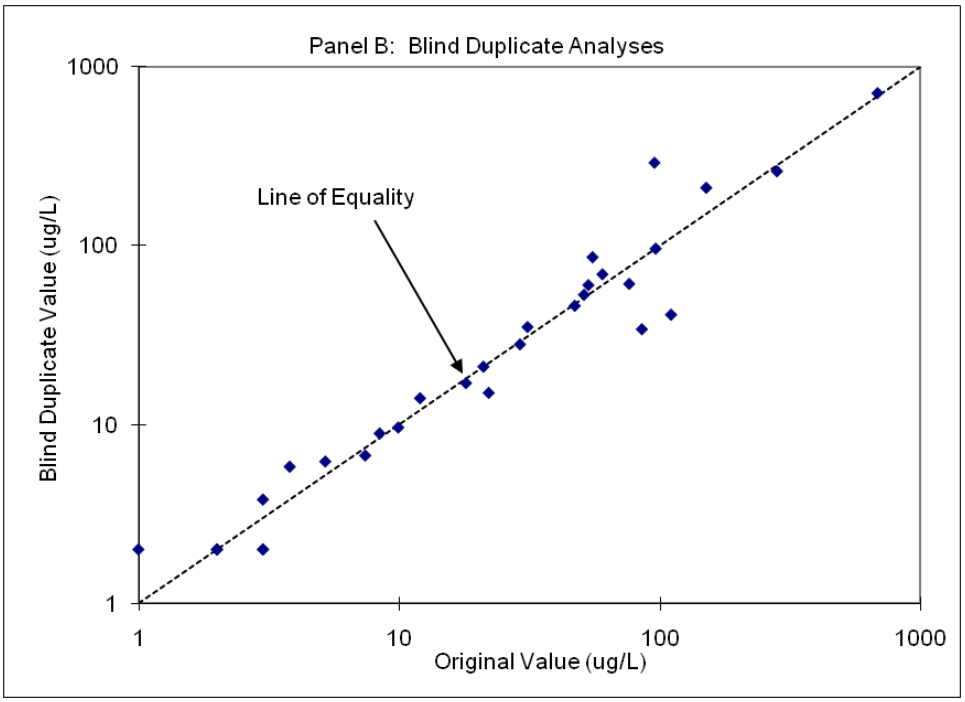
Amount Excreted in Urine (µg) $= Amount\ absorbed \cdot K_u$
$= D \cdot AF_o \cdot K_u$

Urinary Excretion Fraction (UEF) $= Amount\ excreted / Amount\ Ingested$
$= (D \cdot AF_o \cdot K_u) / D$
$= AF_o \cdot K_u$

Relative Bioavailability (x vs. y) $= UEF(x) / UEF(y)$
$= (AF_o(x) \cdot K_u) / (AF_o(y) \cdot K_u)$
$= AF_o(x) / AF_o(y)$

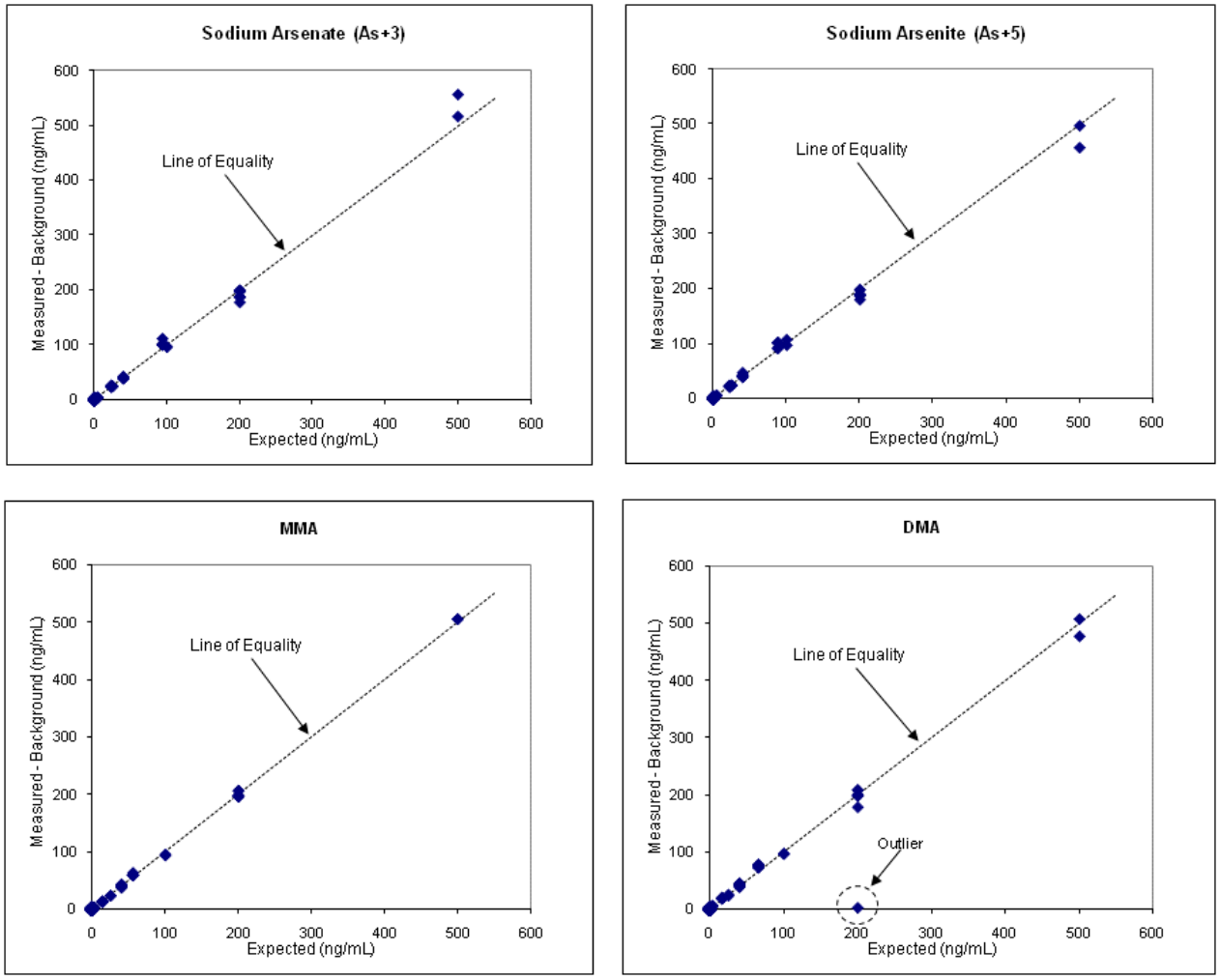
**Figure 2-3. Quality Assurance Data from Phase II Pilot Studies[a]**



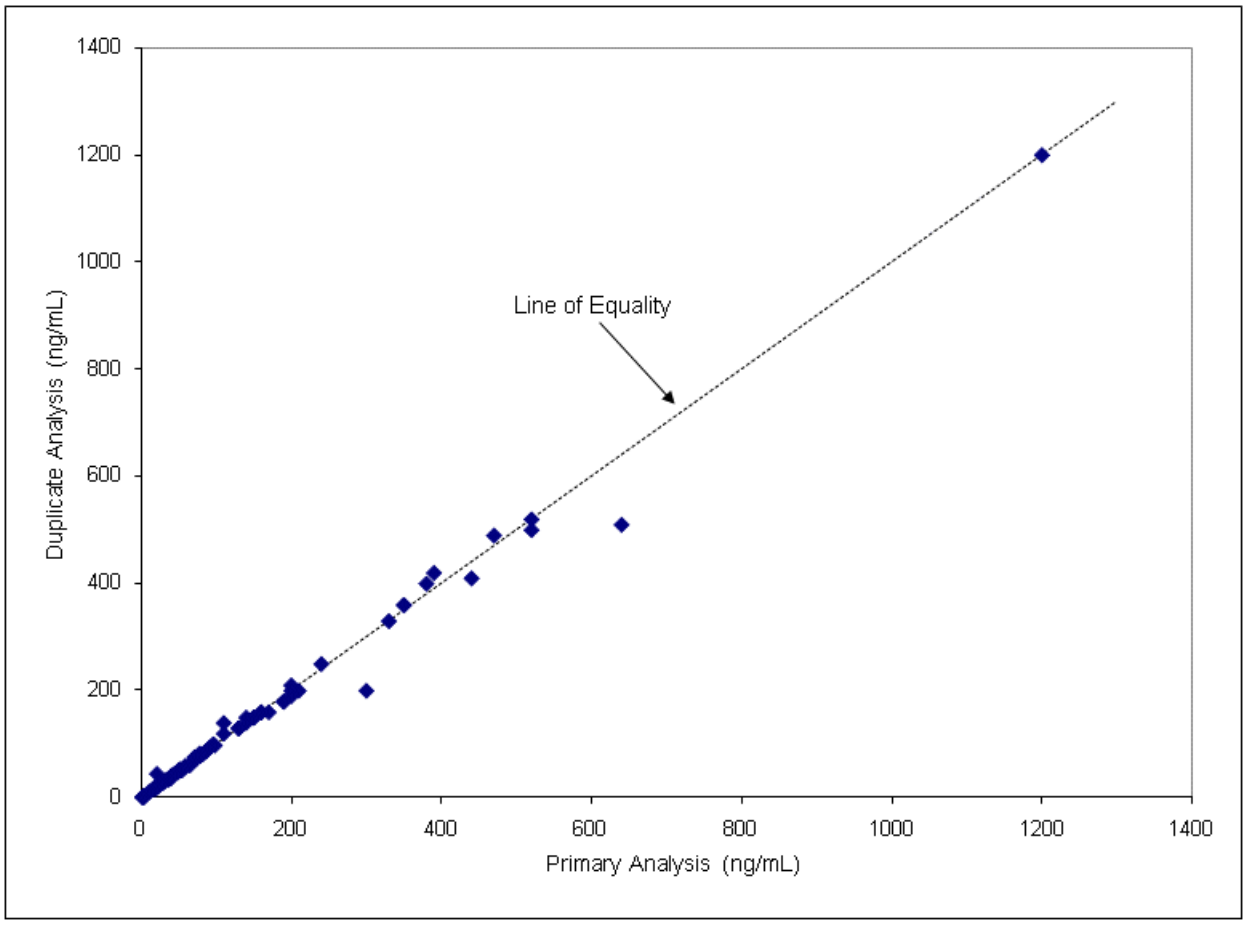

[a]Comparion of measured and actual (nominal) concentrations of performance evaluation (PE) samples for urine (panel A), and between duplicate measurements on the same urine sample (panel B), for Phase II studies. $R^2$ for blind duplicates was 0.91 (n=30).

## Figure 2-4. Phase III Performance Evaluation Samples[a]



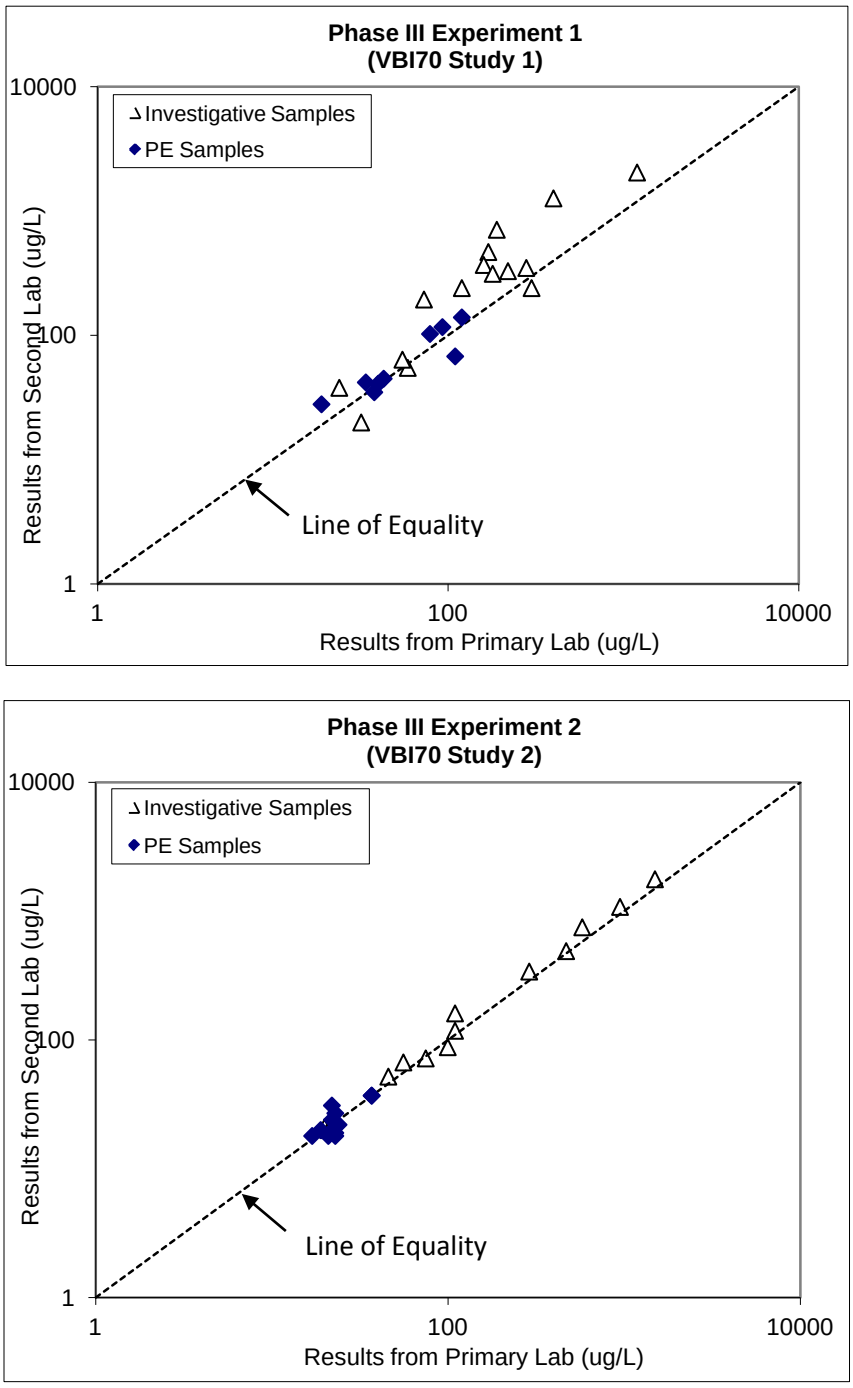

[a]Comparison of measured and actual concentrations of performance evaluation (PE) urine samples for Phase III studies. DMA, dimethylasinic acid; MMA, monomethylarsonic acid. $R^2$ values were <0.99 for the four analytes (N=35–37).

**Figure 2-5. Phase III Blind Duplicate Samples [a]**



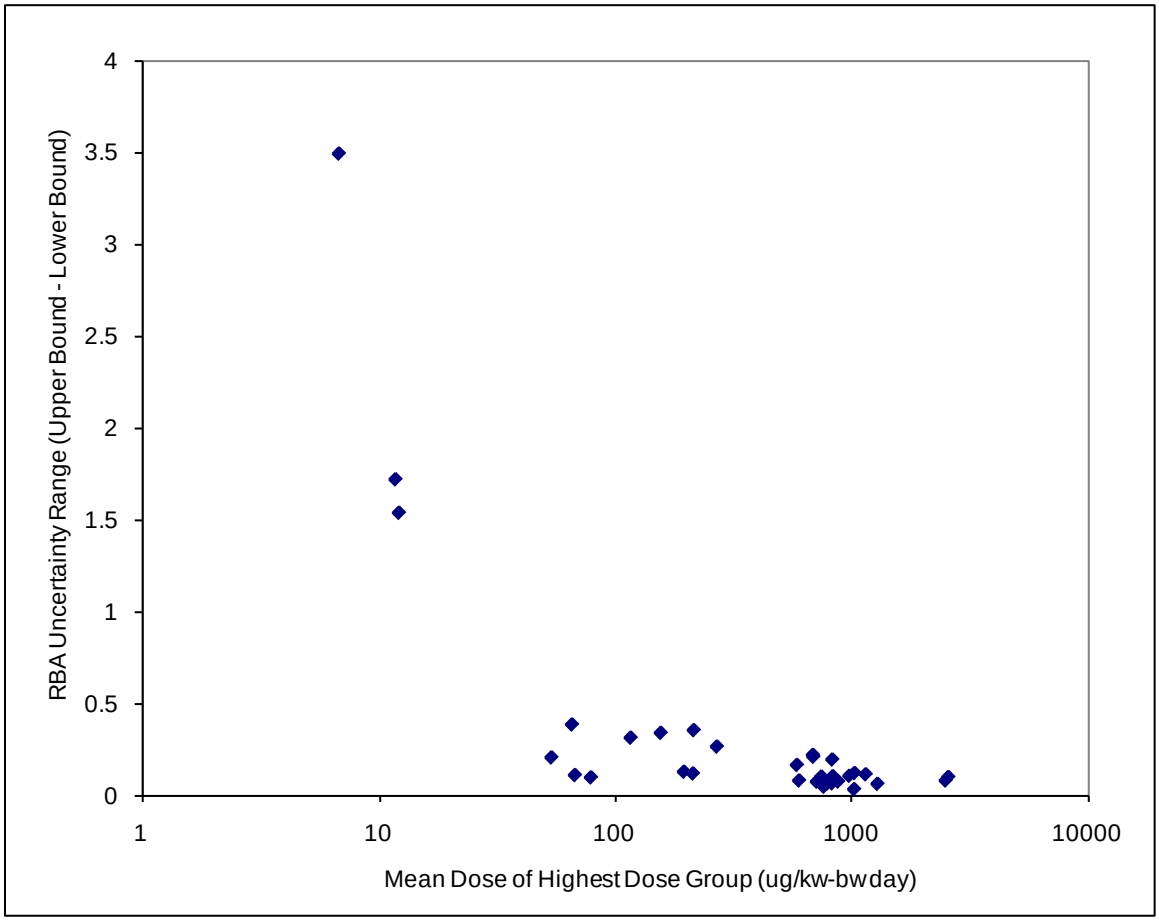

[a]Comparion between duplicate measurements on the same urine sample for Phase III studies. The $R^2$ was 0.98 (n=72).

**Figure 2-6. Phase III Inter-Laboratory Comparison[a]**




[a]Comparison of interlaboratory results of analyses of arsenic in urine in two Phase III studies. Values for $R^2$ were 0.87 (n=24) for Experiment 1 and 1.0 (n=25) for Experiment 2. Samples included urines collected during the RBA assay (investigative samples) and performance evaluation samples (PE).

**Figure 2-7. Uncertainty in RBA Values[a]**




[a]Plot of uncertainty range (90% confidence interval) against administered dose. The dose axis is the group mean dose (µg/kg-day) for the highest dosing group in each study. The confidence interval increases substantially when the administered dose levels are less than 25 µg/kg-day.