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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
Office of Solid Waste and Emergency Response
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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.

1 *Basic Method for Estimating RBA*

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

6
$$RBA(\text{test material}) = UEF(\text{test material}) / UEF(\text{sodium arsenate})$$

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

13 **RESULTS**

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

18

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%
		Aspen Berm ^b	66.9	100% ± 46%
	5	Aspen Residential ^b	16.7	128% ± 52%
		Butte Soil	234	9% ± 3%
	6	Midvale Slag	591	23% ± 4%
		California Gulch Phase I Residential Soil	203	8% ± 3%
	7	California Gulch Fe/Mn PbO	110	57% ± 12%
		California Gulch AV Slag	1050	13% ± 4%
	8	Palmerton Location 2	110	49% ± 10%
		Palmerton Location 4	134	61% ± 11%
	9	California Gulch AV Slag	1050	18% ± 2%
	10	Murray Smelter Soil	310	33% ± 5%
11	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)

^aSame sample as evaluated in Phase II

^bThe 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

1

2

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).

9

Correlation of RBA with Arsenic Geochemistry

10

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

18

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

5

Low Bioavailability	Medium Bioavailability	High Bioavailability
As ₂ O ₃ Sulfosalts	As Phosphate FeAs Oxide PbAs Oxide MnAs Oxide Fe and Zn sulfates	FeAsO

6

7 **CONCLUSION**

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

- 10 1. Juvenile swine constitute a useful and stable animal model for measuring the relative
11 bioavailability of arsenic in a variety of soil or soil-like test materials. The Phase III protocol
12 described in this report is the recommended standard operating procedure (SOP) for the juvenile
13 swine RBA assay.
- 14 2. There are clear differences in the *in vivo* RBA of arsenic between different types of test materials,
15 ranging from less than 10% to more than 60%. Thus, knowledge of the RBA value for different
16 types of test materials at a site can be important for improving arsenic risk assessments at a site.
- 17 3. Available data are not yet sufficient to allow reliable quantitative calculation of the RBA for a test
18 material based only on knowledge of the relative amounts of arsenic mineral phases present.
19 However, tentative qualitative estimates of low, medium, or high bioavailability have been made
20 based on the major phase type of the arsenic containing waste material.
- 21 4. Additional extraction steps were identified and necessary to convert urinary organoarsenic
22 metabolites to inorganic arsenic for analysis of total arsenic in urine.
- 23 5. Due to limitations in detection limits for measurement of arsenic in urine, a minimum arsenic
24 dose of 25 µg/kg bw-day is recommended for the juvenile swine RBA assay, so that the amount
25 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 **1.0 INTRODUCTION**

2 **1.1 Overview**

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

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

7
$$ABA = (Absorbed\ Dose) / (Ingested\ Dose)$$

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

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

11
$$RBA = ABA(test) / ABA(reference)$$

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

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

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

25 **1.2 Using Relative Bioavailability Data to Improve Risk Calculations for Arsenic**

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

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

3

4 **2.0 EXPERIMENTAL METHODS FOR ESTIMATING ARSENIC RBA BY *IN VIVO*** 5 **STUDIES**

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

10 **2.1 Basic Approach for Measuring RBA *In Vivo***

11 Summary of Arsenic Toxicokinetics

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

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

21 Figure 2-1 plots the urinary excretion data from Table 2-1. It is apparent that typical urinary
22 recovery of soluble arsenic in humans (top panel) is dose-independent, and averages about 67% (range =
23 45 to 85%). Urinary recovery of arsenic in rodents (Figure 2-1, lower panel) is similar, with an average
24 value of 70% (range = 36 to 94%). Often the sum of arsenic recovery in urine plus feces is slightly less
25 than 100%. This could be partly due to experimental error, but is more likely due to retention of some
26 arsenic in tissues such as skin and hair.

27 Conceptual Model

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

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

- 4 • If 100% of all absorbed arsenic were excreted in the urine, the UEF would be equal to the oral
5 absorption fraction or ABA. However, some absorbed arsenic is excreted in the feces via the bile
6 and some absorbed arsenic enters tissue compartments (e.g., skin, hair) from which it is cleared
7 very slowly or not at all. Thus, the urinary excretion fraction should not be equated with the
8 absolute absorption fraction.
- 9 • The RBA of two orally administered materials (e.g., a test soil and sodium arsenate) can be
10 calculated from the ratio of the urinary excretion fraction of the two materials. This calculation is
11 independent of the extent of tissue binding or biliary excretion, because the fraction of absorbed
12 arsenic that is excreted in urine (K_u), which does depend on tissue binding and biliary excretion,
13 cancels in the calculation:

$$14 \quad RBA(x \text{ vs. } y) = \frac{UEF(x)}{UEF(y)} = \frac{AF_o(x) \cdot K_u}{AF_o(y) \cdot K_u} = \frac{AF_o(x)}{AF_o(y)}$$

15 where:

16 $RBA(x \text{ vs. } y)$ is the relative bioavailability of As in test material (x) vs. sodium arsenate
17 (y);

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

19 AF_o is the absorption fraction, which is the fraction of the dose absorbed following oral
20 administration; and

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

22
23 Thus, measurement of the urinary excretion fraction (µg/day excreted in urine per µg/day
24 administered) of test material and reference material (sodium arsenate) is the key experimental goal in
25 these arsenic RBA studies.

26 Estimation of UEF

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

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

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

12 **2.2 Experimental Methods**

13 **2.2.1 Study Designs**

14 Phase II Study Designs

15 Measurement of arsenic bioavailability in most Phase II studies was performed in parallel with
16 studies designed to estimate lead bioavailability (U.S. EPA, 2007). Groups of animals (typically 4 or 5
17 per dose group) were given oral doses of a test material (e.g., soil, tailings, slag, sediment) twice daily for
18 15 days, and 24-hour urine samples were collected several times during the study (typically on days 7 and
19 14). Because the main focus of these studies was on lead RBA, these early studies did not include groups
20 of animals that were exposed to an arsenic reference material. Thus, these studies, taken alone, were not
21 sufficient to allow for an estimation of the arsenic RBA of the test materials.

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

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

28 Phase III Study Designs

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

1 test materials, each at two or three different dose levels. In some cases, the doses of arsenic (expressed as
2 $\mu\text{g}/\text{day}$) were held constant over time, rather than being adjusted to account for changing body weight.
3 This is because the basic computational approach used to estimate RBA (described above) compares the
4 mass of arsenic excreted in urine ($\mu\text{g}/\text{day}$) to the mass of arsenic ingested ($\mu\text{g}/\text{day}$), so body weight
5 adjustments are not needed.

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

7 **2.2.2 Experimental Animals**

8 Juvenile swine were selected for use in these studies because their gastrointestinal physiology is
9 more similar to humans than most other animal models (Weis and LaVelle, 1991). All animals were
10 young males of the Pig Improvement Corporation genetically defined Line 26, and were purchased from
11 Chinn Farms, Clarence, MO. All studies used intact animals, except for one (the second VBI70 study),
12 which used castrated animals. The number of animals purchased for each study was typically 6–8 more
13 than required by the protocol. These animals were usually purchased at age 4–5 weeks (weaning occurs
14 at age 3 weeks), and they were then held under quarantine for one week to observe their health before
15 beginning exposure to test materials. Any animals that appeared to be in poor health during this
16 quarantine period were excluded. To minimize weight variations between animals and groups, extra
17 animals most different in body weight (either heavier or lighter) four days prior to exposure (day-4) were
18 also excluded from the study. The remaining animals were assigned to dose groups at random. When
19 exposure began (day zero), the animals were about 5–6 weeks old and weighed an average of about 7–
20 12 kg.

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

26 **2.2.3 Diet**

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

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

7 Drinking water was provided *ad libitum* via self-activated watering nozzles within each cage.
8 Periodic analysis of samples from randomly selected drinking water nozzles indicated the arsenic
9 concentration was less than the detection limit (about 1 µg/L). Assuming water intake of about
10 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
11 a 10 kg animal).

12 **2.2.4 Dosing**

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

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

26 **2.2.5 Collection and Preservation of Urine**

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

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

3 **2.2.6 Arsenic Analysis**

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

10 **2.2.6.1 Sample Digestion**

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

18 Digestion Method 1

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

25 Digestion Method 2

26 A 25 mL aliquot of acidified urine was removed and placed in a clean 100 mL beaker. 3.0 mL of
27 methanol, 10.0 mL of 40% (w/v) magnesium nitrate hexahydrate, and 10.0 mL of concentrated trace
28 metal grade nitric acid (HNO₃) were then added. The beaker was covered with a watch glass and placed
29 on a hot plate to reflux for 8–12 hours at 70–80°C. After this, the temperature was increased to 200°C,
30 and the watch glass was moved back to allow faster evaporation. The sample was then heated to
31 complete dryness (8–12 hours), covered with a watch glass, and allowed to cool. Dried samples were

1 transferred to a cool muffle furnace which was heated at a rate of 1 degree/minute to a temperature of
2 500°C, and then held at 500°C for 3 hours before cooling. Ashed samples were dissolved by adding 5 mL
3 distilled water and 5 mL concentrated trace metal grade hydrochloric acid (HCl), and boiling gently until
4 the white residue was completely dissolved. After cooling, the dissolved sample was diluted with
5 distilled water to 50.0 mL and held until analysis.

6 **2.2.6.2 Arsenic Analysis by Hydride Generation**

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

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

19 **2.2.7 Quality Assurance**

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

22 Blanks

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

25 Spike Recovery

26 Randomly selected samples were spiked with known amounts of inorganic arsenic (5–20 µg) and
27 the recovery of the added arsenic was measured. In Phase II, recovery of arsenic from spiked samples
28 typically ranged from 95 to 105%, with an average across all analyses of 99.8%. In Phase III, recovery of
29 arsenic from spiked samples typically ranged from 83 to 120%, with an average across all analyses of
30 103%.

1 Laboratory Duplicates

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

5 Laboratory Control Standards

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

8

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)

9

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

3 PE Samples and Blind Duplicates

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

7 The combined results for samples evaluated during the Phase II pilot studies are shown in
8 Figure 2-3. As seen in Panel A, there was good accuracy on sodium arsenate PE samples (10, 30, and
9 1000 µg/L) throughout the duration of each study. As shown in Panel B, there was also good
10 reproducibility between blind duplicate samples.

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

17

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

18

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

25

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

1

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

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

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

12 Inter-laboratory Comparison

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

18 Conclusion

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

1 **2.2.8 Test Material Characterization**

2 Table 2-3 describes the test materials for which RBA was measured in this program and provides
3 the analytical results for arsenic. Data on other Target Analyte List (TAL) metals, if available, are
4 provided in Appendix C. As seen, 27 different test materials were investigated (two in duplicate). In all
5 cases, these samples were sieved prior to analysis and dosing, and only materials which passed through a
6 60-mesh screen (corresponding to particles smaller than about 250 μm) were used. This is because it is
7 believed that soil particles less than about 250 μm are most likely to adhere to the hands and be ingested
8 by hand-to-mouth contact, especially in young children.

9 Many of the test materials¹ were characterized with regard to arsenic mineral phase, particle size
10 distribution, and matrix association using electron microprobe analysis (EMPA). In this procedure, an
11 electron microprobe with combined energy dispersive spectrometer (EDS) and multiple wavelength
12 dispersive spectrometers (WDS) was used to evaluate the elemental composition of arsenic-bearing
13 particles. A 1 to 2 gram split of dried sample was placed in a 2.5 cm plastic mold and impregnated with
14 epoxy. Once the sample was hardened, it was polished and carbon coated for EMPA. The EMPA was
15 operated at 15 kV accelerating voltage, with a 20 nA current and a 1 micron focused beam. Instrument
16 response was calibrated using certified mineral or pure metal standards and counting times were chosen to
17 provide 3-sigma detection limits of between 100–200 ppm. Elemental concentrations were corrected
18 using ZAF factors and concentration errors were generally less than 5% relative. For a more detailed
19 explanation of the EMPA method of analyses see Birks (1971) or Heinrich (1981).

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

¹Arsenic 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).

1 phase was an oxide, carbonate, sulfide, sulfate, or phosphate. Secondly, with the exception of the
2 “phosphates,” the major cation associated with the phase was identified. Therefore, phases such as
3 Fe-sulfate, FeOOH, MnOOH, PbMO, AsMO, or PbMSO₄ were identified (where M represents “metal”).
4 Some of these phases could represent a stoichiometric mineral form, but most are likely to be metastable
5 and/or amorphous and have some quantity of arsenic sorbed to their surface.

6 The “phosphate” group is even more generic in that the only common dominant ion is PO₄.
7 Although arsenic and phosphorous are both oxy-anions, a number of particles that contain both arsenic
8 and phosphate have been identified. As above, these might include minerals that contain mixtures of
9 phosphate and arsenate such as walentaite (Ca,Mn,Fe)Fe₃(AsO₄,PO₄)₄·7H₂O, morelandite (Ba,Ca,Pb)₅
10 Cl[AsO₄,PO₄]₃, or turneaureite Ca₅(Cl)[(AsO₄, PO₄)₃], but more likely represent arsenic adsorbed onto
11 other phosphate-containing particles.

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

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

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

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

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

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

8 **2.3 Results**

9 **2.3.1 RBA Estimates**

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

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

1 Figure 2-7 shows that the uncertainty in the RBA value for a test material (as reflected by the
2 difference between the upper bound and the lower bound) depends on the dose of arsenic administered in
3 the study. As seen, three of the test materials (Aspen Berm, Aspen Residential, and Jasper County High
4 Lead Mill) were administered only at low dose levels (less than 20 $\mu\text{g}/\text{kg}$ bw-day) and have extremely
5 wide uncertainty bounds around the RBA estimates. This is due mainly to the fact that the concentrations
6 of arsenic in the urine were very low and, hence, were difficult to quantify with good accuracy and also
7 difficult to distinguish from baseline. Because of the high uncertainty in these results, the data from these
8 three test materials are not considered further. Thus, based on these results, a minimum daily As dose of
9 25 $\mu\text{g}/\text{kg}$ -bw/day is recommended to ensure the amount if excreted in urine reaches a measurable quantity
10 and, that is to minimize uncertainty in RBA estimates.

11 **2.3.2 Effect of Low Analytical Recovery on Phase II RBA Values**

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

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

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

31

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.

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

$$3 \quad RBA = \sum (f_i \cdot RBA_i)$$

4 where:

5 f_i = Fraction of total arsenic present in phase “*i*”

6 RBA_i = Inherent RBA of phase “*i*”
7

8 However, because a total of 28 different phases were identified and reliable RBA results were
9 obtained for only 20 different samples, it is clear that the existing data are not sufficient to perform a
10 robust regression analysis. Instead, a screening-level analysis was performed, as follows. First, in order
11 to reduce the number of independent variables, the 28 different phases were grouped into 9 categories as
12 described in Table 2-9. These categories were based on professional judgment regarding the expected
13 degree of similarity between members of a group, along with information on the relative abundance of
14 each phase (see Table 2-4). Phases with low relative arsenic mass (maximum relative mass in any test
15 material less than 15%) were grouped together under “Minor Constituents;” these phases included AsMO,
16 AsMSO₄, Clays, Paint, Pb Solder, Pb-As Vanadate, PbAsMO, PbAsSbCuO, PbCrO₄, PbMO, PbMS,
17 PbMSO₄, Pyrite, TiO₂, and ZnSiO₄. Next, the fraction of arsenic present in each group was calculated by
18 summing the relative arsenic mass for each phase in the group. Based on the expectation that particles
19 that are totally *included* (fully enclosed or encased in mineral or vitreous matrices) are not likely to
20 contribute significantly to the observed RBA value of a sample, only the relative arsenic mass in partially
21 or entirely *liberated* particles (partially or entirely exposed on their outer surfaces) was included in the
22 sum. The results are shown in Table 2-10.

23 Group-specific RBA values were then estimated by fitting the grouped data to the model using
24 minimization of square errors. Two different options were employed. In the first option, each fitting
25 parameter (group-specific RBA) was fully constrained to be between zero and one, inclusive. In the
26 second option, all parameters were unconstrained. Because the minor constituents do not contribute
27 significantly to the total arsenic mass in any of the tested materials, a reasonable estimate of their specific
28 RBA cannot be obtained. Therefore, an arbitrary coefficient of 0.5 was assumed for this group and the
29 coefficient was not treated as a fitting parameter. The resulting estimates of the group-specific average
30 RBA values for the remaining groups are shown in Table 2-11 (these values apply only to *liberated*
31 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 ₂ O ₃ Sulfosalts	As Phosphate FeAs Oxide PbAs Oxide MnAs Oxide 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.

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

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

12 **3.0 CONCLUSIONS**

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

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

1 4.0 REFERENCES

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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 $\mu\text{g/day}$	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 ₂ O ₃	1000	1	85	1.4	5	
Human	M	1	As ₂ O ₃	760	5	70	NA	22	Mappes 1977
Human	M	1	Mixture	63	1	80	NA	3	Creelius 1977
Human	M	1	Na ₂ HAsO ₄	200	1	50	NA	3	
Human	M	6	Na ₂ HAsO ₄	0.01	1	58	NA	6	Tam et al. 1979
Human	M	2	Na ₂ HAsO 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 ₂ O ₃	1000	1	73	NA	14	
Monkey	M	5	Na ₂ HAsO ₄	360	1	49	2	4	Roberts et al. 2002
Monkey	M	7	Na ₂ HAsO ₄	50-200	1	40	42	4	Roberts et al. 2007
Hamster	M	5	As ₂ O ₃	4500	1	49	11	5	Yamauchi and Yamamura 1985
Hamster	NS	4	Na ₂ HAsO ₄	2000	1	74	12	3	Marafante and Vahter 1987
Mouse	M	5	Na ₂ HAsO ₄	400	1	77	8	2	Vahter and Norin 1980
Mouse	M	5	Na ₂ HAsO ₄	4000	1	89	6	2	
Mouse	M	5	Na ₂ HAsO ₄	40	1	94	NA	2	Vahter 1981
Mouse	M	5	Na ₂ HAsO ₄	400	1	93	NA	2	
Mouse	M	5	Na ₂ HAsO ₄	2000	1	92	NA	2	
Mouse	M	5	Na ₂ HAsO 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

^aValues 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 ₂ O ₃	AsMO	AsSbO	Pb-As Vanadate	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 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)	
			<i>Liberated</i>	<i>Liberated</i>	<i>Included</i>
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%

^aValues 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- $\text{CaAl}_3\text{AsPO}_4\text{-OH}_6$), 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 ($\text{KFe}_3(\text{OH})_6(\text{SO}_4)_2$) or plumbojarosite ($\text{PbFe}_3(\text{OH})_6(\text{SO}_4)_2$). 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 gauge 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 ($(\text{AgCu})_2\text{As}_2\text{S}_{11}$), or polybasite ($(\text{AgCu})_{16}(\text{Sb,As})_2\text{S}_{11}$).
	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 ($\text{Cu}_{12}\text{As}_4\text{S}_{13}$) 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 pyrometallurgically 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 pyrometallurgically 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 Vanadate	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

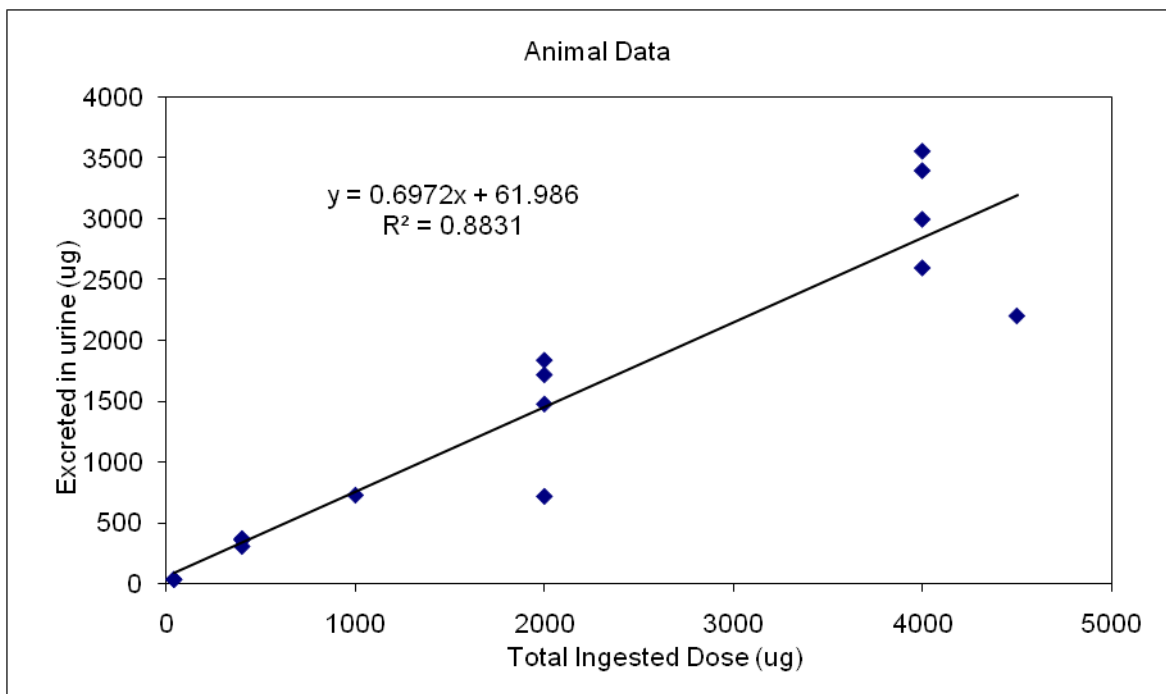
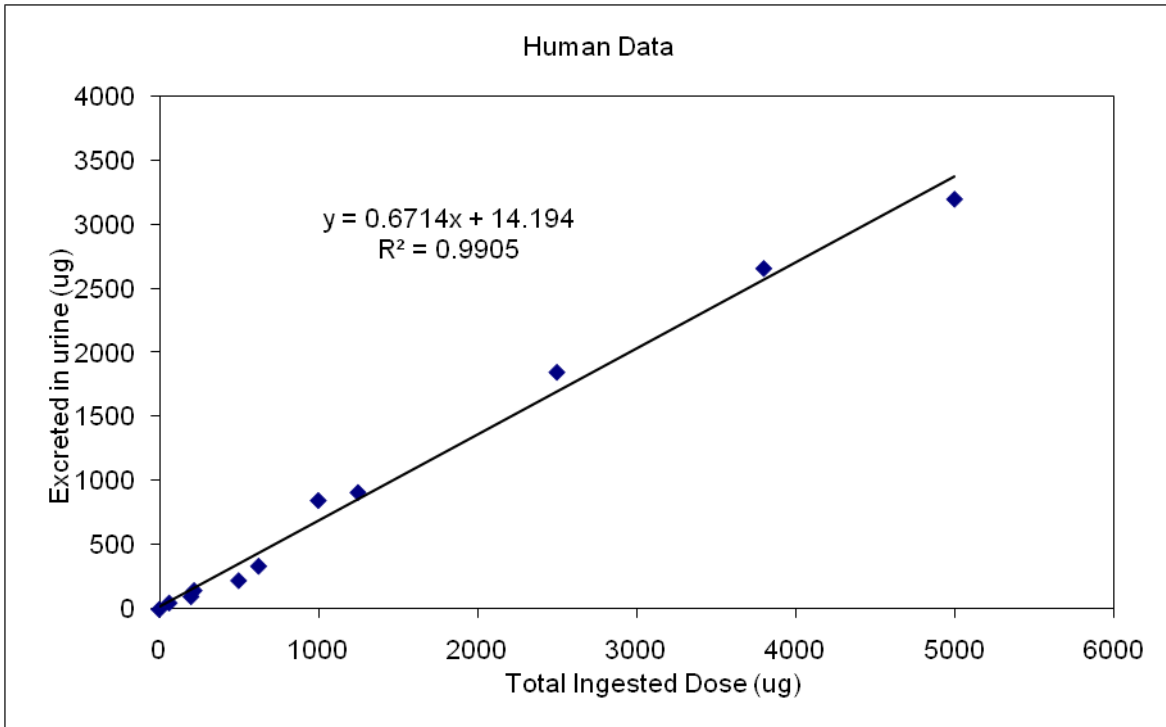
Phase	Experiment	Sample	RBA	Arsenic Conc. (ppm)	Number of Particles Counted	Phase (Liberated/Included)																	
						As Phosphate		As ₂ O ₃		FeAs Oxide		Fe & Zn Sulfates		FeAsO		MnAs Oxide		PbAs Oxide		Sulfosalts		Minor Constit.	
II	2	Bingham Creek Channel Soil	39.3%	149	430	8%	<1%			11%	<1%	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%	<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

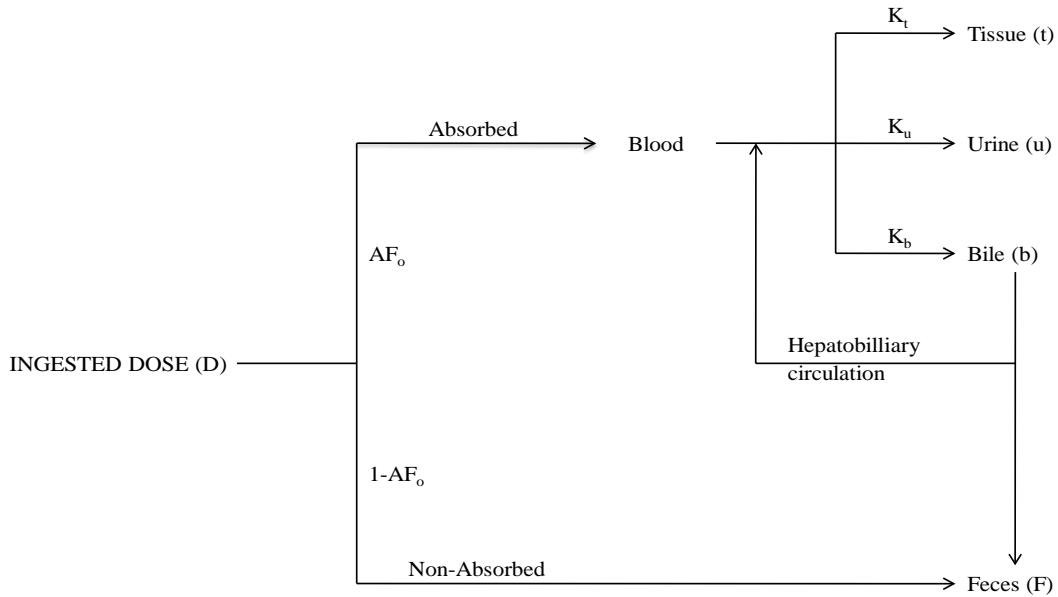
Group Name	Estimated Group-Specific RBA¹		RBA Category²
	Method 1	Method 2	
FeAsO	1.00	1.42	High
As Phosphate	0.55	0.59	Medium
FeAs Oxide	0.45	0.44	Medium
Fe & Zn Sulfates	0.40	0.40	Medium
PbAs Oxide	0.38	0.38	Medium
MnAs Oxide	0.38	0.35	Medium
As ₂ O ₃	0.25	0.25	Low
Sulfosalts	0.02	0.01	Low

Figure 2-1. Excretion of Soluble As in Humans and Animals^a



^aSee 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:

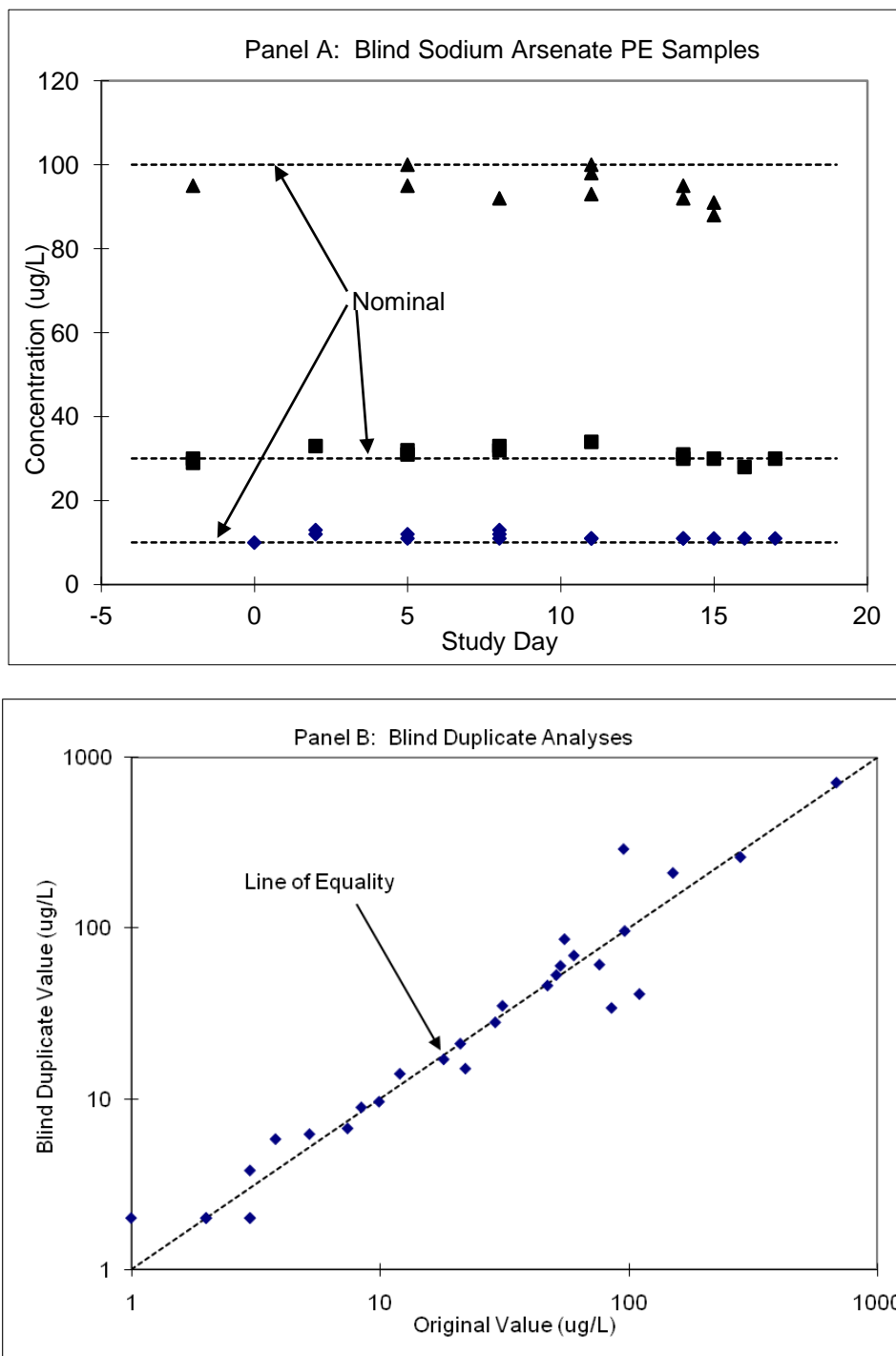
Amount Absorbed (μg) $= D \cdot AF_o$

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

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

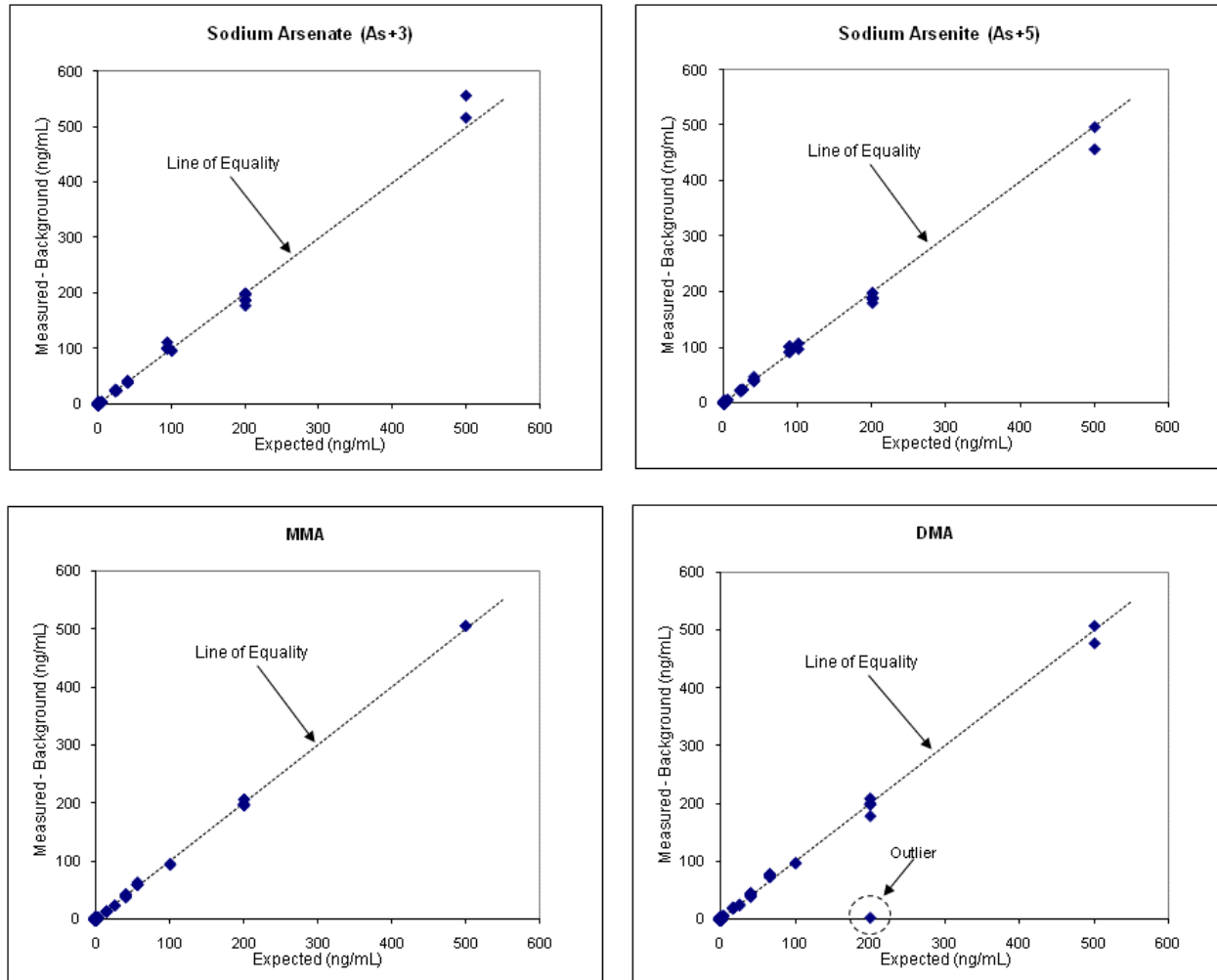
Relative Bioavailability (x vs. y) $= \text{UEF}(x) / \text{UEF}(y)$
 $= (AF_o(x) \cdot K_u) / (AF_o(y) \cdot K_u)$
 $= AF_o(x) / AF_o(y)$

Figure 2-3. Quality Assurance Data from Phase II Pilot Studies^a



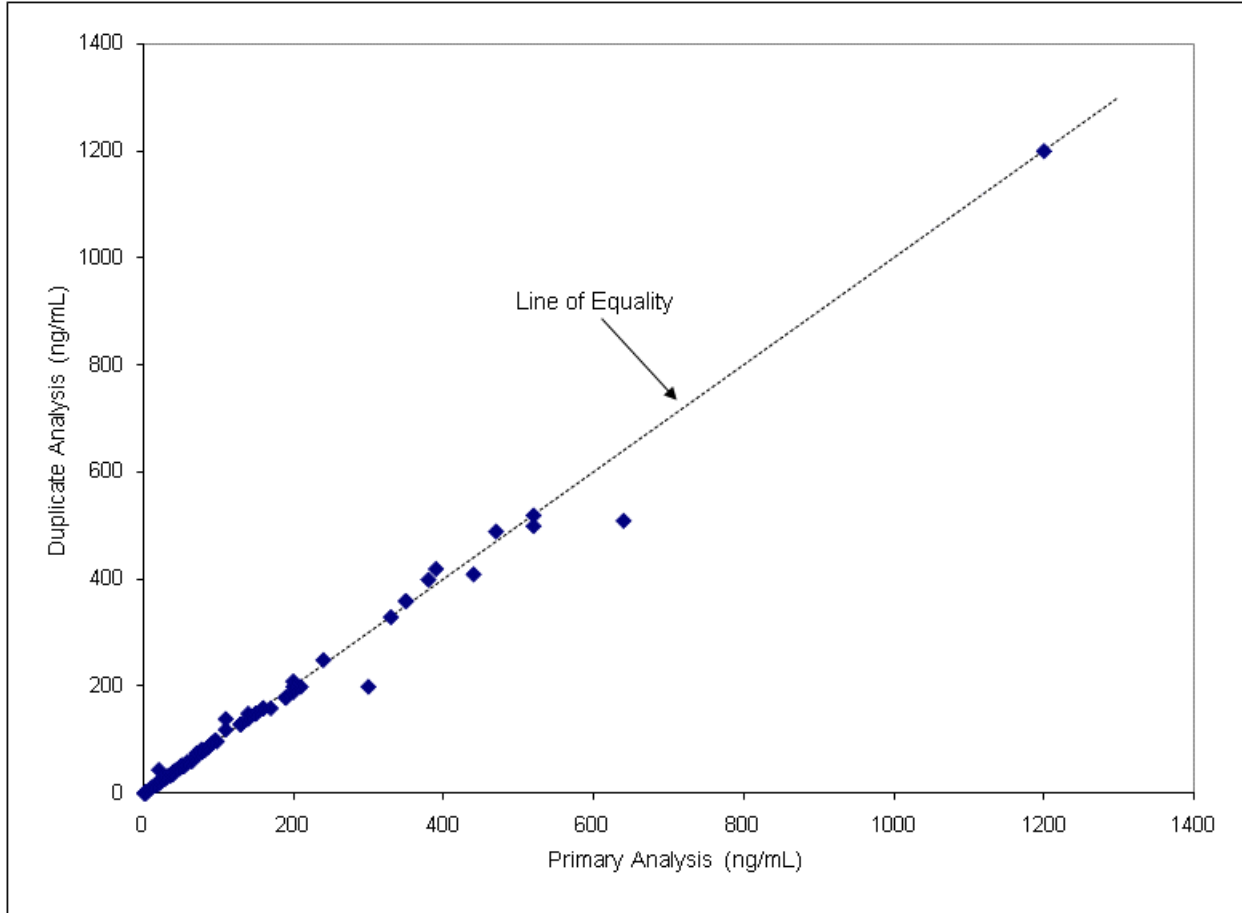
^aComparison of measured and actual (nominal) concentrations of performance evaluation (PE) samples for urine (panel A), and between duplicate measurements on the same urine sample (panel B), for Phase II studies. R^2 for blind duplicates was 0.91 (n=30).

Figure 2-4. Phase III Performance Evaluation Samples^a



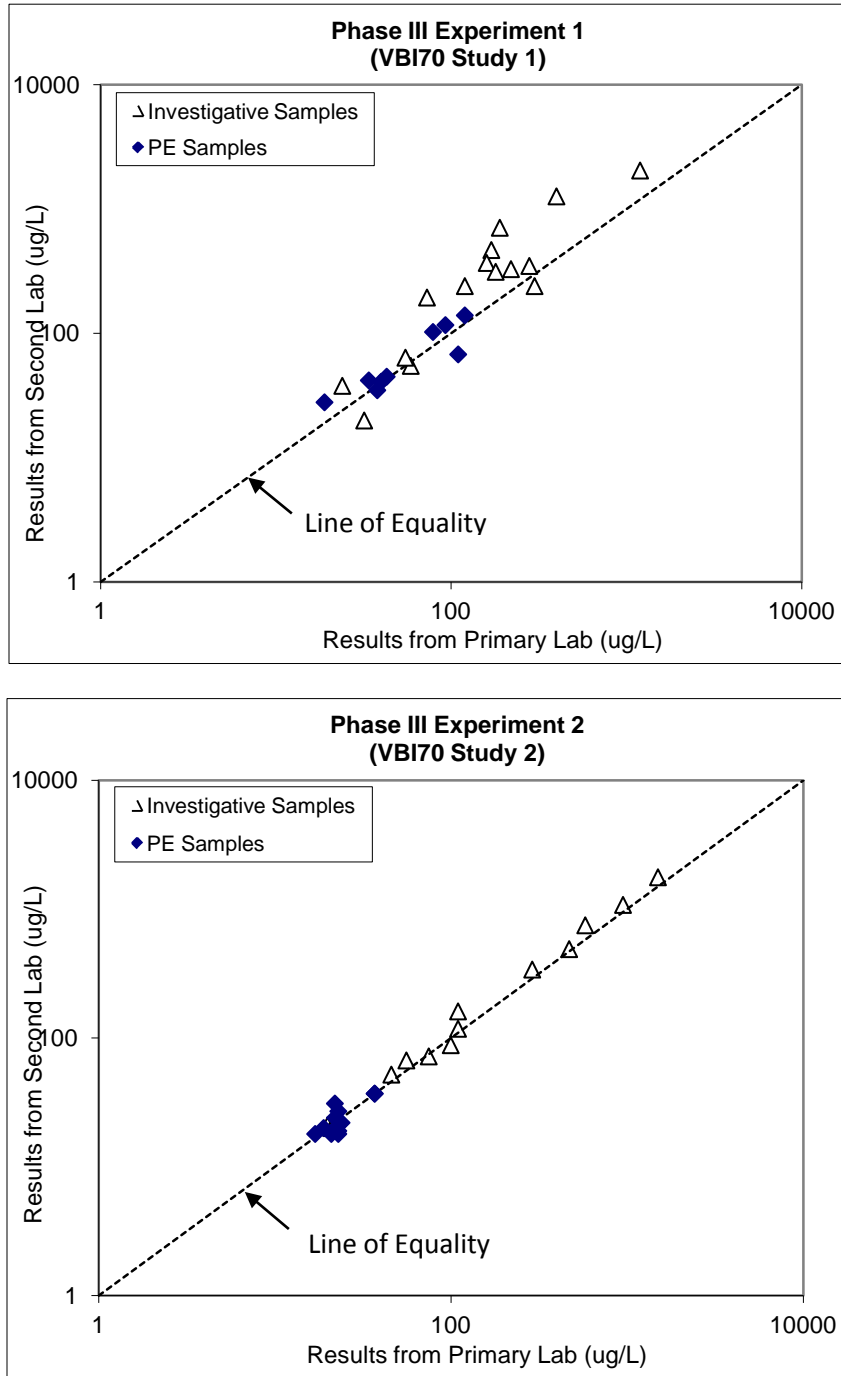
^aComparison of measured and actual concentrations of performance evaluation (PE) urine samples for Phase III studies. DMA, dimethylarsinic acid; MMA, monomethylarsonic acid. R^2 values were <0.99 for the four analytes ($N=35-37$).

Figure 2-5. Phase III Blind Duplicate Samples ^a



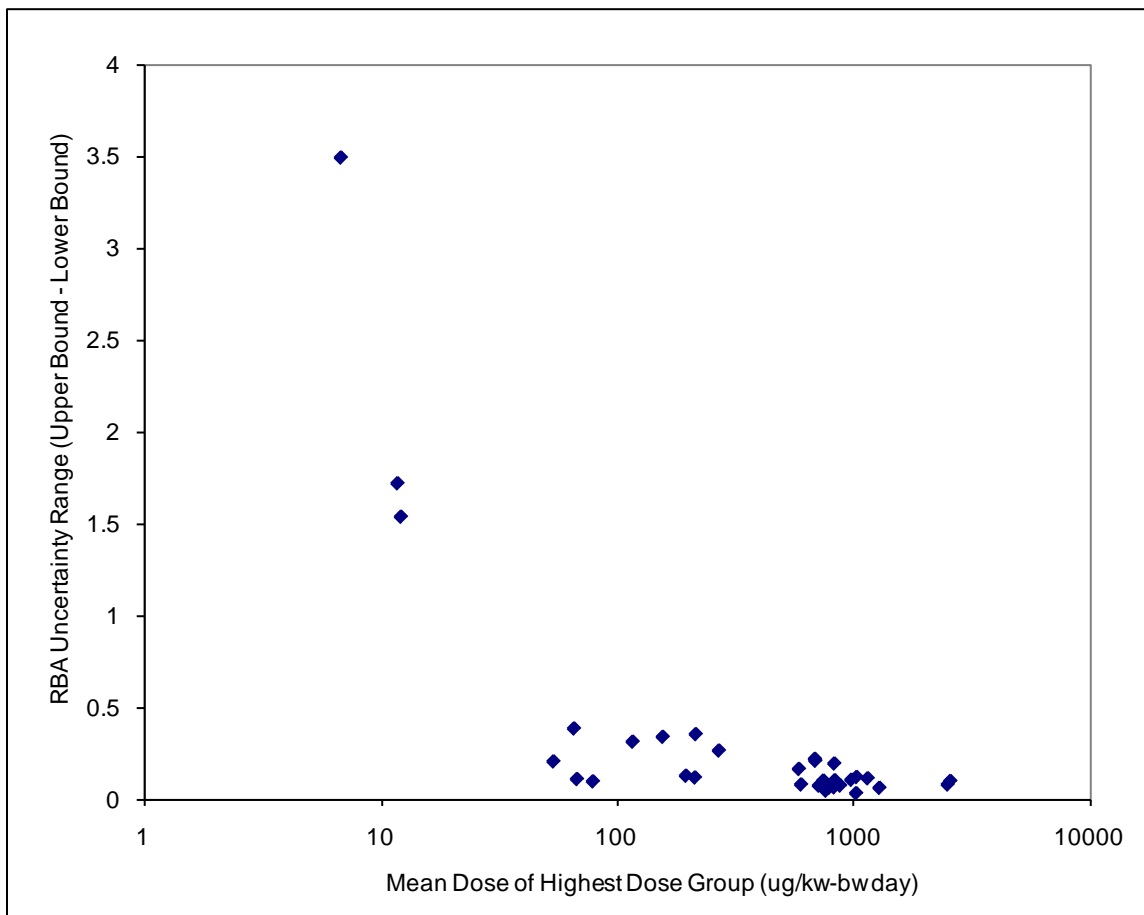
^aComparison between duplicate measurements on the same urine sample for Phase III studies. The R^2 was 0.98 (n=72).

Figure 2-6. Phase III Inter-Laboratory Comparison^a



^aComparison 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



^aPlot of uncertainty range (90% confidence interval) against administered dose. The dose axis is the group mean dose ($\mu\text{g}/\text{kg}\text{-day}$) for the highest dosing group in each study. The confidence interval increases substantially when the administered dose levels are less than $25 \mu\text{g}/\text{kg}\text{-day}$.