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RELATIVE BIOAVAILABILITY OF ARSENIC IN AN ASARCO AND A HAWAII SOIL

Prepared for:

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EXECUTIVE SUMMARY

A study using juvenile swine as test animals was performed to measure the gastrointestinal absorption of arsenic from an ASARCO and a Hawaii soil sample. The ASARCO material was collected from a stockpile of soil from a former smelter site near Tacoma, Washington. The Hawaii material was collected from a school garden located near Kea'au town, Hawaii that had been impacted by arsenic associated with herbicide use in former sugar mill plantation land. The arsenic concentrations (mean ± SD) of the ASARCO and Hawaii soil samples are 181.9 ± 6.3 and 768.85 ± 32.3 mg/kg, respectively.

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

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

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

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

Estimated RBA for Asarco and Hawaii Soils

Measurement Interval	Estimated RBA (90% Confidence Interval)	
	Test Material 1 (ASARCO)	Test Material 2 (Hawaii)
Days 6/7	0.52 (0.44–0.61)	0.34 (0.29–0.40)
Days 9/10	0.49 (0.43–0.56)	0.31 (0.28–0.36)
Days 12/13	0.46 (0.39–0.54)	0.33 (0.28–0.39)
All Days	**0.49 (0.45–0.53)**	**0.33 (0.30–0.36)**

The best fit point estimate RBA of arsenic in an ASARCO and Hawaii soil sample observed was 49 and 33%, respectively.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

APPENDICES

ACRONYMS AND ABBREVIATIONS

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

1.0 INTRODUCTION

1.1 Overview of Bioavailability

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

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

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

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

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

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

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

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

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

1.2 Using RBA Data to Improve Risk Calculations

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

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

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

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

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

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

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

1.3 Purpose of this Study

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

2.0 STUDY DESIGN

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

Table 2-1. Study Design and Dosing Information

Group	Group Name Abbreviation	Dose Material Administered	Number of Swine in Group	Arsenic Dose		
				Target (µg/kg BW-day)	Actual [a] (µg/kg BW-day)	Actual [b] (µg-day)
1	NaAs	Sodium arsenate	4	25	25	339
2	NaAs	Sodium arsenate	4	50	50	678
3	NaAs	Sodium arsenate	4	100	100	1,354
4	TM1	ASARCO	4	40	40	542
5	TM1	ASARCO	4	60	60	813
6	TM1	ASARCO	4	120	120	1,625
7	TM2	Hawaii	4	40	40	833
8	TM2	Hawaii	4	60	60	1,250
9	TM2	Hawaii	4	120	120	2,499
10	Control	Negative control	3	0	0	0

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

2.1 Test Materials

2.1.1 Sample Description

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

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

2.1.2 Sample Preparation and Analysis

2.1.2.1 Hawaii

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

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

The Hawaii soil arsenic concentration was determined by instrumental neutron activation analysis (INAA). Three replicates of the Hawaii soil were analyzed and the arsenic concentration was 768.85 ± 32.3 mg/kg (mean ± SD).

2.1.2.2 ASARCO

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

The ASARCO soil arsenic concentration was determined by INAA. An aliquot of the ASARCO soil was analyzed in duplicate and the arsenic concentration was 181.9 ± 6.3 mg/kg (mean ± SD).

2.2 Experimental Animals

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

The number of animals purchased for the study was several more than required by the protocol. These animals were purchased at an age of about 5–6 weeks (weaning occurs at age 3 weeks) and housed in individual stainless steel cages. The animals were then held under quarantine for one week to observe their health before beginning exposure to dosing materials. Each animal

was examined by a certified veterinary clinician (swine specialist) and any animals that appeared to be in poor health during this quarantine period were excluded from the study. To minimize weight variations among animals and groups, extra animals most different in body weight (either heavier or lighter) five days prior to exposure (day 5) were also excluded from the study. The remaining animals were assigned to dose groups at random (group assignments are presented in Appendix A).

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

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

2.3 Diet

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

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

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

Table 2-2. Typical Feed Composition

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

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

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

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

2.4 Dosing

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

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

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

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

2.5 Collection and Preservation of Urine Samples

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

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

2.6 Arsenic Analysis

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

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

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

2.7 Quality Control

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

Blind Duplicates (Sample Preparation Replicates)

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

Spike Recovery

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

Laboratory Duplicates

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

Laboratory Control Standards

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

Performance Evaluation Samples

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

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

Blanks

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

Summary of QC Results

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

3.0 DATA ANALYSIS

3.1 Overview

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

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

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

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

where:

D = ingested dose (μg)

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

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

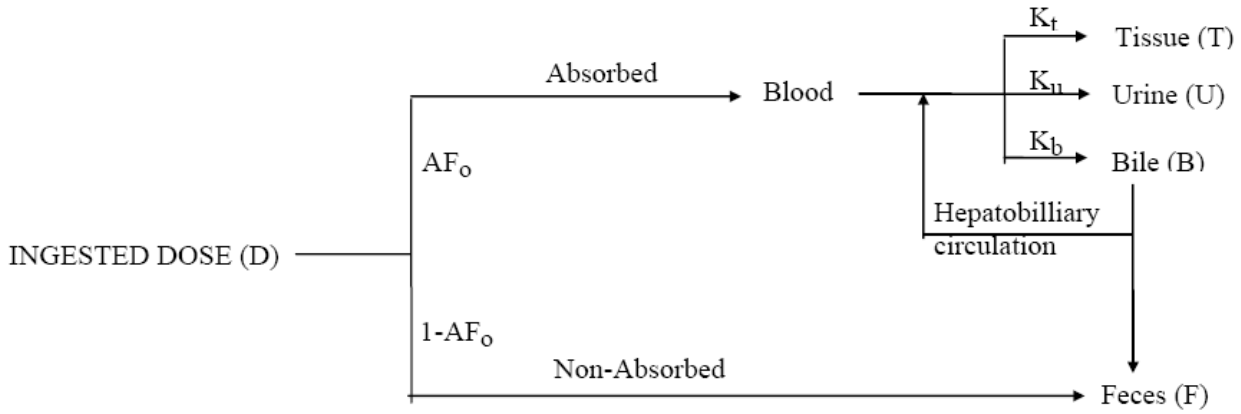
1. Plot the amount of arsenic excreted in the urine (μg per 48 hours) as a function of the administered amount of arsenic (μg per 48 hours), both for reference material and for test material.

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

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

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

Figure 3-1. Conceptual Model for Arsenic Toxicokinetics



where:

AF_o = Oral Absorption Fraction

K_t = Fraction of absorbed arsenic which is retained in tissues

K_u = Fraction of absorbed arsenic which is excreted in urine

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

BASIC EQUATIONS:

Amount in Urine

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

Urinary Excretion Fraction (UEF)

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

Relative Bioavailability

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

3.2 Data Fitting

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

Simultaneous Regression

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

Separate Models:

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

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

Combined Model

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

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

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

Weighted Regression

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

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

where:

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

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

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

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

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

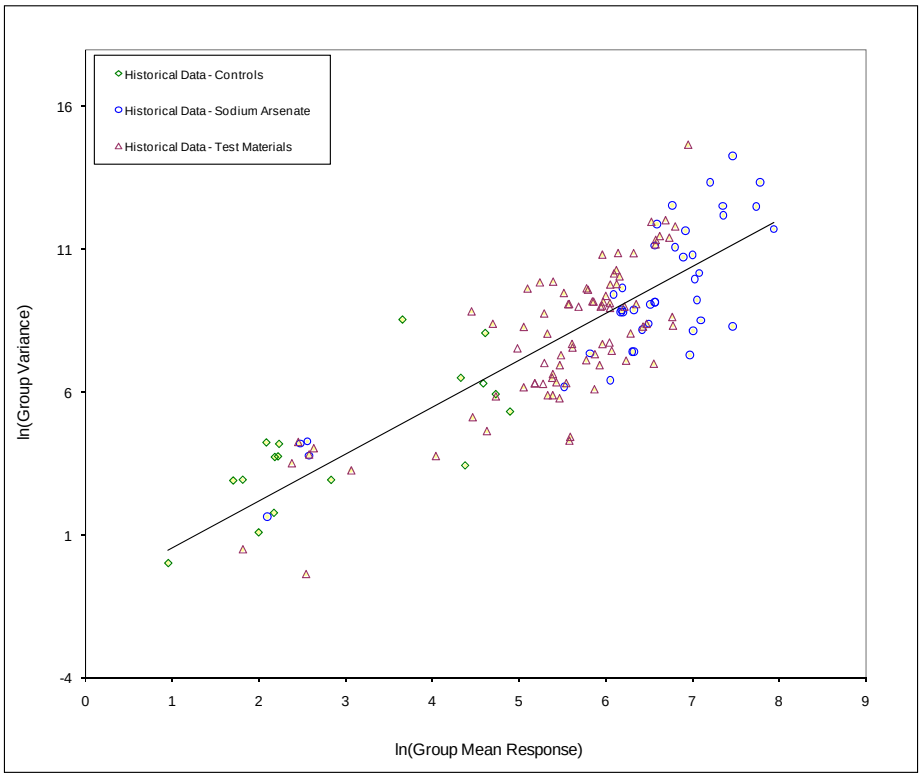
where:

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

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

Based on these data, values of $k1$ and $k2$ were derived using ordinary least squares minimization. The resulting values were -1.10 for $k1$ and 1.64 for $k2$.

Figure 3-2. Urinary Arsenic Variance Model



Goodness of Fit

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

Assessment of Outliers

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

3.3 Calculation of RBA Estimates

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

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

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

4.0 RESULTS

4.1 Clinical Signs

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

4.2 Dosing Deviations

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

4.3 Background Arsenic Excretion

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

Table 4-1. Background Urinary Arsenic

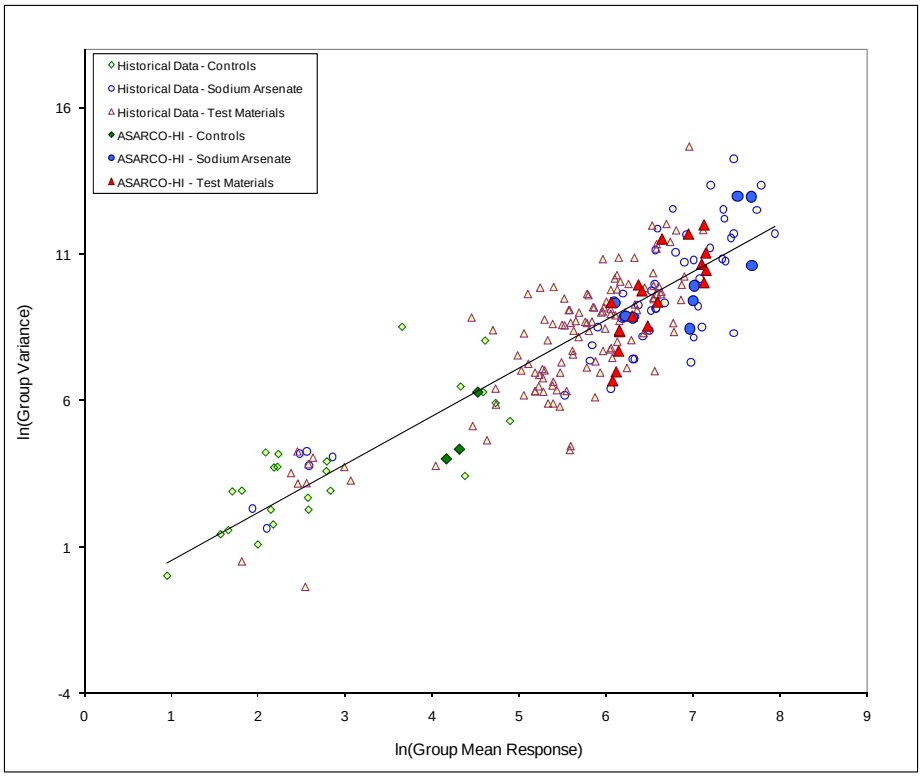
Swine Number	Urine Collection Period (days)	As Dose (µg per collection period)	As Concentration in Urine (µg/L)	Urine Volume (mL)	Total As Excreted (µg/48 hrs)
703	6/7	0	120	600	72
727	6/7	0	34	1680	57
729	6/7	0	56	1140	64
703	9/10	0	65	1260	82
727	9/10	0	23	3360	77
729	9/10	0	55	1180	65
703	12/13	0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

4.4 Urinary Arsenic Variance

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

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

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



4.5 Dose-Response Modeling

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

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

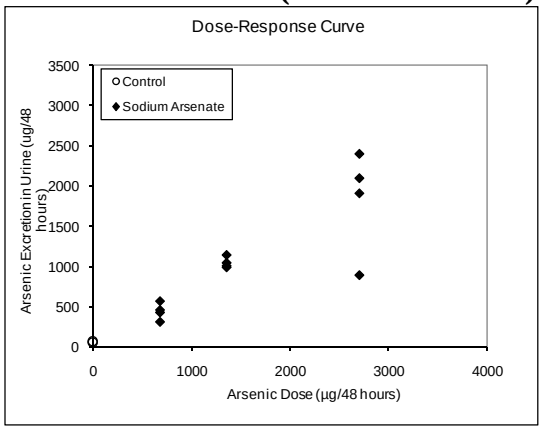
Table 4-2. Urine Excretion Fraction (UEF) Estimates

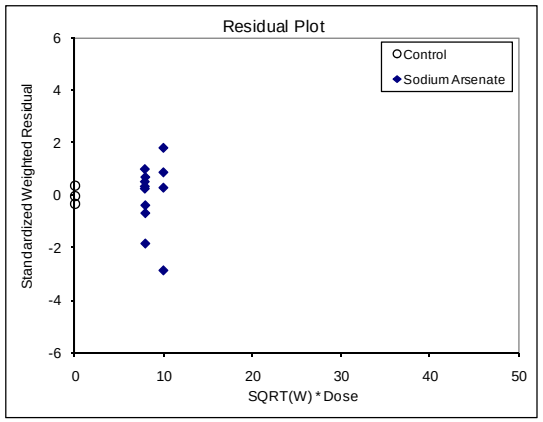
Urine Collection Period (days)	Outliers Excluded	Slopes (UEF Estimates)		
		b_r	b_{t1}	b_{t2}
Days 6/7	0	0.65	0.34	0.22
Days 9/10	0	0.73	0.36	0.23
Days 12/13	0	0.74	0.34	0.25
All Days	0	0.70	0.34	0.23

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

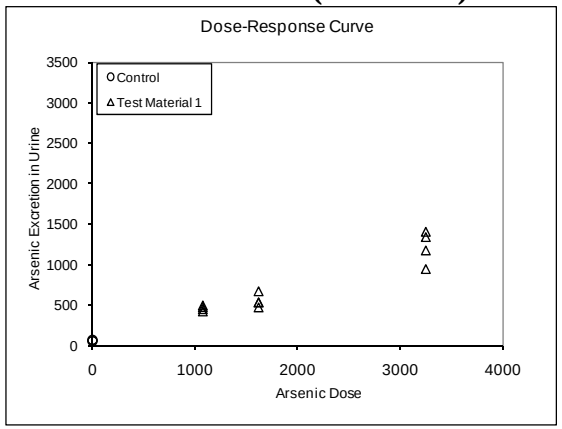
Figure 4-2. ASARCO and Hawaii Urinary Excretion of Arsenic: Days 6/7

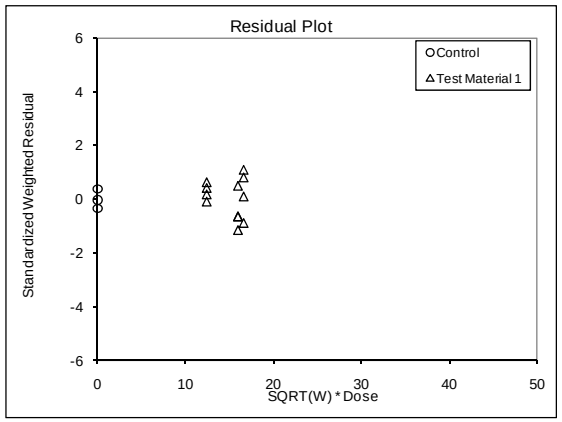
Reference Material (Sodium Arsenate)



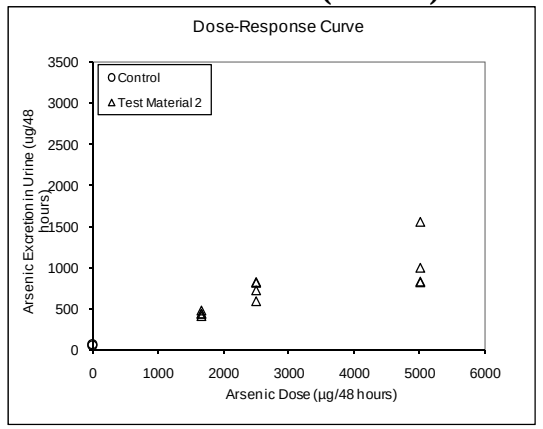


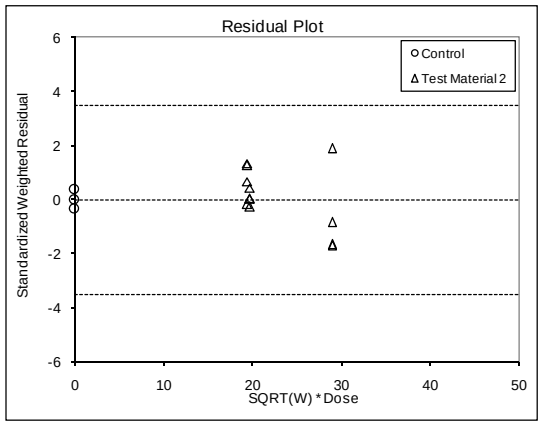
Test Material 1 (ASARCO)





Test Material 2 (Hawaii)





Summary of Fitting [a]

Parameter	Estimate	Standard Error
a	64.3	12.2
b_r	0.65	0.04
b_{t1}	0.34	0.02
b_{t2}	0.22	0.02
Covariance (b_r,b_{t1})	0.0620	–
Covariance (b_r,b_{t2})	0.0584	–
Degrees of Freedom	36	–

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

ANOVA

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

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

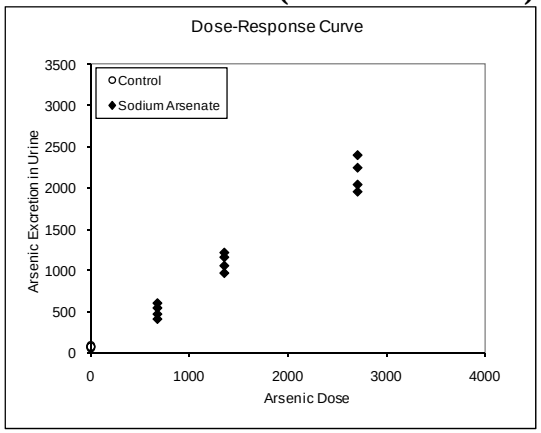
RBA and Uncertainty

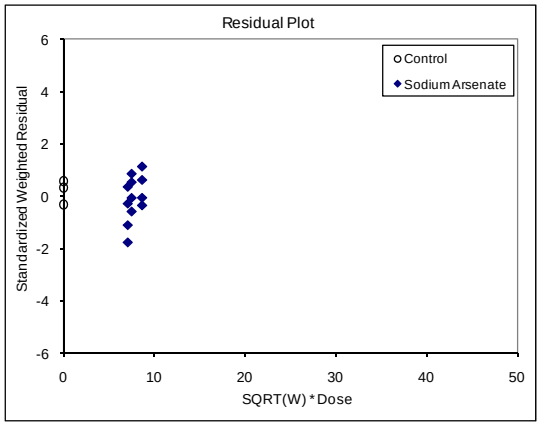
	Test Material 1	Test Material 2
RBA	0.52	0.34
Lower bound [b]	0.44	0.29
Upper bound [b]	0.61	0.40
Standard Error	0.049	0.032

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

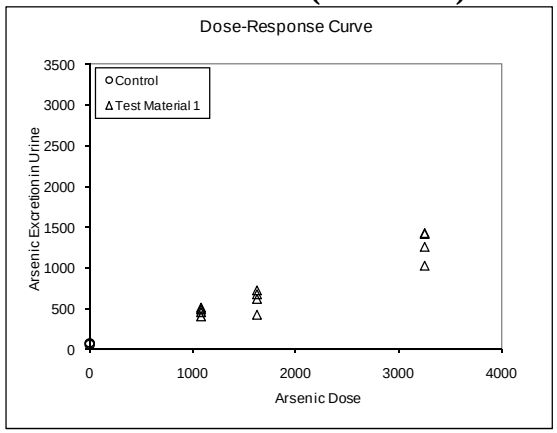
Figure 4-3. ASARCO and Hawaii Urinary Excretion of Arsenic: Days 9/10

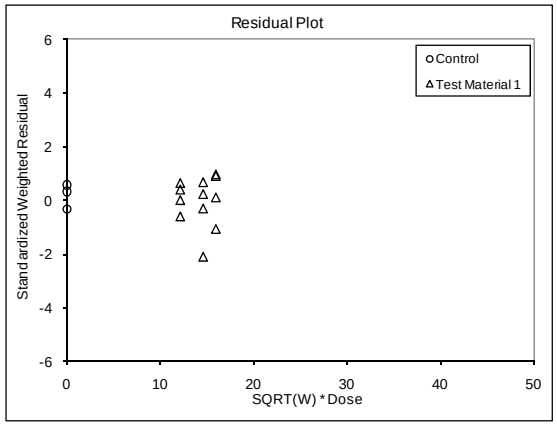
Reference Material (Sodium Arsenate)



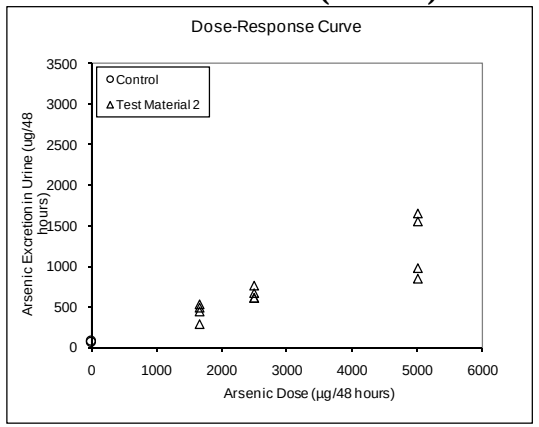


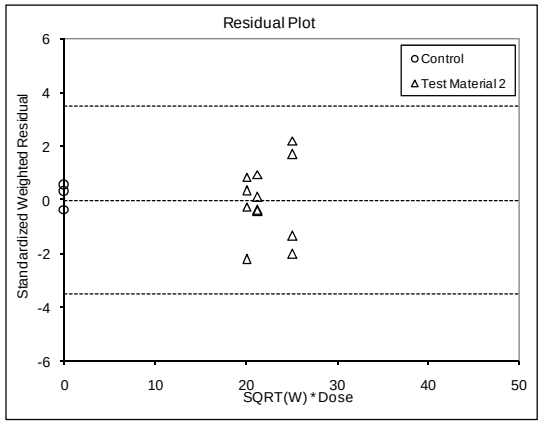
Test Material 1 (ASARCO)





Test Material 2 (Hawaii)





Summary of Fitting [a]

Parameter	Estimate	Standard Error
a	71.0	11.0
b_r	0.73	0.04
b_{t1}	0.36	0.02
b_{t2}	0.23	0.01
Covariance (b_r,b_{t1})	0.0684	–
Covariance (b_r,b_{t2})	0.0698	–
Degrees of Freedom	36	–

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

ANOVA

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

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

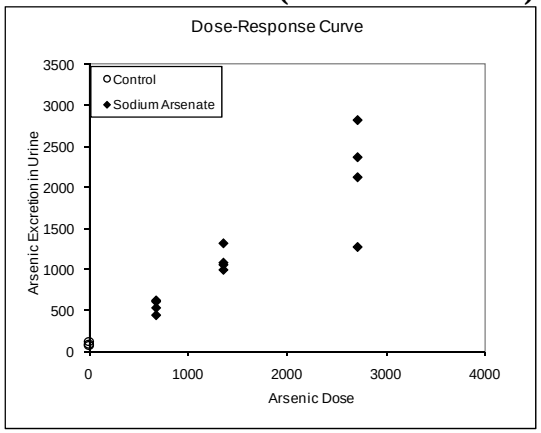
RBA and Uncertainty

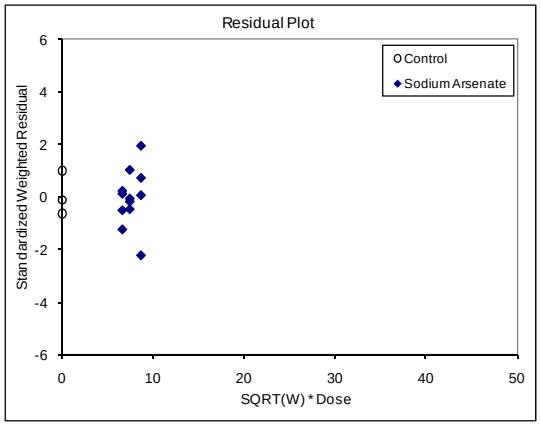
	Test Material 1	Test Material 2
RBA	0.49	0.31
Lower bound [b]	0.43	0.28
Upper bound [b]	0.56	0.36
Standard Error	0.037	0.024

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

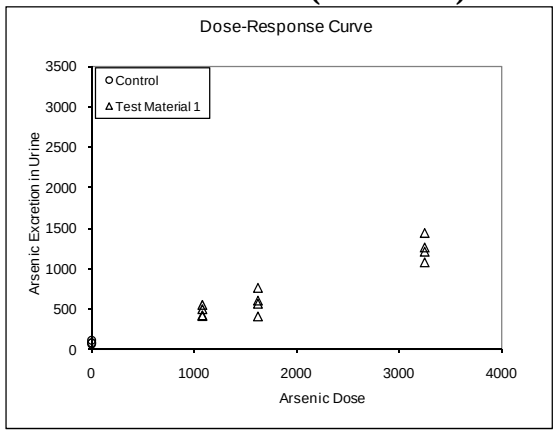
Figure 4-4. ASARCO and Hawaii Urinary Excretion of Arsenic: Days 12/13

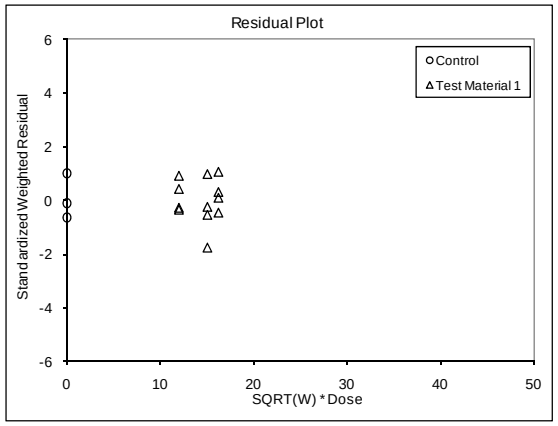
Reference Material (Sodium Arsenate)



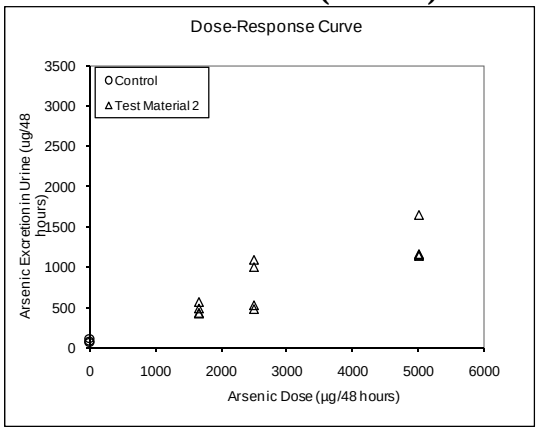


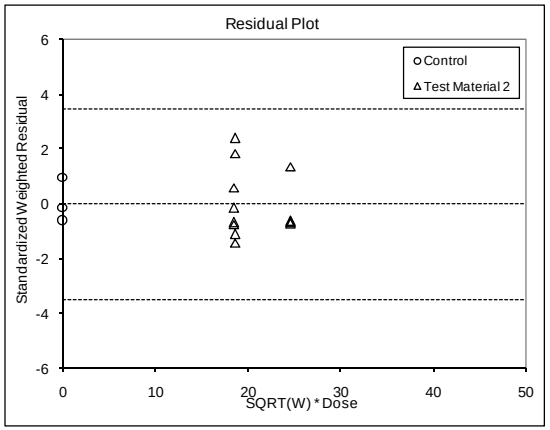
Test Material 1 (ASARCO)





Test Material 2 (Hawaii)





Summary of Fitting [a]

Parameter	Estimate	Standard Error
a	89.7	16.3
b_r	0.74	0.05
b_{t1}	0.34	0.03
b_{t2}	0.25	0.02
Covariance (b_r,b_{t1})	0.0882	–
Covariance (b_r,b_{t2})	0.0819	–
Degrees of Freedom	36	–

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

ANOVA

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

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

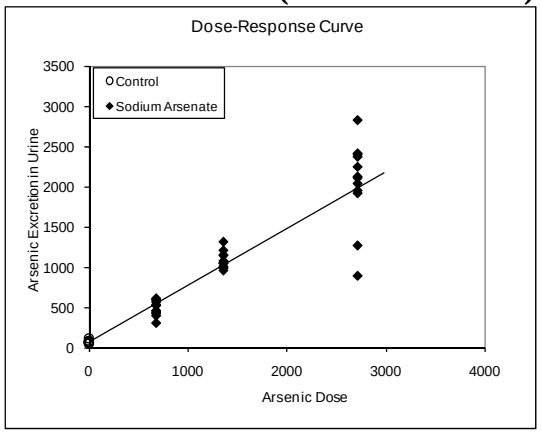
RBA and Uncertainty

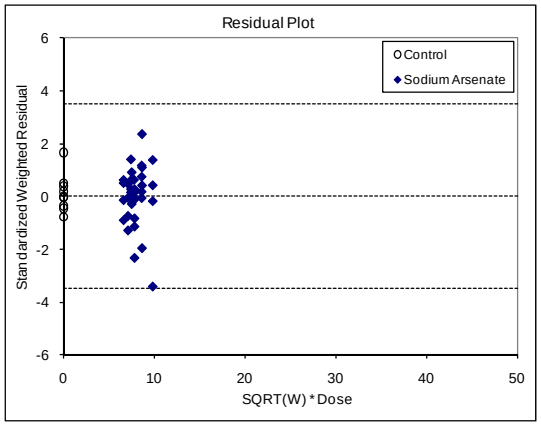
	Test Material 1	Test Material 2
RBA	0.46	0.33
Lower bound [b]	0.39	0.28
Upper bound [b]	0.54	0.39
Standard Error	0.045	0.032

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

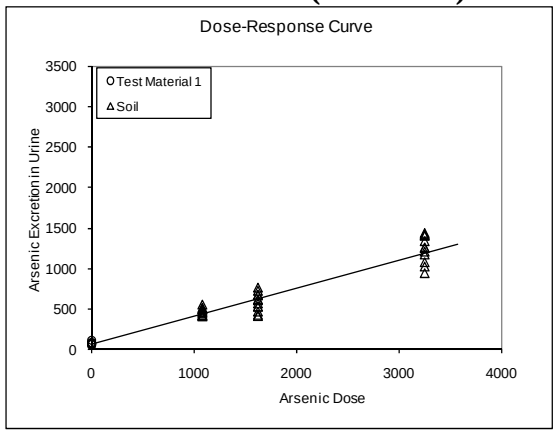
Figure 4-5. ASARCO and Hawaii Urinary Excretion of Arsenic: All Days

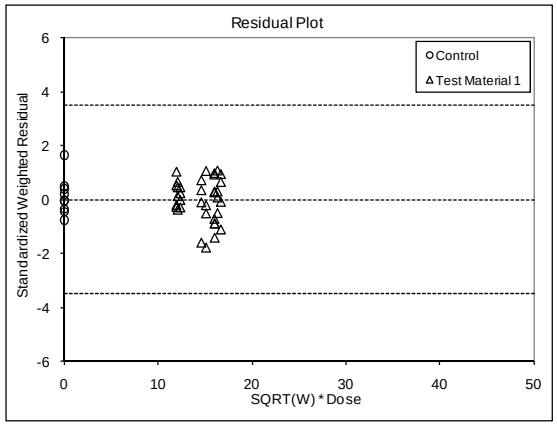
Reference Material (Sodium Arsenate)



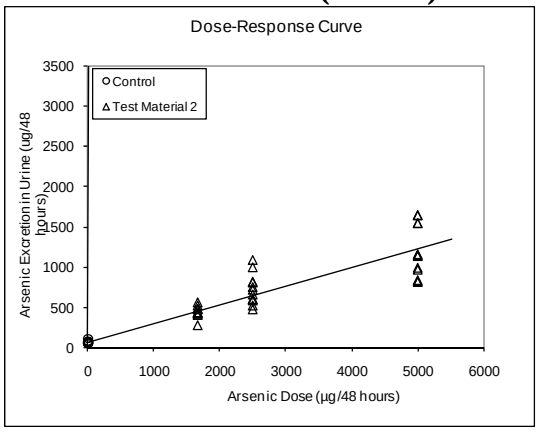


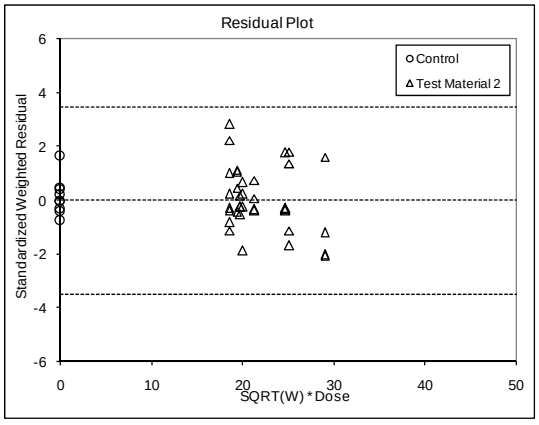
Test Material 1 (ASARCO)





Test Material 2 (Hawaii)





Summary of Fitting [a]

Parameter	Estimate	Standard Error
a	72.7	7.5
b_r	0.70	0.02
b_{t1}	0.34	0.01
b_{t2}	0.23	0.01
Covariance (b_r,b_{t1})	0.0706	–
Covariance (b_r,b_{t2})	0.0680	–
Degrees of Freedom	114	–

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

ANOVA

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

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

RBA and Uncertainty

	Test Material 1	Test Material 2
RBA	0.49	0.33
Lower bound [b]	0.45	0.30
Upper bound [b]	0.53	0.36
Standard Error	0.025	0.017

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

4.6 Calculated RBA Values

Estimated RBA values (mean and 90% confidence interval) are shown in Table 4-3. As shown, the best fit point estimate RBA of arsenic in an ASARCO and Hawaii soil sample observed was is 49 and 33%, respectively.

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

Urine Collection Period (days)	Estimated RBA (90% Confidence Interval)	
	TM1 (ASARCO)	TM2 (Hawaii)
Days 6/7	0.52 (0.44–0.61)	0.34 (0.29–0.40)
Days 9/10	0.49 (0.43–0.56)	0.31 (0.28–0.36)
Days 12/13	0.46 (0.39–0.54)	0.33 (0.28–0.39)
All Days	**0.49 (0.45–0.53)**	**0.33 (0.30–0.36)**

4.7 Uncertainty

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

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

5.0 REFERENCES

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

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

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

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

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

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

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

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

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

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

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

Appendix A: Group Assignments

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

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

Appendix B: Body Weights

Table B-1. Body Weights

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

BW = body weight

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

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

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

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

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

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

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

Appendix D: Analytical Results for Quality Control Samples

Table D-1. Blind Duplicate Samples

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

RPD = relative percent difference

Table D-2. Laboratory Spikes

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

Table D-3. Laboratory Duplicates

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

RPD = relative percent difference

Table D-4. Laboratory Quality Control Standards

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

TABLE D-5. ARSENIC PERFORMANCE EVALUATION SAMPLES

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

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

TABLE D-6. BLANKS

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

Figure D-1. Urinary Arsenic Blind Duplicates

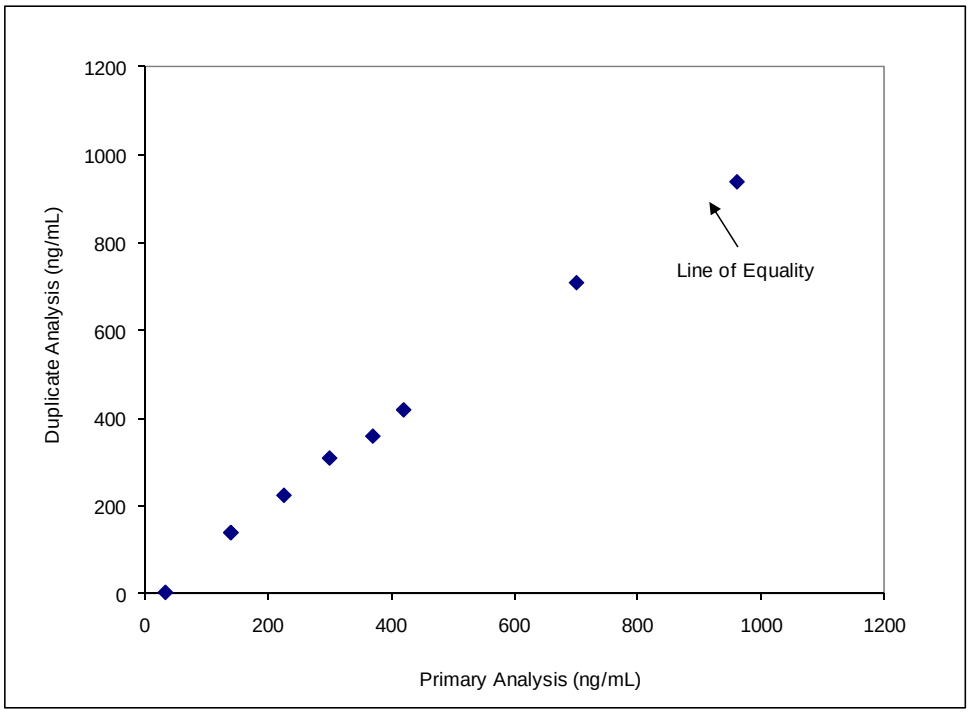


Figure D-2. Performance Evaluation Samples