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Relative Bioavailability of Arsenic in the Flat Creek Soil Reference Material

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ACRONYMS AND ABBREVIATIONS

ABA	Absolute bioavailability
AF_o	Oral absorption fraction
ANOVA	Analysis of variance
As	Arsenic
As^{+3}	Trivalent inorganic arsenic
As^{+5}	Pentavalent inorganic arsenic
DMA	Dimethyl arsenic
D	Ingested dose
DF	Degrees of freedom
FCRM	Flat Creek Soil Reference Material
g	Gram
GLP	Good Laboratory Practices
ICP-MS	Inductively coupled plasma-mass spectrometry
ICP-OES	Inductively coupled plasma-optical emission spectrometry
K_b	Fraction of absorbed arsenic that is excreted in the bile
kg	Kilogram
K_t	Fraction of absorbed arsenic that is retained in tissues
K_u	Fraction of absorbed arsenic that is excreted in urine
MBW	Mean body weight
mL	Milliliter
MMA	Monomethyl arsenic
MSE	Mean squared error
N	Number of data points
NRC	National Research Council
ORD	Office of Research and Development
OSRTI	Office of Superfund Remediation and Technical Innovation
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
SSE	Sum of squared standard error
TM	Test material
UEF	Urinary excretion fraction
U.S. EPA	United States Environmental Protection Agency
USGS	United States Geological Survey
μg	Microgram
°C	Degrees Celsius
°F	Degrees Fahrenheit

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EXECUTIVE SUMMARY

A study using juvenile swine as test animals was performed to measure the gastrointestinal absorption of arsenic (As) from a sample of the Flat Creek Soil Reference Material (FCRM). In conjunction with the United States Environmental Protection Agency (U.S. EPA) Office of Superfund Remediation and Technology Innovation (OSRTI), FCRM was developed by the United States Geological Survey (USGS) from soil containing high concentrations of metals due to mining activity near an abandoned lead mine in Montana. The measured arsenic concentration of FCRM is 740 ± 57 mg/kg (mean ± standard deviation [SD]).

The relative oral bioavailability of arsenic in FCRM was assessed by comparing the absorption of arsenic from FCRM ("test material") to that of a reference material, sodium arsenate. Groups of swine (five per dose group) were given oral doses of the reference material or the test material twice a day for 14 days at three target dose levels (40, 80, and 120 mg As/kg body weight/day). A group of three untreated swine served as a control for the arsenic test groups.

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

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

Estimated arsenic RBA values (mean and 90% confidence interval) are as follows:

Estimated RBA for FCRM	
Measurement Interval	**Estimated Arsenic RBA (90% Confidence Interval)**
Days 6/7	0.16 (0.14–0.19)
Days 9/10	0.17 (0.14–0.20)
Days 12/13	0.17 (0.15–0.19)
All Days	**0.17 (0.15–0.19)**

The best fit point estimate for the arsenic RBA for FCRM soil is 17%.

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 the exposure medium of interest (e.g., soil, dust, water, food, air, paint), intake rates of each exposure 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 exposure 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 matrices such as rock or slag of variable sizes, shapes, and compositions. 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 ("*ref*") 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):

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

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 was 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 Refine Risk Calculations

When reliable data are available on the RBA of a chemical in an exposure medium (e.g., soil), the information can be used to refine the accuracy of exposure and risk calculations at that site. RBA data can be used to adjust default oral toxicity values (reference dose [RfD] and slope factor [SF]) to account for differences in absorption between the chemical ingested as a soluble form of arsenic (As) and the chemical ingested in the exposure media, assuming that the toxicity factors are also based on a readily soluble form of the chemical. For noncancer 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 (e.g., mg/kg body weight/day) 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 Flat Creek Soil Reference Material (FCRM) compared to a soluble form of arsenic (sodium arsenate).

2.0 STUDY DESIGN

The test and reference materials were administered to groups of five juvenile swine at three different dose levels for 14 days (doses were administered in two increments each day). 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 with the dosing material mixed into a small portion of feed, which was hand fed to the animals (see Section 2.4). The study was performed as nearly as possible within guidelines of Good Laboratory Practices (GLP: 40 CFR 792).

Table 2-1. Study Design and Dosing Information

Group	Group Name	Dose Material Administered	Number of Swine in Group	Arsenic Dose[a] Target (µg/kg Body Weight-Day)	Actual[b] (µg/kg Body Weight-Day)
4	Test material	FCRM	5	40	42
5	Test material	FCRM	5	80	85
6	Test material	FCRM	5	120	125
7	Sodium arsenate	Sodium arsenate	5	40	42
8	Sodium arsenate	Sodium arsenate	5	80	83
9	Sodium arsenate	Sodium arsenate	5	120	125
10	Control	Negative control	3	0	0

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

2.1 Test Materials

2.1.1 Sample Description

The test soil used in this investigation was a sample of FCRM. The FCRM was developed by the United States Geological Survey (USGS), in conjunction with the United States Environmental Protection Agency (U.S. EPA) Office of Superfund Remediation and Technical Innovation (OSRTI), from soil containing high concentrations of metals due to mining activity near an abandoned lead mine in Montana.

2.1.2 Sample Preparation and Analysis

The USGS reported the arsenic soil concentration of FCRM sample as 740 ± 57 mg/kg soil (mean ± standard deviation [SD]), determined using inductively coupled plasma-optical emission spectrometry (ICP-OES) and inductively coupled plasma-mass spectrometry (ICP-MS).

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 1 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 that were most different in body weight (either heavier or lighter) 5 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 3 days during the course of the study. In each study, the rate of weight gain was comparable in all dosing groups. Body weight data are presented in Appendix B.

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

2.3   Diet

Animals were weaned onto standard swine chow (purchased from MFA Inc., Columbia, Missouri) by the supplier. The feed was nutritionally complete and met all requirements of the National Institutes of Health (NRC, 1988). The ingredients and nutritional profile of the feed are presented in Appendix C. The measured arsenic concentration in a randomly selected feed sample was 0.11 µg/g feed.

Beginning 5 days before the first day of dosing, each animal was given a daily amount of feed equal to 4.0% of the mean body weight of all animals on study. Feed was reduced to 3.7% body weight starting on day 8 of the study. Feed amounts were adjusted every 3 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. The arsenic concentration measured in six water samples from randomly selected drinking water nozzles averaged 1.1 µg/L.

2.4   Dosing

Animals were exposed to dosing materials (sodium arsenate or test material) for 14 days, with the dose for each day being administered in two equal portions beginning at 9:00 AM and 3:00 PM (2 hours before feeding). Swine were dosed 2 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 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 1 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 3 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 were adjusted accordingly. Actual doses (µg arsenic/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 6–7 (U-1), 9–10 (U-2), and 12–13 (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 separate plastic bottles to ensure that there was no loss of sample due to overflow.

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

2.6   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 heated 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 D.

2.7   Quality Control

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

Blind Duplicates (Sample Preparation Replicates)

A random selection of about 8% of all urine samples generated during the study were prepared for laboratory analysis in duplicate and submitted to the laboratory in a blind fashion. Results are shown in Appendix E (see Table E-1 and Figure E-1).

Six of nine urine duplicate samples had relative percent differences (RPD) values that were <5%. Values for the remaining three duplicates were 20, 29, and 180% (see Appendix E).

Spike Recovery

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

Laboratory Duplicates

No duplicate urine samples were analyzed.

Laboratory Control Standards

Internal laboratory control standards were tested periodically during sample analysis. Recovery of arsenic from these standards was generally good and within the acceptable range (see Table E-3).

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 E (see Table E-4 and Figure E-2). All sample results were close to the expected values, indicating that there was good recovery of the arsenic in all cases.

Blanks

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 were <1 µg/L). Results are shown in Table E-5.

Summary of QC Results

Based on the results of all of the QC samples and the 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

Figure 3-1. Conceptual Model for Arsenic Toxicokinetics
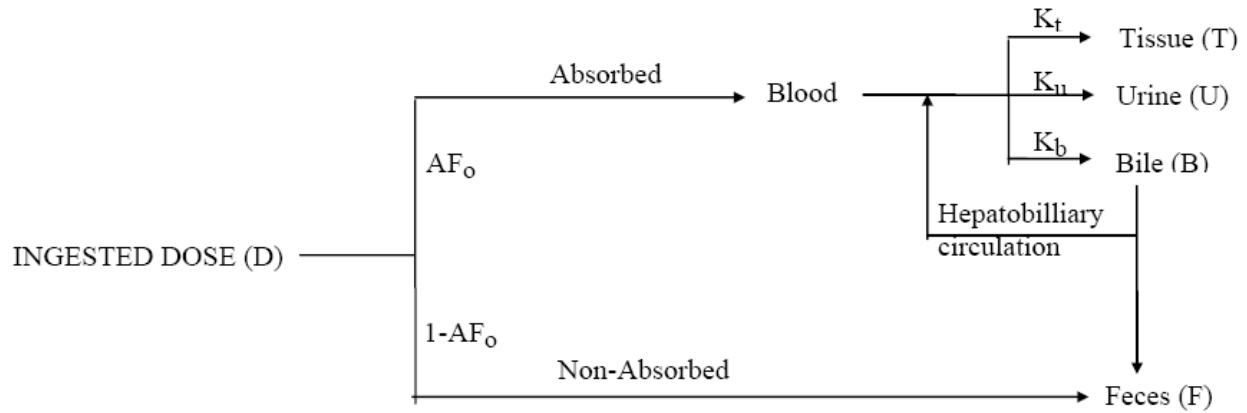
where:

AF$_o$ = oral absorption fraction

K$_t$ = fraction of absorbed arsenic that is retained in tissues

K$_u$ = fraction of absorbed arsenic that is excreted in urine

K$_b$ = fraction of absorbed arsenic that is excreted in the bile

BASIC EQUATIONS:

Amount in Urine

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

UEF

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

RBA

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

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

1. Plot the amount of arsenic excreted in the urine (µg per 48 hours) as a function of the administered amount of arsenic (µg per 48 hours) for both the reference material and the 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 the RBA for each test material as the ratio of the UEF for the test material compared to UEF for the reference material:

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

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 is zero (Finney, 1978).

<u>Weighted Regression</u>

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) (U.S. EPA, 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, the weighted regression is equivalent to the maximum likelihood method.

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

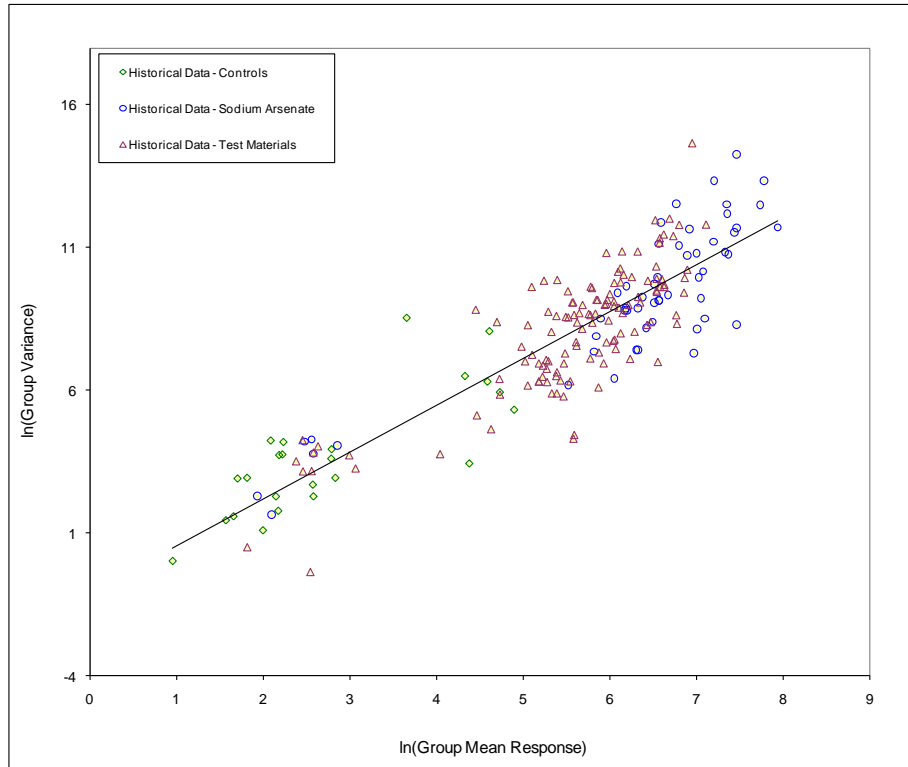$$\ln(s_i^2) = k1 + k2 \cdot \ln(\overline{y}_i)$$

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

Based on these data, values of $k1$ 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 determinations (Adj R^2) as described by Draper and Smith (1998). A fit is considered acceptable if the p-value is <0.05.

Data Assessment

Arsenic data were assessed in two parts. First, the urine volumes and arsenic concentrations were reviewed. A large volume of urine is typically indicative that a swine spilled its drinking water into the urine collection trays. In these instances, the arsenic concentration in the diluted urine will become very small and will be difficult to measure with accuracy. Furthermore, because the response of the swine to arsenic dose is calculated from the product of urine concentration and volume, the result becomes highly uncertain when the concentration is multiplied by a volume that is not representative of the total urine volume. For this reason, in cases where total urine volume per 24-hour period was >5 liters (more than twice the average urine output of swine) and the measured urine concentration of arsenic was at or below the quantitation limit (<2 µg/L), the samples were judged to be unreliable and were excluded from the quantitative analysis. No samples met these criteria for exclusion.

The full dataset was modeled and analyzed for individual measured responses that appeared atypical compared to the responses from other animals in the same dose group. Responses that

yielded standardized weighted residuals >3.5 or <-3.5 were considered to be potential outliers (Canavos, 1984).

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. However, one swine died prior to initiating dosing. This pig showed no signs of illness and was replaced before dosing began. Five swine received 1 cc Naxcel once per day for several days during the study (Table 4-1) to treat a systemic bacterial infection (swine were found with fever ≥104°F).

Table 4-1. NAXCEL Treatments

Swine Number	Days of Treatment
927	-4 – -2
908	-4 – -2
944	-3 – -1
946	1–3
934	2–4

4.2   Dosing Deviations

One pig (Swine #946) missed the initial dose on day 0. 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 4-2. Urinary arsenic concentration (mean ± SD) was 84 ± 130 µg/L (42 ± 37 µg/L after excluding the outlier for swine 916, days 9 and 10). The values shown are generally within the range of typical endogenous background urinary arsenic levels reported from other studies (see

Figure 3-2), although at the higher end of the detected range. This supports the view that the animals were not exposed to any significant exogenous sources of arsenic throughout the study.

Table 4-2. Background Urinary Arsenic

Swine Number	Urine Collection Period (Days)	Arsenic Dose (µg per Collection Period)	Arsenic Concentration in Urine (µg/L)	Urine Volume (mL)	Total Arsenic Excreted (µg/48 Hours)
911	6/7	0	32	3,520	112
940	6/7	0	34	3,400	114
916	6/7	0	37	2,520	92
911	9/10	0	19	4,085	76
940	9/10	0	21	3,340	71
916	9/10	0	419	3,300	1,383
911	12/13	0	27	4,600	124
940	12/13	0	33	3,940	130
916	12/13	0	132	1,320	174

4.4   Urinary Arsenic Variance

As discussed in Section 3.2, the urinary arsenic dose-response data are analyzed using weighted least squares regression and the weights are assigned using an "external" variance model. To ensure that the variance model was valid, the variance values from each of the dose groups were superimposed on the historic data set (see Figure 4-1). As shown, aside from the control pig that was identified as an outlier, the variance of the urinary arsenic data from this study is consistent with the data used to generate the variance model.

4.5   Dose-Response Modeling

Urinary data for collection days 9 and 10 for control pig 916 were identified as outliers (see Section 3.2) and were excluded from analysis. The remaining data set was analyzed (Figures 4-2 through 4-5).

All of the dose-response curves were approximately linear, with the slope of the best-fit straight line being equal to the best estimate of the UEF. The resulting slopes (UEF estimates) for the final fittings of the test material and corresponding reference material are shown in Table 4-3.

Figure 4-1. FCRM Data Compared to Urinary Arsenic Variance Model
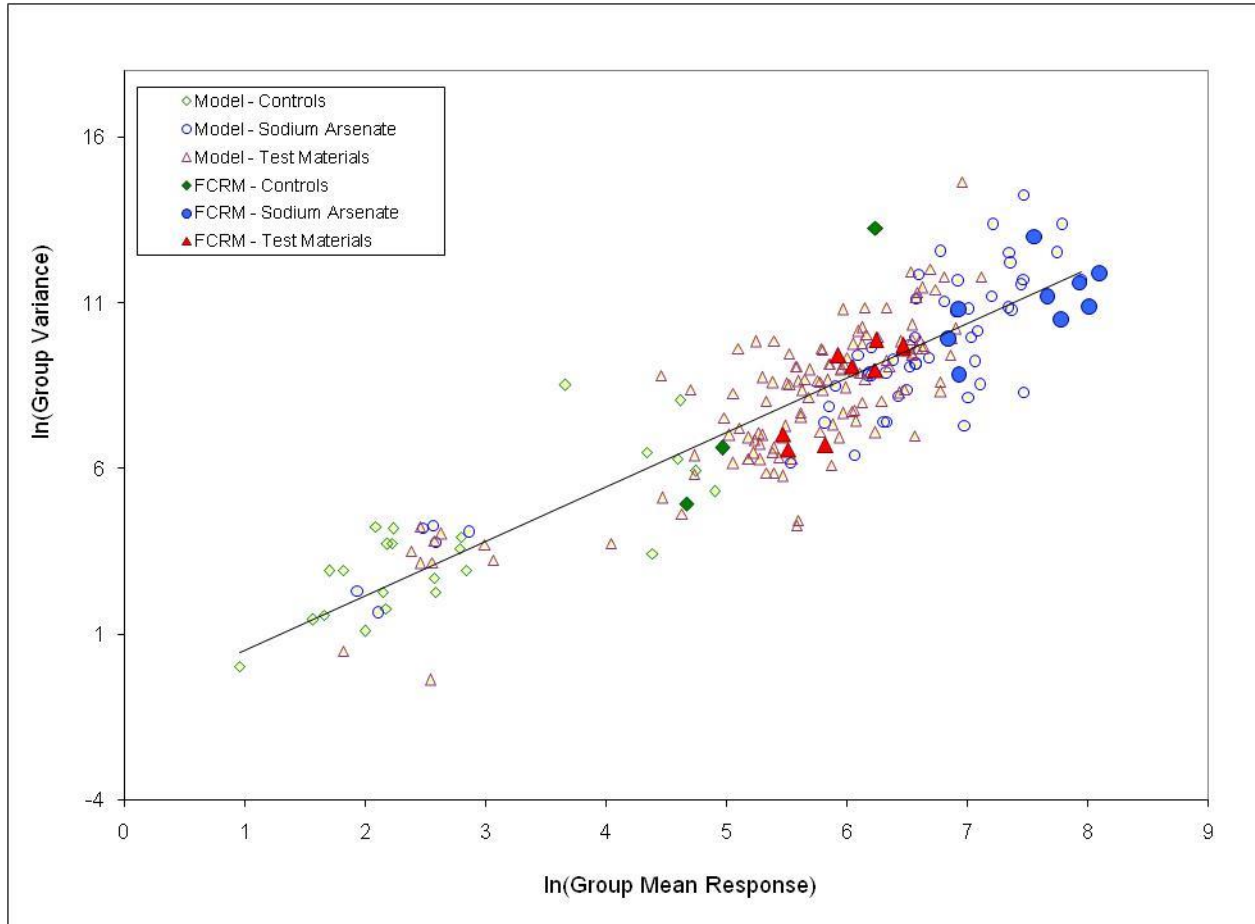
Table 4-3. Urine Excretion Fraction (UEF) Estimates

Urine Collection Period (Days)	Outliers Excluded	Slopes (UEF Estimates)	
		b_r	b_t
Days 6/7	0	0.77	0.13
Days 9/10	1	0.70	0.04
Days 12/13	0	0.74	0.13
All days	0	0.74	0.12

b_r = slope for reference material (sodium arsenate) dose-response; b_t = slope for test material 1 (FCRM) dose-response

Figure 4-2. FCRM Urinary Excretion of Arsenic: Days 6/7

Reference Material (Sodium Arsenate)

Test Material (FCRM)
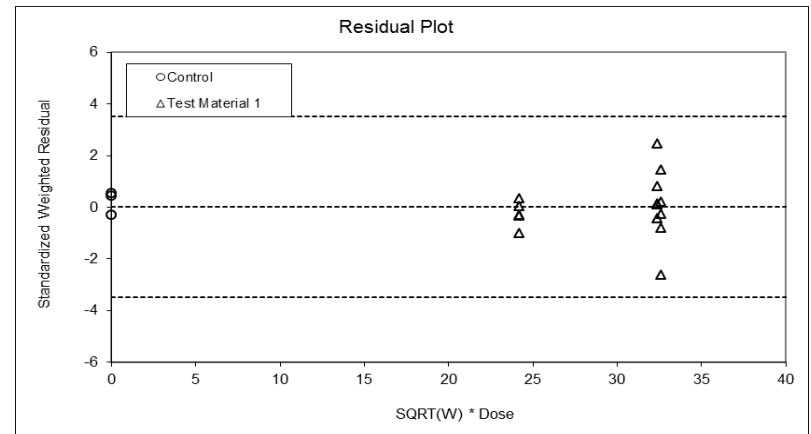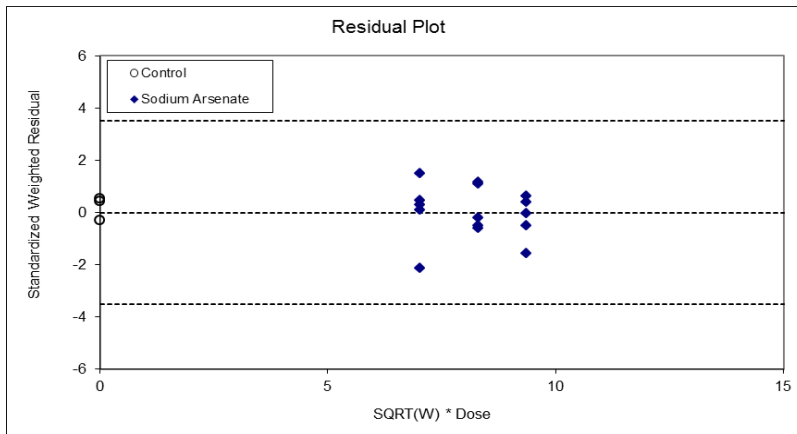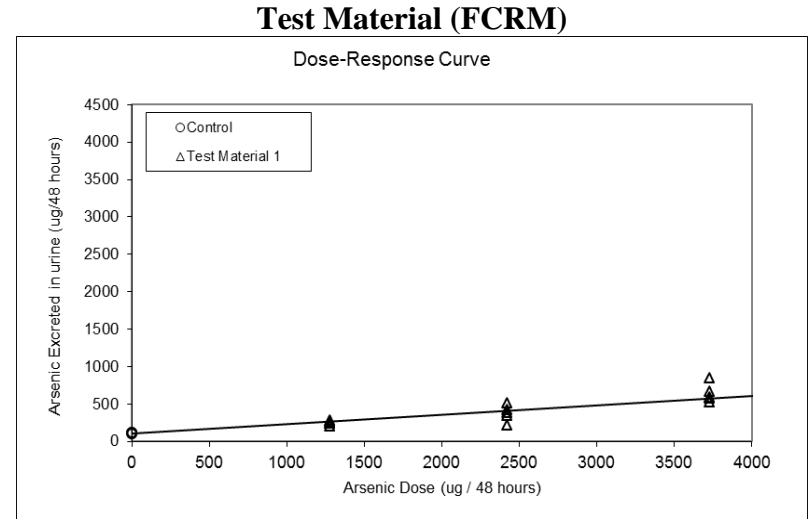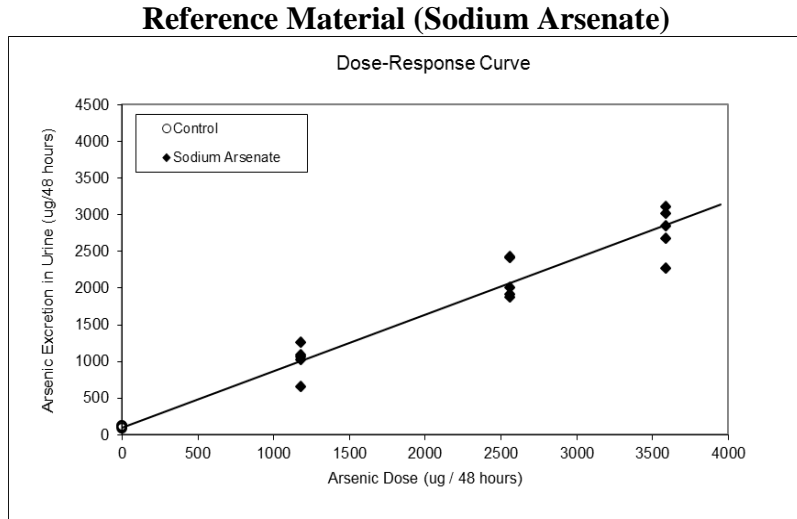
Summary of Fitting[a]

Parameter	Estimate	Standard Error
a	100.5	14.8
b_r	0.77	0.03
b_{t1}	0.13	0.01
Covariance (b_r,b_t)	0.1197	–
Degrees of freedom	31	–

[a] $y = a + b_r \cdot x_r + b_t \cdot x_t$

where r = Reference Material, t = Test Material

ANOVA

Source	SSE	DF	MSE
Fit	662.31	2	331.16
Error	31.91	30	1.06
Total	694.23	32	21.69

ANOVA = analysis of variance;

DF = degrees of freedom;

MSE = mean squared error;

SSE = sum of squared standard error

Statistic	Estimate
F	311.291
P	<0.001
Adjusted R^2	0.9510

RBA and Uncertainty

	Test Material
RBA	0.16
Lower bound[b]	0.14
Upper bound[b]	0.19
Standard error[b]	0.015

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

Figure 4-3. FCRM Urinary Excretion of Arsenic: Days 9/10

Reference Material (Sodium Arsenate)
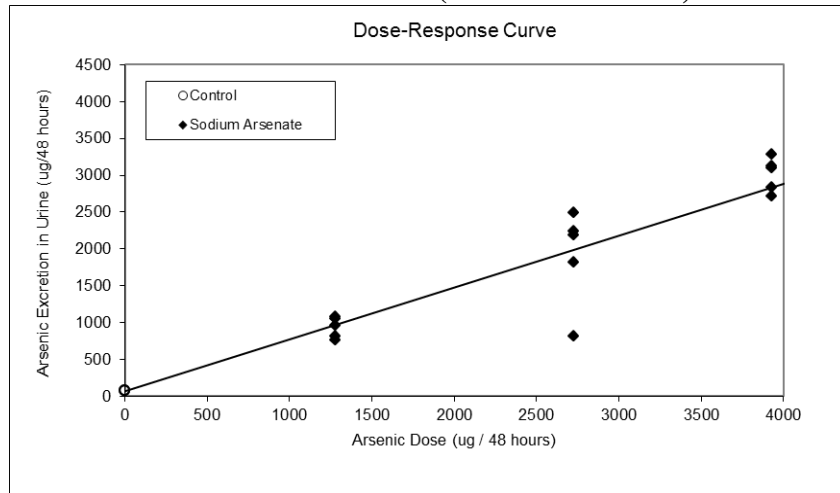
Test Material (FCRM)
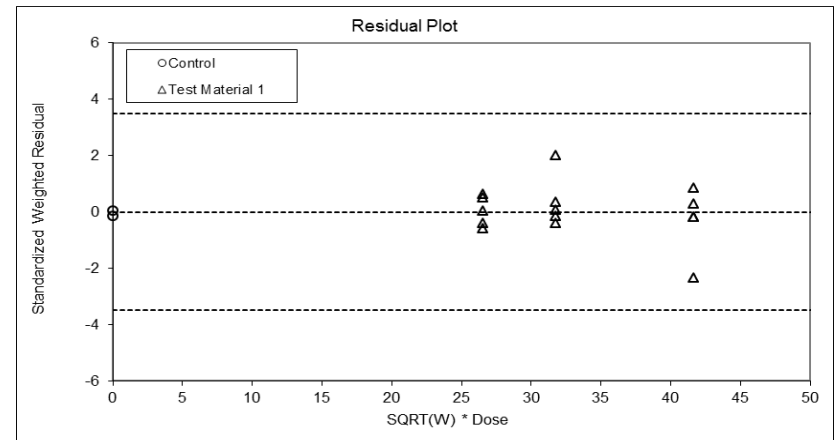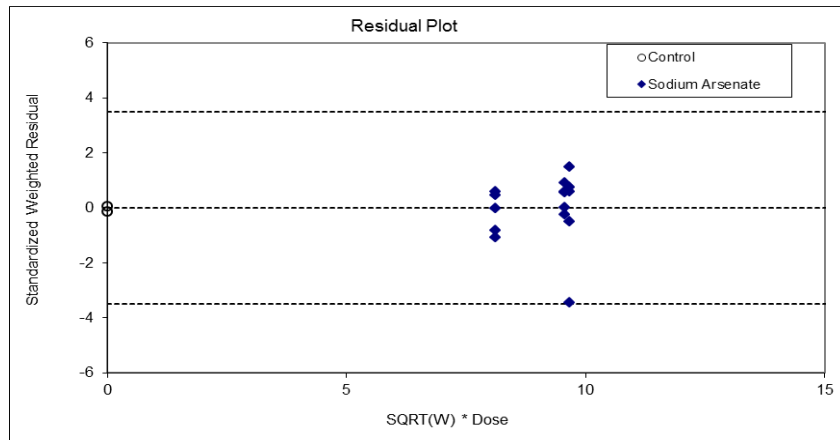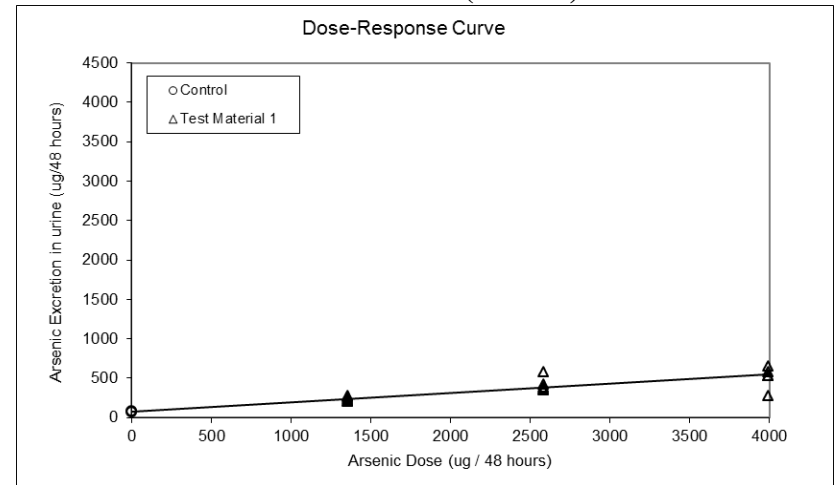
Summary of Fitting[a]

Parameter	Estimate	Standard Error
a	74.6	16.3
b_r	0.70	0.04
b_{t1}	0.12	0.01
Covariance (b_r,b_t)	0.1090	–
Degrees of freedom	30	–

[a]$y = a + b_r*x_r + b_t*x_t$
where r = Reference Material, t = Test Material

ANOVA

Source	SSE	DF	MSE
Fit	699.09	2	349.55
Error	45.51	29	1.57
Total	744.60	31	24.02

ANOVA = analysis of variance;
DF = degrees of freedom;
MSE = mean squared error;
SSE = sum of squared
standard error

	Statistic	Estimate
	F	222.734
	P	<0.001
	Adjusted R^2	0.937

RBA and Uncertainty

	Test Material
RBA	0.17
Lower bound[b]	0.14
Upper bound[b]	0.20
Standard error[b]	0.018

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

Figure 4-4. FCRM Urinary Excretion of Arsenic: Days 12/13

Reference Material (Sodium Arsenate)
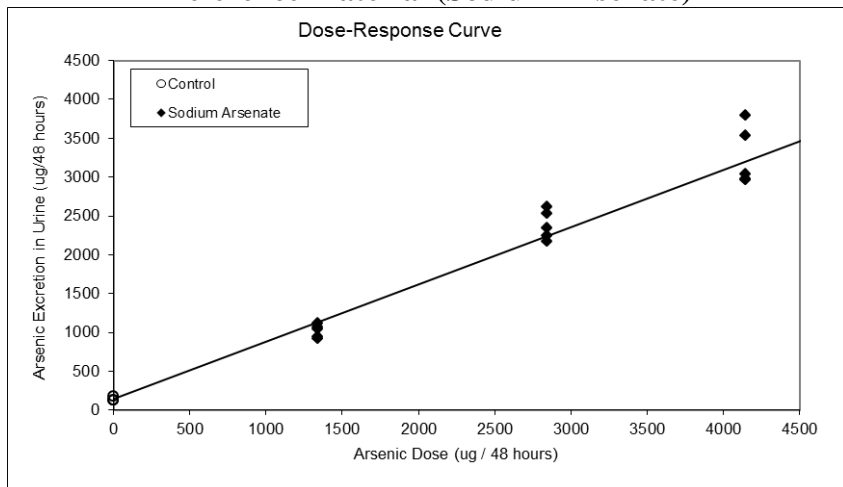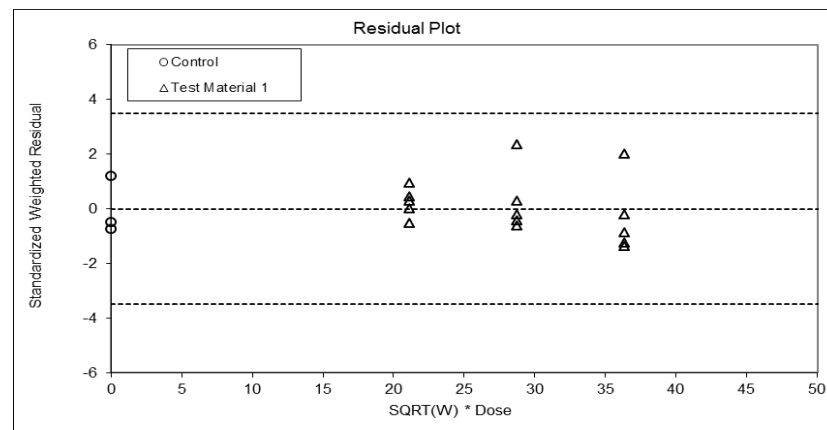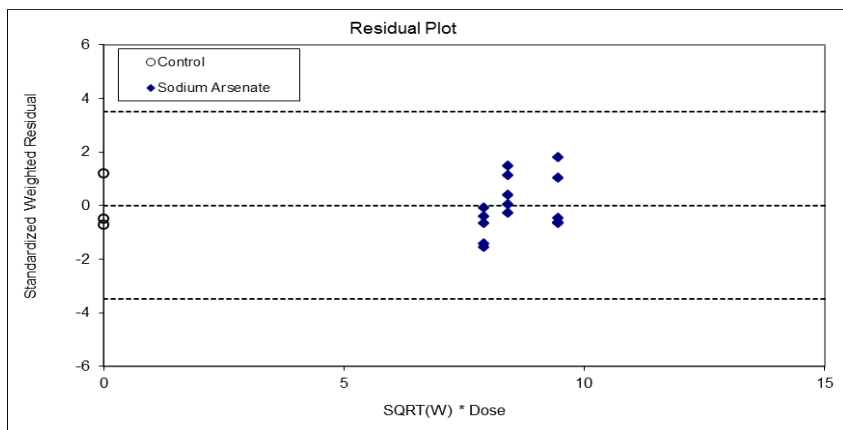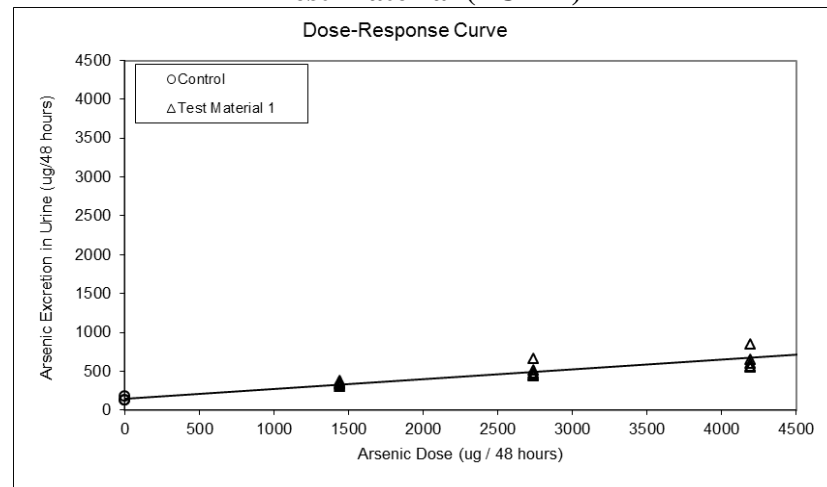
Test Material (FCRM)

Summary of Fitting[a]

Parameter	Estimate	Standard Error
a	143.3	14.4
b_r	0.74	0.02
b_t	0.13	0.01
Covariance (b_r,b_t)	0.1459	–
Degrees of freedom	31	–

[a]$y = a + b_r*x_r + b_t*x_t$

where r = Reference Material, t = Test Material

ANOVA

Source	SSE	DF	MSE
Fit	633.96	2	316.98
Error	19.03	30	0.63
Total	625.99	32	20.41

ANOVA = analysis of variance;
DF = degrees of freedom;
MSE = mean squared error;
SSE = sum of squared standard error

	Statistic	Estimate
	F	499.679
	P	<0.001
	Adjusted R^2	0.9689

RBA and Uncertainty

|	Test Material
RBA	0.17
Lower bound[b]	0.15
Upper bound[b]	0.19
Standard error[b]	0.012

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

Figure 4-5. FCRM Urinary Excretion of Arsenic: All Days

Reference Material (Sodium Arsenate)
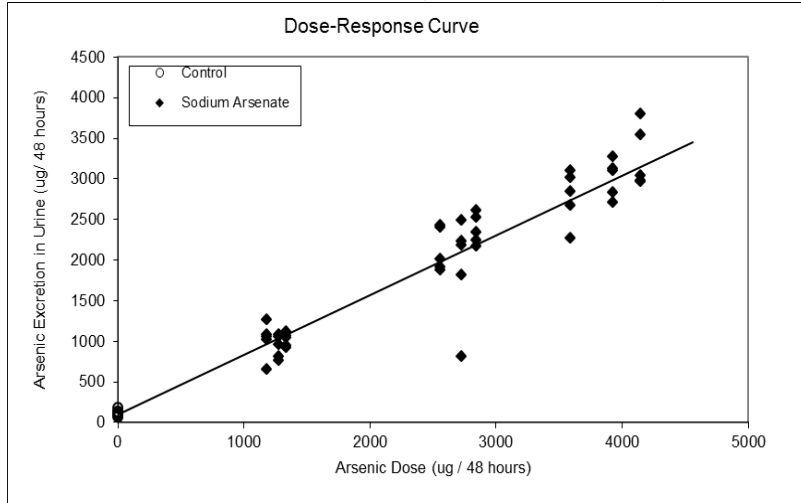
Test Material (FCRM)
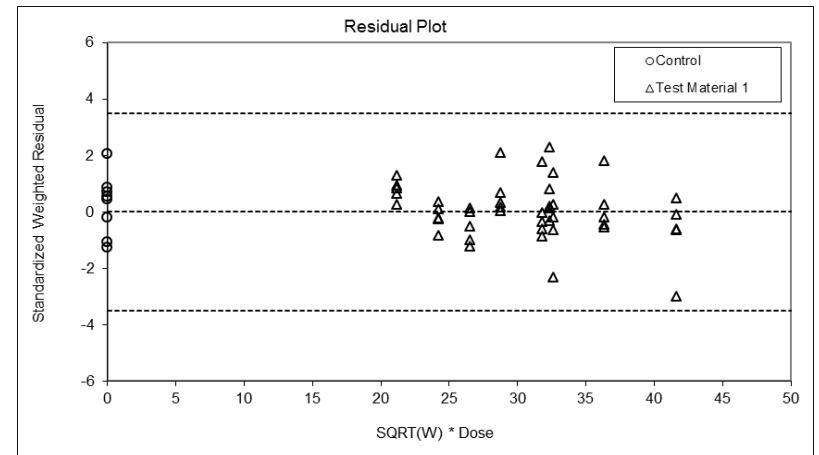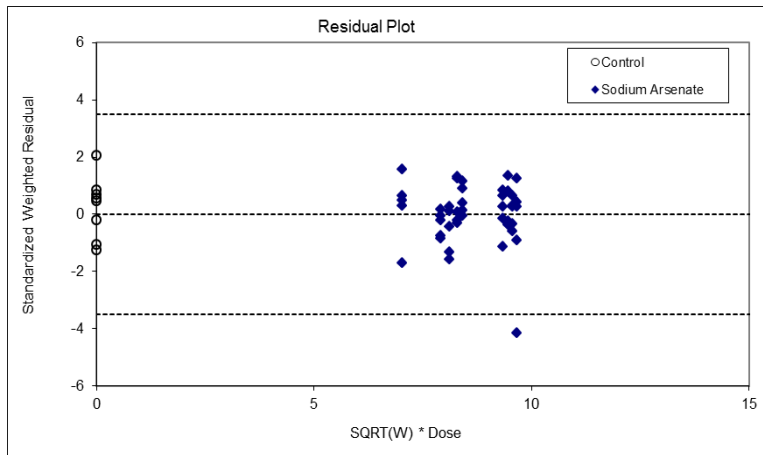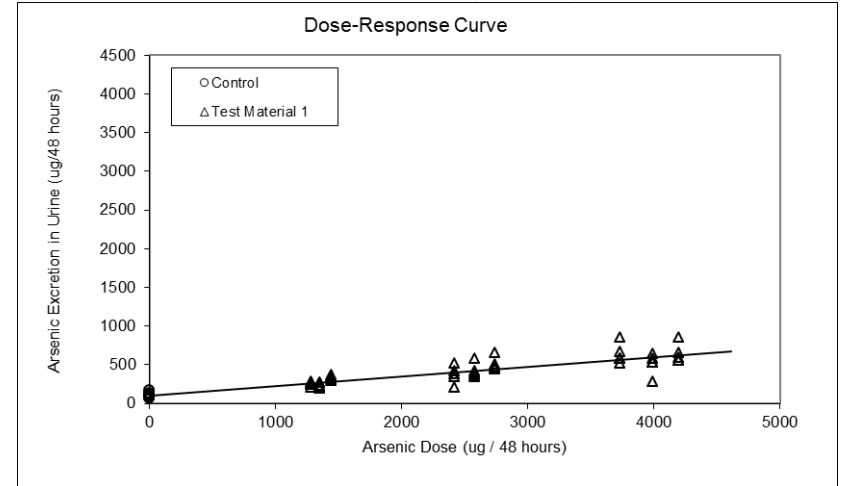
Summary of Fitting[a]

Parameter	Estimate	Standard Error
a	98.4	9.5
b_r	0.74	0.02
b_t	0.12	0.01
Covariance (b_r,b_t)	0.1208	–
Degrees of freedom	96	–

[a]$y = a + b_r*x_r + b_t*x_t$

where r = Reference Material, t = Test Material

ANOVA

Source	SSE	DF	MSE
Fit	2022.20	2	1011.10
Error	118.20	95	1.24
Total	2140.40	97	22.07

ANOVA = analysis of variance;
DF = degrees of freedom;
MSE = mean squared error;
SSE = sum of squared standard error

Statistic	Estimate
F	812.643
P	<0.001
Adjusted R^2	0.9436

RBA and Uncertainty

	Test Material
RBA	0.17
Lower bound[b]	0.15
Upper bound[b]	0.19
Standard error[b]	0.009

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

4.6   Calculated RBA Values

Estimated RBA values (mean and 90% confidence interval) are shown in Table 4-4. As shown, the best fit point estimate RBA of arsenic in FCRM is 17%.

**Table 4-4. Estimated Arsenic Relative Bioavailability (RBA)
for FCRM**

Urine Collection Period (days)	Estimated RBA (90% Confidence Interval)
Days 6/7	0.16 (0.14–0.19)
Days 9/10	0.17 (0.14–0.20)
Days 12/13	0.17 (0.15–0.19)
All Days	**0.17 (0.15–0.19)**

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

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Appendix A: Group Assignments

Table A-1. Group Assignments for FCRM Arsenic Study

Swine Number	Group	Treatment	Target Arsenic Dose (µg/kg-day)
914	4	FCRM	40
948			
929			
952			
905			
906	5	FCRM	80
949			
942			
907			
946			
904	6	FCRM	120
917			
934			
939			
924			
903	7	Sodium arsenate	40
927			
945			
909			
935			
908	8	Sodium arsenate	80
910			
902			
912			
922			
944	9	Sodium arsenate	120
919			
928			
943			
951			
911	10	Control	0
940			
916			

Appendix B: Body Weights

Table B-1. Body Weights

Group Info	Animal Ear Tag	Day -5 4/4/12	Group MBW	Day -1 4/8/12	Group MBW	Day 2 4/11/12	Group MBW	Day 5 4/14/12	Group MBW	Day 8 4/17/12	Group MBW	Day 11 4/20/12	Group MBW	Day 14 4/23/12	Group MBW
4 TM1 40 (As)	914	11.2		12.1		13.2		14		15		16		17	
	948	13.2		14		14.9		15.8		16.5		17.8		19	
	929	12.6		13.1		14		15.2		15.7		17		18	
	952	12.8		13.3		14.4		15.3		16.5		17.4		18.4	
	905	12.1	12.38	12.7	13.04	13.7	14.04	14.6	14.98	15.8	15.90	16.7	16.98	17.9	18.06
5 TM1 80 (As)	906	12.1		13.1		14		15		15.7		17.1		18	
	949	12.3		12.8		13.9		15		16		16.9		18.1	
	942	12		12.6		13.8		14.5		15.3		16.3		18.3	
	907	12.2		14.2		14.8		16		17		17.5		17.8	
	946	10.5	11.82	10	12.54	9.6	13.22	10.2	14.14	11.7	15.14	12.8	16.12	14.3	17.30
6 TM1 120 (As)	904	12.5		13.3		14.7		15.3		16.2		17		18.3	
	917	13.9		14.1		15.2		15.8		16.8		18		19.2	
	934	12.1		12.7		12.6		13.3		14.9		15.4		16	
	939	11.2		11.8		12.7		13.4		14.5		15.4		16.6	
	924	12.2	12.38	13	12.98	13.9	13.82	14.9	14.54	15.7	15.62	16.6	16.48	18	17.62
7 NaAs 40	903	12.2		13.1		13		13.5		14.2		14.8		16.1	
	927	10.2		11.3		11.8		12.5		13.6		17.3		15.5	
	945	13.1		13.7		14.7		15.3		16.8		17.2		18.5	
	909	12.5		13.2		14		14.8		16.3		14.5		18.3	
	935	10.1	11.62	11.1	12.48	11.8	13.06	12.8	13.78	14	14.98	14.8	15.72	16	16.88
8 NaAs 80	908	12.3		13.2		14		14.8		15.9		16.3		17.5	
	910	12.7		13.1		14.2		15.1		15.9		16.8		18	
	902	11		12.2		13		14		15.3		16.2		17.1	
	912	13.4		14.5		14.8		15.6		16.6		17.4		18.2	
	922	13.1	12.50	13.9	13.38	14.7	14.14	15.5	15.00	16.5	16.04	17.2	16.78	18.4	17.84
9 NaAs 120	944	12.5		12.8		13.6		14.1		15		16.2		17.7	
	919	13.4		14.4		14.9		15.3		16.6		18		18.7	
	928	10.7		12.1		12.8		13.6		14.6		15.5		16.4	
	943	11.9		12.5		13.4		13.3		14.8		16.1		18	
	951	10.9	11.88	11.7	12.70	12.4	13.42	13.5	13.96	15.8	15.36	15.5	16.26	18.2	17.80
10 Control 0	911	11.9		12.7		13.6		14		15.1		15.8		16.6	
	940	10.5		11.2		12.8		12		13.1		14.3		15	
	916	12.1	11.50	12.6	12.17	13.5	13.30	14.5	13.50	15.4	14.53	16.6	15.57	17.4	16.33

Group MBW = Mean body weight of each group.

Appendix C: Typical Feed Composition

Table C-1. Procine Grower Produced by the University of Missouri Feed Mill

Corn	1528 lbs
Bean Mill	350 lbs
Fat	50 lbs
Dicalcium phosphate	34 lbs
Limestone	18 lbs
Salt	6 lbs
Vitamins	4 lbs
Minerals	3 lbs
Zenepro	2 lbs
Biotin	2 lbs

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

**Table D-1. Urinary Arsenic Analytical Results and Urine Volumes for
FCRM Study Samples**

Group	Material	Collection Period (days)	Sample ID	Swine Number	Urinary Arsenic Concentration (µg/L)	Urine Volume (mL)
4	TM	6/7	USGS-573	914	7.38	33120
			USGS-618	948	65.7	4240
			USGS-627	929	57.5	4220
			USGS-594	952	146	1420
			USGS-608	905	153	1720
		9/10	USGS-646	914	7.21	29040
			USGS-667	948	60.2	3940
			USGS-642	929	51.1	5220
			USGS-669	952	125	1580
			USGS-666	905	171	1600
		12/13	USGS-719	914	15.6	19040
			USGS-732	948	88.7	3660
			USGS-721	929	52.3	6480
			USGS-729	952	189	1980
			USGS-695	905	123	2820
5	TM	6/7	USGS-605	906	219	1580
			USGS-592	949	224	1880
			USGS-596	942	221	2320
			USGS-619	907	36.6	10500
			USGS-607	946	113	1860
		9/10	USGS-660	906	226	1840
			USGS-658	949	171	2000
			USGS-653	942	54.2	7160
			USGS-638	907	88.8	4115
			USGS-652	946	689	840
		12/13	USGS-722	906	108	4220
			USGS-710	949	248	1780
			USGS-733	942	1100	600
			USGS-694	907	91.4	5560
			USGS-736	946	343	1380
6	TM	6/7	USGS-600	904	217	3920
			USGS-599	917	80.8	6440
			USGS-621	934	91.6	6380
			USGS-611	939	94.8	6115
			USGS-583	924	102	6500
		9/10	USGS-639	904	75.1	7000
			USGS-649	917	136	4280
			USGS-659	934	82.7	6380
			USGS-631	939	78.8	3500
			USGS-681	924	96.8	6660
		12/13	USGS-728	904	100	8500
			USGS-693	917	117	4700

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

Group	Material	Collection Period (days)	Sample ID	Swine Number	Urinary Arsenic Concentration (µg/L)	Urine Volume (mL)
			USGS-715	934	110	5920
			USGS-731	939	117	4800
			USGS-708	924	60.3	9860
7	Sodium arsenate	6/7	USGS-576	903	208	3140
			USGS-580	927	338	3140
			USGS-597	945	133	7700
			USGS-595	909	650	1940
			USGS-586	935	512	2125
		9/10	USGS-650	903	238	3220
			USGS-663	927	375	2820
			USGS-628	945	96.4	10000
			USGS-680	909	305	2660
			USGS-641	935	694	1560
		12/13	USGS-702	903	277	3340
			USGS-690	927	436	2560
			USGS-724	945	112	8420
			USGS-720	909	527	1980
			USGS-716	935	413	2600
8	Sodium arsenate	6/7	USGS-624	908	274	6860
			USGS-612	910	1150	2110
			USGS-623	902	1770	1360
			USGS-622	912	628	3200
			USGS-591	922	261	7320
		9/10	USGS-647	908	405	2000
			USGS-634	910	799	3120
			USGS-635	902	1930	1160
			USGS-630	912	696	3140
			USGS-668	922	240	7580
		12/13	USGS-697	908	371	5840
			USGS-712	910	972	2600
			USGS-704	902	834	3140
			USGS-711	912	623	3760
			USGS-707	922	234	9600
9	Sodium arsenate	6/7	USGS-606	944	782	3640
			USGS-581	919	427	6260
			USGS-572	928	985	2300
			USGS-616	943	697	4320
			USGS-582	951	1470	2110
		9/10	USGS-636	944	432	6560
			USGS-656	919	475	6540
			USGS-655	928	853	3180
			USGS-675	943	361	8660
			USGS-665	951	1690	1940
		12/13	USGS-700	944	419	7080
			USGS-709	919	372	8000
			USGS-730	928	1320	2300

**Table D-1. Urinary Arsenic Analytical Results and Urine Volumes for
FCRM Study Samples**

Group	Material	Collection Period (days)	Sample ID	Swine Number	Urinary Arsenic Concentration (µg/L)	Urine Volume (mL)
			USGS-738	943	1180	3000
			USGS-734	951	2040	1860
10	Control	6/7	USGS-604	911	31.7	3520
			USGS-617	940	33.6	3400
			USGS-609	916	36.7	2520
		9/10	USGS-651	911	18.5	4085
			USGS-676	940	21.3	3340
			USGS-657	916	419	3300
		12/13	USGS-713	911	27	4600
			USGS-698	940	33	3940
			USGS-723	916	132	1320

Appendix E: Analytical Results for Quality Control Samples

Table E-1. Blind Duplicate Samples

Blind Duplicate Sample ID	Sample Type	Swine Number	Collection Days	Original Sample Concentration (µg/L)	Duplicate Sample Concentration (µg/L)	RPD
USGS-574	Urine	942	6/7	221	165	29%
USGS-584	Urine	940	6/7	33.6	33.2	1.2%
USGS-789	Urine	934	6/7	91.6	91.3	0.3%
USGS-790	Urine	944	9/10	432	23.3	180%
USGS-791	Urine	911	9/10	18.5	15.2	20%
USGS-645	Urine	949	9/10	171	169	1.2%
USGS-699	Urine	912	12/13	623	648	3.9%
USGS-684	Urine	922	12/13	234	231	1.3%
USGS-792	Urine	929	12/13	52.3	54.4	3.9%

Table E-2. Laboratory Spikes

Spike Sample ID	Sample Type	Original Sample Concentration (µg/L)	Added Spike Concentration (µg/L)	Measured Sample Concentration (µg/L)	Recovery (%)[a]
P206030-MS1	Water	15.6	300	309	98%
P206030-MS2	Water	371	300	688	106%
P206030-MS3	Water	24	300	349	108%
P206031-MS1	Water	1.24	30	37.4	121%
P206029-MS1	Water	7.38	300	295	96%
P206029-MS2	Water	274	300	580	102%
P206029-MS3	Water	42.7	300	351	103%
P206029-MS4	Water	694	300	1040	117%
P206029-MS5	Water	447	300	779	111%

[a]Values reported by laboratory.

Table E-3. Laboratory Quality Control Standards

Sample ID	Associated Sample Type	Measured Concentration (µg/L)	Detection Limit (µg/L)	Analysis Date	True Concentration	Recovery (%)
P206029-BS1	Water	58.7	1	06/16/2012	60	98%
P206030-BS1	Water	59.7	1	06/16/2012	60	99%
P206031-BS1	Water	61.4	1	06/17/2012	60	102%

Table E-4. Arsenic Performance Evaluation Samples

| Sample ID	PE ID	PE Standard	PE Concentration (µg/L)	Sample Concentration (µg/L)	Adjusted Concentration (µg/L)	RPD
USGS-643	as3.100	Sodium arsenite	100	151	109.3	9%
USGS-687	as3.20	Sodium arsenite	20	60.6	18.9	6%
USGS-593	As3.400	Sodium arsenite	400	498	456.3	13%
USGS-620	as5.100	Sodium arsenate	100	144	102.3	2%
USGS-662	as5.20	Sodium arsenate	20	57.1	15.4	26%
USGS-735	as5.400	Sodium arsenate	400	493	451.3	12%
USGS-737	ctrl	Control urine	0	24	-17.7	-200%
USGS-625	ctrl	Control urine	0	34.9	-6.8	-200%
USGS-678	dma100	Disodium methylarsenate	100	139	97.3	3%
USGS-626	dma20	Disodium methylarsenate	20	44.1	2.4	158%
USGS-691	dma400	Disodium methylarsenate	400	455	413.3	3%
USGS-706	mma100	Dimethyl arsenic acid	100	149	107.3	7%
USGS-577	mma20	Dimethyl arsenic acid	20	42.7	0.98	181%
USGS-654	mma400	Dimethyl arsenic acid	400	447	405.3	1%

PE = performance evaluation. Sample concentration adjusted by subtracting mean of background arsenic (~41.7 µg/L) from sample concentration (excluding outlier for swine 916, days 9 and 10); RPD = relative percent difference

Table E-5. Blanks

Sample ID	Associated Sample Type	Measured Concentration	Detection Limit	Units
P206029-BLK1	Water	<1	1	µg/L
P206030-BLK1	Water	<1	1	µg/L

Figure E-1. Urinary Arsenic Blind Duplicates
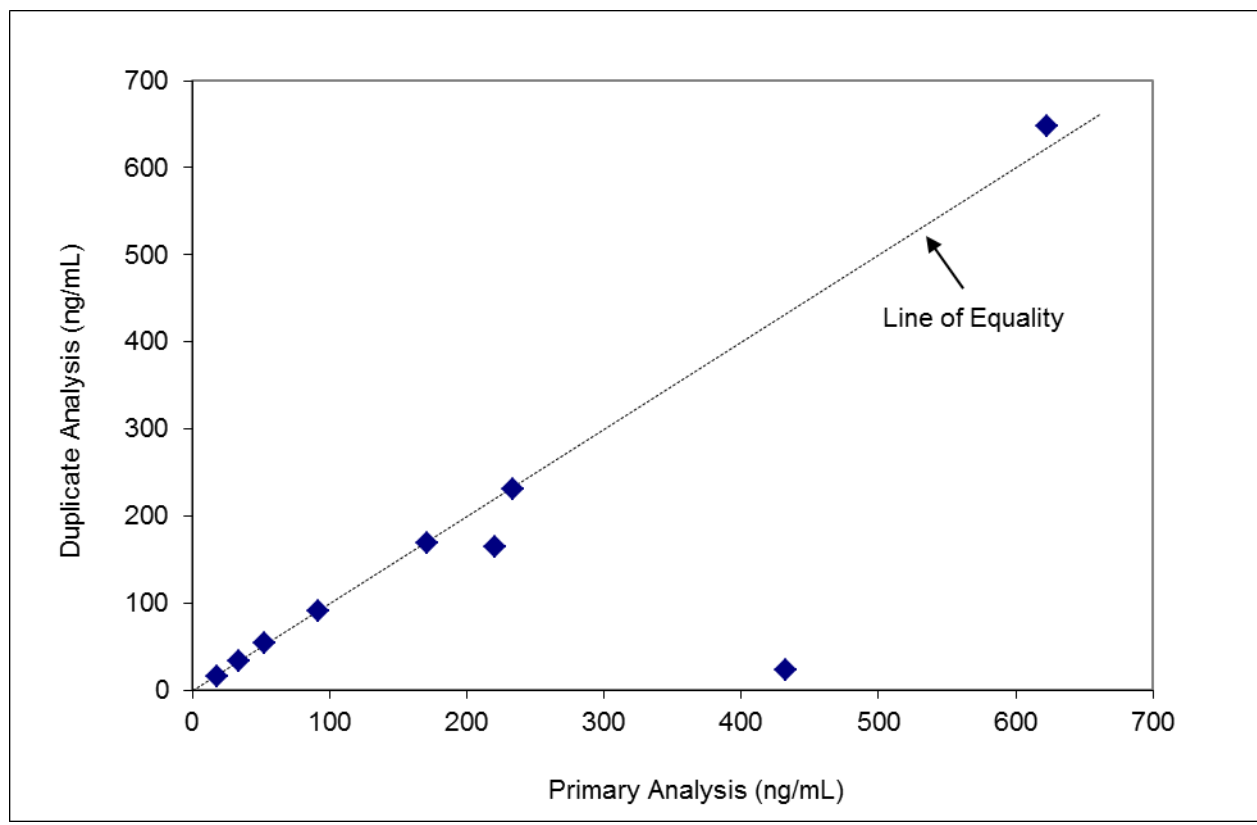
Figure E-2. Performance Evaluation Samples