RELATIVE BIOAVAILABILITY OF ARSENIC IN TWO SOILS FROM THE IRON KING MINE

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 two soil samples collected from the Iron King mine – Humboldt Smelter Superfund Site. The mine operated from 1906 until the 1960's and was active in gold, silver, copper, lead, and zinc mining. The Humboldt Smelter performed custom smelting for many mines in the area and was active from 1870 to 1937. The soil samples (HSJ583 and IKJ583) were collected from the Chaparral Gulch near a residential area (HSJ583) and a tailings pile (IKJ583). The arsenic concentrations (mean ± standard deviation) of the soil samples are 200.4 ± 5.3 (HSJ583, TM1) and 3957.2 ± 332.7 (IKJ583, TM2) mg/kg.

The relative oral bioavailability of arsenic was assessed by comparing the absorption of arsenic from the Iron King soils ("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 5, 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 each test material 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:

Collection Interval	Estimated RBA (90% Confidence Interval)	
	Test Material 1 (HSJ583)	Test Material 2 (IKJ583)
Days 5/6	0.57 (0.50–0.65)	0.18 (0.16–0.21)
Days 9/10	0.70 (0.59–0.82)	0.21 (0.18–0.25)
Days 12/13	0.57 (0.51–0.63)	0.17 (0.16–0.19)
All Days	0.60 (0.56–0.65)	0.19 (0.17–0.20)

The best fit point estimate RBAs for the Iron King soil samples are 60% and 19% for TM1 and TM2, respectively.

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ACRONYMS AND ABBREVIATIONS

ABA	Absolute bioavailability
AF_o	Oral absorption fraction
As^{+3}	Trivalent inorganic arsenic
As^{+5}	Pentavalent inorganic arsenic
DMA	Dimethyl arsenic
D	Ingested dose
g	Gram
GLP	Good Laboratory Practices
ICP MS	Inductively coupled plasma mass spectrometry
kg	Kilogram
K_u	Fraction of absorbed arsenic which is excreted in urine
mL	Milliliter
MMA	Monomethyl arsenic
N	Number of data points
NIST	National Institute of Standards and Technology
ORD NERL	Office of Research and Development National Exposure Research Laboratory
PE	Performance Evaluation
QC	Quality control
RBA	Relative bioavailability
ref	Reference material
RfD	Reference dose
RPD	Relative percent difference
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 some appropriate reference material (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 (e.g., arsenic) 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 a 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 amount of the same chemical absorbed from 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 in water and the chemical ingested in site media, assuming the toxicity factors are based on a readily soluble form of the chemical. For non-cancer effects, the default reference dose ($RfD_{default}$) can be adjusted ($RfD_{adjusted}$) as follows:

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

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

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

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

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

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

1.3 Purpose of this Study

The objective of this study was to use juvenile swine as a test system in order to determine the RBA of arsenic in two Iron King soil samples compared to a soluble form of arsenic (sodium arsenate).

2.0 STUDY DESIGN

The test materials and a reference material (sodium arsenate) 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).

2.1 Test Materials

2.1.1 Sample Description

The Iron King Mine – Humboldt Smelter Superfund Site is located near Humboldt Arizona. The site operated from 1906 to the 1960's and was active gold, silver, copper, lead, and zinc. The Humboldt Smelter performed custom smelting for many mines in the area and was active from

1870 to 1937. Arsenic and lead have been detected in site materials, including tailings deposits, at elevated concentrations. These materials are migrating off-site. Residential properties and the town of Humboldt are located immediately adjacent to the site and between the mine and smelter. Samples were collected from the Chaparral Gulch near a residential area (HSJ583) and a tailings pile (sample IKJ583). The arsenic concentrations (mean ± standard deviation) of the soil samples are 200.4 ± 5.3 (HSJ583, TM1) and 3957.2 ± 332.7 (IKJ583, TM2) mg/kg.

2.1.2 Sample Preparation and Analysis

USEPA Region 9 collected the soil from Iron King Mine – Humboldt Smelter Superfund Site. Soil was sieved to remove large chunks and rocks and shipped to the EPA Office of Research and Development National Exposure Research Laboratory (ORD NERL) where the soils were then sieved to <250 μm and homogenized using a vortex mixer. For arsenic analysis, sieved soil samples were digested following EPA Method 3051A (microwave digestion) and analyzed following EPA Method 6020 (inductively coupled plasma mass spectrometry [ICP MS]); four replicates of each sample were analyzed.

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 were weaned onto standard swine chow (made at the University of Missouri Animal Science Feed Mill). The feed was nutritionally complete (NRC 1988). The ingredients of the

feed are presented in Appendix C. Arsenic concentration in a randomly selected feed sample measured <0.1 µg/g.

Prior to the start of dosing and throughout the dosing period, each day every animal was given an 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 <0.6 µg/L.

2.4 Dosing

Animals were exposed to dosing materials (sodium arsenate or sieved test material) for 14 days, with the dose for each day being administered in two equal portions beginning at 9:00 AM and 3:00 PM (two hours before feeding). 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 5g) and the dough was pinched shut. This was then placed in the feeder at dosing time.

Target arsenic doses (expressed as µg of arsenic per kg of body weight per day) for animals in each group were determined prior to the study and are shown in the study design (see Table 2-1). Based on the target arsenic dose, a daily mass of arsenic administered (either as sodium arsenate or as sieved test material) to animals in each group is 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 is 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.

In planning for this study, the soil concentration for TM2 was reported incorrectly in the file used to calculate study doses. As a result, soil doses administered to swine in the TM2 groups were larger than needed, and actual doses were about 3-fold greater than the target dose (see Section 4.2 for further discussion).

After completion of the study, the true mean body weight of all swine combined was calculated using the actual body weights (measured every three days during the study), and the resulting true mean body weight was used to calculate the actual doses achieved. Any missed or late doses were recorded and the actual doses adjusted accordingly. Actual doses (µg arsenic per day) for each group are shown in Table 2-1.

2.5 Collection and Preservation of Urine Samples

Samples of urine were collected from each animal for 48-hour periods on days 5 to 6 (U-1), 9 to 10,(U-2), and 12 to 13 (U-3) of the study. Collection began at 8:00 AM and ended 48 hours later. The urine was collected in a plastic bucket placed beneath each cage, which was emptied into a plastic storage bottle. Aluminum screens were placed under the cages to minimize contamination with feces or spilled food. Due to the length of the collection period, collection containers were emptied periodically (typically twice daily) into a separate plastic bottles to ensure that there was no loss of sample due to overflow.

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

Analytical results for the urine samples are presented in 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 (i.e., two separate subsamples of urine were digested) and submitted to the laboratory in a blind fashion. Results are shown in Appendix E (see Table E-1 and Figure E-1). There was generally good agreement between results for the duplicate pairs.

Spike Recovery

During arsenic analysis, one feed sample and every tenth urine sample was spiked with known amounts of arsenic (sodium arsenate) and the recovery of the added arsenic was measured.

Results (see Table E-2) show that mean arsenic concentrations recovered from spiked samples were usually within 10% of actual arsenic concentrations.

Laboratory Duplicates

During arsenic analysis, every tenth sample was analyzed in duplicate. Duplicate results for urine samples (see Table E-3) typically agreed within 10% relative percent difference (RPD). The duplicate water and feed samples were below the detection limit.

Laboratory Control Standards

National Institute of Standards and Technology (NIST) Standard Reference Materials® (SRM), (2003) for which a certified concentration of specific analytes has been established, were tested periodically during sample analysis. Recovery of arsenic from these standards was generally good and within the acceptable range (see Table E-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 Table E-5 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

Blank samples were run along with each batch of samples (n=8). Blanks never yielded a measurable level of arsenic (all results <1 µg/L). Results are shown in Table E-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:

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

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

3.2 Dose-Response Model

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:

s_i^2 = 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 $k2$.

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 this study, responses that yielded standardized weighted residuals greater than 3.5 or less than -3.5 were considered to be potential outliers (Canavos, 1984). Such a data point was encountered in the data set for this study. Therefore, RBA values were calculated both for all the data (outliers included) and without the outlier, and the result with the outlier excluded was used as the preferred estimate.

3.3 Calculation of RBA Estimates

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

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

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

4.0 RESULTS

4.1 Clinical Signs

The doses of arsenic administered in this study are below a level that is expected to cause toxicological responses in swine. No clinical signs of arsenic-induced toxicity were noted in any of the animals used in the studies. Three swine received 1 cc Naxcel once per day on days 2, 3, and 4 (swines 606 and 609) or days 11, 12, and 13 (swine 636) during the study to treat a systemic bacterial infection (swine were found with fever ≥104°).

4.2 Dosing Deviations

Missed doses are summarized in Table 4-1. Most missed doses occurred on the first two days of dosing and were not specific to any particular group.

As noted in Section 2, the soil concentration for TM2 was reported incorrectly in the file used to calculate study doses (reported values were lower than actual). As a result, soil doses administered to swine in the TM2 groups were about 3-fold larger than targeted, and therefore the actual doses administered were greater than the target doses specified in the study design (see Table 2-1).

Although the administered arsenic doses for TM2 were higher than the target doses, this did not affect the study outcome because the dose-response pattern remained approximately linear. Since it is the ratio of administered arsenic to excreted arsenic between test and reference materials that is used to compute relative bioavailability, differences in administered doses between groups is accounted for in the calculations. Additionally, there were no observed signs of toxicity in any of the groups. Therefore, the higher doses administered in the TM2 group compared to target doses did not impact study performance or outcome.

4.3 Background Arsenic Excretion

Measured values for urinary arsenic excretion (mean and standard deviation) for control animals from days 5 to 13 are shown in Table 4-2. Mean urinary arsenic concentration (± standard deviation) was 49.8 ± 10.0 µg/L. The values shown are representative of levels in urine due to endogenous background levels in food and water and support the view that the animals were not exposed to any significant exogenous sources of arsenic throughout the study.

4.4 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 in Figure 4-1, the variances of the urinary arsenic data from this study are consistent with the data used to generate the variance model.

4.5 Dose-Response Modeling

The dose-response data for arsenic in urine were modeled using all of the data (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 4-3.

4.6 Calculated RBA Values

Estimated RBA values (mean and 90% confidence interval) are shown in Table 4-4. The best fit point estimate RBA for the Iron King soil samples is 60% and 19% for TM1 and TM2, respectively.

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 and absorption 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.

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TABLES & FIGURES

TABLE 2-1. Study Design and Dosing Information

Group	Group Name Abbreviation	Dose Material Administered	As Concentration of the Material (µg/g or µg/µL)	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	2	4	25	25	307
2	NaAs	Sodium arsenate	10	4	50	50	614
3	NaAs	Sodium arsenate	10	4	100	100	1228
4	TM1	Iron King TM1 HSJ583	200	4	40	40	492
5	TM1	Iron King TM1 HSJ584	200	4	60	60	736
6	TM1	Iron King TM1 HSJ585	200	4	120	120	1476
7	TM2	Iron King TM2 IKJ583	3957	4	40	116	1425
8	TM2	Iron King TM2 IKJ584	3957	4	60	175	2137
9	TM2	Iron King TM2 IKJ585	3957	4	120	349	4274
10	Control	None (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 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).

TABLE 4-1. Missed Dose Consumption

Study Day	Swine Number	Note
0	601	Day 0 – Swine 601 did not eat AM or PM dose. Daily dose adjusted to 0%.
	605	Day 0 – Swine 605 did not eat AM or PM dose. Daily dose adjusted to 0%.
	606	Day 0 – Swine 606 did not eat AM dose. Daily dose adjusted to 50%.
	609	Day 0 – Swine 609 did not eat AM dose. Daily dose adjusted to 50%.
	615	Day 0 – Swine 615 did not eat AM dose. Daily dose adjusted to 50%.
	628	Day 0 – Swine 628 did not eat AM dose. Daily dose adjusted to 50%.
	635	Day 0 – Swine 635 did not eat AM or PM dose. Daily dose adjusted to 0%.
	643	Day 0 – Swine 643 did not eat AM dose. Daily dose adjusted to 50%.
1	601	Day 1 – Swine 601 did not eat AM or PM dose. Daily dose adjusted to 0%.
	605	Day 1 – Swine 605 did not eat AM or PM dose. Daily dose adjusted to 0%.
	606	Day 1 – Swine 606 did not eat PM dose. Daily dose adjusted to 50%.
	609	Day 1 – Swine 609 did not eat AM or PM dose. Daily dose adjusted to 0%.
	635	Day 1 – Swine 635 did not eat AM dose. Daily dose adjusted to 50%.
10	636	Day 10 – Swine 636 did not eat AM dose and only 50% of PM dose. Daily dose adjusted to 25%.

TABLE 4-2. Background Urinary Arsenic

Swine Number	Urine Collection Period (days)	As Dose (µg per collection period)	As Concentration in Urine (µg/L)	Urine Volume (µL)	Total As Excreted (µg/48 hours)
608	5/6	0	51	880	44.88
612	5/6	0	46	800	36.8
640	5/6	0	43	1110	47.73
608	9/10	0	45	1710	76.95
612	9/10	0	52	1400	72.8
640	9/10	0	57	1310	74.67
608	12/13	0	43	1810	77.83
612	12/13	0	72	900	64.8
640	12/13	0	39	1360	53.04

TABLE 4-3. Urine Excretion Fraction (UEF) Estimates

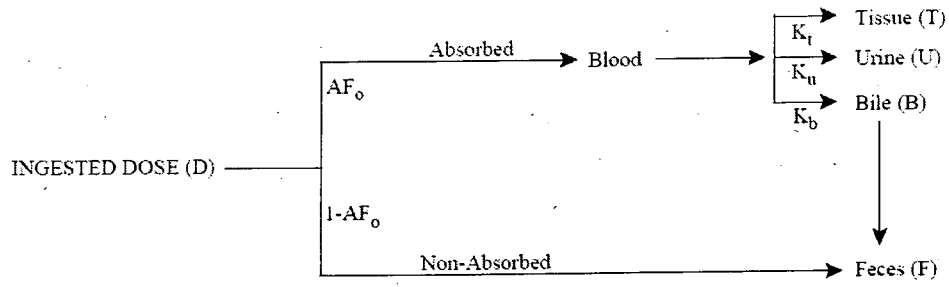
Urine Collection Period (days)	Outliers Excluded	Slopes (UEF Estimates)		
		b_r	b_{t1}	b_{t2}
Days 5/6	0	0.67	0.38	0.12
Days 9/10	0	0.64	0.45	0.14
Days 12/13	0	0.76	0.43	0.13
All Days	0	0.68	0.41	0.13

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

TABLE 4-4. Estimated RBA for Iron King Soils

Urine Collection Period (days)	Estimated RBA (90% Confidence Interval)	
	Test Material 1 (HSJ583)	Test Material 2 (IKJ583)
Days 5/6	0.57 (0.50 - 0.65)	0.18 (0.16 - 0.21)
Days 9/10	0.70 (0.59 - 0.82)	0.21 (0.18 - 0.25)
Days 12/13	0.57 (0.51 - 0.63)	0.17 (0.16 - 0.19)
All Days	0.60 (0.56 -0.65)	0.19 (0.17 -0.20)

FIGURE 3-1. Conceptual Model for Arsenic Toxicokinetics



where:

D = ingested dose (μg)
AF_o = oral absorption fraction
K_t = fraction of absorbed arsenic which is retained in tissues
K_u = fraction of absorbed arsenic which is excreted in urine
K_b = fraction of absorbed arsenic which is excreted in the bile

Basic equations:

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

Amount Excreted (μg) $=$ Amount absorbed $\times K_u = D \times AF_o \times K_u$

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

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

FIGURE 3-2. Urinary Arsenic Variance Model

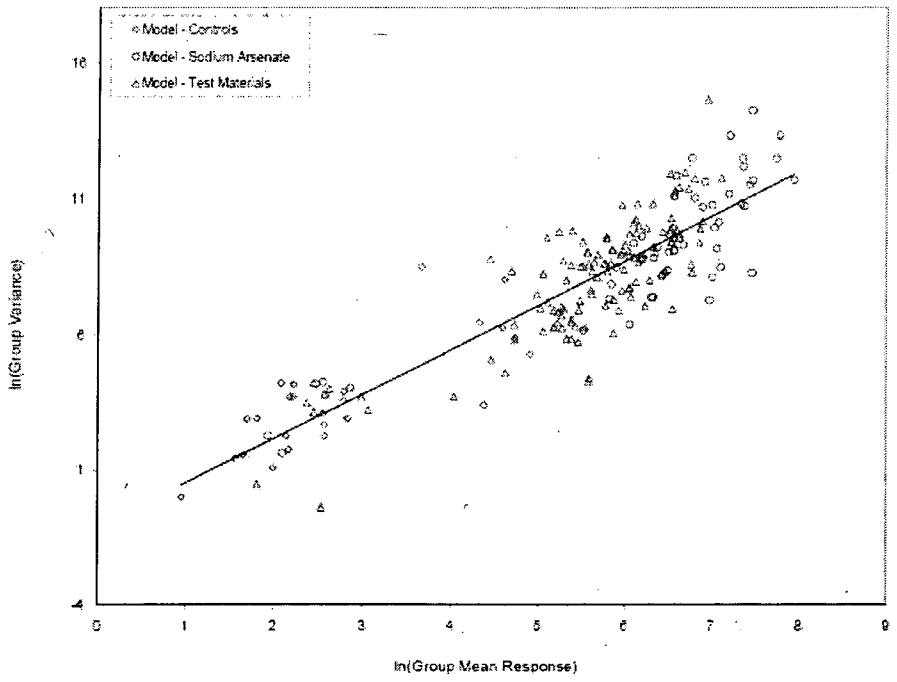


FIGURE 4-1. Iron King Data Compared to Urinary Arsenic Variance Model

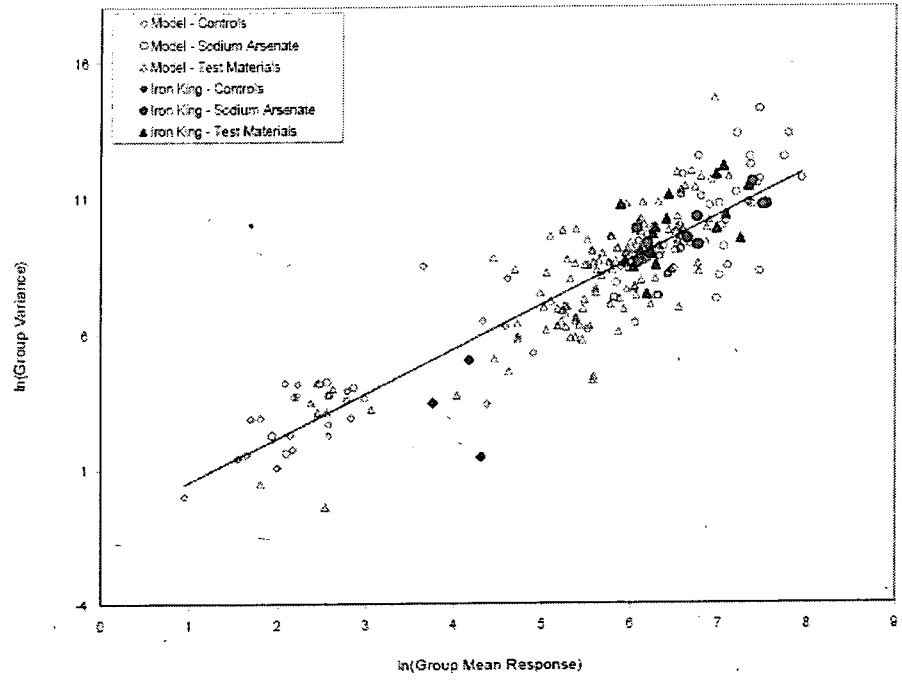
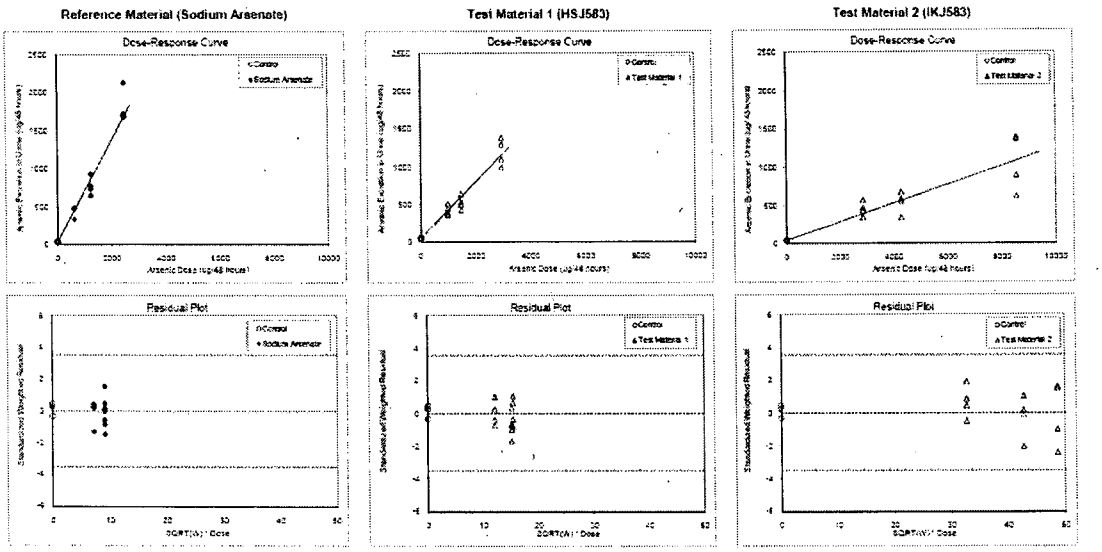


FIGURE 4-2. Iron King Urinary Excretion of Arsenic: Days 5/6



Summary of Fitting[a]

Parameter	Estimate	Standard Error
a	43.1	27.0
b_1	0.67	0.04
b_{r1}	0.38	0.03
b_{r2}	0.12	0.01
Covariance (b_1,b_{r1})	0.2870	--
Covariance (b_1,b_{r2})	0.2967	--
Degrees of Freedom	36	--

[a] $y = a + b_1 * x + b_{r1} * x_{r1} + b_{r2} * x_{r2}$

where r = Reference Material, t1 = Test Material 1, and t2 = Test Material 2

ANOVA

Source	SSE	DF	MSE
Fit	937.74	3	312.58
Error	46.75	35	1.31
Total	983.49	38	25.88

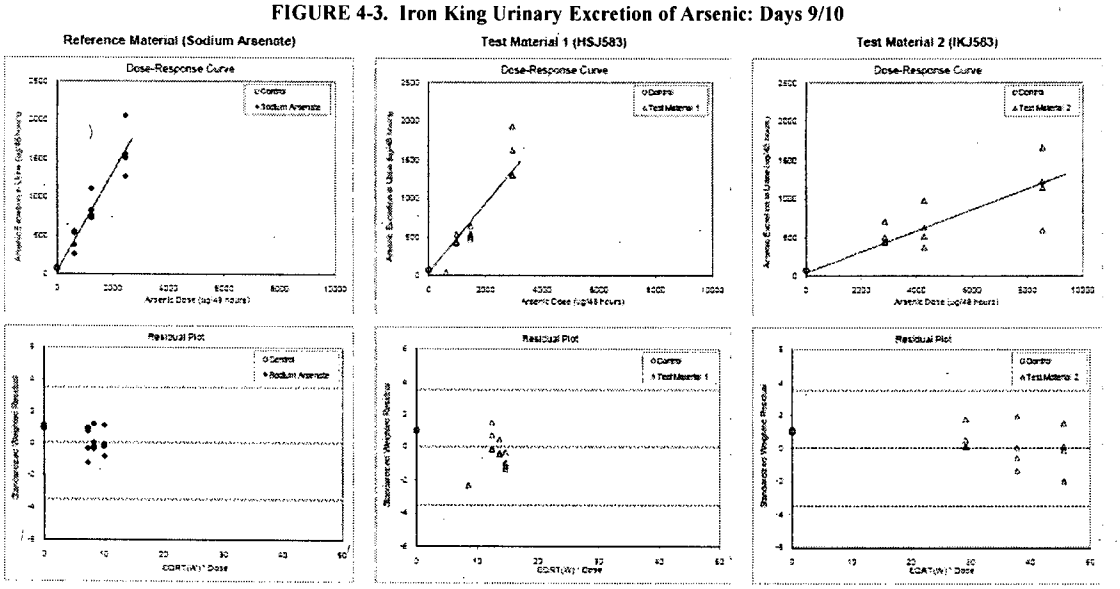
Statistic	Estimate
F	239.116
p	< 0.001
Adjusted R^2	0.9495

RBA and Uncertainty

	Test Material 1	Test Material 2
RBA	0.57	0.18
Lower bound[a]	0.50	0.16
Upper bound[a]	0.65	0.21
Standard Error[a]	0.043	0.015

[a] Calculated using Fieller's theorem

FIGURE 4-3. Iron King Urinary Excretion of Arsenic: Days 9/10



Summary of Fitting[a]

Parameter	Estimate	SE
a	42.3	37.7
b_1	0.64	0.05
b_{r1}	0.45	0.04
b_{r2}	0.16	0.01
Covariance (b_1,b_{r1})	0.3372	--
Covariance (b_1,b_{r2})	0.2837	--
Degrees of Freedom	36	--

[a] $y = a + b_1 * x + b_{r1} * x_{r1} + b_{r2} * x_{r2}$

where r = Reference Material, t1 = Test Material 1, and t2 = Test Material 2

ANOVA

Source	SSE	DF	MSE
Fit	866.64	3	288.88
Error	101.90	35	2.91
Total	968.54	38	25.49

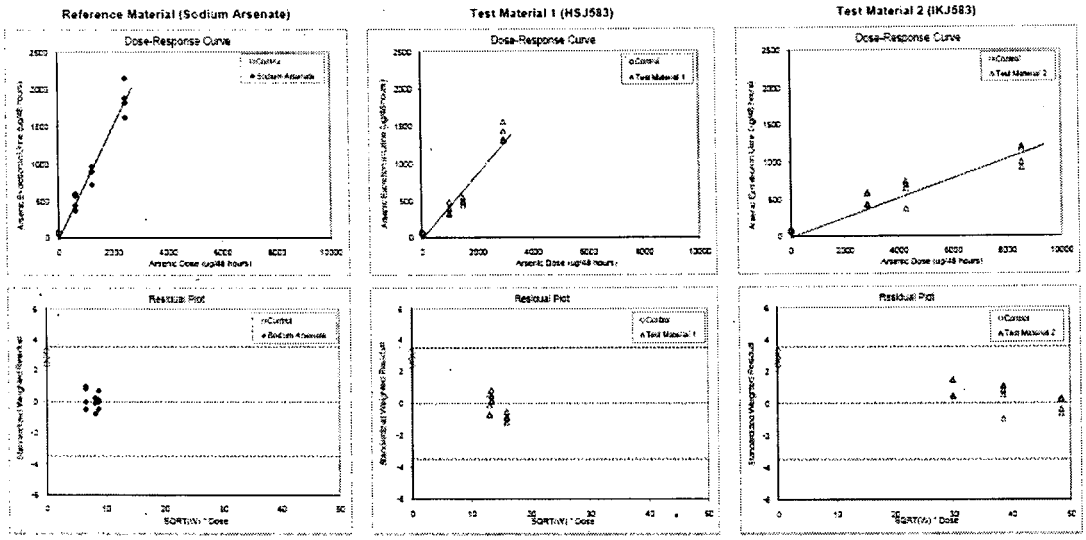
Statistic	Estimate
F	99.224
p	< 0.001
Adjusted R^2	0.8958

RBA and Uncertainty

	Test Material 1	Test Material 2
RBA	0.70	0.21
Lower bound[a]	0.59	0.18
Upper bound[a]	0.82	0.26
Standard Error[a]	0.066	0.022

[a] Calculated using Fieller's theorem

FIGURE 4-4. Iron King Urinary Excretion of Arsenic: Days 12/13



Summary of Fitting*

Parameter	Estimate	SE
a	-17.3	42.3
b_1	0.76	0.04
b_{t1}	0.43	0.03
b_{t2}	0.13	0.01
Covariance (b, b_{t1})	0.3835	--
Covariance (b, b_{t2})	0.4574	--
Degrees of Freedom	38	--

* $y = a + b_1 * x_i + b_{t1} * x_{t1} + b_{t2} * x_{t2}$

where r = Reference Material, t1 = Test Material 1, and t2 = Test Material 2

ANOVA

Source	SSE	DF	MSE
Fit	1044.08	3	348.03
Error	112.11	35	3.20
Total	1156.20	38	30.43

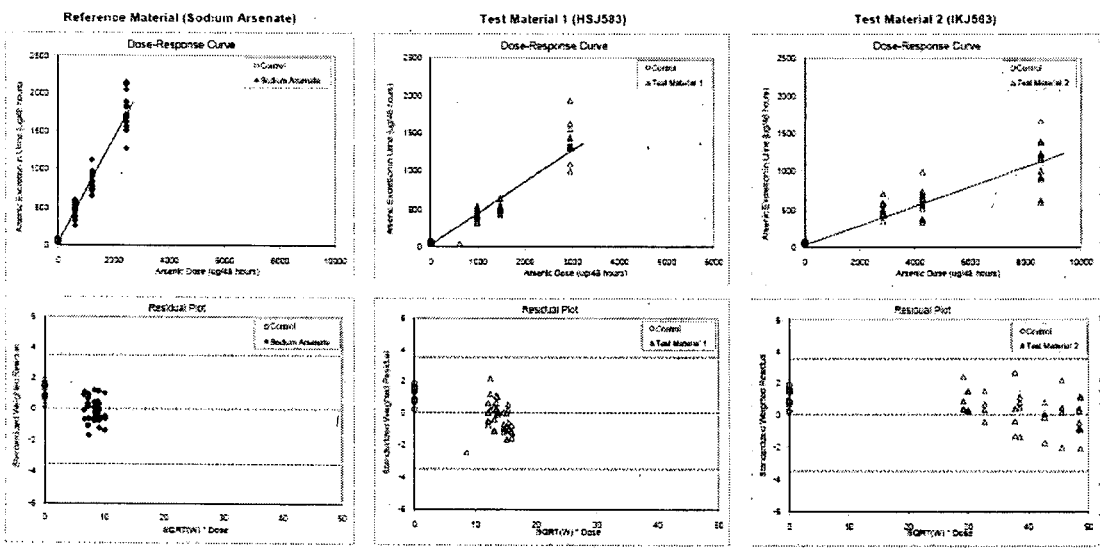
Statistic	Estimate
F	108.649
p	< 0.001
Adjusted R^2	0.9947

RBA and Uncertainty

	Test Material 1	Test Material 2
RBA	0.57	0.17
Lower bound*	0.51	0.16
Upper bound*	0.63	0.19
Standard Error*	0.036	0.012

* Calculated using Fieller's theorem

FIGURE 4-5. Iron King Urinary Excretion of Arsenic: All Days



Summary of Fitting*

Parameter	Estimate	SE
a	33.4	19.7
b_1	0.55	0.02
b_{t1}	0.41	0.02
b_{t2}	0.13	0.01
Covariance (b, b_{t1})	0.3459	--
Covariance (b, b_{t2})	0.3290	--
Degrees of Freedom	114	--

* $y = a + b_1 * x_i + b_{t1} * x_{t1} + b_{t2} * x_{t2}$

where r = Reference Material, t1 = Test Material 1, and t2 = Test Material 2

ANOVA

Source	SSE	DF	MSE
Fit	2790.43	3	930.14
Error	218.50	113	1.93
Total	3025.93	116	26.91

Statistic	Estimate
F	487.726
p	< 0.001
Adjusted R^2	0.9264

RBA and Uncertainty

	Test Material 1	Test Material 2
RBA	0.40	0.19
Lower bound*	0.36	0.17
Upper bound*	0.45	0.22
Standard Error*	0.022	0.009

* Calculated using Fieller's theorem

APPENDIX A: Group Assignments for the Iron King Arsenic RBA Study November 2009

Swine Number	Group	Treatment	Actual Arsenic Dose [a] µg/kg bw-day
604 613 615 638	1	NaAs	25
611 626 635 641	2	NaAs	50
603 605 628 631	3	NaAs	100
619 633 636 643	4	TM1	40
616 622 627 629	5	TM1	60
602 602 607 609 623	66	TM1	120
606 624 625 639	7	TM2	116
601 610 620 637	8	TM2	175
614 630 632 634	9	TM2	349
608 612 640	10	Control	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.

APPENDIX B: Body Weights

Group	Swine No.	Weight														
		Day -5 11/3/09	Group MBW	Day -1 11/8/09	Group MBW	Day 2 11/11/09	Group MBW	Day 5 11/14/09	Group MBW	Day 8 11/17/09	Group MBW	Day 11 11/20/09	Group MBW	Day 14 11/23/09	Group MBW
1 NaAs	604	8.9		9.2		9.6		10.2		10.5		11.8		12.5	
	613	8.2		8.1		8.4		8.9		9.5		10		10.3	
	615	7.8		8.8		9		9.3		10.2		11.1		11.7	
	638	9.2	8.53 ±0.64	9.1	8.80 ±0.50	9.7	9.18 ±0.60	10.3	9.68 ±0.68	11.1	10.33 ±0.67	11.8	11.18 ±0.83	12.3	11.70 ±0.99
2 NaAs	611	7.7		7.5		8.4		9		9.7		10.2		10.7	
	626	9		9.1		9.3		10.2		10.8		11.7		12.5	
	635	8.4		8.4		9.2		9.7		10.5		11.2		11.5	
	641	8.2	8.33 ±0.54	8.3	8.33 ±0.66	8.9	8.95 ±0.40	9.5	9.60 ±0.50	10.3	10.33 ±0.46	11	11.03 ±0.62	11.7	11.60 ±0.74
3 NaAs	603	8.5		8.5		9.1		9.6		10.4		11.3		11.9	
	605	8.5		8.5		8.8		9.5		10.2		11.2		11.8	
	628	9		9		9.6		10.2		11		11.4		12.4	
	631	9.1	8.78 ±0.32	9.6	8.90 ±0.52	10.5	9.50 ±0.74	11	10.08 ±0.69	11.7	10.83 ±0.68	12.8	11.68 ±0.75	13.3	12.35 ±0.69
4 TM1	619	9		9		9.8		10		10.8		11.6		12.1	
	633	7.9		8.3		9		9.6		10.2		10.8		11.1	
	636	9		9.1		9.6		9.9		11		10.3		11.2	
	643	8.2	8.53 ±0.56	8	8.60 ±0.54	8.8	9.30 ±0.48	9.4	9.73 ±0.28	10	10.50 ±0.48	10.9	10.90 ±0.54	11.4	11.45 ±0.45
5 TM1	616	8.8		8.8		9.2		10.1		10.6		11.4		12.1	
	622	8.9		9		9.3		9.9		10.5		10.9		11.3	
	627	7.6		7.9		8.7		9.1		9.9		10.4		10.8	
	629	8	8.33 ±0.63	7.9	8.40 ±0.58	8.4	8.90 ±0.42	8.9	9.50 ±0.59	9.5	10.13 ±0.52	10.1	10.70 ±0.57	10.7	11.23 ±0.64
6 TM1	602	8.4		8		8.9		9.4		10		11		11.8	
	607	8		8		8.7		9.1		9.7		10.7		11.6	
	609	7.9		7.7		7.8		8.8		9.4		10.1		10.7	
	623	9.3	8.40 ±0.64	9.1	8.20 ±0.62	9.8	8.80 ±0.82	10.5	9.45 ±0.74	11	10.03 ±0.69	12.1	10.98 ±0.84	12.6	11.68 ±0.78
7 TM2	606	9.2		9.2		9.6		10.3		11		11.9		12.6	
	624	8.4		8.1		8.5		9.1		9.8		10.7		10.9	
	625	8.8		8.7		8.5		9.4		10.4		11.5		11.8	
	639	9.5	8.98 ±0.48	9.3	8.83 ±0.55	9.8	9.10 ±0.70	10.1	9.73 ±0.57	10.3	10.38 ±0.49	10.8	11.23 ±0.57	11.5	11.70 ±0.71
8 TM2	601	8.3		8.4		8.5		9.3		10.1		10.9		11.4	
	610	8.6		8.1		8.9		9.3		9.8		11.1		11.7	
	620	9.4		8.7		8.7		8.9		9.7		10.3		10.9	
	637	8.3	8.65 ±0.52	8.1	8.33 ±0.29	8.3	8.60 ±0.26	8.8	9.08 ±0.26	9.5	9.78 ±0.23	10.5	10.70 ±0.37	10.7	11.18 ±0.46

Group	Swine No.	Weight																					
		Day -5 11/3/09	Group MBW	Day -1 11/8/09	Group MBW	Day 2 11/11/09	Group MBW	Day 5 11/14/09	Group MBW	Day 8 11/17/09	Group MBW	Day 11 11/20/09	Group MBW	Day 14 11/23/09	Group MBW
9 TM2	614	9.1		8.5		9.3		10		10.8		11.7		12.2	
	630	8.7		8.8		9.5		10		10.6		11.8		12.3	
	632	8.4		8.2		8.5		9.1		9.6		10.5		11.2	
	634	8.7	8.73 ±0.29	8.4	8.48 ±0.25	8.5	8.95 ±0.53	9.3	9.60 ±0.47	10.1	10.28 ±0.54	10.8	11.20 ±0.65	11.6	11.83 ±0.52
10 Control	608	9.4		9		9.7		10.3		11.1		11.9		12.6	
	612	7.7		7.8		8.3		9.1		9.6		10.4		11	
	640	8.4	8.55 ±0.70	8.7	8.48 ±0.51	9.2	8.93 ±0.64	9.9	9.65 ±0.55	10.6	10.60 ±0.50	11.6	11.18 ±0.69	12.1	11.83 ±0.68

Group MBW = means and standard deviations of each group's body weight.

APPENDIX C: 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			
Tryptophan, %	0.32	**Fiber (max), %**		**6.8**
Valine, %	1.16			
Alanine, %	0.95	**Carbohydrates, %**		**62.2**
Aspartic Acid, %	2.33			
Glutamic Acid, %	4.96	**Energy (kcal/g) [c]**		**3.62**
Glycine, %	0.79	*From:*	*kcal*	*%*
Proline, %	1.83	Protein	0.84	23.1
Serine, %	1.25	Fat (ether extract)	0.315	8.7
Taurine, %	0	Carbohydrates	2.487	68.3
Minerals		**Vitamins**		
Calcium, %	0.8	Vitamin A, IU/g		1.7
Phosphorus, %	0.72	Vitamin 0-3 (added), IU/g		0.2
Phosphorus (available), %	0.4	Vitamin E, IU/kg		11
Potassium, %	0.27	Vitamin K (as menadione), ppm		0.52
Magnesium, %	0.04	Thiamin Hydrochloride, ppm		1
Sodium, %	0.3	Ribonavin, ppm		3.1
Chlorine, %	0.31	Niacin, ppm		13
Fluorine, ppm	0	Pantothenic Acid, ppm		9
Iron, ppm	82	Folic Acid, ppm		0.3
Zinc, ppm	84	Pyridoxine, ppm		1.7
Manganese, ppm	3	Biotin, ppm		0.1
Copper, ppm	4.9	Vitamin B-12, mcg/kg		15
Cobalt, ppm	0.1	Choline Chloride, ppm		410
Iodine, ppm	0.15	Ascorbic Acid, ppm		0
Chromium, ppm	0			
Molybdenum, ppm	0.01			
Selenium, ppm	0.26			

[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 As Fed basis except where otherwise indicated.
[c] Energy (kcal/gm) - Sum of decimal fractions of protein, fat and carbohydrate x 4,9,4 kcal/gm respectively.

APPENDIX D: Urinary Volumes and Urinary Arsenic Analytical Results for Iron King Study Samples

Group	Material	Collection Period (days)	Sample ID	Swine Number	Urinary As concentration (µg/L)	Urine Volume (mL)
1	NaAs	5/6	IK-109	604	280	1180
1	NaAs	5/6	IK-146	613	380	1240
1	NaAs	5/6	IK-126	615	220	2240
1	NaAs	5/6	IK-102	638	140	3420
1	NaAs	9/10	IK-193	604	150	1740
1	NaAs	9/10	IK-149	613	150	2560
1	NaAs	9/10	IK-178	615	190	2960
1	NaAs	9/10	IK-148	638	150	3540
1	NaAs	12/13	IK-212	604	150	2470
1	NaAs	12/13	IK-206	613	210	2100
1	NaAs	12/13	IK-228	615	140	4240
1	NaAs	12/13	IK-204	638	82	6940
2	NaAs	5/6	IK-112	611	495	1490
2	NaAs	5/6	IK-147	626	330	2360
2	NaAs	5/6	IK-128	635	290	2240
2	NaAs	5/6	IK-116	641	240	3880
2	NaAs	9/10	IK-174	611	404	2055
2	NaAs	9/10	IK-160	626	220	3480
2	NaAs	9/10	IK-185	635	435	1680
2	NaAs	9/10	IK-166	641	230	4840
2	NaAs	12/13	IK-227	611	240	3720
2	NaAs	12/13	IK-235	626	150	6440
2	NaAs	12/13	IK-226	635	340	2125
2	NaAs	12/13	IK-224	641	180	4980
3	NaAs	5/6	IK-118	603	960	1750
3	NaAs	5/6	IK-139	605	575	3000
3	NaAs	5/6	IK-127	628	300	5660
3	NaAs	5/6	IK-103	631	1300	1640
3	NaAs	9/10	IK-151	603	990	1580
3	NaAs	9/10	IK-158	605	488	3100
3	NaAs	9/10	IK-190	628	170	7460
3	NaAs	9/10	IK-163	631	950	2160
3	NaAs	12/13	IK-239	603	700	2700
3	NaAs	12/13	IK-208	605	290	5600
3	NaAs	12/13	IK-236	628	230	7940
3	NaAs	12/13	IK-240	631	1100	1960
4	TM1	5/6	IK-108	619	57	8880
4	TM1	5/6	IK-105	633	400	1100
4	TM1	5/6	IK-124	636	730	480
4	TM1	5/6	IK-120	643	140	2720
4	TM1	9/10	IK-172	619	72	7440
4	TM1	9/10	IK-155	633	260	1640
4	TM1	9/10	IK-150	636	140	320

Group	Material	Collection Period (days)	Sample ID	Swine Number	Urinary As concentration (µg/L)	Urine Volume (mL)
4	TM1	9/10	IK-173	643	54	8110
4	TM1	12/13	IK-220	619	47	10260
4	TM1	12/13	IK-215	633	290	1100
4	TM1	12/13	IK-232	636	492	660
4	TM1	12/13	IK-229	643	96	4130
5	TM1	5/6	IK-142	616	84	7560
5	TM1	5/6	IK-143	622	150	3300
5	TM1	5/6	IK-123	627	89	4720
5	TM1	5/6	IK-122	629	90	5860
5	TM1	9/10	IK-183	616	160	4015
5	TM1	9/10	IK-167	622	180	3000
5	TM1	9/10	IK-177	627	110	4600
5	TM1	9/10	IK-176	629	96	4980
5	TM1	12/13	IK-195	616	110	3990
5	TM1	12/13	IK-197	622	120	4000
5	TM1	12/13	IK-209	627	70	7020
5	TM1	12/13	IK-203	629	87	6220
6	TM1	5/6	IK-137	602	77	16860
6	TM1	5/6	IK-125	607	960	1440
6	TM1	5/6	IK-144	609	2600	420
6	TM1	5/6	IK-101	623	566	1750
6	TM1	9/10	IK-159	602	160	8130
6	TM1	9/10	IK-188	607	461	2820
6	TM1	9/10	IK-168	609	3400	570
6	TM1	9/10	IK-189	623	720	2260
6	TM1	12/13	IK-221	602	130	11040
6	TM1	12/13	IK-222	607	370	3590
6	TM1	12/13	IK-237	609	3000	520
6	TM1	12/13	IK-200	623	423	3090
7	TM2	5/6	IK-121	606	94	6060
7	TM2	5/6	IK-113	624	446	970
7	TM2	5/6	IK-135	625	67	7050
7	TM2	5/6	IK-115	639	100	3440
7	TM2	9/10	IK-186	606	81	8740
7	TM2	9/10	IK-184	624	190	2660
7	TM2	9/10	IK-165	625	57	7740
7	TM2	9/10	IK-171	639	80	5490
7	TM2	12/13	IK-234	606	66	8800
7	TM2	12/13	IK-233	624	100	5870
7	TM2	12/13	IK-199	625	66	6560
7	TM2	12/13	IK-214	639	72	5880
8	TM2	5/6	IK-117	601	89	7610
8	TM2	5/6	IK-131	610	320	1060
8	TM2	5/6	IK-130	620	730	800
8	TM2	5/6	IK-119	637	210	2640
8	TM2	9/10	IK-157	601	100	6310
8	TM2	9/10	IK-191	610	180	2075
8	TM2	9/10	IK-152	620	543	950
8	TM2	9/10	IK-156	637	390	2520

Group	Material	Collection Period (days)	Sample ID	Swine Number	Urinary As concentration (µg/L)	Urine Volume (mL)
8	TM2	12/13	IK-213	601	110	5820
8	TM2	12/13	IK-207	610	250	1480
8	TM2	12/13	IK-196	620	840	880
8	TM2	12/13	IK-238	637	150	4610
9	TM2	5/6	IK-107	614	580	2400
9	TM2	5/6	IK-106	630	230	2700
9	TM2	5/6	IK-111	632	700	1960
9	TM2	5/6	IK-134	634	390	2300
9	TM2	9/10	IK-164	614	517	3220
9	TM2	9/10	IK-162	630	360	3190
9	TM2	9/10	IK-181	632	640	1920
9	TM2	9/10	IK-175	634	390	1530
9	TM2	12/13	IK-219	614	290	3440
9	TM2	12/13	IK-231	630	250	4840
9	TM2	12/13	IK-230	632	512	2300
9	TM2	12/13	IK-198	634	451	2040
10	Control	5/6	IK-129	608	51	880
10	Control	5/6	IK-104	612	46	800
10	Control	5/6	IK-138	640	43	1110
10	Control	9/10	IK-179	608	45	1710
10	Control	9/10	IK-192	612	52	1400
10	Control	9/10	IK-154	640	57	1310
10	Control	12/13	IK-217	608	43	1810
10	Control	12/13	IK-225	612	72	900
10	Control	12/13	IK-205	640	39	1360

APPENDIX E: Analytical Results for Quality Control Samples

TABLE E-1. Blind Duplicate Samples

Blind Duplicate Sample ID	Sample Type	Swine Number	Urine Collection Days	Original Sample Concentration (µg/L)	Duplicate Concentration (µg/L)	RPD
IK-114	Urine	611	6/7	495	506	2%
IK-133	Urine	609	6/7	2600	2500	4%
IK-136	Urine	601	6/7	89	85	5%
IK-161	Urine	612	9/10	52	51	2%
IK-170	Urine	625	9/10	57	58	2%
IK-187	Urine	613	9/10	150	160	6%
IK-201	Urine	614	12/13	290	280	4%
IK-210	Urine	643	12/13	96	100	4%
IK-211	Urine	602	12/13	130	130	0%

RPD = relative percent difference.

TABLE E-2. Laboratory Spikes

Spike Sample ID	Sample Type	Original Sample Concentration (ppb)	Added Spike Concentration (ppb)	Measured Sample concentration (ppb)	Recovered Spike (ppb)	Recovery
IK-110	Urine	140	200	320	180	90%
IK-120	Urine	140	200	330	190	95%
IK-130	Urine	730	200	880	150	75%
IK-140	Urine	52	200	240	188	94%
IK-150	Urine	140	200	330	190	95%
IK-160	Urine	220	200	413	193	97%
IK-170	Urine	58	200	250	192	96%
IK-180	Urine	436	200	700	264	132%
IK-190	Urine	170	200	360	190	95%
IK-200	Urine	423	200	700	277	139%
IK-210	Urine	100	200	300	200	100%
IK-220	Urine	4747	200	250	203	102%
IK-230	Urine	512	200	790	278	139%
IK-240	Urine	1100	200	1300	200	100%
IK-276	Feed	<0.25	55.9	56	55.7	100%
IK-277	Water	<0.05	9.9	11	11	110%

TABLE E-3. Laboratory Duplicates

Duplicate Sample ID	Sample Type	Original Sample Concentration (ppb)	Duplicate Concentration (ppb)	RPD	Absolute Difference
IK-105	Urine	400	400	0%	0
IK-115IK-115	Urine	100	100	0%	0
IK-125	Urine	960	1000	4%	40
IK-135	Urine	67	67	0%	0
IK-145	Urine	70	68	3%	2
IK-155	Urine	260	280	7%	20
IK-165	Urine	57	58	2%	1
IK-175	Urine	390	436	11%	46
IK-185	Urine	435	486	11%	51
IK-195	Urine	110	120	9%	10
IK-206	Urine	210	210	0%	0
IK-215	Urine	290	280	4%	10
IK-225	Urine	72	74	3%	2
IK-235	Urine	150	150	0%	0
IK-273	Feed	<0.25	<0.25	0%	0
IK-277	Water	<0.05	<0.05	0%	0

RPD = relative percent difference.

TABLE E-4. Laboratory Quality Control Standards

Sample ID	Measured Arsenic Concentration (ppb)	Detection Limit (ppb)	Reference Material ID	Certified Mean ± Standard Deviation	Recovery
QC 1	200	10	NIST 2670a-H	220 ± 10	91%
QC-2	210	10	NIST 2670a-H	220 ± 10	95%
QC-3	210	10	NIST 2670a-H	220 ± 10	95%
QC-4	230	10	NIST 2670a-H	220 ± 10	105%
QC-5	210	10	NIST 2670a-H	220 ± 10	95%
QC-6	220	10	NIST 2670a-H	220 ± 10	100%
QC-7	<5	5	NIST 2670a-L	3	83%
QC-8	57	1	NIST 1643e	58.98 ± 0.7	97%
QC-9	7.5	0.2	NIST 1566b	7.65 ± 0.65	98%

TABLE E-5. Performance Evaluation Samples

Sample ID	PE ID	PE Standard	PE Concentration	Sample Concentration	Adjusted Concentration	RPD
IK-140	ctrl	Control Urine	0	52	2	
IK-218	ctrl	Control Urine	0	39	0	0%
IK-141	mma20	Dimethyl arsenic acid	20	64	14	34%
IK-180	mma400	Dimethyl arsenic acid	400	436	386	4%
IK-216	mma100	Dimethyl arsenic acid	100	180	130	26%
IK-145	dma20	Disodium methylarsenate	20	70	20	1%
IK-169	dma100	Disodium methylarsenate	100	170	120	18%
IK-223	dma400	Disodium methylarsenate	400	462	412	3%
IK-110	as5.100	Sodium arsenate	100	140	90	10%
IK-182	as5.20	Sodium arsenate	20	64	14	34%
IK-202	as5.400	Sodium arsenate	400	408	358	11%
IK-132	as3.400	Sodium arsenite	400	414	364	9%
IK-153	as3.100	Sodium arsenite	100	130	80	22%
IK 194	as3.20	Sodium arsenite	20	60	10	65%

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

TABLE E-6. Blanks

Sample ID	Measured Arsenic Concentration (ppb)	Detection Limit (ppb)
Blank-1	<1	1
Blank-2	<1	1
Blank-3	<1	1
Blank-4	<1	1
Blank-5	<1	1
Blank-6	<1	1
Blank-7	<1	1
Blank-8	<0.5	0.5
Blank-9	<0.1	0.1

FIGURE E-1. Urinary Arsenic Blind Duplicates

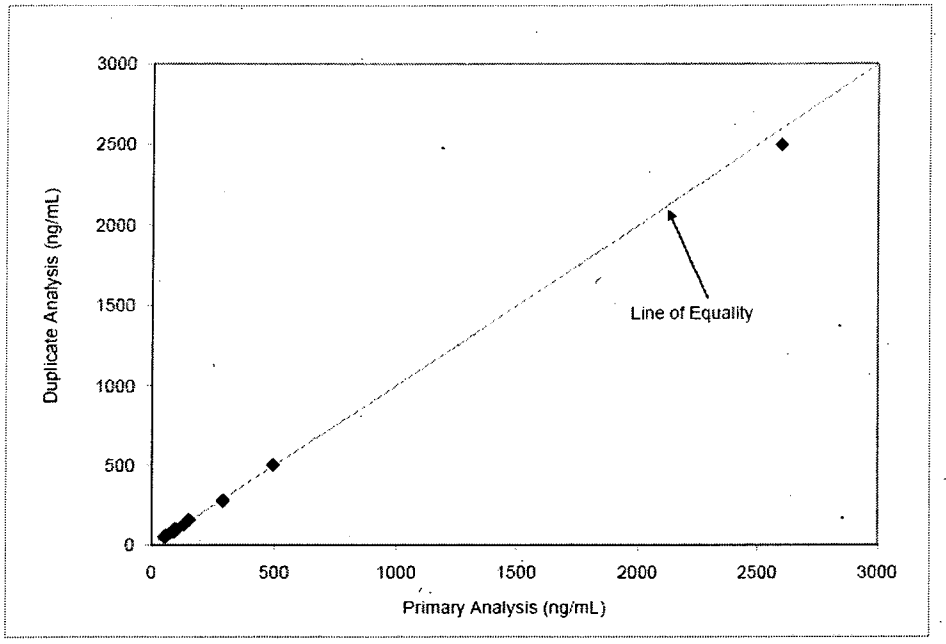


FIGURE E-2. Performance Evaluation Samples