

**RELATIVE BIOAVAILABILITY OF ARSENIC IN
NIST SRM 2710 (MONTANA SOIL)**

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EXECUTIVE SUMMARY

A study using juvenile swine as test animals was performed to measure the gastrointestinal absorption of arsenic from a sample of National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 2710. This is a soil from Montana that is contaminated by mine tailings deposits. The relative bioavailability of arsenic was assessed by comparing the absorption of arsenic from NIST SRM 2710 to that of sodium arsenate. Groups of four swine were given oral doses of sodium arsenate or the test soil twice a day for 14 days. A group of three non-treated swine served as a control.

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

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

The results are summarized below:

Time Interval	Estimated RBA (90% Confidence Interval)
Days 6/7	0.41 (0.36 - 0.47)
Days 9/10	0.42 (0.39 - 0.47)
Days 12/13	0.50 (0.40 - 0.62)
All Days	0.44 (0.40 - 0.48)

Arsenic in NIST SRM 2710 is absorbed about 44% as well as arsenic from sodium arsenate.

TABLE OF CONTENTS

1.0	INTRODUCTION	1
1.1	Overview of Bioavailability.....	1
1.2	Using Bioavailability Data to Improve Risk Calculations.....	2
1.3	Purpose of this Study	2
2.0	STUDY DESIGN.....	3
2.1	Test Materials.....	3
2.1.1	Sample Description.....	3
2.1.2	Sample Preparation	3
2.1.3	Arsenic Concentration	4
2.2	Experimental Animals	4
2.3	Diet.....	4
2.4	Dosing	5
2.5	Collection and Preservation of Urine Samples	5
2.6	Arsenic Analysis	6
2.7	Quality Assurance.....	6
3.0	DATA ANALYSIS.....	9
3.1	Overview	9
3.2	Dose-Response Model	10
3.3	Calculation of RBA Estimates	11
4.0	RESULTS	13
4.1	Clinical Signs	13
4.2	Background Arsenic Excretion.....	13
4.3	Dose-Response Patterns.....	13
4.4	Calculated RBA Values	14
4.5	Uncertainty.....	15
5.0	REFERENCES	16

LIST OF TABLES

Table 2-1	Dosing Protocol
Table 2-2	Typical Feed Composition

LIST OF FIGURES

Figure 2-1	Body Weight Gain
Figure 2-2	Urinary Arsenic Blind Duplicates
Figure 2-3	Performance Evaluation Samples
Figure 3-1	Conceptual Model for Arsenic Toxicokinetics
Figure 4-1	Urinary Arsenic Variance
Figure 4-2	Urinary Excretion of Arsenic: Days 6/7 (All Data)
Figure 4-3	Urinary Excretion of Arsenic: Days 9/10 (All Data)
Figure 4-4	Urinary Excretion of Arsenic: Days 12/13 (All Data)
Figure 4-5	Urinary Excretion of Arsenic: All Days (All Data)
Figure 4-6	Urinary Excretion of Arsenic: Days 6/7 (Outlier Excluded)
Figure 4-7	Urinary Excretion of Arsenic: All Days (Outlier Excluded)

APPENDIX

Appendix A Detailed Results

Table A-1	Schedule
Table A-2	Certified Values
Table A-3	Group Assignments
Table A-4	Body Weights and Actual Administered Doses, by Day
Table A-5	Late Dose Consumption
Table A-6	Urine Volumes
Table A-7	Urinary Arsenic Analytical Results for Study Samples
Table A-8	Arsenic Analytical Results for Quality Control Samples

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
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
QA	Quality assurance
RBA	Relative bioavailability
ref	Reference material
RfD	Reference dose
SD	Standard deviation
SF	Slope factor
SRM	Standard reference material
test	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

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. Bioavailability is a measure of the amount of chemical that is absorbed by the body from an ingested medium. The amount of bioavailable chemical 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 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:

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

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*):

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

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 Bioavailability Data to Improve Risk Calculations

When reliable data are available on the bioavailability of a chemical in a site medium (e.g., soil), this information can be used to improve the accuracy of exposure and risk calculations at that site. For example, the basic equation for estimating the site-specific ABA of a test soil is as follows:

$$ABA_{soil} = ABA_{soluble} \cdot RBA_{soil}$$

where:

$$\begin{aligned} ABA_{soil} &= \text{Absolute bioavailability of the chemical in soil ingested by a human} \\ ABA_{soluble} &= \text{Absolute bioavailability of some dissolved or fully soluble form of the chemical in children} \\ RBA_{soil} &= \text{Relative bioavailability of the chemical in soil as measured in swine} \end{aligned}$$

Available bioavailability 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 a sample of National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 2710 compared to a soluble form of arsenic (sodium arsenate).

2.0 STUDY DESIGN

This investigation of arsenic RBA was performed according to the basic design presented in Table 2-1. The study investigated arsenic absorption from sodium arsenate (NaAs) and a test material (TM1). Each material was administered to groups of four animals at one or two different dose levels for 14 days (a detailed schedule is presented in Appendix A, Table A-1). Additionally, the study included a non-treated group of two animals to serve as a control for determining background arsenic levels. All doses were administered orally.

The study design was based on the standardized study protocol for measuring lead relative bioavailability (USEPA 2007) using the juvenile swine model. The basic model for estimating arsenic RBA differed from lead in that the urinary excretion fraction (UEF) of arsenic administered in test material and in reference material (sodium arsenate) was measured, and the ratio of the two UEF values then calculated:

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

The UEF for each material (test soil, sodium arsenate) was estimated by plotting the mass of arsenic excreted by each animal as a function of the dose administered, and then fitting a linear regression line to the combined data. The process of deriving the best fit linear regression were fit using simultaneous weighted linear regression.

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 test soil used in this investigation was a sample of NIST SRM 2710. The sample consists of soil collected from the top 4 inches of pasture land along Silver Bow Creek near Butte, Montana. The soil is a native Montana soil that has been contaminated with mine tailings deposits. The collection site is approximately 6.5 miles south of settling ponds that feed the creek. The creek periodically floods, depositing mine tailings with high concentrations of copper, manganese, and zinc at the collection site (NIST 2003).

2.1.2 Sample Preparation

NIST SRM 2710 was prepared by air drying in an oven for three days at room temperature. The material was then passed over a vibrating 2 mm screen to remove plant material, rocks, and large chunks of aggregated soil. Material remaining on the screen was deaggregated and rescreened. The combined material passing the screen was ground in a ball mill to pass a 74 micrometer (μm) screen, radiation sterilized, and blended for 24 hours to achieve a high degree of homogeneity (NIST 2003). This prepared soil as provided by NIST was used as-is for the bioavailability study, without further preparation.

2.1.3 Arsenic Concentration

The certified concentration value for arsenic in NIST SRM 2710 is 626 ± 38 mg/kg (NIST 2003). This value is a weighted mean of results from two independent analytical methods, hydride generation atomic absorption spectrometry and radiochemical neutron activation analysis – mixed acid digestion. The stated uncertainties include allowances for measurement imprecision, material variability, and differences among analytical methods (NIST 2003). Certified values of additional elements are shown in Table A-2 of Appendix A.

2.2 Experimental Animals

Juvenile swine were selected for use in this study 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, Table A-3).

When exposure began (day zero), the animals were about 6-7 weeks old and weighed an average of about 9.3 kilograms (kg). The animals were weighed every three days during the course of the study. On average, animals gained about 0.26 kg/day and the rate of weight gain was comparable in all dosing groups, ranging from 0.23 to 0.32 kg/day. These body weight data are presented in Appendix A, Table A-4, and summarized in Figure 2-1.

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 pig chow (purchased from MFA Inc., Columbia, Missouri) by the supplier. The animals were gradually transitioned from the MFA feed to a special purified diet originally developed for lead RBA studies (purchased from Purina TestDiet[®], Richmond, IN) several days before dosing began, and this feed was maintained for the duration of the study. The feed was nutritionally complete and met all requirements of the National Institutes of Health–National Research Council (NRC 1988); the ingredients and nutritional profile of the feed are presented in Table 2-2.

Prior to the start of dosing, each day every animal was given an amount of feed equal to 4.0% of the mean body weight of all animals on study. After dosing began (beginning with the evening feeding of Day 1), the amount of feed per day was reduced to 3.5% of the mean body weight to encourage consumption of the dose materials. 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. Analysis of random feed samples indicated that the arsenic levels did not exceed 0.1 µg/g.

Drinking water was provided *ad libitum* via self-activated watering nozzles within each cage. Analysis of samples from randomly selected drinking water nozzles indicated the arsenic concentrations were below a level of detection (1 µg/L).

2.4 Dosing

The protocol for exposing animals to arsenic is shown in Table 2-1. Animals were exposed to dosing materials (sodium arsenate or test soil) 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). To facilitate dose administration, dosing materials were placed in a small depression in a ball of dough consisting of moistened TestDiet[®] feed (typically about 5 g) and the dough was pinched shut. This was then placed in the feeder at dosing time.

Occasionally, some animals did not consume their entire dose. In these instances, the missed doses were estimated and recorded and the time-weighted average dose calculation for each animal was adjusted downward accordingly (see Appendix A, Table A-4). Doses that were consumed late are noted in Table A-5, although no dose adjustments are required in these cases.

Administered amounts of dose materials were held constant throughout the study and were determined using the expected mean body weight during the exposure interval (14 days). The expected mean body weight was estimated as the mean of the actual measured weights on day -1 and the predicted weights for day 14, which were extrapolated from the day -1 weights assuming a weight gain of 1.5 kg every 3 days. The resulting estimated mean body weight was 12.86 kg.

After completion of the study, body weights were estimated by interpolation for those days when measurements were not collected. The actual administered doses were then calculated for each day and averaged across all days. The actual mean doses for each dosing group are included in Table 2-1; the actual daily doses administered to each animal are presented in Appendix A, Table A-4.

2.5 Collection and Preservation of Urine Samples

Samples of urine were collected from each animal for 48-hour periods on days 6 to 7 (U-1), 9 to 10 (U-2), and 12 to 13 (U-3) of the study. Collection began at 9:00 AM and ended 48 hours later. The urine was collected in a plastic bucket placed beneath each cage, which was emptied into a plastic storage bottle. Aluminum screens were placed under the cages to minimize contamination with feces, spilled food, or other debris. Due to the length of the collection

period, collection containers were emptied periodically (typically twice daily) into a separate plastic bottles to ensure that there was no loss of sample due to overflow.

At the end of each collection period, the total urine volume for each animal was measured (see Appendix A, Table A-6) and three 60-milliliter (mL) portions were removed and acidified with 0.6 mL concentrated nitric acid¹. All samples were refrigerated. Two of the aliquots were archived in the refrigerator 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. Preliminary 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.

Urine analytical results are presented in Appendix A, Table A-7. All responses below the quantitation limit were evaluated at one-half the quantitation limit. Quality assurance samples are described in the following section (2.7).

2.7 Quality Assurance

A number of quality assurance (QA) steps were taken during this project to evaluate the accuracy of the analytical procedures. The results for quality assurance samples are presented in Appendix A, Table A-8, and are summarized below.

Blind Duplicates (Sample Preparation Replicates)

A random selection of about 20% of all urine samples generated during the study were prepared for laboratory analysis in duplicate (i.e., two separate subsamples of urine were prepared for analysis) and submitted to the laboratory in a blind fashion. The results for the blind duplicates are shown in Figure 2-2. As seen, there was good agreement between results for the duplicate pairs in all cases.

¹ Urine samples EP3-1-134 and EP3-1-160 (pigs 312 and 318 from group 2, U-2 urine collection) were inadvertently combined into a single sample prior to analysis. Thus, results for these two samples were excluded from the data evaluation.

Performance Evaluation Samples

A number of Performance Evaluation (PE) samples (urines of known arsenic concentration) were submitted to the laboratory in a blind fashion. The PE samples included several different concentrations each of four different types of arsenic (As+3, As+5, MMA, and DMA). The results for the PE samples are shown in Figure 2-3. As seen, there was good recovery of the arsenic in all cases.

Spike Recovery

During arsenic analysis, every tenth sample was spiked with known amounts of arsenic (sodium arsenate) and the recovery of the added arsenic was measured. Arsenic recovery for individual samples ranged from 95% to 106%, with an average of $103 \pm 3.3\%$ (N = 10).

Laboratory Duplicates

During arsenic analysis, every tenth sample was analyzed in duplicate. All duplicate results (N = 12) agreed within ± 1 times the detection limit or less than 10% relative percent difference (RPD).

Laboratory Control Standards

Laboratory control standards (samples of reference materials for which a certified concentration of specific analytes has been established) were tested periodically during sample analysis. Results are summarized below:

Standard	Description	Certified Mean \pm SD (ng/mL)	Mean (ng/mL)	Range (ng/mL)	SD (ng/mL)	Mean % Recovery	N
NIST 2670a-H	Freeze-dried human urine, spiked	220 +/-10	237	230 - 240	5.8	108%	3
NIST 2670a-L	Freeze-dried human urine, unspiked	3*	4	3 - 5	1.4	133%	2
NIST 1640	Natural fresh water (unspiked)	26.7 +/-0.41	26	--	--	97%	1
NIST 1566b	Freeze-dried oyster tissue	7.65 +/-0.65	7.5	--	--	98%	1

*Note that the arsenic concentration in NIST 2670a-L as provided by NIST is a reference value, not a certified value. Reference values are non-certified values that are the best estimate of the true value but do not meet the NIST criteria for certification.

SD = Standard deviation

N = Number of samples analyzed

Recovery of arsenic from these standards was generally good and within the acceptable range.

Blanks

Blank samples run along with each batch of samples never yielded a measurable level of arsenic (N = 7).

Based on the results of all of the quality assurance 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 urinary excretion fraction 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 urinary excretion fraction 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 ($\mu\text{g}/\text{day}$) as a function of the administered amount of arsenic ($\mu\text{g}/\text{day}$), both for reference material (sodium arsenate) and for test material.
2. Find the best fit linear regression line through each data set. The slope of each line ($\mu\text{g}/\text{day}$ excreted per $\mu\text{g}/\text{day}$ ingested) is the best estimate of the urinary excretion fraction (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).

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). 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). 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. Log-variance increases as an approximately linear function of log-mean response:

$$\ln(s_i^2) = k_1 + k_2 \cdot \ln(\bar{y}_i)$$

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

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). When such data points were encountered in a data set, the RBA values were calculated both with and without the potential outlier(s) excluded, and the result with the outlier(s) 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 study.

However, four animals exhibited signs of illness (e.g., gastrointestinal distress, elevated temperature) in the early days of the study and were treated with 1 cubic centimeter Naxcel, an injectable antibiotic given for transient illness. Pigs 319 (group 6) and 308 (group 3) were treated for three days beginning on day 0 and day 1, respectively, and pigs 318 (group 2) and 309 (group 4) were treated for one day on day 1. Symptoms promptly went away and the animals were retained on study.

4.2 Background Arsenic Excretion

The urinary excretion results for control animals from days 6-13 ranged from 2.2 to 11.1 $\mu\text{g}/48$ hours with a mean of 6.2. These values are representative of endogenous background levels in food and water and support the view that the animals were not exposed to any significant exogenous sources of arsenic throughout the study.

4.3 Dose-Response Patterns

Urinary Arsenic Variance

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. The data used to derive the variance model are shown in Figure 4-1. This data was gathered from previous RBA studies on swine. Based on these data, values of k_1 and k_2 were derived using ordinary least squares minimization. The resulting values were -1.10 for k_1 and 1.64 for k_2 .

Superimposed on Figure 4-1 is the variance data from this study (as indicated by the solid symbols) on top of the historic data set (open symbols). As seen, the variance of the urinary arsenic data from this study is consistent with the data used to generate the variance model.

Urinary Arsenic

The dose-response data for arsenic in urine were modeled using a linear equation (see Section 3.2). The results of these fittings are shown in Figures 4-2 (days 6/7), 4-3 (days 9/10), 4-4 (days 12/13), and 4-5 (all days combined)². One outlier was identified in the fittings, from group 6 on

² Urine samples EP3-1-134 and EP3-1-160 (pigs 312 and 318 from group 2, U-2 urine collection) were inadvertently combined into a single sample prior to analysis. Thus, results for these two samples were excluded from the data evaluation.

days 6/7. This outlier was excluded from the final evaluation for arsenic RBA; see Figures 4-6 (days 6/7) and 4-7 (all days combined) for the revised fittings.

4.4 Calculated RBA Values

As seen in Figures 4-2 through 4-7, all of the dose-response curves are approximately linear, with the slope of the best-fit straight line being equal to the best estimate of the UEF. The following table summarizes the resulting slopes (outliers excluded when applicable):

Time Interval	Outliers Excluded ^a	Slope (UEF Estimate)	
		b _r	b _{t1}
Days 6/7	1	0.83	0.34
Days 9/10	0	0.82	0.35
Days 12/13	0	0.81	0.40
All Days	1	0.82	0.36

^a As indicated in Figures 4-2 and 4-5

b_r = slope term for the reference material data set

b_{t1} = slope term for the Test Material 1 data set

As discussed previously (Section 3), the relative bioavailability of arsenic in a specific test material is calculated as follows:

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

The following table summarizes the estimated RBA values:

Time Interval	Estimated RBA (90% Confidence Interval)
Days 6/7	0.41 (0.36 - 0.47)
Days 9/10	0.42 (0.39 - 0.47)
Days 12/13	0.50 (0.40 - 0.62)
All Days	0.44 (0.40 - 0.48)

As shown, using sodium arsenate as a relative frame of reference, the RBA estimate is approximately 44% for NIST SRM 2710.

4.5 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. This between-animal variability in response 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 children, it is possible that there are differences in physiological parameters that may influence RBA and, so, 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. In this regard, it is important to recall that 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 AND FIGURES

TABLE 2-1 DOSING PROTOCOL

Group	Number of Animals	Dose Material Administered	Arsenic Dose ($\mu\text{g}/\text{kg}\cdot\text{day}$)	
			Target	Actual ^a
1	3	Control	0	0.0
2	4	Sodium Arsenate	25	24.1
3	4	Sodium Arsenate	50	47.5
4	4	Sodium Arsenate	100	95.9
5	4	Test Material 1	60	58.2
6	4	Test Material 1	120	114.5

^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.

Doses were administered in two equal portions given at 9:00 AM and 3:00 PM each day. Doses were held constant based on a body weight of 12.86 kg, the expected mean weight during the exposure interval (14 days).

TABLE 2-2 TYPICAL FEED COMPOSITION

Purina TestDiet® 5TXP: Porcine Grower Purified Diet with Low Lead¹

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 PROFILE²

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)³	3.62
Glycine, %	0.79	<i>From:</i>	<i>kcal %</i>
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		

FOOTNOTES

¹ This special purified diet was originally developed for lead RBA studies.

² 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.

³ Energy (kcal/gm) - Sum of decimal fractions of protein, fat and carbohydrate x 4,9,4 kcal/gm respectively.

FIGURE 2-1 BODY WEIGHT GAIN

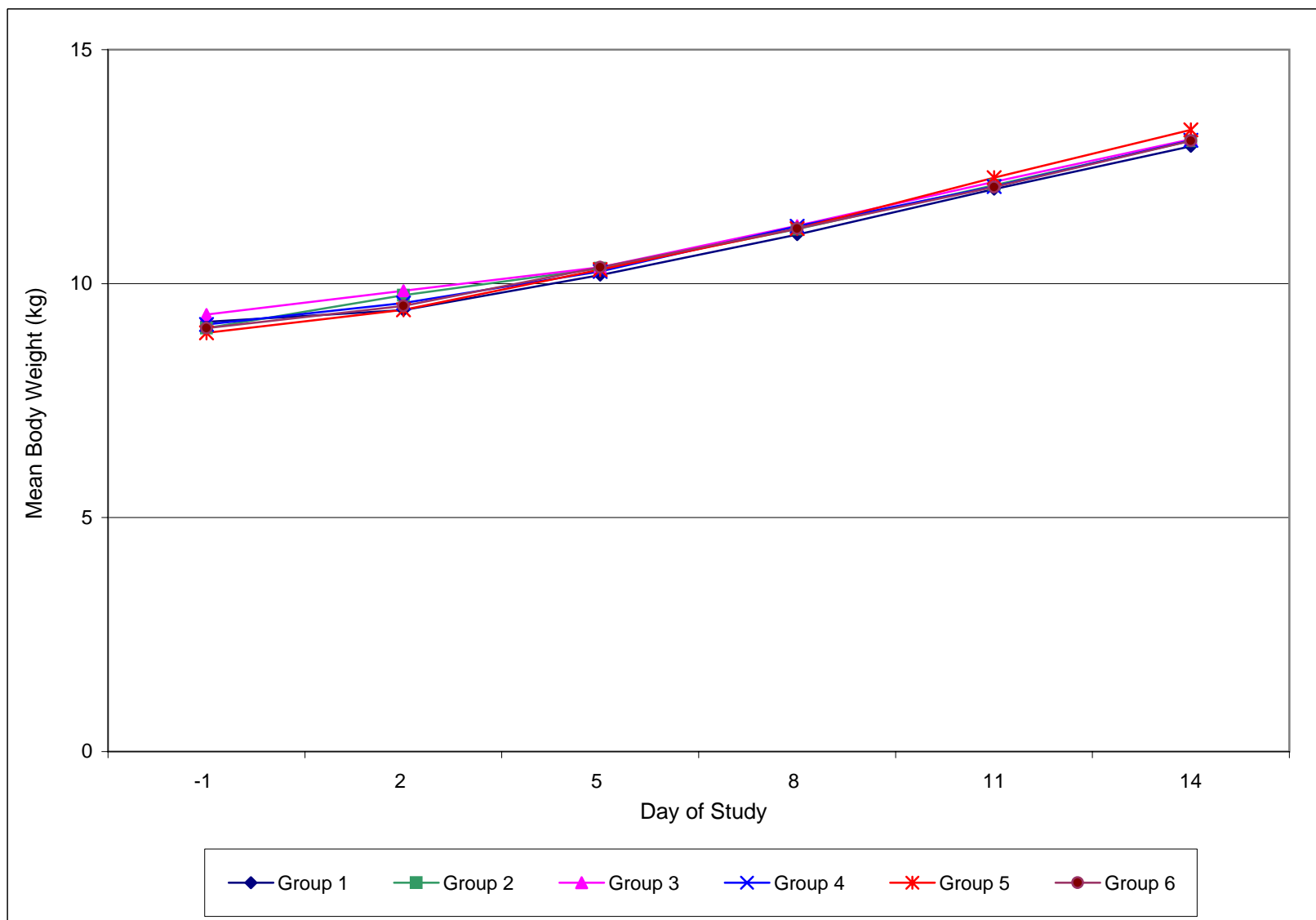


FIGURE 2-2 URINARY ARSENIC BLIND DUPLICATES

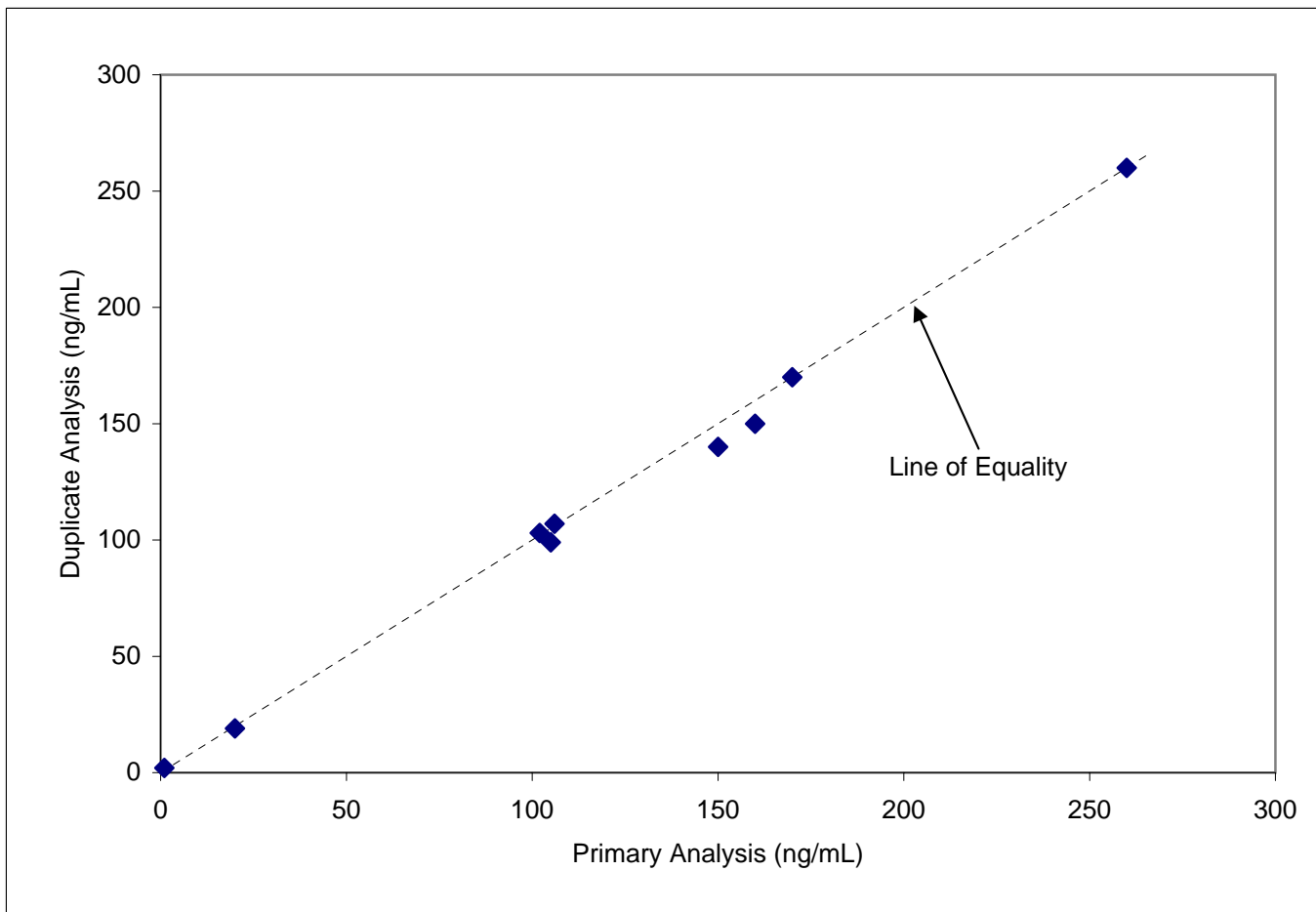


FIGURE 2-3 PERFORMANCE EVALUATION SAMPLES

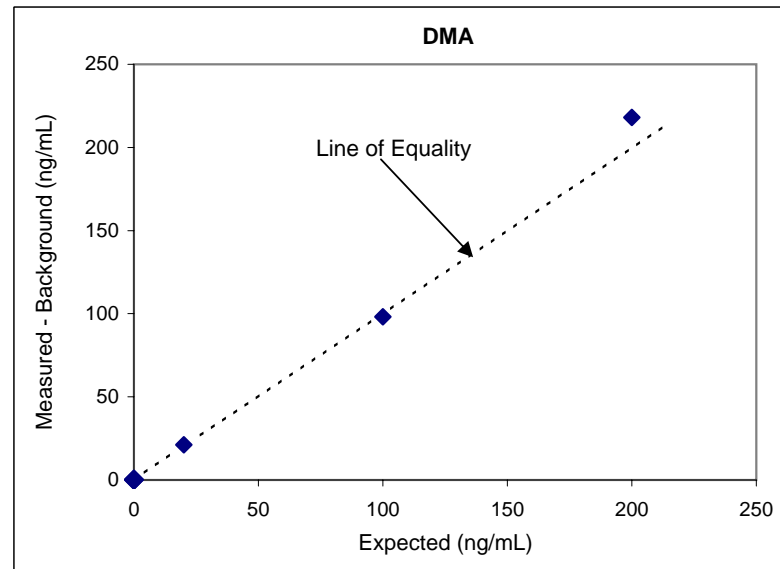
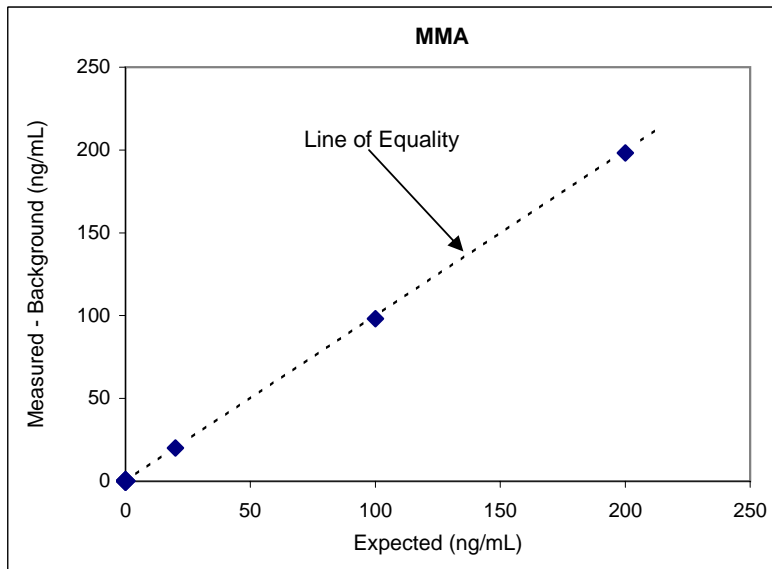
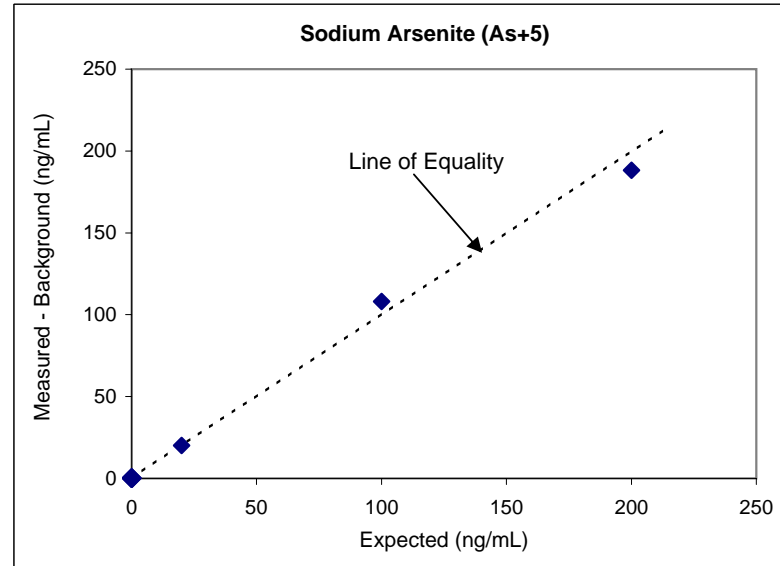
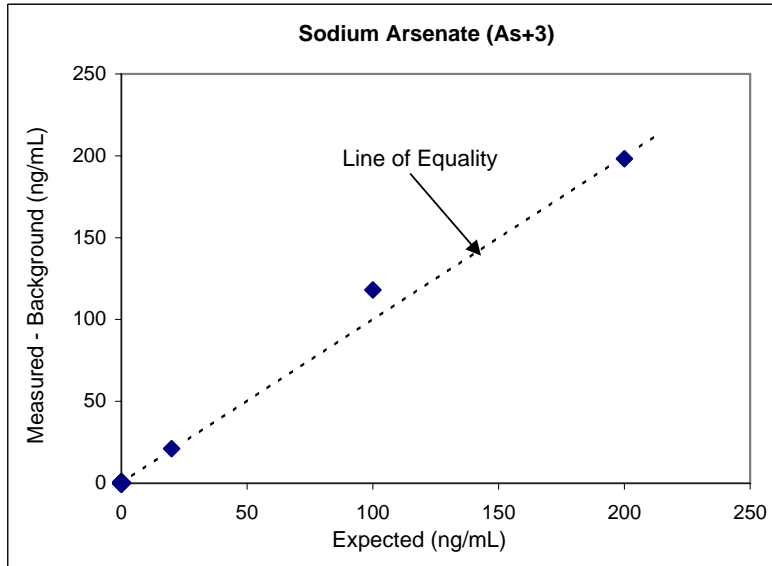
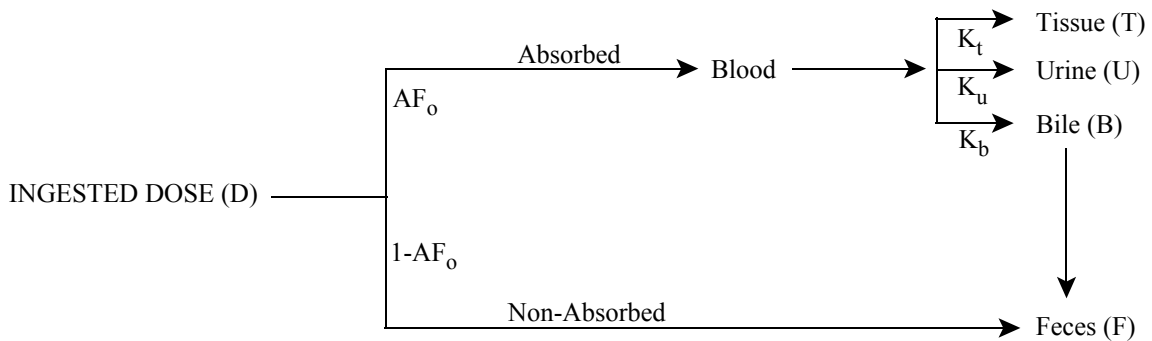


Figure 3-1. Conceptual Model for Arsenic Toxicokinetics



where:

D = Ingested dose (ug)

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:

$$\text{Amount Absorbed (ug)} = D \times AF_o$$

$$\begin{aligned} \text{Amount Excreted (ug)} &= \text{Amount absorbed} \times K_u \\ &= D \times AF_o \times K_u \end{aligned}$$

$$\begin{aligned} \text{Urinary Excretion Fraction (UEF)} &= \text{Amount excreted} / \text{Amount Ingested} \\ &= (D \times AF_o \times K_u) / D \\ &= AF_o \times K_u \end{aligned}$$

$$\begin{aligned} \text{Relative Bioavailability (x vs. y)} &= \text{UEF}(x) / \text{UEF}(y) \\ &= (AF_o(x) \times K_u) / (AF_o(y) \times K_u) \\ &= AF_o(x) / AF_o(y) \end{aligned}$$

FIGURE 4-1 URINARY ARSENIC VARIANCE MODEL

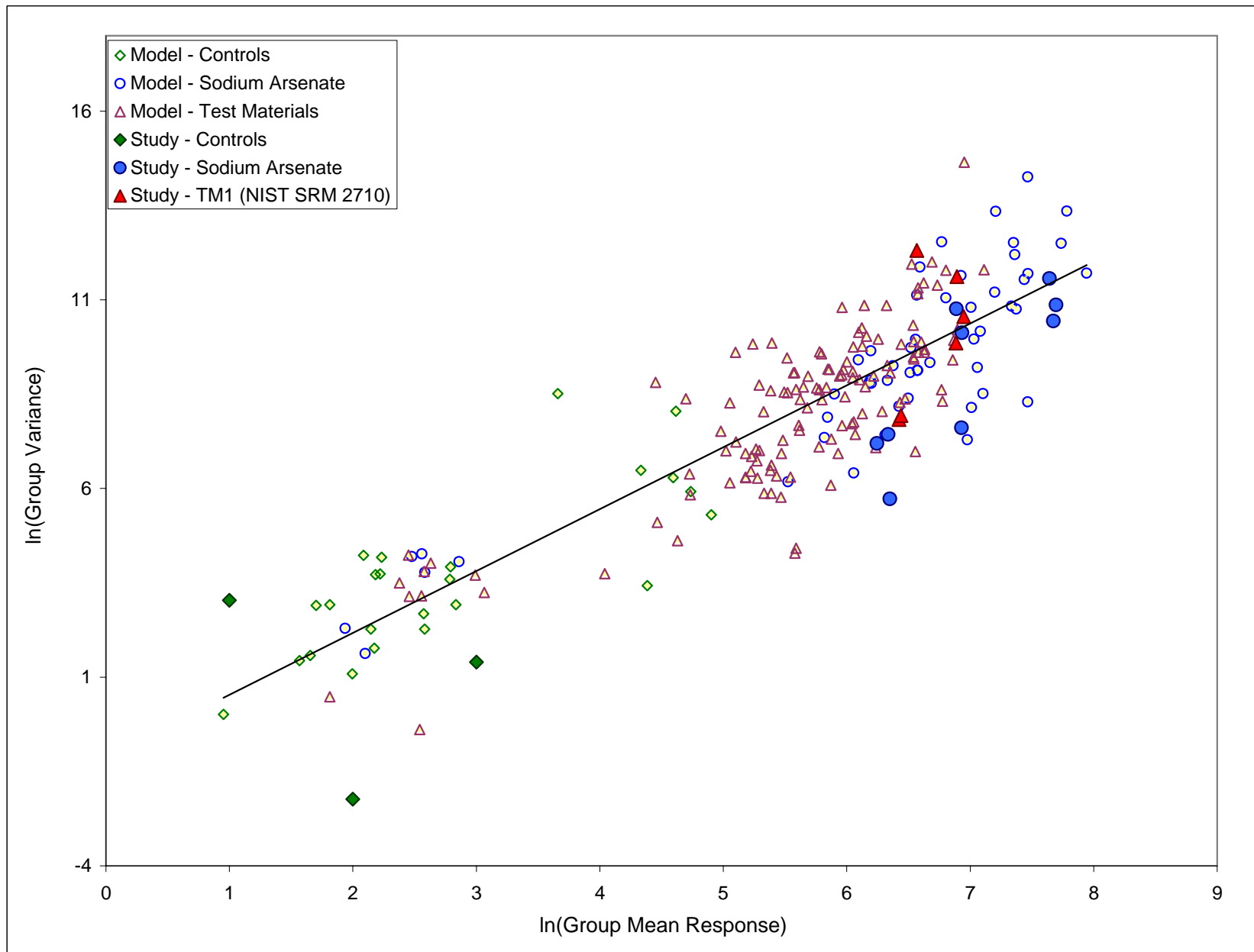
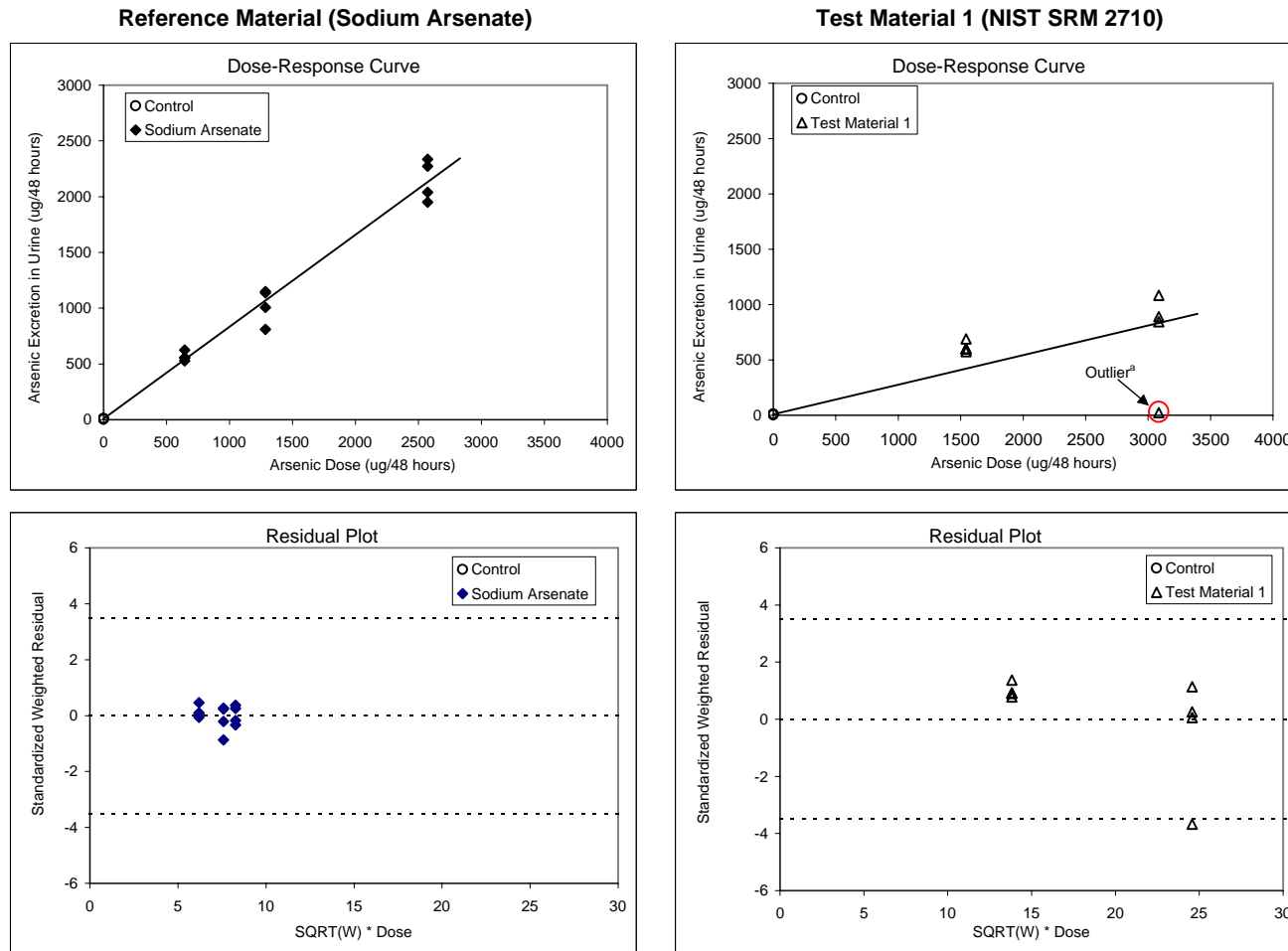


FIGURE 4-2 URINARY EXCRETION OF ARSENIC: Days 6/7 (All Data)



^a Note that the data from this figure were refitted with the outlier excluded (see Figure 4-6); this outlier was excluded from the final evaluation for arsenic F

Summary of Fitting^b

Parameter	Estimate	Standard Error
a	7.3	3.1
b _r	0.83	0.07
b _{t1}	0.27	0.03
Covariance (b _r , b _{t1})	0.0013	--
Degrees of Freedom	21	--

$$^b y = a + b_r * x_r + b_{t1} * x_{t1}$$

where r = Reference Material, t1 = Test Material 1

ANOVA

Source	MSE
Fit	337.45
Error	3.43
Total	33.79

Statistic	Estimate
F	98.508
p	< 0.001
Adjusted R ²	0.8986

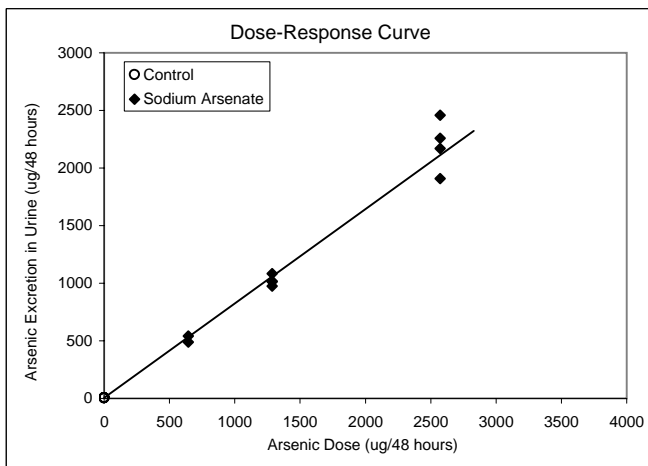
RBA and Uncertainty

	Test Material 1
RBA	0.32
Lower bound ^c	0.25
Upper bound ^c	0.42
Standard Error ^c	0.049

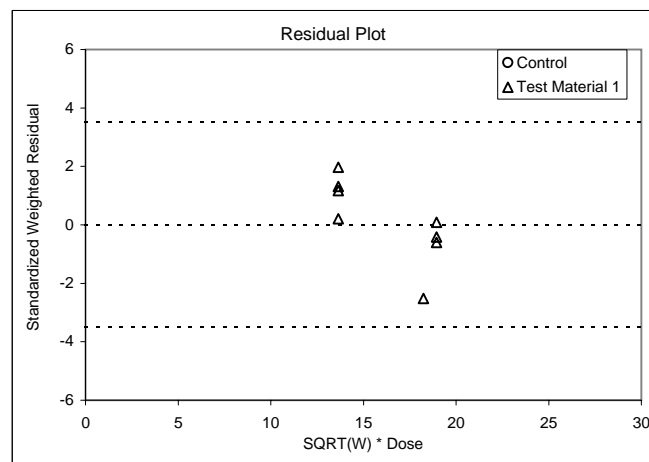
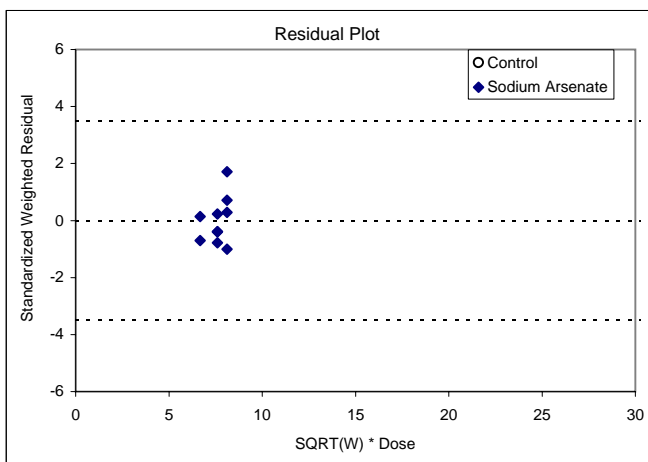
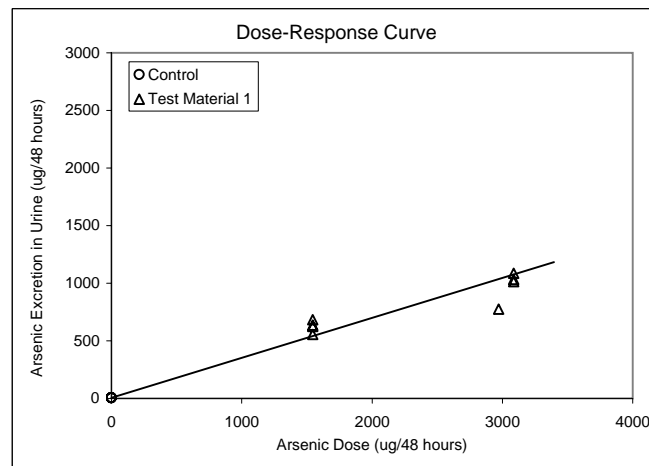
^c Calculated using Fieller's theorem

FIGURE 4-3 URINARY EXCRETION OF ARSENIC: Days 9/10 (All Data)

Reference Material (Sodium Arsenate)



Test Material 1 (NIST SRM 2710)



Summary of Fitting^a

Parameter	Estimate	SE
a	5.6	0.9
b _r	0.82	0.03
b _{t1}	0.35	0.01
Covariance (b _r , b _{t1})	0.0007	--
Degrees of Freedom	19	--

^a $y = a + b_r \cdot x_r + b_{t1} \cdot x_{t1}$

where r = Reference Material, t1 = Test Material 1

ANOVA

Source	MSE
Fit	324.90
Error	0.46
Total	32.90

Statistic	Estimate
F	713.819
p	< 0.001
Adjusted R ²	0.9862

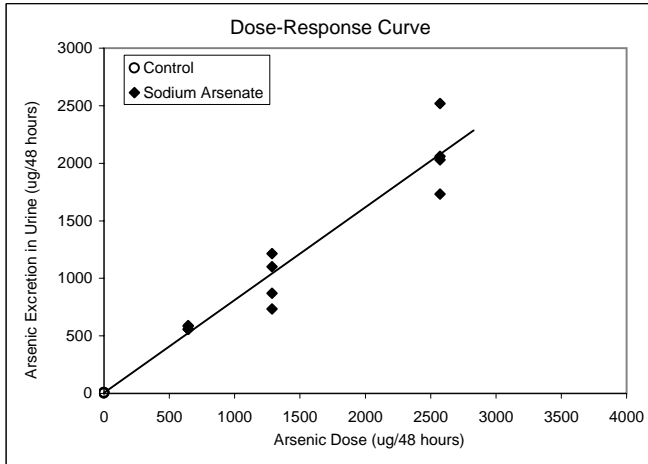
RBA and Uncertainty

	Test Material 1
RBA	0.42
Lower bound ^b	0.39
Upper bound ^b	0.47
Standard Error ^b	0.023

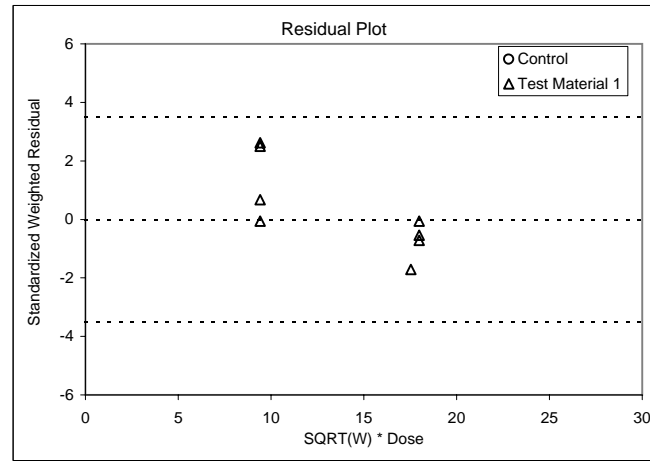
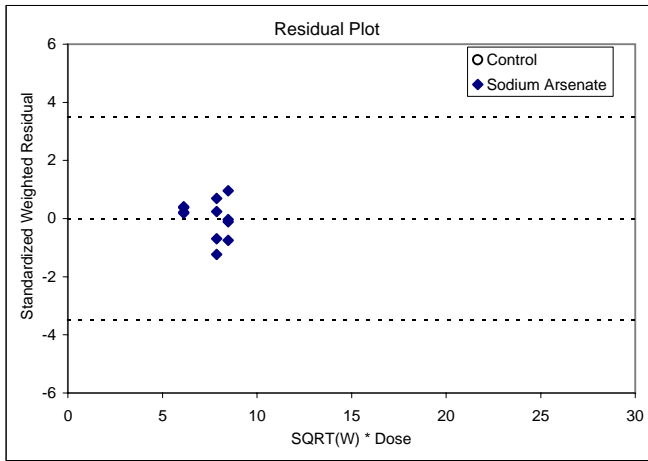
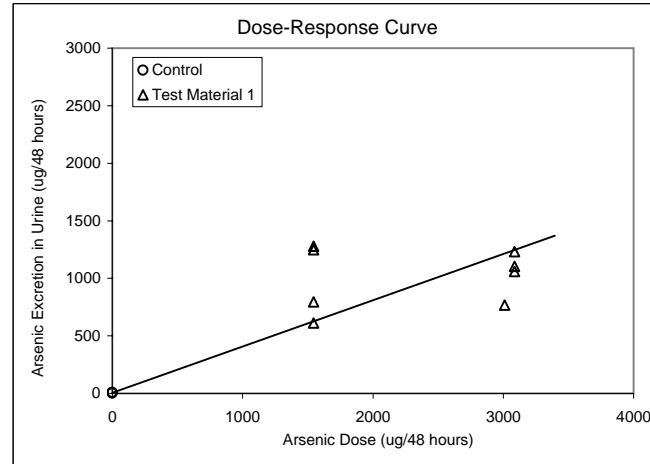
^b Calculated using Fieller's theorem

FIGURE 4-4 URINARY EXCRETION OF ARSENIC: Days 12/13 (All Data)

Reference Material (Sodium Arsenate)



Test Material 1 (NIST SRM 2710)



Summary of Fitting^a

Parameter	Estimate	SE
a	5.9	2.3
b _r	0.81	0.06
b _{t1}	0.40	0.04
Covariance (b _r , b _{t1})	0.0006	--
Degrees of Freedom	21	--

$$^a y = a + b_r \cdot x_r + b_{t1} \cdot x_{t1}$$

where r = Reference Material, t1 = Test Material 1

ANOVA

Source	MSE
Fit	353.82
Error	2.58
Total	34.52

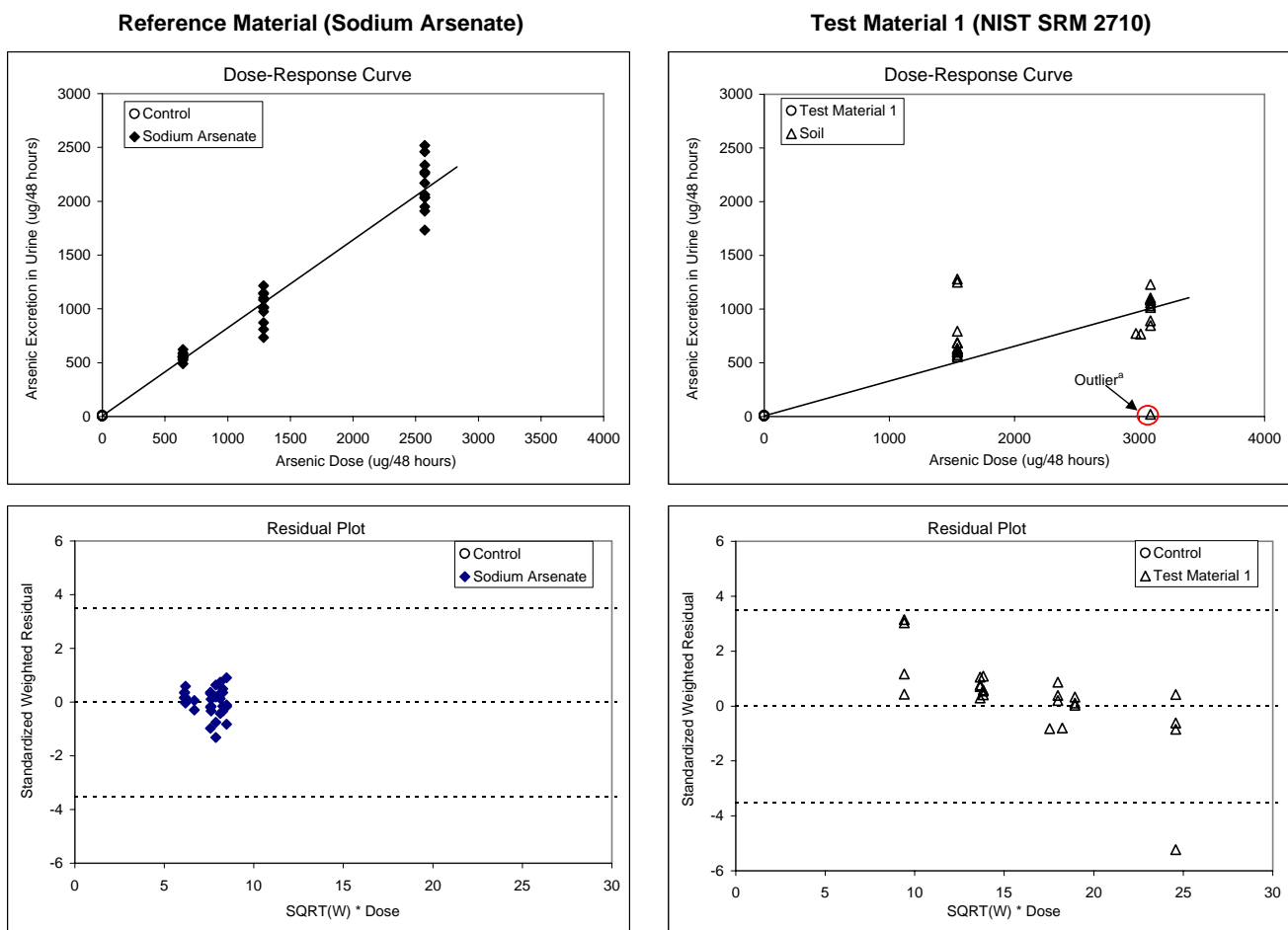
Statistic	Estimate
F	136.884
p	< 0.001
Adjusted R ²	0.9251

RBA and Uncertainty

	Test Material 1
RBA	0.50
Lower bound ^b	0.40
Upper bound ^b	0.62
Standard Error ^b	0.062

^b Calculated using Fieller's theorem

FIGURE 4-5 URINARY EXCRETION OF ARSENIC: All Days (All Data)



^a Note that the data from this figure were refitted with the outlier excluded (see Figure 4-7); this outlier was excluded from the final evaluation for arsenic RBA.

Summary of Fitting^b

Parameter	Estimate	SE
a	6.1	1.3
b _r	0.82	0.03
b _{t1}	0.32	0.02
Covariance (b _r , b _{t1})	0.0008	--
Degrees of Freedom	65	--

^b $y = a + b_r \cdot x_r + b_{t1} \cdot x_{t1}$

where r = Reference Material, t1 = Test Material 1

ANOVA

Source	MSE
Fit	1006.11
Error	2.34
Total	32.76

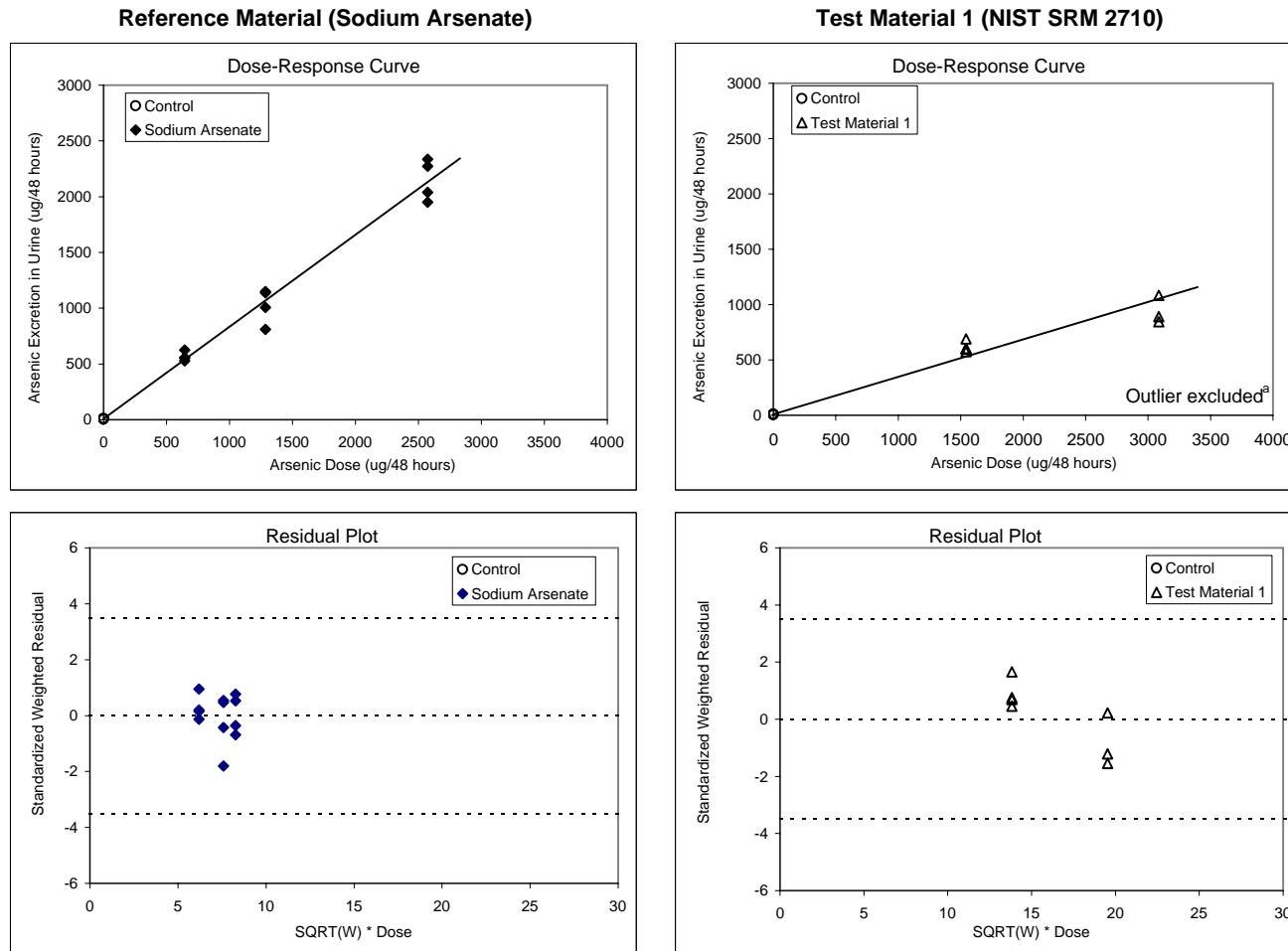
Statistic	Estimate
F	429.067
p	< 0.001
Adjusted R ²	0.9284

RBA and Uncertainty

	Test Material 1
RBA	0.40
Lower bound ^c	0.35
Upper bound ^c	0.45
Standard Error ^c	0.028

^c Calculated using Fieller's theorem

FIGURE 4-6 URINARY EXCRETION OF ARSENIC: Days 6/7 (Outliers Excluded)



^a The outlier was identified in the initial fitting (see Figure 4-2); the data are plotted here (Figure 4-6) with the outlier excluded. These results, with the outlier excluded, were used in the final evaluation for arsenic RBA.

Summary of Fitting^b

Parameter	Estimate	Standard Error
a	7.2	1.5
b _r	0.83	0.04
b _{t1}	0.34	0.02
Covariance (b _r , b _{t1})	0.0011	--
Degrees of Freedom	20	--

^b $y = a + b_r \cdot x_r + b_{t1} \cdot x_{t1}$

where r = Reference Material, t1 = Test Material 1

ANOVA

Source	MSE
Fit	333.25
Error	0.81
Total	32.47

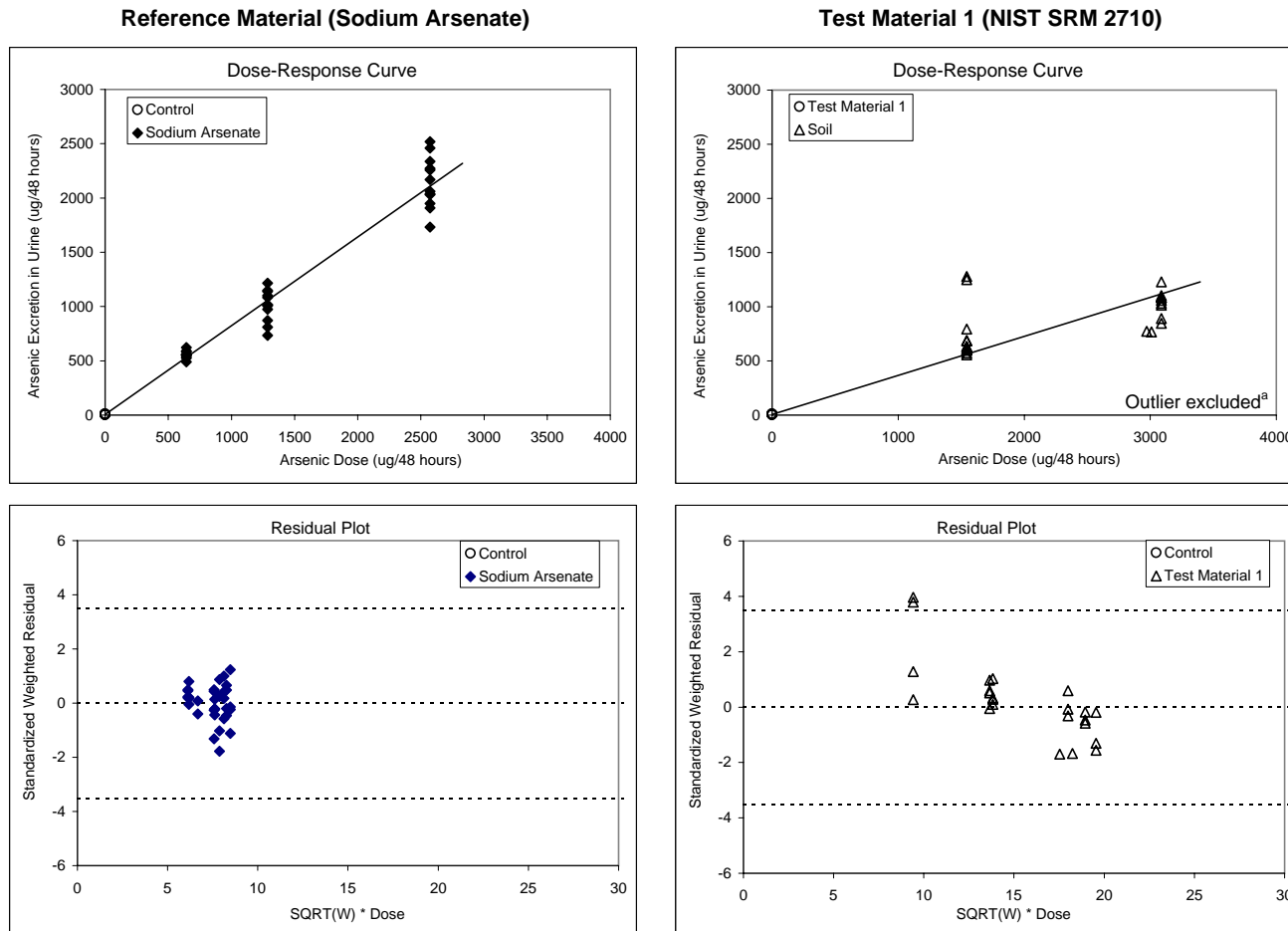
Statistic	Estimate
F	411.586
p	< 0.001
Adjusted R ²	0.9751

RBA and Uncertainty

	Test Material 1
RBA	0.41
Lower bound ^c	0.36
Upper bound ^c	0.47
Standard Error ^c	0.030

^c Calculated using Fieller's theorem

FIGURE 4-7 URINARY EXCRETION OF ARSENIC: All Days (Outliers Excluded)



^a The outlier was identified in the initial fitting (see Figure 4-5); the data are plotted here (Figure 4-7) with the outlier excluded. These results, with the outlier excluded, were used in the final evaluation for arsenic RBA.

Summary of Fitting^b

Parameter	Estimate	SE
a	6.1	1.0
b _r	0.82	0.03
b _{t1}	0.36	0.01
Covariance (b _r , b _{t1})	0.0008	--
Degrees of Freedom	64	--

$$y = a + b_r * x_r + b_{t1} * x_{t1}$$

where r = Reference Material, t1 = Test Material 1

ANOVA

Source	MSE
Fit	1010.24
Error	1.27
Total	32.32

Statistic	Estimate
F	794.939
p	< 0.001
Adjusted R ²	0.9607

RBA and Uncertainty

	Test Material 1
RBA	0.44
Lower bound ^c	0.40
Upper bound ^c	0.48
Standard Error ^c	0.023

^c Calculated using Fieller's theorem

APPENDIX A

DETAILED RESULTS

TABLE A-1 SCHEDULE

Study Day	Day	Date	Cull Pigs/ Assign Dose Group	Feed Special Diet	Weigh	Dose Preparation	Dose Administration	Urine Collection ^a	Sacrifice/ Necropsy
-6	Tuesday	04/10/07							
-5	Wednesday	04/11/07	Cull Pigs		X				
-4	Thursday	04/12/07		transition					
-3	Friday	04/13/07	Assign Dose Groups	transition					
-2	Saturday	04/14/07		transition					
-1	Sunday	04/15/07		transition	X	X			
0	Monday	04/16/07		X			X		
1	Tuesday	04/17/07		X			X		
2	Wednesday	04/18/07		X	X		X		
3	Thursday	04/19/07		X		X	X		
4	Friday	04/20/07		X			X		
5	Saturday	04/21/07		X	X		X		
6	Sunday	04/22/07		X			X	U-1 ↑ ↓	
7	Monday	04/23/07		X			X		
8	Tuesday	04/24/07		X	X	X	X		
9	Wednesday	04/25/07		X			X	U-2 ↑ ↓	
10	Thursday	04/26/07		X			X		
11	Friday	04/27/07		X	X		X		
12	Saturday	04/28/07		X			X	U-3 ↑ ↓	
13	Sunday	04/29/07		X			X		
14	Monday	04/30/07		X	X				X

^a Urine was collected over a period of 48 hours.

TABLE A-2 CERTIFIED VALUES

Element	Mass Fraction (%)
Aluminum	6.44 ± 0.08
Calcium	1.25 ± 0.03
Iron	3.38 ± 0.10
Magnesium	0.853 ± 0.042
Manganese	1.01 ± 0.04
Phosphorus	0.106 ± 0.015
Potassium	2.11 ± 0.11
Silicon	28.97 ± 0.18
Sodium	1.14 ± 0.06
Sulfur	0.240 ± 0.006
Titanium	0.283 ± 0.010

Element	Mass Fraction (mg/kg)
Antimony	38.4 ± 3
Arsenic	626 ± 38
Barium	707 ± 51
Cadmium	21.8 ± 0.2
Copper	2950 ± 130
Lead	5532 ± 80
Mercury	32.6 ± 1.8
Nickel	14.3 ± 1.0
Silver	35.3 ± 1.5
Vanadium	76.6 ± 2.3
Zinc	6952 ± 91

Source: NIST, 2003

TABLE A-3 GROUP ASSIGNMENTS

Pig Number	Dose Group	Material Administered	Target Dose of Arsenic ($\mu\text{g}/\text{kg}\text{-day}$)
317 320 326	1	Control	0
304 312 318 327	2	Sodium Arsenate	25
308 310 314 315	3	Sodium Arsenate	50
302 305 309 313	4	Sodium Arsenate	100
301 311 321 328	5	Test Material 1	60
303 306 307 319	6	Test Material 1	120

TABLE A-4 BODY WEIGHTS AND ACTUAL ADMINISTERED DOSES, BY DAY

Body weights were measured on days -1, 2, 5, 8, 11, and 14. Weights for other days are estimated, based on linear interpolation between measured values.

Group	Pig #	Day -1		Day 0		Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7		Day 8		Day 9		Day 10		Day 11		Day 12		Day 13		Day 14		Mean As Dose (µg/kg-d)
		BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)	BW (kg)	As Dose (µg/kg-d)			
1	317	9.4	0.00	9.5	0.00	9.6	0.00	9.7	0.00	10.0	0.00	10.3	0.00	10.6	0.00	10.7	0.00	10.9	0.00	11.1	0.00	11.4	0.00	11.8	0.00	12.1	0.00	12.3	0.00	12.6	0.00	12.8	0.00	0.00
1	320	8.9	0.00	8.9	0.00	8.9	0.00	8.9	0.00	9.2	0.00	9.5	0.00	9.8	0.00	10.1	0.00	10.5	0.00	10.8	0.00	11.1	0.00	11.4	0.00	11.8	0.00	12.1	0.00	12.4	0.00	12.7	0.00	0.00
1	326	9.4	0.00	9.5	0.00	9.6	0.00	9.7	0.00	9.9	0.00	10.0	0.00	10.2	0.00	10.6	0.00	10.9	0.00	11.3	0.00	11.6	0.00	11.9	0.00	12.2	0.00	12.6	0.00	13.0	0.00	13.4	0.00	0.00
2	304	8.8	0.00	9.0	35.60	9.3	34.70	9.5	33.85	9.7	33.21	9.9	32.59	10.1	32.00	10.3	31.17	10.6	30.39	10.9	29.64	11.2	28.75	11.5	27.92	11.9	27.14	12.1	26.54	12.4	25.97	12.7	25.42	30.92
2	312	8.5	0.00	8.7	37.10	8.9	36.20	9.1	35.34	9.4	34.33	9.6	33.38	9.9	32.48	10.2	31.53	10.5	30.63	10.8	29.78	11.1	28.93	11.4	28.13	11.8	27.37	12.1	26.61	12.4	25.90	12.8	25.22	31.54
2	318	9.4	0.00	9.6	33.56	9.8	32.76	10.1	32.00	10.2	31.53	10.4	31.07	10.5	30.63	10.8	29.78	11.1	28.97	11.4	28.21	11.7	27.45	12.0	26.72	12.4	26.04	12.7	25.35	13.0	24.70	13.4	24.09	29.39
2	327	9.6	0.00	9.9	32.65	10.1	31.84	10.4	31.07	10.5	30.58	10.7	30.10	10.9	29.64	11.1	28.97	11.4	28.33	11.6	27.72	11.9	27.06	12.2	26.43	12.5	25.83	12.8	25.16	13.1	24.52	13.5	23.91	28.71
3	308	9.8	0.00	9.9	65.07	10.0	48.16	10.2	63.36	10.3	62.24	10.5	61.16	10.7	60.11	11.0	58.38	11.3	56.75	11.7	55.21	12.0	53.82	12.3	52.50	12.6	51.25	12.8	50.12	13.1	49.03	13.4	48.00	56.46
3	310	9.6	0.00	9.7	66.30	9.8	65.63	9.9	64.96	10.0	64.10	10.2	63.26	10.3	62.44	10.6	60.58	10.9	58.82	11.3	57.17	11.6	55.60	11.9	54.12	12.2	52.72	12.5	51.59	12.7	50.51	13.0	49.47	59.57
3	314	8.5	0.00	8.7	73.78	9.0	71.59	9.3	69.53	9.4	68.18	9.6	66.88	9.8	65.63	10.1	63.78	10.4	62.04	10.7	60.39	11.0	58.38	11.4	56.50	11.8	54.74	12.1	53.08	12.5	51.52	12.9	50.05	63.04
3	315	9.6	0.00	9.7	66.08	9.9	64.86	10.1	63.68	10.3	62.64	10.4	61.64	10.6	60.67	10.9	59.19	11.1	57.77	11.4	56.42	11.7	55.13	11.9	53.90	12.2	52.72	12.5	51.45	12.8	50.25	13.1	49.10	58.64
4	302	8.6	0.00	8.7	147.57	8.8	72.81	9.0	143.72	9.2	140.58	9.4	137.57	9.6	134.69	9.9	129.93	10.3	125.49	10.6	121.35	10.8	119.10	11.0	116.94	11.2	114.85	11.6	110.89	12.0	107.19	12.4	103.73	122.96
4	305	9.0	0.00	9.2	140.32	9.3	137.82	9.5	135.40	9.7	133.30	9.8	131.26	10.0	129.28	10.2	125.90	10.5	122.70	10.8	119.66	11.0	116.58	11.3	113.66	11.6	110.89	11.9	108.09	12.2	105.43	12.5	102.90	124.34
4	309	9.0	0.00	9.1	141.87	9.2	140.07	9.3	138.31	9.6	134.46	9.8	130.81	10.1	127.36	10.4	123.29	10.8	119.47	11.1	115.88	11.4	112.83	11.7	109.94	12.0	107.19	12.3	104.72	12.6	102.36	12.9	100.10	123.22
4	313	10.0	0.00	10.2	126.52	10.4	123.88	10.6	121.35	10.9	118.19	11.2	115.19	11.5	112.34	11.8	109.16	12.1	106.16	12.5	103.32	12.8	100.49	13.2	97.82	13.5	95.28	13.8	92.99	14.2	90.80	14.5	88.71	109.05
5	301	8.6	0.00	8.7	88.37	8.9	86.56	9.1	84.81	9.4	82.10	9.7	79.57	10.0	77.18	10.2	75.66	10.4	74.21	10.6	72.81	10.9	70.70	11.2	68.70	11.6	66.82	11.9	65.13	12.2	63.52	12.5	61.99	75.99
5	311	9.4	0.00	9.5	81.24	9.6	76.37	9.7	79.57	10.1	76.79	10.4	74.21	10.8	71.79	11.0	69.95	11.3	68.20	11.6	66.53	12.1	63.96	12.5	61.58	13.0	59.37	13.4	57.67	13.8	56.06	14.2	54.54	69.41
5	321	9.0	0.00	9.1	84.66	9.2	83.59	9.4	82.54	9.7	79.98	10.0	77.57	10.3	75.30	10.6	73.04	10.9	70.91	11.2	68.91	11.6	66.72	11.9	64.67	12.3	62.75	12.6	61.09	13.0	59.52	13.3	58.03	72.93
5	328	8.9	0.00	9.1	84.81	9.4	82.54	9.6	80.39	9.8	78.75	10.0	77.18	10.2	75.66	10.6	73.04	10.9	70.59	11.3	68.30	11.6	66.53	11.9	64.86	12.2	63.26	12.6	61.50	12.9	59.83	13.3	58.25	72.57
6	303	9.1	0.00	9.3	166.87	9.4	164.21	9.6	157.59	9.9	156.71	10.2	152.08	10.5	147.71	10.8	143.59	11.1	139.69	11.4	122.40	11.6	129.55	11.9	123.40	12.2	120.69	12.5	120.72	12.8	117.73	13.1	117.83	142.26
6	306	9.2	0.00	9.2	167.48	9.3	166.27	9.4	165.09	9.6	161.35	9.8	157.77	10.0	154.36	10.3	150.59	10.5	147.01	10.8	143.59	11.1	139.69	11.4	136.00	11.7	132.49	12.0	129.17	12.3	126.01	12.6	122.99	149.31
6	307	8.8	0.00	9.0	171.83	9.2	167.48	9.5	163.34	9.7	159.40	9.9	155.65	10.2	152.08	10.4	148.18	10.7	144.48	11.0	140.96	11.3	136.60	11.7	132.49	12.0	128.63	12.3	125.15	12.7	121.86	13.0	118.74	147.43
6	319	9.2	0.00	9.4	82.25	9.6	161.35	9.8	158.31	10.1	152.83	10.5	147.71	10.8	142.92	11.1	139.27	11.4	135.80	11.7	132.49	11.9	129.53	12.2	126.69	12.5	123.98	12.8	120.43	13.2	117.08	13.6	113.92	133.70

Missed Doses:

Day 0 - Pig 319 did not eat entire PM dose (ate approximately 0%). Daily dose adjusted to 50%.

Day 1 - Pig 302 did not eat entire AM dose (ate approximately 0%). Daily dose adjusted to 50%.

Day 1 - Pig 308 did not eat entire AM dose (ate approximately 50%). Daily dose adjusted to 75%.

Day 1 - Pig 311 did not eat entire AM or PM dose (ate approximately 95% of each). Daily dose adjusted to 95%.

Day 2 - Pig 303 did not eat entire PM dose (ate approximately 95%). Daily dose adjusted to 97.5%.

Day 8 - Pig 303 did not eat entire PM dose (ate approximately 80%). Daily dose adjusted to 90%.

Day 9 - Pig 303 did not eat entire PM dose (ate approximately 95%). Daily dose adjusted to 97.5%.

Day 10 - Pig 303 did not eat entire AM or PM dose (ate approximately 95% of each). Daily dose adjusted to 95%.

Day 11 - Pig 303 did not eat entire AM or PM dose (ate approximately 95% of each). Daily dose adjusted to 95%.

Day 12 - Pig 303 did not eat entire PM dose (ate approximately 95%). Daily dose adjusted to 97.5%.

Day 13 - Pig 303 did not eat entire PM dose (ate approximately 95%). Daily dose adjusted to 97.5%.

Instances of late consumption of doses are shown in Table A-5 (no adjustments necessary).

TABLE A-5 LATE DOSE CONSUMPTION

Study Day	Pig	Notes
Day 0	303	PM dose was finished between 3 PM and 5 PM.
	307	AM dose was finished by 2 PM; 80% of PM dose was eaten at dosing; dose was finished by 5:30 PM.
	311	PM dose was finished with PM feeding.
	317	PM dose was finished with PM feeding.
Day 1	302	AM and PM doses were finished overnight.
	303	AM dose was finished by 3 PM.
	306	AM dose was finished by 3 PM.
	308	AM and PM doses were finished overnight.
	311	95% of AM dose was eaten by 3 PM.*
	317	AM dose was finished by Noon.
Day 2	303	AM dose was finished by 3 PM; 95% of PM dose was eaten by 6 PM.*
	310	PM dose was finished by 5 PM.
	311	AM dose was finished by 3 PM; 75% of PM dose was eaten by PM feeding; dose was finished overnight.
Day 3	303	AM dose was finished by 11 AM.
	310	75% of PM dose was eaten at dosing; dose was finished overnight.
	311	95% of PM dose was eaten by 6 PM; dose was finished by 10 PM.
Day 4	302	AM dose was finished by 3 PM; 80% of PM dose was eaten by 5 PM; dose was finished overnight.
	303	AM dose was finished by 3 PM; PM dose was finished by 5 PM.
	310	AM dose was finished by 3 PM; 90% of PM dose was eaten by 5 PM.*
Day 5	301	PM dose was eaten at 4:30 PM (doughball had been caught up in the feeder).
	303	AM dose was finished by 11:30 AM; PM dose was finished by 4:30 PM.
	310	AM dose was finished by 11:30 AM; PM dose was finished by 4:30 PM.
Day 6	303	AM dose was finished by 11 AM.
	310	AM dose was finished by 11 AM.
Day 7	303	AM dose was finished by 3 PM; PM dose was finished by 5 PM.
	310	PM dose was finished by 4:30 PM.
Day 8	303	AM dose was finished by 3 PM; PM dose was finished by 5 PM.
Day 9	303	AM dose was finished by 3 PM; 80% of PM dose was eaten by 5 PM; 95% was eaten by the next morning.*
Day 10	303	AM dose was finished by 3 PM; 80% of PM dose was eaten by 5 PM; 95% was eaten by the next morning.*
Day 11	303	AM dose was finished by 3 PM; 80% of PM dose was eaten by 5 PM; 95% was eaten by the next morning.*
Day 12	303	AM dose was finished by 3 PM; 50% of PM dose was eaten by 5 PM; 95% was eaten by the next morning.*
	310	AM dose was finished by 3 PM.
Day 13	303	AM dose was finished by 3 PM; 50% of PM dose was eaten by 5 PM; 95% was eaten by the next morning.*

*Incomplete dose is accounted for in Table A-4.
See Table A-4 for missed doses.

TABLE A-6 URINE VOLUMES

Group	Pig Number	Urine Collection ^a		
		U-1 Days 6-7	U-2 Days 9-10	U-3 Days 12-13
1	317	4320	5320	7140
	320	5530	5580	7480
	326	1860	1990	1990
2	304	8360	7115	6270
	312	5260	10690	4660
	318	8560	6220	8860
	327	32800	24460	19820
3	308	3960	2850	2900
	310	7630	6092	4900
	314	10840	8470	7140
	315	3150	3760	3960
4	302	4840	5780	5640
	305	11470	10850	10500
	309	14600	9460	10850
	313	8420	6100	4560
5	301	7180	6620	6000
	311	13570	13040	16200
	321	5580	4510	4030
	328	4920	3800	4740
6	303	5840	4840	4800
	306	5940	5740	5860
	307	19030	16700	16480
	319	18800	7920	10800

Units = milliliters

^a Urine was collected over 48-hour periods.

TABLE A-7 URINARY ARSENIC ANALYTICAL RESULTS FOR STUDY SAMPLES

Sample Number	Tag Number	Pig Number	Group	Material Administered	Urine Collection Days	48-hr Dose (ug/48hr)	48-hr BWAdj Dose (ug/kg-48hr)	Reported As Conc (ng/mL)	DL	AdjConc* (ng/mL)	Urine Volume (mL)	Total Excreted (ug/48hrs)
EP3-1-320-U1	EP3-1-107	320	1	Control	6/7	0	0	2	1	2	5530	11
EP3-1-326-U1	EP3-1-106	326	1	Control	6/7	0	0	4.5	1	4.5	1860	8
EP3-1-317-U1	EP3-1-131	317	1	Control	6/7	0	0	<1	1	0.5	4320	2
EP3-1-304-U1	EP3-1-111	304	2	Sodium Arsenate	6/7	643.15	61.56	63	1	63	8360	527
EP3-1-312-U1	EP3-1-102	312	2	Sodium Arsenate	6/7	643.15	62.15	105	1	105	5260	552
EP3-1-318-U1	EP3-1-125	318	2	Sodium Arsenate	6/7	643.15	58.75	65	1	65	8560	556
EP3-1-327-U1	EP3-1-126	327	2	Sodium Arsenate	6/7	643.15	57.3	19	1	19	32800	623
EP3-1-308-U1	EP3-1-120	308	3	Sodium Arsenate	6/7	1286.3	115.13	290	4	290	3960	1148
EP3-1-315-U1	EP3-1-118	315	3	Sodium Arsenate	6/7	1286.3	116.95	320	4	320	3150	1008
EP3-1-310-U1	EP3-1-113	310	3	Sodium Arsenate	6/7	1286.3	119.4	106	1	106	7630	809
EP3-1-314-U1	EP3-1-101	314	3	Sodium Arsenate	6/7	1286.3	125.82	105	1	105	10840	1138
EP3-1-302-U1	EP3-1-127	302	4	Sodium Arsenate	6/7	2572.61	255.42	421	4	421	4840	2038
EP3-1-305-U1	EP3-1-114	305	4	Sodium Arsenate	6/7	2572.61	248.6	170	2	170	11470	1950
EP3-1-309-U1	EP3-1-122	309	4	Sodium Arsenate	6/7	2572.61	242.76	160	2	160	14600	2336
EP3-1-313-U1	EP3-1-115	313	4	Sodium Arsenate	6/7	2572.61	215.32	270	4	270	8420	2273
EP3-1-321-U1	EP3-1-104	321	5	Test Material 1	6/7	1543.57	143.95	103	1	103	5580	575
EP3-1-311-U1	EP3-1-116	311	5	Test Material 1	6/7	1543.57	138.15	44	1	44	13570	597
EP3-1-301-U1	EP3-1-110	301	5	Test Material 1	6/7	1543.57	149.87	84	1	84	7180	603
EP3-1-328-U1	EP3-1-129	328	5	Test Material 1	6/7	1543.57	143.63	140	2	140	4920	689
EP3-1-303-U1	EP3-1-128	303	6	Test Material 1	6/7	3087.13	283.28	3.7	1	3.7	5840	22
EP3-1-306-U1	EP3-1-119	306	6	Test Material 1	6/7	3087.13	297.6	150	2	150	5940	891
EP3-1-307-U1	EP3-1-105	307	6	Test Material 1	6/7	3087.13	292.67	57	1	57	19030	1085
EP3-1-319-U1	EP3-1-123	319	6	Test Material 1	6/7	3087.13	275.07	45	1	45	18800	846
EP3-1-320-U2	EP3-1-140	320	1	Control	9/10	0	0	1	1	1	5580	6
EP3-1-326-U2	EP3-1-138	326	1	Control	9/10	0	0	3	1	3	1990	6
EP3-1-317-U2	EP3-1-150	317	1	Control	9/10	0	0	1	1	1	5320	5
EP3-1-327-U2	EP3-1-137	327	2	Sodium Arsenate	9/10	643.15	53.49	20	1	20	24460	489
EP3-1-304-U2	EP3-1-158	304	2	Sodium Arsenate	9/10	643.15	56.68	76	1	76	7115	541
EP3-1-315-U2	EP3-1-151	315	3	Sodium Arsenate	9/10	1286.3	109.02	270	4	270	3760	1015
EP3-1-308-U2	EP3-1-149	308	3	Sodium Arsenate	9/10	1286.3	106.32	380	4	380	2850	1083
EP3-1-310-U2	EP3-1-148	310	3	Sodium Arsenate	9/10	1286.3	109.73	160	2	160	6092	975
EP3-1-314-U2	EP3-1-143	314	3	Sodium Arsenate	9/10	1286.3	114.88	120	1	120	8470	1016
EP3-1-302-U2	EP3-1-159	302	4	Sodium Arsenate	9/10	2572.61	236.04	330	4	330	5780	1907
EP3-1-309-U2	EP3-1-157	309	4	Sodium Arsenate	9/10	2572.61	222.77	260	4	260	9460	2460
EP3-1-313-U2	EP3-1-133	313	4	Sodium Arsenate	9/10	2572.61	198.31	370	4	370	6100	2257
EP3-1-305-U2	EP3-1-152	305	4	Sodium Arsenate	9/10	2572.61	230.25	200	2	200	10850	2170
EP3-1-328-U2	EP3-1-135	328	5	Test Material 1	9/10	1543.57	131.39	180	2	180	3800	684
EP3-1-301-U2	EP3-1-161	301	5	Test Material 1	9/10	1543.57	139.4	84	1	84	6620	556
EP3-1-311-U2	EP3-1-155	311	5	Test Material 1	9/10	1543.57	125.54	48	1	48	13040	626
EP3-1-321-U2	EP3-1-145	321	5	Test Material 1	9/10	1543.57	131.4	141	1	141	4510	636
EP3-1-319-U2	EP3-1-139	319	6	Test Material 1	9/10	3087.13	256.22	128	1	128	7920	1014
EP3-1-307-U2	EP3-1-147	307	6	Test Material 1	9/10	3087.13	269.09	65	1	65	16700	1086
EP3-1-303-U2	EP3-1-141	303	6	Test Material 1	9/10	2971.36	252.95	160	2	160	4840	774
EP3-1-306-U2	EP3-1-146	306	6	Test Material 1	9/10	3087.13	275.69	180	2	180	5740	1033
EP3-1-317-U3	EP3-1-176	317	1	Control	12/13	0	0	<1	1	0.5	7140	4
EP3-1-320-U3	EP3-1-163	320	1	Control	12/13	0	0	1	1	1	7480	7
EP3-1-326-U3	EP3-1-178	326	1	Control	12/13	0	0	3.2	1	3.2	1990	6
EP3-1-312-U3	EP3-1-186	312	2	Sodium Arsenate	12/13	643.15	52.51	120	1	120	4660	559
EP3-1-318-U3	EP3-1-166	318	2	Sodium Arsenate	12/13	643.15	50.06	66	1	66	8860	585
EP3-1-327-U3	EP3-1-177	327	2	Sodium Arsenate	12/13	643.15	49.67	28	1	28	19820	555
EP3-1-304-U3	EP3-1-165	304	2	Sodium Arsenate	12/13	643.15	52.51	94	1	94	6270	589
EP3-1-308-U3	EP3-1-171	308	3	Sodium Arsenate	12/13	1286.3	99.15	380	4	380	2900	1102
EP3-1-310-U3	EP3-1-192	310	3	Sodium Arsenate	12/13	1286.3	102.1	150	2	150	4900	735
EP3-1-314-U3	EP3-1-167	314	3	Sodium Arsenate	12/13	1286.3	104.6	170	2	170	7140	1214
EP3-1-315-U3	EP3-1-190	315	3	Sodium Arsenate	12/13	1286.3	101.7	220	4	220	3960	871
EP3-1-309-U3	EP3-1-164	309	4	Sodium Arsenate	12/13	2572.61	207.08	190	2	190	10850	2062
EP3-1-313-U3	EP3-1-170	313	4	Sodium Arsenate	12/13	2572.61	183.78	380	4	380	4560	1733
EP3-1-305-U3	EP3-1-175	305	4	Sodium Arsenate	12/13	2572.61	213.53	240	4	240	10500	2520
EP3-1-302-U3	EP3-1-183	302	4	Sodium Arsenate	12/13	2572.61	218.08	360	4	360	5640	2030
EP3-1-311-U3	EP3-1-173	311	5	Test Material 1	12/13	1543.57	113.73	49	1	49	16200	794
EP3-1-321-U3	EP3-1-169	321	5	Test Material 1	12/13	1543.57	120.61	310	4	310	4030	1249
EP3-1-328-U3	EP3-1-174	328	5	Test Material 1	12/13	1543.57	121.32	270	4	270	4740	1280
EP3-1-301-U3	EP3-1-168	301	5	Test Material 1	12/13	1543.57	128.65	102	1	102	6000	612
EP3-1-319-U3	EP3-1-189	319	6	Test Material 1	12/13	3087.13	237.52	98	1	98	10800	1058
EP3-1-303-U3	EP3-1-179	303	6	Test Material 1	12/13	3009.95	238.45	160	2	160	4800	768
EP3-1-306-U3	EP3-1-185	306	6	Test Material 1	12/13	3087.13	255.17	210	4	210	5860	1231
EP3-1-307-U3	EP3-1-182	307	6	Test Material 1	12/13	3087.13	247.01	67	1	67	16480	1104

NOTE: Urine samples EP3-1-134 and EP3-1-160 were inadvertently combined into a single sample prior to analysis. Thus, results shown here represent the mean concentration of the two samples combined.

EP3-1-312-U2	EP3-1-134	312	2	Sodium Arsenate	9/10	643.15	57.05	60	1	60	10690	641
EP3-1-318-U2	EP3-1-160	318	2	Sodium Arsenate	9/10	643.15	54.17	61	1	61	6220	379

*Non-detects taken at one-half the detection limit.

TABLE A-8 ARSENIC ANALYTICAL RESULTS FOR QUALITY CONTROL SAMPLES

Blind Duplicates

Tag Number	Reported As Conc	DL	Units	Pig Number	Original Pig #	Group	Event/Day
EP3-1-130	99	1	Ng/ml	2312	312	2	U1
EP3-1-112	107	1	Ng/ml	2310	310	3	U1
EP3-1-108	140	2	Ng/ml	2306	306	6	U1
EP3-1-136	2	1	Ng/ml	2317	317	1	U2
EP3-1-154	19	1	Ng/ml	2327	327	2	U2
EP3-1-156	260	4	Ng/ml	2309	309	4	U2
EP3-1-191	170	2	Ng/ml	2314	314	3	U3
EP3-1-172	103	1	Ng/ml	2301	301	5	U3
EP3-1-162	150	2	Ng/ml	2303	303	6	U3

Performance Evaluation Samples

Tag Number	Reported As Conc	DL	Units	QC Sample	Nominal PE Conc
EP3-1-181	2	1	Ng/ml	Control Urine	0
EP3-1-103	2	1	Ng/ml	Control Urine	0
EP3-1-184	200	4	Ng/ml	Sodium arsenate	200
EP3-1-132	23	1	Ng/ml	Sodium arsenate	20
EP3-1-124	120	2	Ng/ml	Sodium arsenate	100
EP3-1-180	22	1	Ng/ml	Sodium arsenite	20
EP3-1-153	110	2	Ng/ml	Sodium arsenite	100
EP3-1-121	190	2	Ng/ml	Sodium arsenite	200
EP3-1-187	100	2	Ng/ml	Dimethyl arsenic acid	100
EP3-1-144	200	4	Ng/ml	Dimethyl arsenic acid	200
EP3-1-117	22	1	Ng/ml	Dimethyl arsenic acid	20
EP3-1-188	220	4	Ng/ml	Disodium methylarsenate	200
EP3-1-142	100	2	Ng/ml	Disodium methylarsenate	100
EP3-1-109	23	1	Ng/ml	Disodium methylarsenate	20

Laboratory Spikes

Tag Number	Spiked As Conc	DL	Units	Nominal Spike Amount
EP3-1-110	290	4	Ng/ml	200
EP3-1-120	489	4	Ng/ml	200
EP3-1-130	310	4	Ng/ml	200
EP3-1-140	210	4	Ng/ml	200
EP3-1-150	210	4	Ng/ml	200
EP3-1-160	270	4	Ng/ml	200
EP3-1-170	589	4	Ng/ml	200
EP3-1-180	230	4	Ng/ml	200
EP3-1-186	330	4	Ng/ml	200
EP3-1-192	360	4	Ng/ml	200
EP3-1-409	10	0.2	mcg/g	9.96
EP3-1-412	39	1	Ng/ml	40

Laboratory Duplicates

Tag Number	Duplicate As Conc	DL	Units
EP3-1-105	57	1	Ng/ml
EP3-1-115	270	4	Ng/ml
EP3-1-125	65	1	Ng/ml
EP3-1-135	180	2	Ng/ml
EP3-1-145	140	1	Ng/ml
EP3-1-155	50	1	Ng/ml
EP3-1-165	93	1	Ng/ml
EP3-1-175	220	4	Ng/ml
EP3-1-183	370	4	Ng/ml
EP3-1-189	97	1	Ng/ml
EP3-1-407	0.06	0.05	mcg/g
EP3-1-410	<1	1	Ng/ml

Laboratory Control Standards

Tag Number	Reported As Conc	DL	Units	SRMID	Certified Mean
QC-1	5	3	Ng/ml	NIST 2670a-L	3
QC-2	<3	3	Ng/ml	NIST 2670a-L	3
QC-3	240	10	Ng/ml	NIST 2670a-H	220 ± 10
QC-4	230	10	Ng/ml	NIST 2670a-H	220 ± 10
QC-5	240	10	Ng/ml	NIST 2670a-H	220 ± 10
EP3-1-1566	7.5	0.1	mcg/g	NIST 1566b	7.65 ± 0.65
EP3-1-415	26	1	Ng/ml	NIST 1640	26.7 ± 0.41

Blanks

Tag Number	Reported As Conc	DL	Units
Blank-1	<1	1	Ng/ml
Blank-2	<1	1	Ng/ml
Blank-3	<1	1	Ng/ml
Blank-4	<1	1	Ng/ml
Blank-5	<1	1	Ng/ml
Blank-6	<0.05	0.05	mcg/g
Blank-7	<1	1	Ng/ml