RELATIVE BIOAVAILABILITY OF ARSENIC AND VANADIUM IN SOIL FROM A SUPERFUND SITE IN PALESTINE, TEXAS

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EXECUTIVE SUMMARY

A study using juvenile swine as test animals was performed to measure the gastrointestinal absorption of arsenic and vanadium from soil collected from a Superfund site in Palestine, Texas. The relative bioavailability of arsenic and vanadium was assessed by comparing the absorption of arsenic or vanadium from the test soil to that of a reference material (sodium arsenate or vanadyl sulfate). Groups of five swine were given oral doses of sodium arsenate, vanadyl sulfate, or the test soil twice a day for 15 days; a group of three non-treated swine served as a control. The arsenic concentration in the test soil was 47 µg/g and the vanadium concentration was 121 µg/g.[1]

Arsenic

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 linear regression analysis. The relative bioavailability (RBA) of arsenic in the test soil compared to that in sodium arsenate was calculated as follows:

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

The results are summarized below:

Measurement Endpoint	Estimated Soil RBA (90% Confidence Interval)
Days 6/7	0.19 (0.17 - 0.21)
Days 9/10	0.16 (0.14 - 0.19)
Days 12/13	0.13 (0.11 - 0.15)
All Days	0.15 (0.14 - 0.16)

Using sodium arsenate as a relative frame of reference, the RBA estimate for the test soil is approximately 15%. This value is markedly lower than the default value range of 80%-100% for arsenic in soil that is usually employed when reliable site-specific data are lacking. This indicates that the arsenic in this soil is not as well absorbed as soluble arsenic.

[1] Due to an insufficient quantity of soil provided at the start of the study, the primary soil sample was used for dosing on days 0-11 only. For the final dose preparation (administered on days 12-14), the remaining soil was mixed with additional soil obtained from the supplier. The arsenic concentration of this combined soil sample was 62 µg/g and the vanadium concentration was 147 µg/g.

Vanadium

The amount of vanadium absorbed by each animal was evaluated by measuring the concentration of vanadium in liver, kidney, and bone (measured on day 15 at study termination). The dose-response data for vanadium in each tissue were modeled using a linear equation. RBA for each tissue was calculated as the ratio of the slope term from the test soil equation to the slope term from the vanadyl sulfate equation. The suggested point estimate is calculated as the simple mean of the three endpoint-specific estimates. The results are summarized below:

Measurement Endpoint	Estimated Soil RBA (90% Confidence Interval)
Liver Vanadium	0.08 (0.06 - 0.10)
Kidney Vanadium	0.06 (0.05 - 0.08)
Bone Vanadium	0.08 (0.06 - 0.10)
Point Estimate	0.08 (0.06 - 0.10)

Using vanadyl sulfate as a relative frame of reference the RBA point estimate for the test soil is approximately 8%. This value indicates that the vanadium in the test soil is not as well absorbed as soluble vanadium.

These relative bioavailability estimates may be used to improve accuracy and decrease uncertainty in estimating human health risks from exposure to this test soil.

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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-AES	Inductively coupled plasma atomic emission spectroscopy
kg	Kilogram
K_u	Fraction of absorbed arsenic which is excreted in urine
mL	Milliliter
MMA	Monomethyl arsenic
N	Number of data points
QA	Quality assurance
RBA	Relative bioavailability
ref	Reference material
RfD	Reference dose
SD	Standard deviation
SF	Slope factor
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:
>
> $$ABA = \frac{Absorbed\ Dose}{Ingested\ Dose}$$
>
> 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 relative bioavailability (RBA) of a chemical in a site medium (e.g., soil), this information can be used to improve the accuracy of exposure and risk calculations at that site. Available RBA data can be used to adjust default oral toxicity values (reference dose and slope factor) to account for differences in absorption between the chemical ingested in water and the chemical ingested in site media, assuming the toxicity factors are based on a readily soluble form of the chemical. 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 to determine the RBA of arsenic and vanadium in soil collected from a Superfund site in Palestine, Texas compared to a soluble form of arsenic (sodium arsenate) and vanadium (vanadyl sulfate).

2.0 STUDY DESIGN

This investigation of arsenic and vanadium RBA was performed according to the basic design presented in Table 2-1. The study investigated arsenic and vanadium absorption from sodium arsenate ($NaHAsO_4$), vanadyl sulfate ($VOSO_4$), and a test material (TM1). Each material was administered to groups of five animals at three different dose levels for 15 days (a detailed schedule is presented in Appendix A, Table A-1). Additionally, the study included a non-treated group of three animals to serve as a control for determining background arsenic and vanadium 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:

RBA(test material) = UEF(test material) / UEF(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 Material

2.1.1 Sample Description

The test material used in this investigation was a soil sample collected from a Superfund site in Palestine, Texas. Due to an insufficient quantity of soil provided at the start of the study, the initial soil sample was only used for dosing on days 0-11. The final dose (administered on days 12-14) used the remaining soil mixed with new, additional soil obtained from the supplier.

2.1.2 Sample Preparation

The soil sample was sieved through a 250 micrometer (μm) sieve prior to test substance analysis and characterization. Only material that passed through the sieve (corresponding to particles smaller than about 250 μm) were used in the bioavailability study. The study was limited to this fine-grained soil fraction because it is believed that soil particles less than about 250 μm are most likely to adhere to the hands and be ingested by hand-to-mouth contact, especially in young children.

2.1.3 Arsenic and Vanadium Concentrations

The dried and sieved soil samples were analyzed for arsenic and vanadium by L. E. T., Inc., (Columbia, Missouri). Arsenic and vanadium concentrations were measured in duplicate by inductively coupled plasma atomic emission spectroscopy (ICP-AES). The resulting mean arsenic values were 47 μg/g for the initial soil sample and 62 μg/g in the supplementary combined soil sample. The resulting mean vanadium values were 121 μg/g in the initial soil sample and 147 μg/g in the supplementary combined soil sample.

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) six days prior to exposure (day -6) 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-2).

When exposure began (day zero), the animals were about 6-7 weeks old and weighed an average of about 10.5 kilograms (kg). The animals were weighed every three days during the course of the study. On average, animals gained about 0.37 kg/day and the rate of weight gain was comparable in all dosing groups, ranging from 0.32 to 0.44 kg/day. These body weight data are presented in Appendix A, Table A-3, and summarized in Figure 2-1.

All animals were examined daily by an attending veterinarian while on study. Most animals (N = 41) exhibited no problems throughout the study. Several animals (N = 7) exhibited elevated temperatures, diarrhea, and/or anorexia and were treated with Naxcel for a duration of 3 days (see Appendix A, Table A-4).

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 feed originally developed for lead RBA studies (purchased from Zeigler Brothers, Inc., Gardners, Pennsylvania), 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. The typical nutritional components and chemical analysis of the feed are

presented in Table 2-2. Each day every animal was given an amount of feed equal to 4% of the mean body weight of all animals on study, except for animals dosed with soil (groups 4-6), which received an amount of feed equal to 3.7% of the mean body weight of all animals (to compensate for the extra feed required when dosing with soil). Feed amounts were adjusted every three days, when pigs 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 0.2 μg/g; vanadium concentrations did not exceed 1.0 ug/g.

Drinking water was provided *ad libitum* (i.e., free feeding) via self-activated watering nozzles within each cage. Analysis of samples from randomly selected drinking water nozzles indicated the arsenic and vanadium concentrations were below a level of detection.

2.4 Dosing

The protocol for exposing animals to arsenic and vanadium is shown in Table 2-1. Animals were exposed to dosing materials (sodium arsenate, vanadyl sulfate, test soil) for 15 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), with two minute intervals allowed for individual pig dosing. 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. The doughballs were administered to the animals by hand.

Occasionally, some animals did not consume their entire dose and there were some difficulties with doughball preparation. 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-3).

Due to an insufficient quantity of soil provided at the start of the study, the initial soil sample was only used for dosing on days 0-11. For the final dose preparation (administered on days 12-14), the remaining soil was mixed with additional soil obtained from the supplier. However, there was still insufficient soil to prepare the second half of the day 14 dosing, so no animals received the 3:00 PM dose on day 14.

Administered amounts of dose materials were based on the arsenic or vanadium concentration in the dosing materials and the measured group mean body weights. Specifically, the amount of dosing material to be administered for the three days following each weighing was based on the group mean body weight adjusted by the addition of 1 kg to account for the expected weight gain over each time interval. After completion of the study, body weights were estimated by interpolation for those days when measurements were not collected and the actual administered doses were calculated for each day and then 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 pig are presented in Appendix A, Tables A-6 (arsenic) and A-7 (vanadium).

2.5 Collection of Biological Samples

Urine

Samples of urine were collected from each animal for 48-hour periods on days 6 to 7 (U1), 9 to 10 (U2), and 12 to 13 (U3) of the study. Collection began at 9:00 AM and ended 48 hours later. The urine was collected in a stainless steel pan placed beneath each cage, which drained into a plastic storage bottle. Each collection pan was fitted with a nylon screen 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 holding container 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-8) and three 60-milliliter (mL) portions were removed and acidified with 0.6 mL concentrated nitric acid. Two of the aliquots were archived in the refrigerator and one aliquot was sent for arsenic analysis. All samples were refrigerated until arsenic analysis.

Liver, Kidney, and Bone

On day 15, all animals were humanely euthanized and samples of liver, kidney, and bone (the right femur, defleshed) were removed and stored at -80 degrees Celsius (°C) in plastic bags for vanadium analysis.

Subsamples of all biological samples collected were archived in order to allow for reanalysis and verification of arsenic or vanadium levels, if needed. All animals were also subjected to detailed examination at necropsy by a certified veterinary pathologist in order to assess overall animal health. All samples were assigned random chain-of-custody tag numbers and submitted to the analytical laboratory for analysis in a blind fashion.

2.6 Analysis of Biological Samples

Urine

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-9.

Liver and Kidney

Five grams of liver were placed in a screw-cap Teflon container with 5 mL of concentrated (70%) nitric acid and heated in an oven to 90°C overnight. After cooling, the digestate was transferred to a clean 50 mL volumetric flask and diluted to volume with deionized distilled water. The same procedure was followed for kidney, except quantities were halved due to less tissue available.

Bone

The right femur of each animal was defleshed, broken, and dried at 100°C overnight. The dried bones were then placed in a muffle furnace and dry-ashed at 450°C for 48 hours. Following dry ashing, the bone was ground to a fine powder using a mortar and pestle, and 200 mg was removed and dissolved in 10.0 mL of 1:1 (volume:volume) concentrated nitric acid/water. After the powdered bone was dissolved and mixed, 5.0 mL of the acid solution was removed and diluted to 25.0 mL in deionized distilled water.

Liver, kidney, and bone samples and other materials (e.g., food, water, reagents, solutions) were analyzed for vanadium by ICP-AES. Vanadium analytical results for study samples are presented in Appendix A, Table A-10. 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 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-11, and are summarized below.

Spike Recovery

Randomly selected samples were spiked with known amounts of arsenic (sodium arsenate) or vanadium (vanadyl sulfate) and the recovery of the added analyte was measured. Arsenic recovery for individual samples ranged from 101% to 113%, with an average of 106 ± 4.1% (N = 9). Vanadium recovery for individual samples ranged from 113% to 134%, with an average of 119 ± 8.3% (N = 6).

Laboratory Duplicates

Periodically during arsenic analysis, urine samples were randomly selected by the analyst for duplicate analysis (i.e., the same prepared sample was analyzed twice). Urinary arsenic duplicates had a percent deviation of 0% to 9.5%, with an average of 2.1% ± 3.3% (N = 11).

In addition, a random selection of about 20% of all tissue samples (liver, kidney, and femur) generated during the study were prepared for vanadium analysis in duplicate (i.e., two separate subsamples of tissue were prepared for analysis); the identity of these samples was known by the

analytical laboratory. Tissue vanadium duplicates had a percent deviation of 0% to 17%, with an average of 8.3% ± 5.2% (N = 9).

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. There was good agreement between results for the duplicate pairs.

No blind duplicates of liver, kidney, or femur samples were submitted to the analytical laboratory for vanadium analysis.

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 for the standards are summarized below:

Analyte	Standard	Certified Mean ± SD	Mean	SD	Mean % Recovery	N
Arsenic	NIST 1566b	7.65 ± 0.65	7.9	0.07	102.6%	2
	NIST 1640	.0267 ± 0.0004	0.030	0.001	110.5%	2
	NRCC TORT-2	21.6 ± 1.8	21.0	0.0	97.2%	2
Vanadium	NIST 1640	.01299 ± 0.0004	0.013	0.0	100.1%	6
	NRCC TORT-2	1.64 ± 0.19	1.70	0.0	103.7%	4

SD = Standard deviation
N = Number of data points used in curve fitting

As seen, recovery of arsenic and vanadium 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 or vanadium (N = 16).

Summary of QA Results

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 and vanadium absorption from the test material.

3.0 DATA ANALYSIS FOR ARSENIC

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 (μg/day) as a function of the administered amount of arsenic (μg/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 (μg/day excreted per μg/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). According to Finney (1978), when the data to be analyzed consist of two dose-response curves (the reference material and the test material), both curves must have the same intercept because 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:

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

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

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, an analysis was made by looking at responses that yielded standardized weighted residuals greater than 3.5 or less than -3.5 (Canavos,1984). When such data points were encountered in a data set, the UEF and 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 Arsenic 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 DATA ANALYSIS FOR VANADIUM

4.1 Overview

The basic approach for measuring vanadium absorption *in vivo* is to administer an oral dose of vanadium to test animals and measure the increase in vanadium levels in one or more body compartments (e.g., soft tissue, bone). In order to calculate the RBA value of a test material, the increase in vanadium in a body compartment is measured both for that test material and a reference material (vanadyl sulfate). Because equal absorbed doses of vanadium will produce equal responses (i.e., equal increases in concentration in tissues) regardless of the source or nature of the ingested vanadium, the RBA of a test material is calculated as the ratio of doses (test material and reference material) that produce equal increases in vanadium concentration in the body compartment. Thus, the basic data reduction task required to calculate an RBA for a test material is to fit mathematical equations to the dose-response data for both the test material and the reference material, and then solve the equations to find the ratio of doses that would be expected to yield equal responses.

The curve-fitting methods and rationale, along with the methods used to quantify uncertainty in the RBA estimates, are summarized below.

4.2 Measurement Endpoints

Three independent measurement endpoints were evaluated based on the concentration of vanadium observed in liver, kidney, and bone (femur). The measurement endpoint was the concentration in the tissue at the time of sacrifice (day 15).

4.3 Dose-Response Model

Basic Equation

Selection of an appropriate dose-response model and weighting factors requires data from multiple studies and, in contrast to arsenic for which multiple studies support the use of a linear dose-response model, data are only available for a single vanadium study. Therefore, the vanadium data set was evaluated using weighted linear regression, which was selected for most endpoints investigated by USEPA, including liver, kidney, and bone lead (USEPA, 2007). Indeed, inspection of the data (see Figures 5-10, 5-11, and 5-12) suggested that they could be well-fit using a linear equation.

Simultaneous Regression

Similar to arsenic analysis, data analysis consists of two dose-response curves for each endpoint (the reference material and test material) and because there is no difference between the curves when the dose is zero, both curves for a given endpoint must have the same intercept. This

requirement is achieved by combining the two dose response equations into one and solving for the parameters simultaneously, resulting in the following equation:

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

where:

y = response
x = dose
a, b = empirical coefficients for reference material (r) and test material (t)

All model fitting was performed using JMP® version 3.2.2, a commercial software package developed by SAS®.

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

As discussed previously for arsenic (Section 3.2), the preferred method for estimating the value of σ_i^2 uses 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. However, because vanadium data are only available from a single study, it was not possible to develop an external variance model. Instead, the observed variance (s_i^2) in the responses of animals in dose group i was used to estimate the value of σ_i^2.

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, an analysis was made by looking at responses that yielded standardized weighted residuals greater than 3.5 or less than -3.5 (Canavos,1984). When such data points were encountered in a data set, the UEF and 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.

4.4 Calculation of Vanadium RBA Estimate

Endpoint-specific RBA Estimates

Vanadium RBA values were estimated using the basic statistical techniques recommended by Finney (1978). Each endpoint-specific RBA value was calculated as the ratio of the slope term for the test material data set (b_t) to 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).

RBA Point Estimate

Because there are three independent estimates of RBA for the test material (one from each measurement endpoint), the final RBA estimate involves combining the three endpoint-specific RBA values into a single value (point estimate) and estimating the uncertainty around that point estimate. As reflected in the coefficient of variation for endpoint-specific RBA estimate, the three endpoint-specific RBA values are all approximately equally reliable. Therefore, the RBA point estimate for each test material was calculated as the simple mean of all three endpoint-specific RBA values.

The uncertainty bounds around the point estimate were estimated using Monte Carlo simulation. Values for RBA were drawn from the uncertainty distributions for each endpoint with equal frequency. Each endpoint-specific uncertainty distribution was assumed to be normal, with the mean equal to the best estimate of RBA and the standard deviation estimated from Fieller's Theorem (Finney, 1978). The uncertainty in the point estimate was characterized as the range from the 5^{th} to the 95^{th} percentile of the mean across endpoints.

5.0 RESULTS

5.1 Clinical Signs

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

5.2 Data Exclusions

Occasionally, the dilution of urine by spilled water is so large that the concentration of arsenic in the urine cannot be quantified. These instances are defined by having a urine arsenic concentration at or below the quantitation limit (2 μg/liter) and a total urine volume greater than 10,000 mL. When both of these conditions are met, the data are deemed unreliable and excluded from further calculations. In this study, one result (pig #709 from group 10 on days 12/13) was deemed unreliable for this reason and excluded from all analyses.

In addition, pig #713 (group 5, middle dose of test soil) spilled a large portion of its dose in its urine bucket on day 6. Therefore, the urine collected from this animal on days 6/7 was excluded *a priori*.

5.3 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 5-1. This data was gathered from previous RBA studies on swine. 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 $k2$.

Superimposed on Figure 5-1 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). All data were used in the initial fittings. The results of the initial fittings are shown in Figures 5-2 (days 6/7), 5-3 (days 9/10), 5-4 (days 12/13), and 5-5 (all days). Two outliers were identified based on the identification process described earlier. Outliers are identified in Figures 5-2 through 5-5. These outliers were subsequently excluded from the final evaluation for arsenic (Figures 5-6 through 5-9).

Tissue Vanadium

The dose-response data for vanadium in liver, kidney, and bone (measured at sacrifice on day 15) were modeled using a linear equation (see Section 4.3). All data were included in the initial fittings. The results of these fittings are shown in Figures 5-10 (liver), 5-11 (kidney), and 5-12 (femur). No outliers were identified in the vanadium data sets.

5.4 Calculated RBA Values

Arsenic

The dose-response curves are approximately linear (Figures 5-6 through 5-9), with the slope of the best-fit straight line being equal to the best estimate of the UEF.

As discussed previously (Section 3.1), the relative bioavailability of arsenic in a specific test material is calculated as follows:

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

The following table summarizes the estimated RBA values:

Measurement Endpoint	Estimated Soil RBA (90% Confidence Interval)
Days 6/7	0.19 (0.17 - 0.21)
Days 9/10	0.16 (0.14 - 0.19)
Days 12/13	0.13 (0.11 - 0.15)
All Days	0.15 (0.14 - 0.16)

As shown, using sodium arsenate as a relative frame of reference, the RBA estimate for the test soil is approximately 15%.

Vanadium

Vanadium RBA values were calculated for each measurement endpoint (liver, kidney, and bone) using the method described in Section 4.4; the suggested point estimate is calculated as the simple mean of the three endpoint-specific estimates. The results are shown below:

Measurement Endpoint	Estimated Soil RBA (90% Confidence Interval)
Liver Vanadium	0.08 (0.06 - 0.10)
Kidney Vanadium	0.06 (0.05 - 0.08)
Bone Vanadium	0.08 (0.06 - 0.10)
Point Estimate	0.08 (0.06 - 0.10)

As shown, using vanadyl sulfate as a relative frame of reference, the RBA estimate for the test soil is approximately 8%.

5.5 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 or vanadium 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 endpoint-specific and the point estimate values of RBA.

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 that RBA values in swine are not 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 or vanadium. 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 soil along with food. The magnitude of this bias is not known.

Dosing Anomalies

There were a few instances where some animals did not consume their entire dose (see Appendix A, Tables A-6 and A-7). During the study, however, the dosing technician observed each animal and attempted to estimate the fraction of dose not consumed; these estimates of missed doses were then used to adjust the time-weighted average dose calculation for each animal downward. Because these estimates of missed doses are subjective, they introduce some uncertainty; however, the magnitude of this uncertainty is thought to be small. All calculations are based on actual administered doses (not target doses) to compensate for dosing errors.

There was insufficient soil to prepare the second half of final dosing (day 14) dosing, so dosing for all animals was terminated after the day 14 morning dosing (i.e., no animals in any group received the 3:00 PM dose on day 14). This could result in a decrease in the magnitude of the measured vanadium concentrations in the endpoint tissues. However, because the animals were dosed for 15 days, the magnitude of this decrease is likely to be small. In addition, because the lack of dosing was applied to all groups, it is expected that any observable effect will be cancelled and it is not expected to introduce a significant error. Urine collections ended on day 13, so arsenic concentrations are unaffected by this dosing anomaly.

6.0 CONCLUSIONS AND RECOMMENDATIONS

Arsenic

When reliable site-specific data are lacking, a default RBA value in the range of 80%-100% is usually employed for arsenic in soil. The RBA estimate of 15% for the test soil used in this study is markedly lower than the default range, indicating that the arsenic in this soil is not as well absorbed as soluble arsenic. It is appropriate to take this into account when evaluating potential risks to humans from incidental ingestion of this soil.

Vanadium

Due to a general lack of data, the RBA typically employed for vanadium in soil is 100%. The RBA estimate of 8% obtained for the test soil used in this study is markedly lower than that default assumption, indicating that the vanadium in this soil is not as well absorbed as soluble vanadium. It is appropriate to take this into account when evaluating potential risks to humans from incidental ingestion of this soil.

Recommendations

These site-specific RBA estimates for arsenic and vanadium are an improvement over the default values and should be considered for use in site-specific risk assessments. However, it important to consider that the values are specific to the soil tested in this study. Use of the RBA estimates may improve accuracy and decrease uncertainty in estimating human health risks from exposure to this test soil, as well as increase confidence in computations of site-specific risk-based cleanup levels.

7.0 REFERENCES

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TABLES AND FIGURES

TABLE 2-1 DOSING PROTOCOL

Group	Number of Animals	Dose Material Administered	Arsenic Dose (µg/kg-day)		Vanadium Dose (µg/kg-day)	
			Target	Actual [a]	Target	Actual [a]
1	5	$NaHAsO_4$	30	30.4	0	0.0
2	5	$NaHAsO_4$	60	60.3	0	0.0
3	5	$NaHAsO_4$	120	121.1	0	0.0
4	5	Soil	40	42.6	103	107.8
5	5	Soil	80	84.8	206	214.3
6	5	Soil	160	165.8	412	418.9
7	5	$VOSO_4$	0	0.0	80	88.3
8	5	$VOSO_4$	0	0.0	160	162.3
9	5	$VOSO_4$	0	0.0	320	322.5
10	3	Control	0	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.

Doses were administered in two equal portions given at 9:00 AM and 3:00 PM each day. Doses were based on the mean weight of the animals in each group, and were adjusted every three days to account for weight gain.

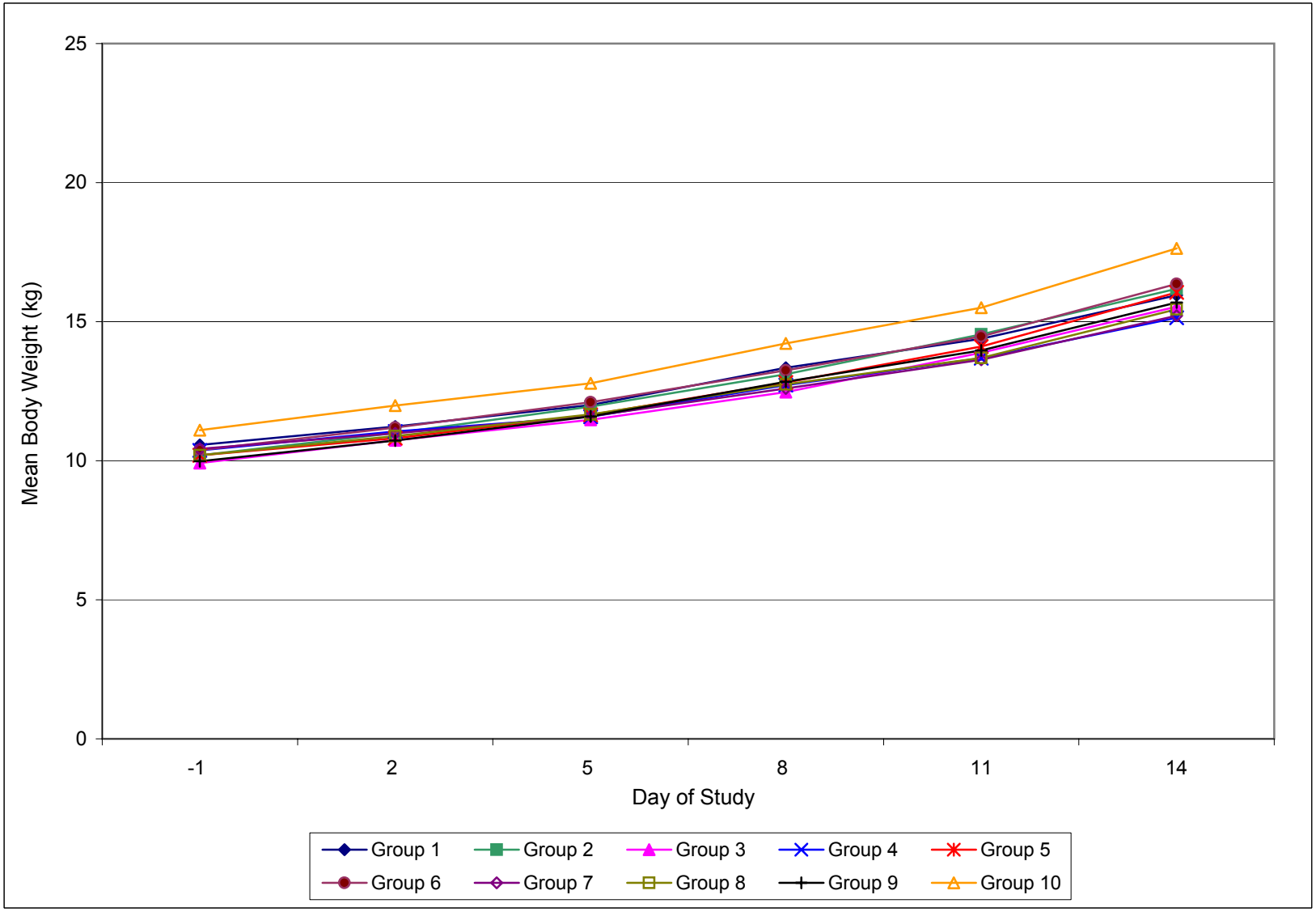
TABLE 2-2 TYPICAL FEED COMPOSITION

Nutrient Name	Amount
Protein	20.1021%
Arginine	1.2070%
Lysine	1.4690%
Methionine	0.8370%
Met+Cys	0.5876%
Tryptophan	0.2770%
Histidine	0.5580%
Leucine	1.8160%
Isoleucine	1.1310%
Phenylalanine	1.1050%
Phe+Tyr	2.0500%
Threonine	0.8200%
Valine	1.1910%
Fat	4.4440%
Saturated Fat	0.5590%
Unsaturated Fat	3.7410%
Linoleic 18:2:6	1.9350%
Linoleic 18:3:3	0.0430%
Crude Fiber	3.8035%
Ash	4.3347%
Calcium	0.8675%
Phos Total	0.7736%
Available Phosphorous	0.7005%
Sodium	0.2448%
Potassium	0.3733%

Nutrient Name	Amount
Chlorine	0.1911%
Magnesium	0.0533%
Sulfur	0.0339%
Manganese	20.4719 ppm
Zinc	118.0608 ppm
Iron	135.3710 ppm
Copper	8.1062 ppm
Cobalt	0.0110 ppm
Iodine	0.2075 ppm
Selenium	0.3196 ppm
Nitrogen Free Extract	60.2340%
Vitamin A	5.1892 kIU/kg
Vitamin D3	0.6486 kIU/kg
Vitamin E	87.2080 IU/kg
Vitamin K	0.9089 ppm
Thiamine	9.1681 ppm
Riboflavin	10.2290 ppm
Niacin	30.1147 ppm
Pantothenic Acid	19.1250 ppm
Choline	1019.8600 ppm
Pyridoxine	8.2302 ppm
Folacin	2.0476 ppm
Biotin	0.2038 ppm
Vitamin B12	23.4416 ppm

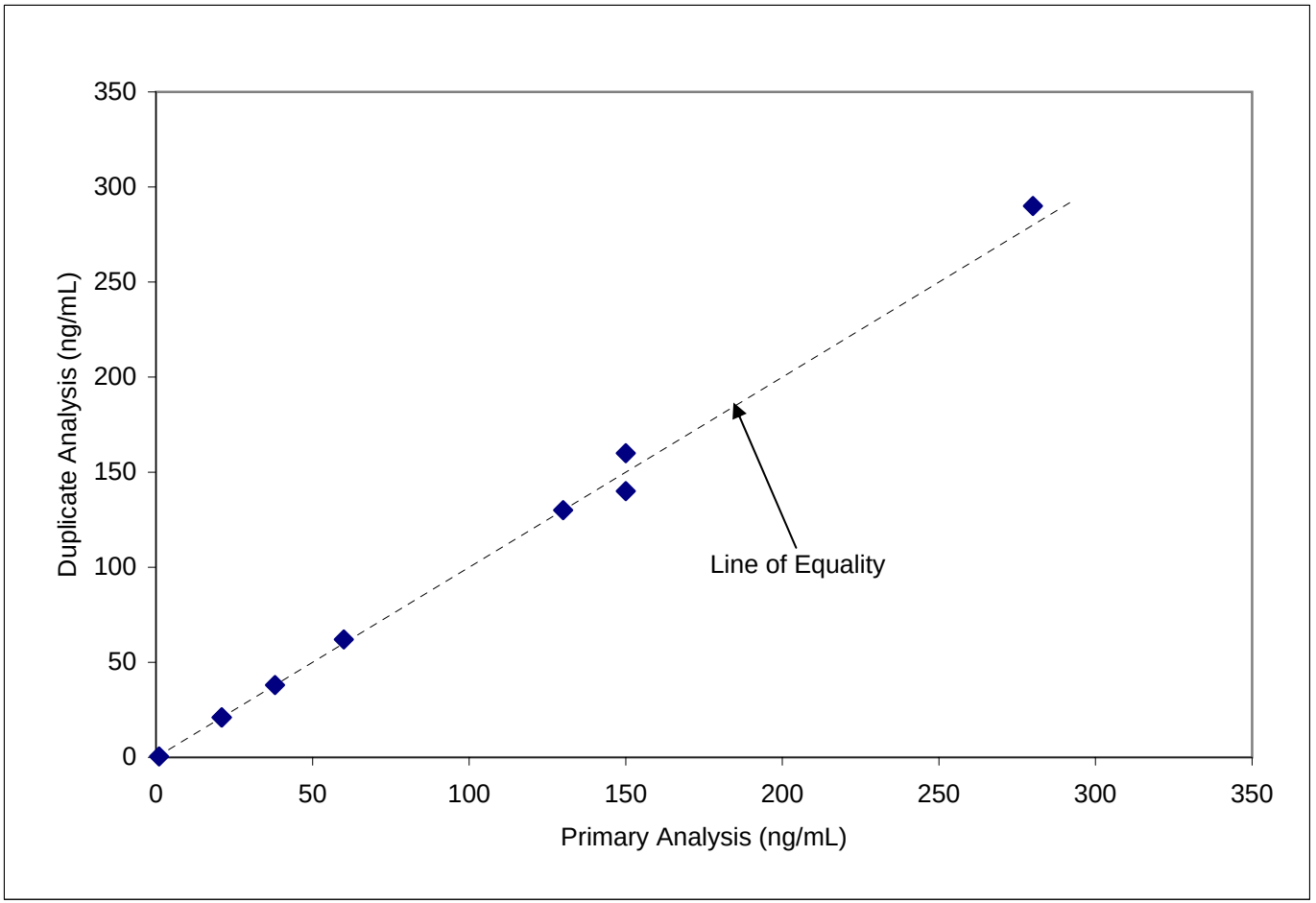
Feed obtained from and nutritional values provided by Zeigler Bros., Inc

FIGURE 2-1 BODY WEIGHT GAIN



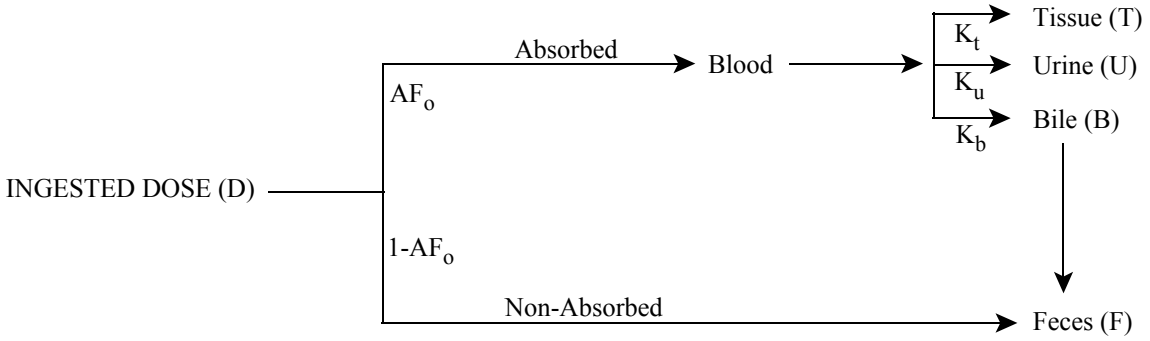
1_PTX Weights.xls (Fig2-1_group weights)

FIGURE 2-2 URINARY ARSENIC BLIND DUPLICATES (SAMPLE PREPARATION



5_PTX Lab QC analysis.xls (Fig2-2_BlindDupes)

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:

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

Amount Excreted (ug) = Amount absorbed $\times K_u$

= $D \times AF_o \times K_u$

Urinary Excretion Fraction (UEF) = Amount excreted / Amount Ingested

= $(D \times AF_o \times K_u) / D$

= $AF_o \times K_u$

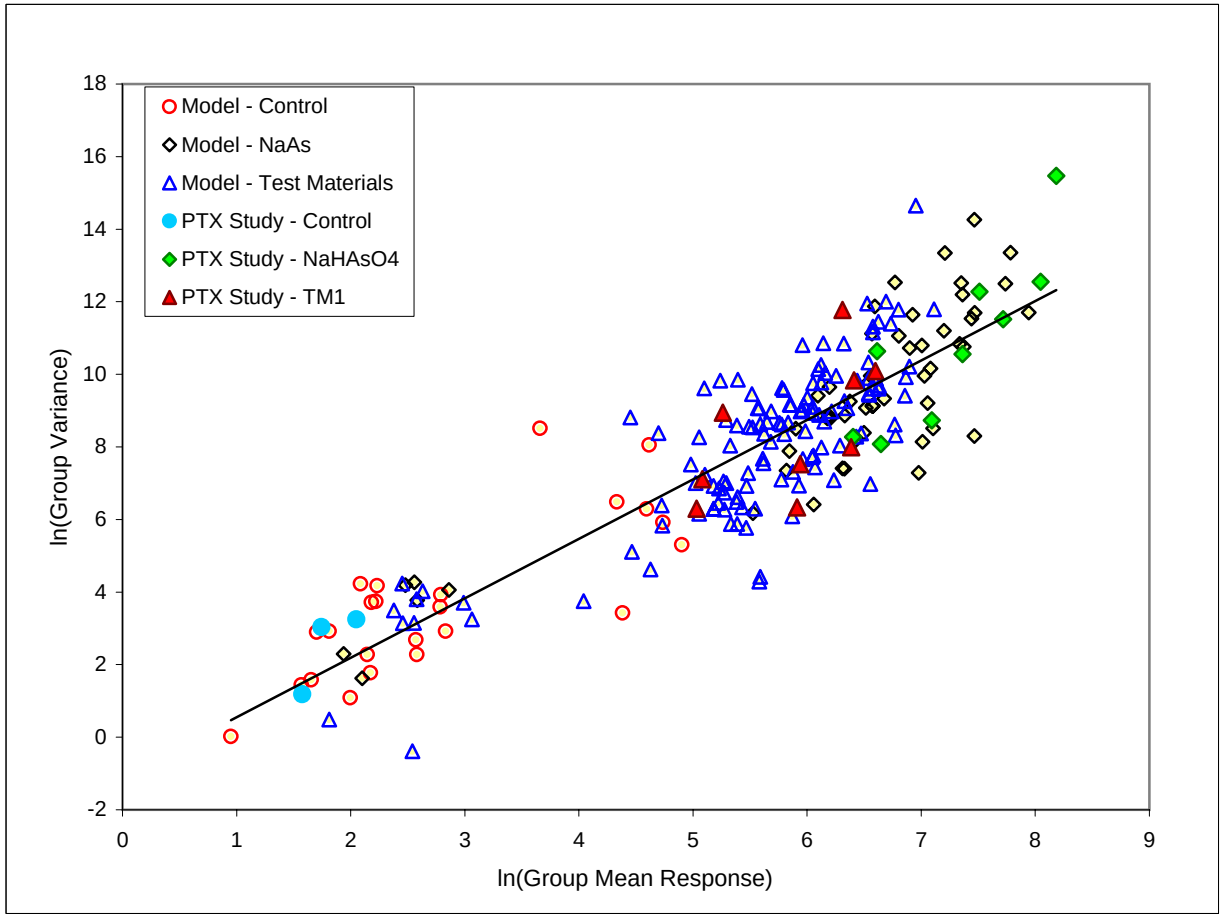
Relative Bioavailability (x vs. y) = UEF(x) / UEF(y)

= $(AF_o(x) \times K_u) / (AF_o(y) \times K_u)$

= $AF_o(x) / AF_o(y)$

Fig 3-1_Toxicokinetics.wpd

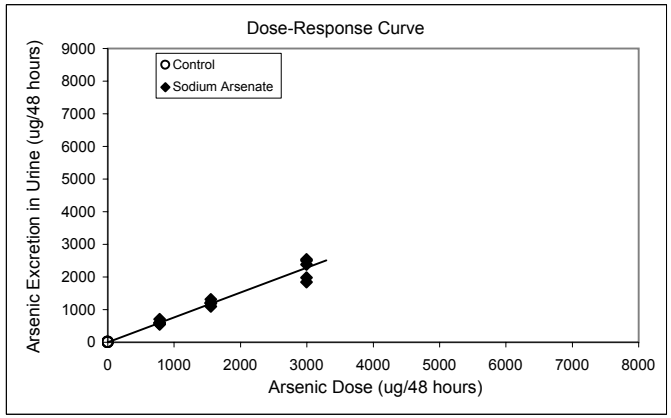
FIGURE 5-1 URINARY ARSENIC VARIANCE



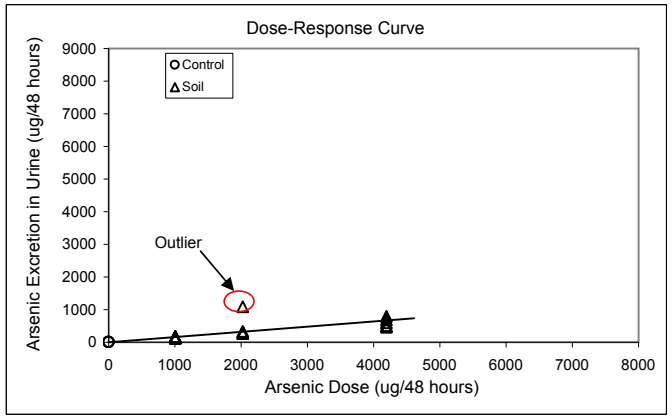
4_PTX Variance.xls (Fig A-5_PhIII Oral)

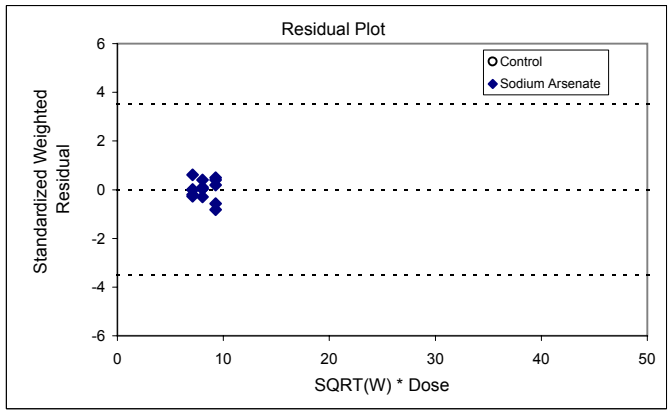
FIGURE 5-2 URINARY EXCRETION OF ARSENIC: Days 6/7 (All Data)

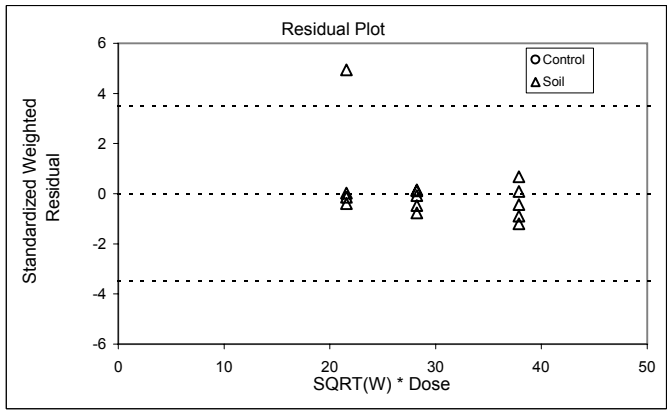
Reference Material (Sodium Arsenate)



Test Material (Soil)







Summary of Fitting[a]

Parameter	Estimate	SE
a	4.9	2.1
b1	0.76	0.05
b2	0.16	0.01
Covariance (b1,b2)	0.0018	--
Degrees of Freedom	30	--

[a] y = a + b1*x1 + b2*x2

ANOVA

Source	SSE	DF	MSE
Fit	909.30	2	454.65
Error	83.85	29	2.89
Total	993.15	31	32.04

Statistic	Estimate
F	157.242
p	< 0.001
Adjusted R^2	0.9097

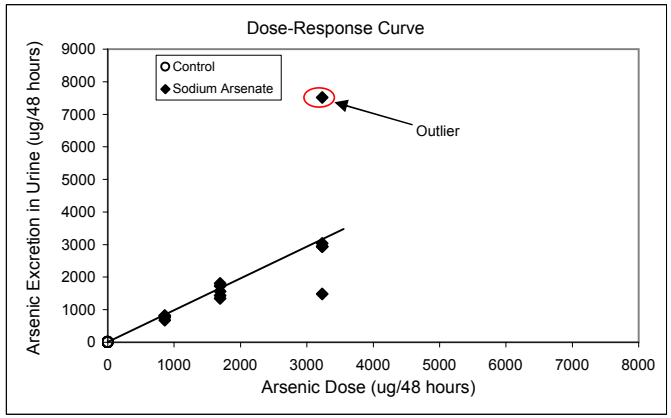
RBA and Uncertainty

Test Material (Soil)	
RBA	0.21
Lower bound[b]	0.17
Upper bound[b]	0.25
Standard Error[b]	0.025

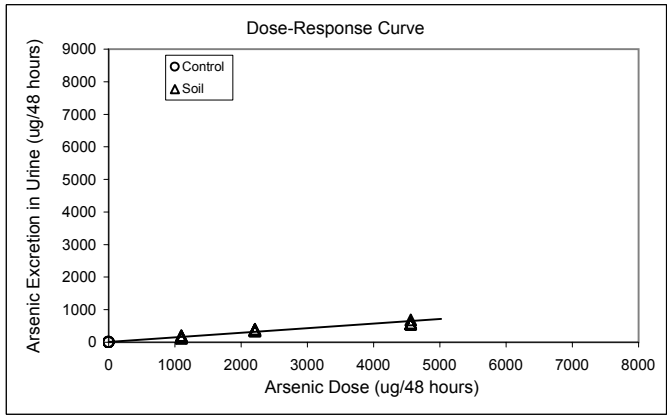
[b] Calculated using Fieller's theorem

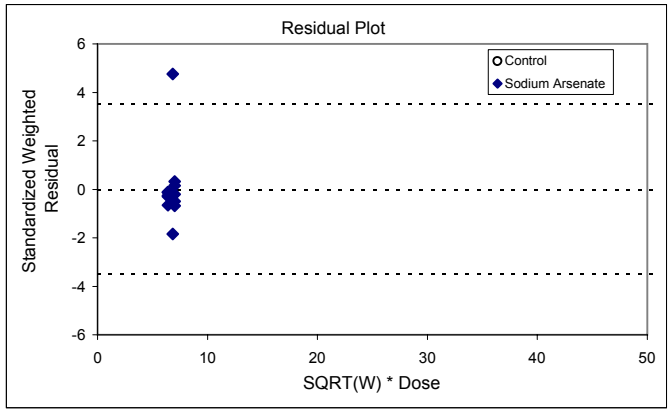
FIGURE 5-3 URINARY EXCRETION OF ARSENIC: Days 9/10 (All Data)

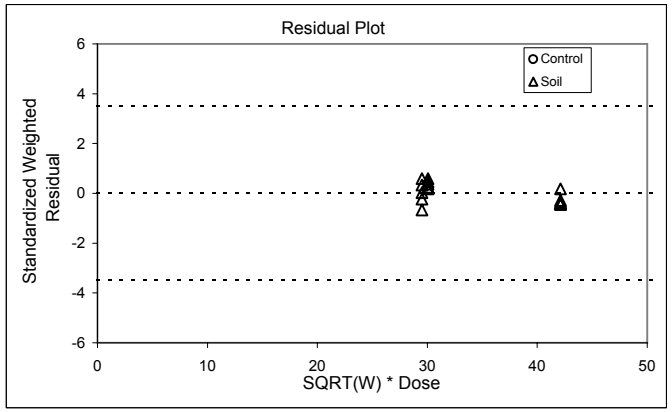
Reference Material (Sodium Arsenate)



Test Material (Soil)







Summary of Fitting[a]

Parameter	Estimate	SE
a	5.7	2.8
b1	0.98	0.08
b2	0.14	0.02
Covariance (b1,b2)	0.0020	--
Degrees of Freedom	31	--

[a] y = a + b1*x1 + b2*x2

ANOVA

Source	SSE	DF	MSE
Fit	1003.04	2	501.52
Error	119.23	30	3.97
Total	1122.27	32	35.07

Statistic	Estimate
F	126.192
p	< 0.001
Adjusted R^2	0.8867

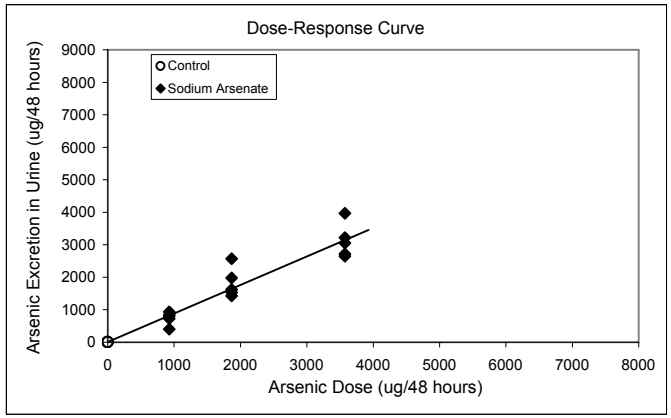
RBA and Uncertainty

Test Material (Soil)	
RBA	0.14
Lower bound[b]	0.11
Upper bound[b]	0.18
Standard Error[b]	0.019

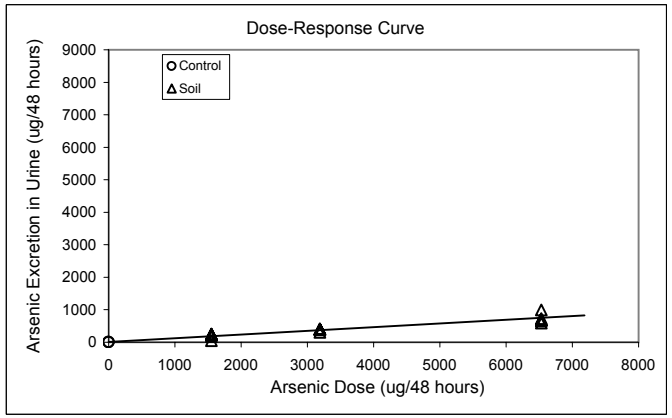
[b] Calculated using Fieller's theorem

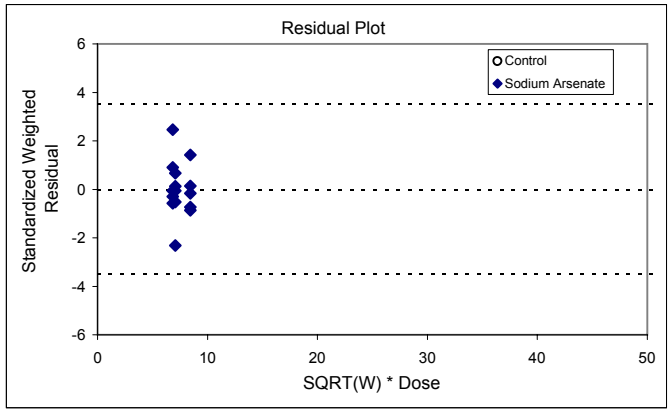
FIGURE 5-4 URINARY EXCRETION OF ARSENIC: Days 12/13 (All Data)

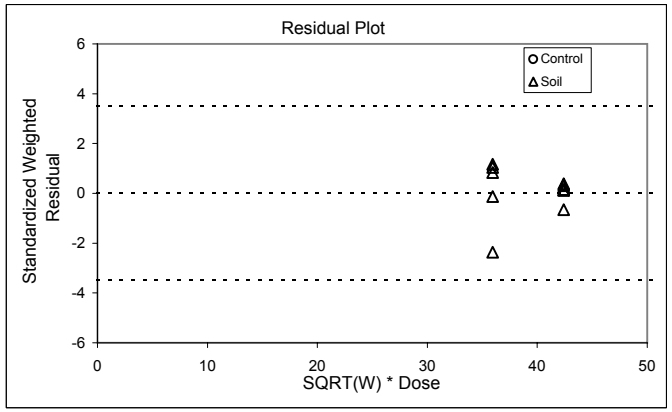
Reference Material (Sodium Arsenate)



Test Material (Soil)







Summary of Fitting[a]

Parameter	Estimate	SE
a	9.2	3.5
b1	0.88	0.05
b2	0.11	0.01
Covariance (b1,b2)	0.0056	--
Degrees of Freedom	30	--

[a] y = a + b1*x1 + b2*x2

ANOVA

Source	SSE	DF	MSE
Fit	1006.66	2	503.33
Error	58.60	29	2.02
Total	1065.26	31	34.36

Statistic	Estimate
F	249.093
p	< 0.001
Adjusted R^2	0.9412

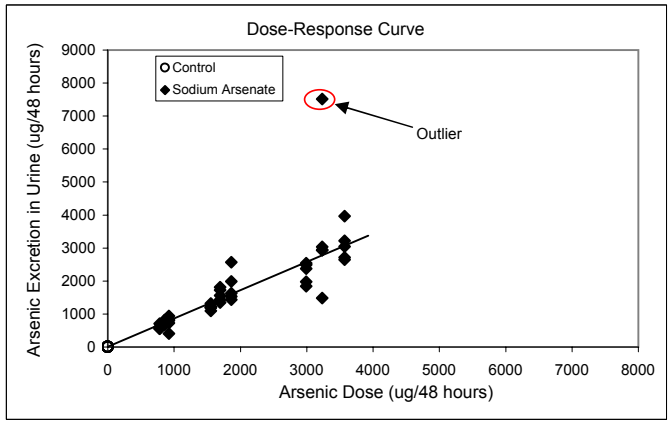
RBA and Uncertainty

Test Material (Soil)	
RBA	0.13
Lower bound[b]	0.11
Upper bound[b]	0.15
Standard Error[b]	0.012

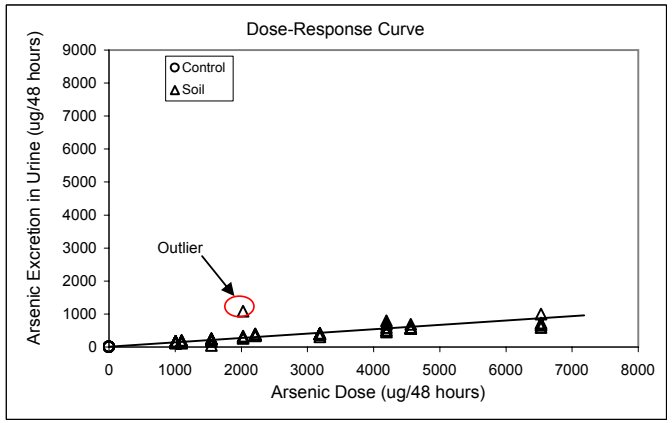
[b] Calculated using Fieller's theorem

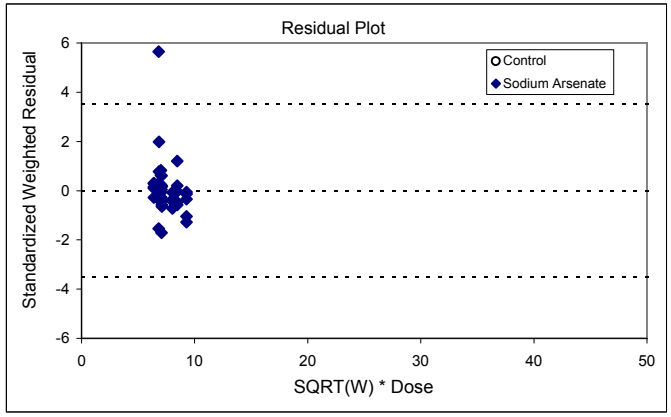
FIGURE 5-5 URINARY EXCRETION OF ARSENIC: All Days (All Data)

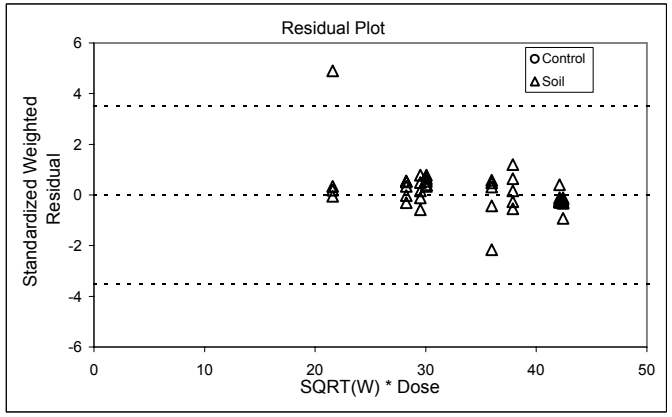
Reference Material (Sodium Arsenate)



Test Material (Soil)







Summary of Fitting[a]

Parameter	Estimate	SE
a	5.8	1.5
b1	0.86	0.04
b2	0.13	0.01
Covariance (b1,b2)	0.0023	--
Degrees of Freedom	95	--

[a] y = a + b1*x1 + b2*x2

ANOVA

Source	SSE	DF	MSE
Fit	2894.30	2	1447.15
Error	302.37	94	3.22
Total	3196.67	96	33.30

Statistic	Estimate
F	449.890
p	< 0.001
Adjusted R^2	0.9034

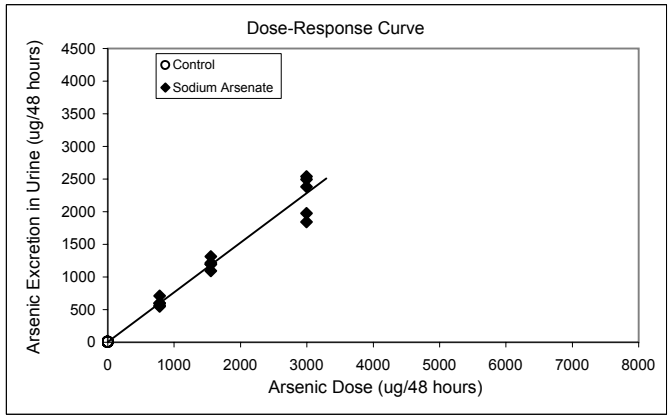
RBA and Uncertainty

Test Material (Soil)	
RBA	0.15
Lower bound[b]	0.14
Upper bound[b]	0.17
Standard Error[b]	0.011

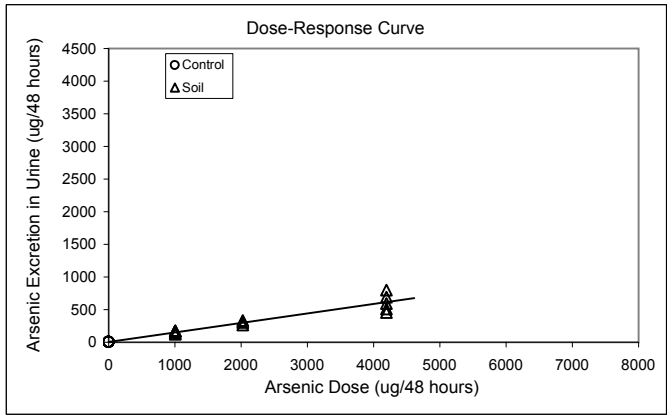
[b] Calculated using Fieller's theorem

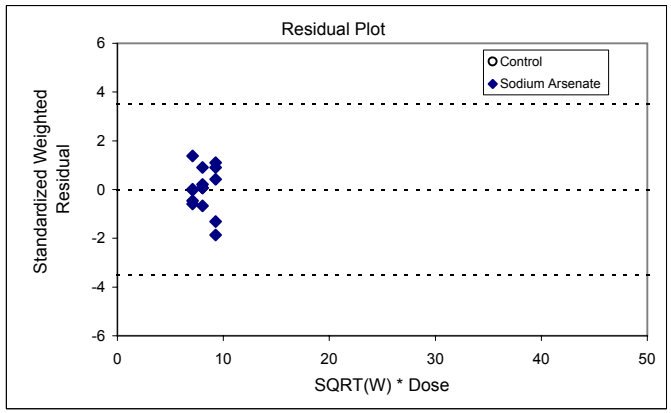
FIGURE 5-6 URINARY EXCRETION OF ARSENIC: Days 6/7 (Outliers Excluded)

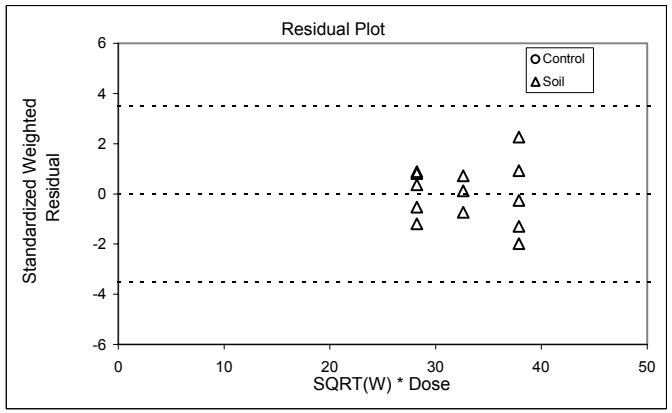
Reference Material (Sodium Arsenate)



Test Material (Soil)







Summary of Fitting[a]

Parameter	Estimate	SE
a	4.9	0.9
b1	0.76	0.02
b2	0.15	0.01
Covariance (b1,b2)	0.0018	--
Degrees of Freedom	29	--

[a] y = a + b1*x1 + b2*x2

ANOVA

Source	SSE	DF	MSE
Fit	882.36	2	441.18
Error	15.82	28	0.57
Total	898.18	30	29.94

Statistic	Estimate
F	780.745
p	< 0.001
Adjusted R^2	0.9811

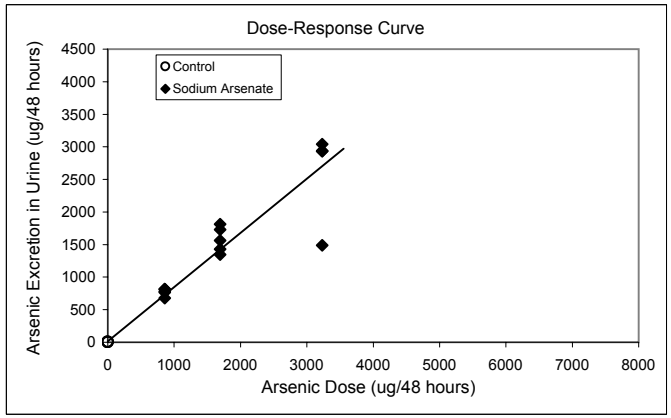
RBA and Uncertainty

Test Material (Soil)	
RBA	0.19
Lower bound[b]	0.17
Upper bound[b]	0.21
Standard Error[b]	0.010

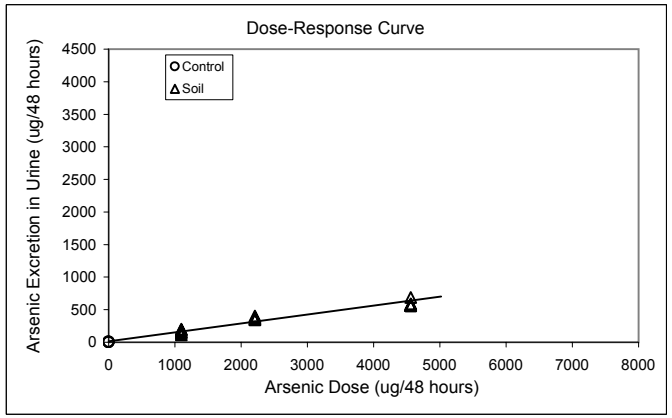
[b] Calculated using Fieller's theorem

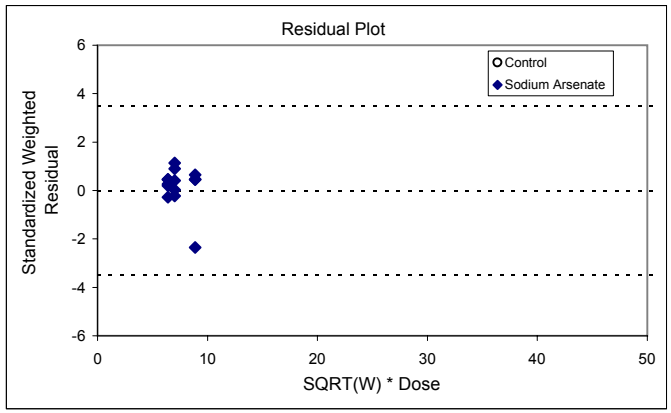
FIGURE 5-7 URINARY EXCRETION OF ARSENIC: Days 9/10 (Outliers Excluded)

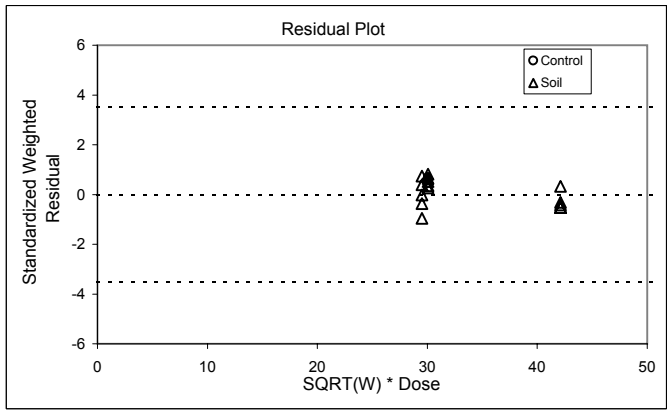
Reference Material (Sodium Arsenate)



Test Material (Soil)







Summary of Fitting[a]

Parameter	Estimate	SE
a	13.2	17.8
b1	0.83	0.04
b2	0.14	0.01
Covariance (b1,b2)	0.1267	--
Degrees of Freedom	30	--

[a] y = a + b1*x1 + b2*x2

ANOVA

Source	SSE	DF	MSE
Fit	887.57	2	443.79
Error	63.32	29	2.18
Total	950.89	31	30.67

Statistic	Estimate
F	203.245
p	< 0.001
Adjusted R^2	0.9288

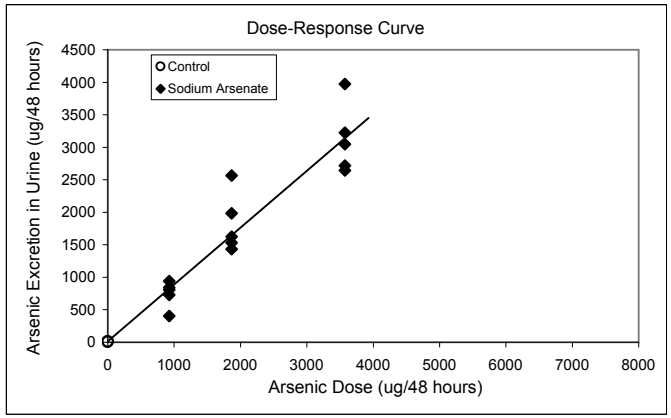
RBA and Uncertainty

Test Material (Soil)	
RBA	0.16
Lower bound[b]	0.14
Upper bound[b]	0.19
Standard Error[b]	0.016

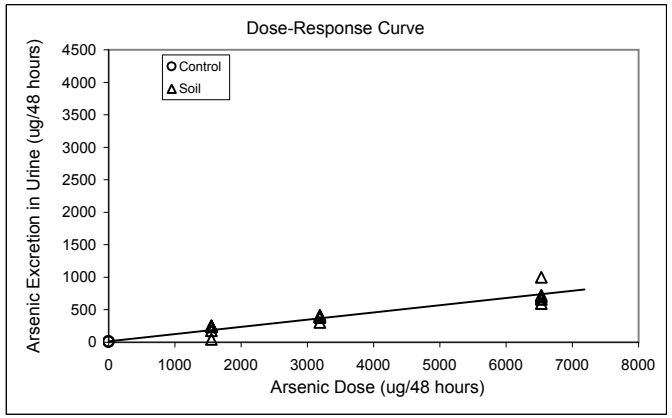
[b] Calculated using Fieller's theorem

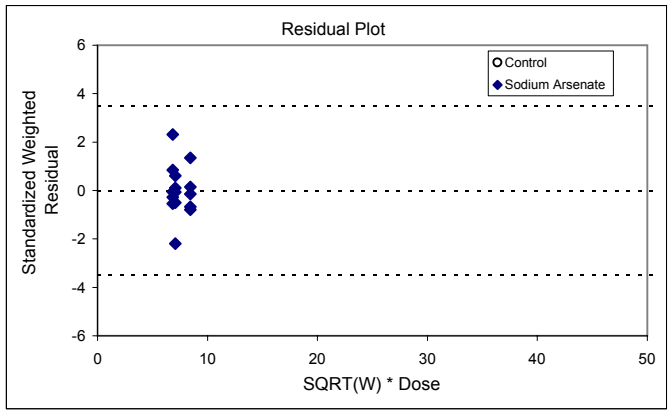
FIGURE 5-8 URINARY EXCRETION OF ARSENIC: Days 12/13 (Outliers Excluded)

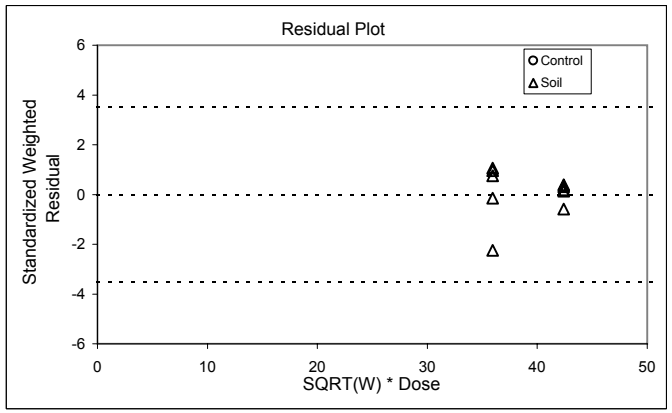
Reference Material (Sodium Arsenate)



Test Material (Soil)







Summary of Fitting[a]

Parameter	Estimate	SE
a	16.3	32.5
b1	0.87	0.04
b2	0.11	0.01
Covariance (b1,b2)	0.2209	--
Degrees of Freedom	30	--

[a] y = a + b1*x1 + b2*x2

ANOVA

Source	SSE	DF	MSE
Fit	987.31	2	493.65
Error	66.72	29	2.30
Total	1054.02	31	34.00

Statistic	Estimate
F	214.578
p	< 0.001
Adjusted R^2	0.9323

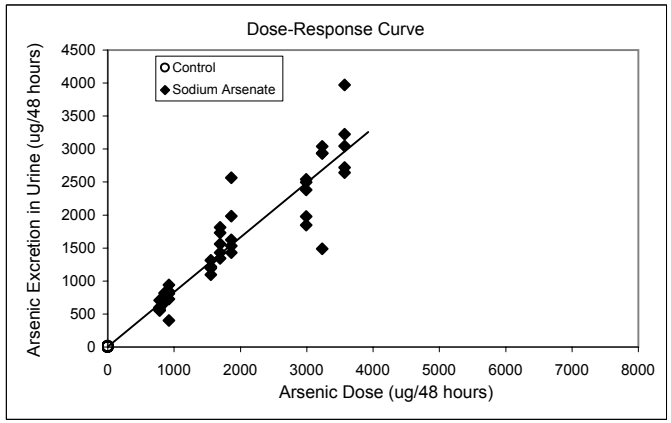
RBA and Uncertainty

Test Material (Soil)	
RBA	0.13
Lower bound[b]	0.11
Upper bound[b]	0.15
Standard Error[b]	0.012

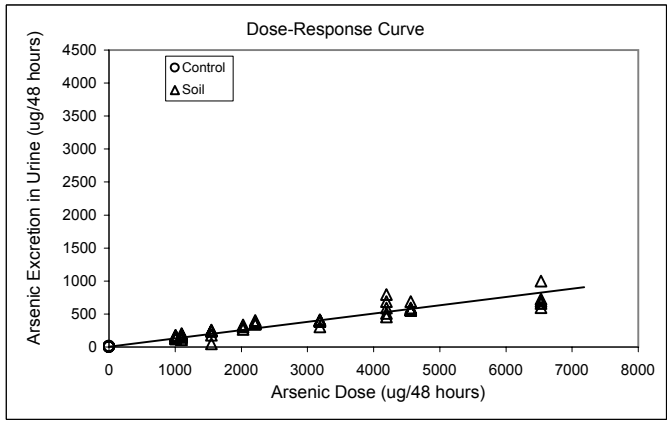
[b] Calculated using Fieller's theorem

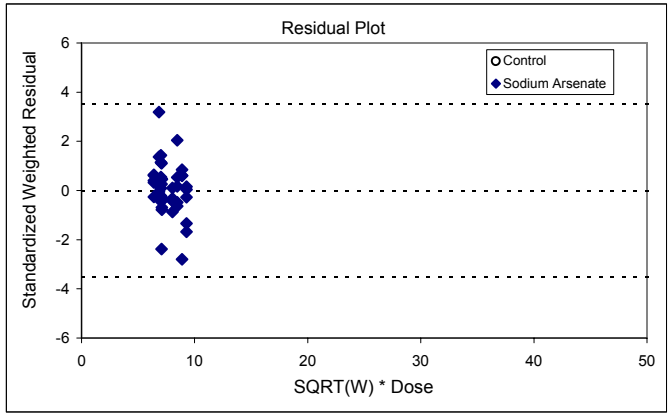
FIGURE 5-9 URINARY EXCRETION OF ARSENIC: All Days (Outliers Excluded)

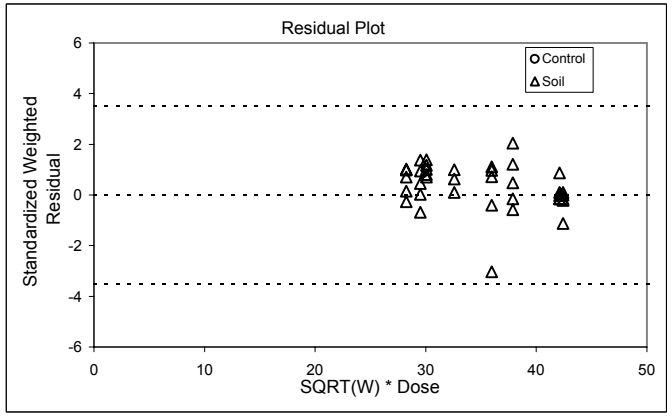
Reference Material (Sodium Arsenate)



Test Material (Soil)







Summary of Fitting[a]

Parameter	Estimate	SE
a	5.1	1.5
b1	0.83	0.02
b2	0.13	0.00
Covariance (b1,b2)	0.0034	--
Degrees of Freedom	93	--

[a] y = a + b1*x1 + b2*x2

ANOVA

Source	SSE	DF	MSE
Fit	2728.52	2	1364.26
Error	132.58	92	1.44
Total	2861.10	94	30.44

Statistic	Estimate
F	946.672
p	< 0.001
Adjusted R^2	0.9527

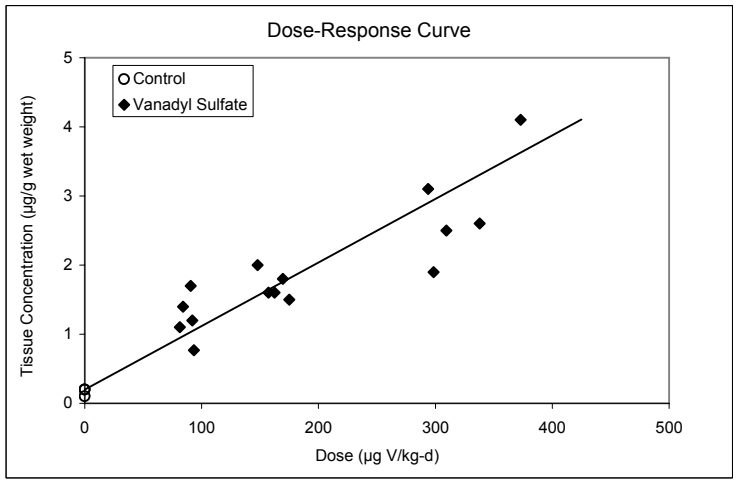
RBA and Uncertainty

Test Material (Soil)	
RBA	0.15
Lower bound[b]	0.14
Upper bound[b]	0.16
Standard Error[b]	0.007

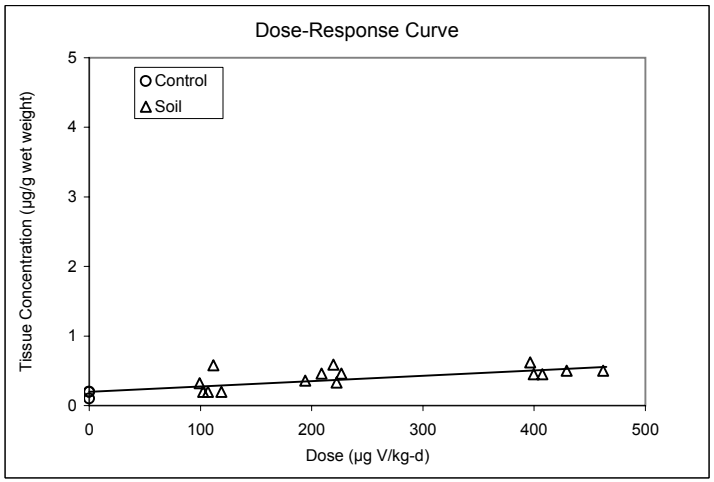
[b] Calculated using Fieller's theorem

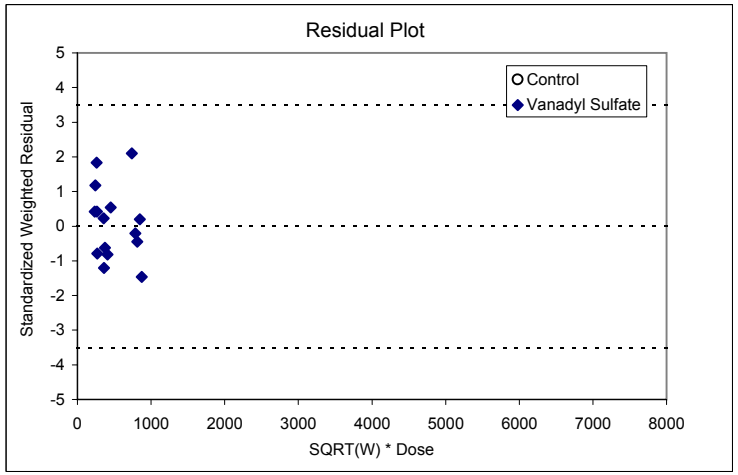
FIGURE 5-10 LIVER VANADIUM DOSE-RESPONSE

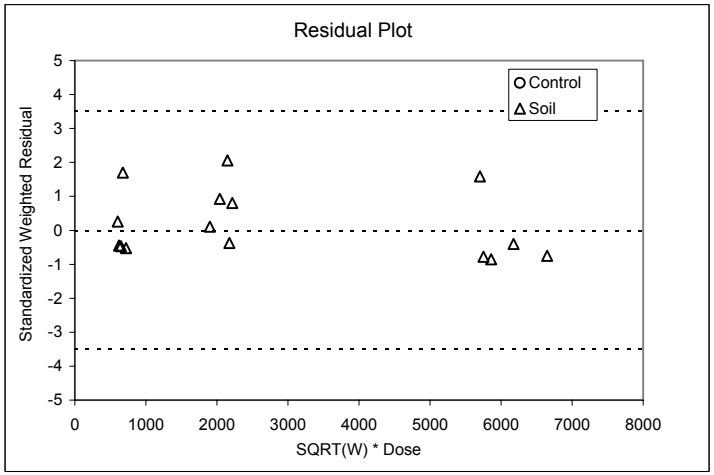
Reference Material (Vanadyl Sulfate)



Test Material (Soil)







Summary of Fitting*

Parameter	Estimate	Standard Error
a	1.99E-01	3.26E-02
b_r	9.20E-03	5.55E-04
b_{tm}	7.69E-04	1.16E-04
Covariance (b_r,b_{tm})	0.2663	--
Degrees of Freedom	30	--

*Data were fit using the linear model: $y = a + b_r \cdot x_r + b_{tm} \cdot x_{tm}$

Goodness of Fit

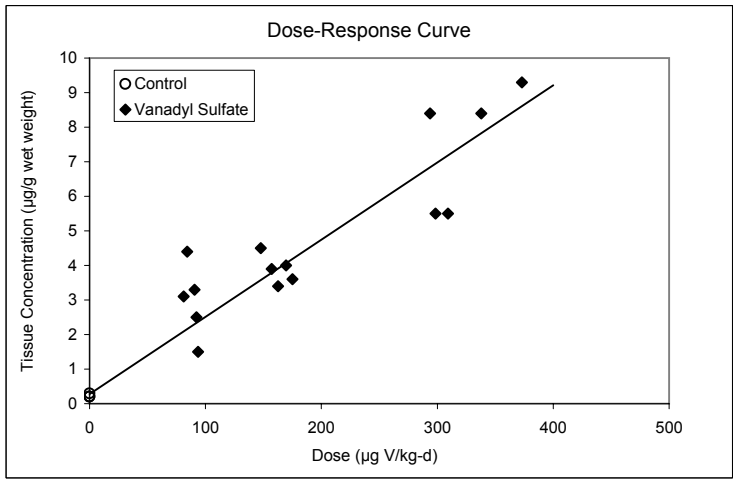
Statistic	Estimate
F	139.966
p	< 0.001
Adjusted R^2	0.8968

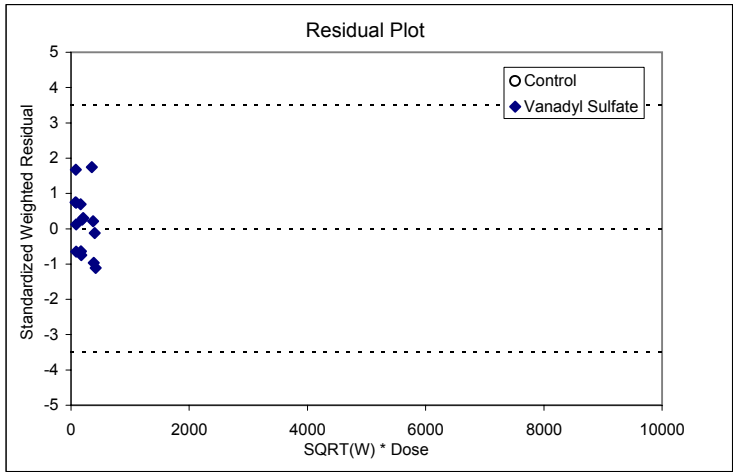
RBA and Uncertainty

Test Material	
RBA	0.08
Lower Bound	0.06
Upper Bound	0.10
Standard Error	0.012

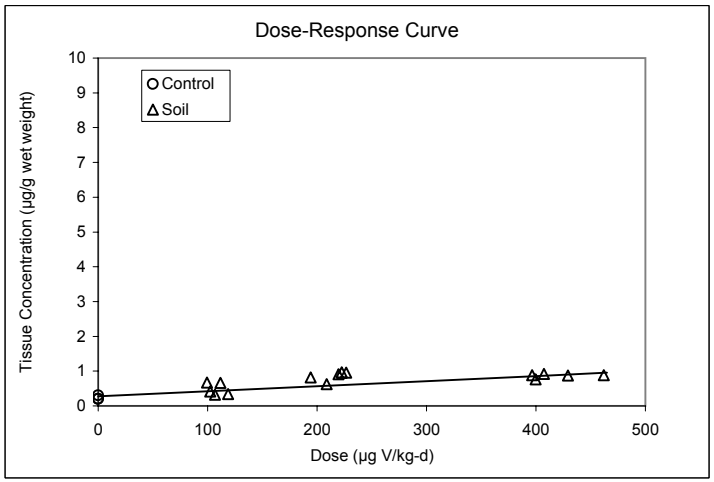
FIGURE 5-11 KIDNEY VANADIUM DOSE-RESPONSE

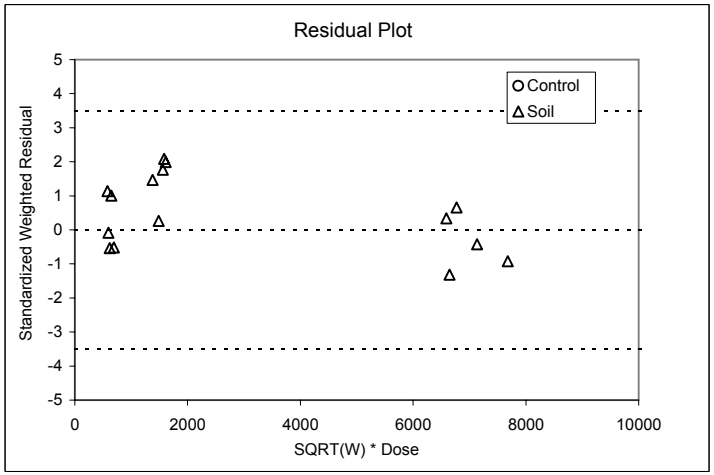
Reference Material (Vanadyl Sulfate)





Test Material (Soil)





Summary of Fitting*

Parameter	Estimate	Standard Error
a	2.80E-01	4.09E-02
b_r	2.23E-02	1.39E-03
b_{tm}	1.45E-03	1.33E-04
Covariance (b_r,b_{tm})	0.1335	--
Degrees of Freedom	30	--

*Data were fit using the linear model: $y = a + b_r \cdot x_r + b_{tm} \cdot x_{tm}$

Goodness of Fit

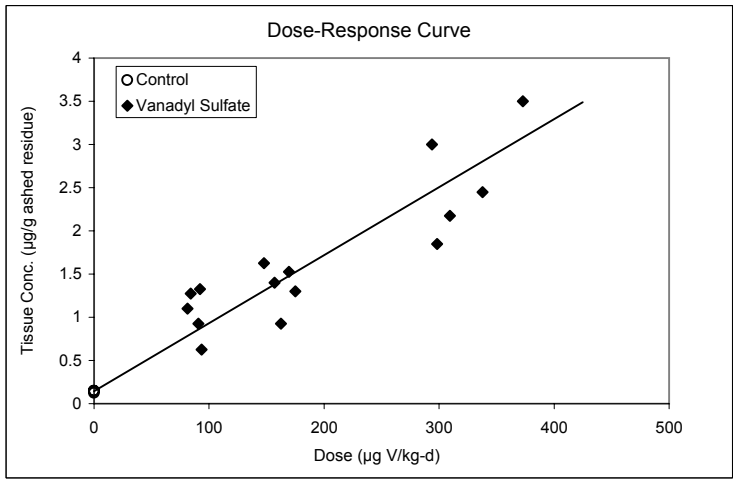
Statistic	Estimate
F	168.998
p	< 0.001
Adjusted R^2	0.9130

RBA and Uncertainty

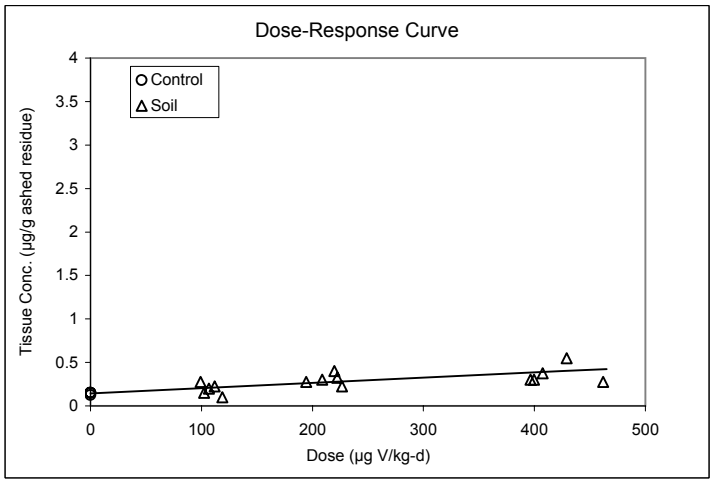
Test Material	
RBA	0.06
Lower Bound	0.05
Upper Bound	0.08
Standard Error	0.007

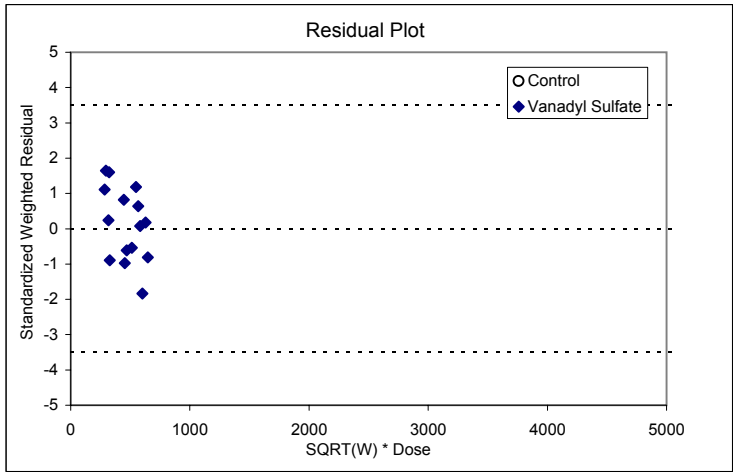
FIGURE 5-12 FEMUR VANADIUM DOSE-RESPONSE

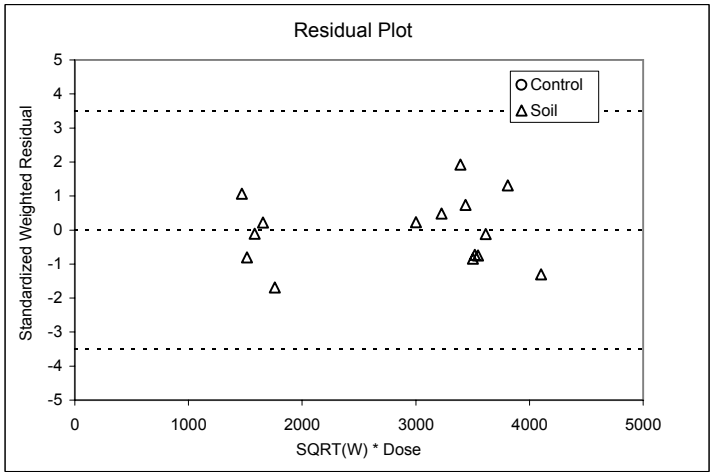
Reference Material (Vanadyl Sulfate)



Test Material (Soil)







Summary of Fitting*

Parameter	Estimate	Standard Error
a	1.43E-01	8.45E-03
b_r	7.87E-03	5.55E-04
b_{tm}	6.04E-04	9.40E-05
Covariance (b_r,b_{tm})	0.0310	--
Degrees of Freedom	30	--

*Data were fit using the linear model: $y = a + b_r \cdot x_r + b_{tm} \cdot x_{tm}$

Goodness of Fit

Statistic	Estimate
F	118.484
p	< 0.001
Adjusted R^2	0.8801

RBA and Uncertainty

Test Material	
RBA	0.08
Lower Bound	0.06
Upper Bound	0.10
Standard Error	0.013

APPENDIX A

DETAILED RESULTS

TABLE A-1 SCHEDULE

Study Day	Day	Date	Feed Special Diet	Cull Pigs/ Assign Dose Group	Weigh	Dose Preparation	Dose Administration	48-hour Urine Collection	Sacrifice/ Necropsy
-6	Tuesday	02/08/05	transition	X	X				
-5	Wednesday	02/09/05	transition						
-4	Thursday	02/10/05	transition						
-3	Friday	02/11/05	X						
-2	Saturday	02/12/05	X						
-1	Sunday	02/13/05	X		X	X			
0	Monday	02/14/05	X				X		
1	Tuesday	02/15/05	X				X		
2	Wednesday	02/16/05	X		X	X	X		
3	Thursday	02/17/05	X				X		
4	Friday	02/18/05	X				X		
5	Saturday	02/19/05	X		X	X	X		
6	Sunday	02/20/05	X				X	U-1	
7	Monday	02/21/05	X				X		
8	Tuesday	02/22/05	X		X	X	X		
9	Wednesday	02/23/05	X				X	U-2	
10	Thursday	02/24/05	X				X		
11	Friday	02/25/05	X		X	X	X		
12	Saturday	02/26/05	X				X	U-3	
13	Sunday	02/27/05	X				X		
14	Monday	02/28/05	X		X		X		
15	Tuesday	03/01/05							X

TABLE A-2 GROUP ASSIGNMENTS

Pig Number	Dose Group	Material Administered	Target Dose of Arsenic (µg/kg-day)	Target Dose of Vanadium (µg/kg-day)
705 727 732 742 749	1	$NaHAsO_4$	30	0
718 721 722 726 751	2	$NaHAsO_4$	60	0
701 707 724 734 748	3	$NaHAsO_4$	120	0
704 708 712 719 735	4	Soil	40	103
713 714 715 731 750	5	Soil	80	206
723 738 739 747 752	6	Soil	160	412
703 710 717 740 746	7	$VOSO_4$	0	80
716 720 736 737 743	8	$VOSO_4$	0	160
702 728 733 744 745	9	$VOSO_4$	0	320
709 711 730	10	Control	0	0

TABLE A-3 BODY WEIGHTS 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.
All weights shown in kilograms (kg).

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
1	705	10.1	10.1	10.1	10.2	10.3	10.4	10.6	11.0	11.4	11.9	12.1	12.4	12.7	12.9	13.2	13.4
1	727	9.9	10.2	10.5	10.8	11.1	11.5	11.8	12.3	12.7	13.2	13.5	13.9	14.3	14.9	15.5	16.1
1	732	11.1	11.3	11.5	11.8	12.0	12.3	12.6	13.0	13.4	13.8	14.2	14.6	15.0	15.5	16.0	16.6
1	742	10.4	10.7	11.0	11.3	11.5	11.7	12.0	12.4	12.9	13.3	13.7	14.2	14.6	15.2	15.9	16.5
1	749	11.5	11.7	12.0	12.2	12.5	12.8	13.2	13.7	14.2	14.7	14.9	15.2	15.4	16.0	16.6	17.2
2	718	11.3	11.5	11.7	11.9	12.2	12.5	12.8	13.2	13.6	14.0	14.6	15.2	15.8	16.3	16.8	17.3
2	721	10.0	10.2	10.5	10.8	11.2	11.5	11.9	12.2	12.6	12.9	13.4	13.8	14.3	14.8	15.3	15.9
2	722	10.5	10.8	11.1	11.5	11.7	11.9	12.2	12.7	13.2	13.7	14.1	14.6	15.0	15.5	16.0	16.6
2	726	9.7	9.9	10.2	10.4	10.7	11.1	11.4	11.8	12.2	12.6	13.1	13.6	14.1	14.6	15.1	15.7
2	751	9.6	9.9	10.2	10.5	10.8	11.2	11.5	11.8	12.2	12.5	12.9	13.2	13.6	14.2	14.9	15.6
3	701	9.6	9.7	9.9	10.1	10.5	10.9	11.3	11.5	11.7	11.9	12.4	12.9	13.4	14.1	14.7	15.4
3	707	9.6	9.9	10.3	10.6	10.7	10.9	11.0	11.4	11.7	12.1	12.5	13.0	13.5	13.9	14.3	14.7
3	724	9.9	10.1	10.4	10.6	10.9	11.2	11.5	11.9	12.2	12.6	13.1	13.6	14.1	14.6	15.1	15.6
3	734	10.5	10.8	11.2	11.5	11.7	12.0	12.2	12.6	13.0	13.4	13.8	14.1	14.5	15.2	15.8	16.5
3	748	10.1	10.4	10.7	11.0	11.1	11.3	11.4	11.8	12.1	12.5	13.0	13.5	14.0	14.5	15.1	15.6
4	704	9.8	10.0	10.3	10.6	10.8	10.9	11.1	11.6	12.0	12.5	12.8	13.1	13.5	14.0	14.6	15.1
4	708	11.2	11.4	11.7	12.0	12.2	12.4	12.7	13.0	13.4	13.8	14.2	14.6	15.1	15.7	16.4	17.0
4	712	10.9	11.1	11.3	11.6	11.8	12.1	12.4	12.7	13.1	13.5	13.8	14.2	14.6	15.2	15.8	16.5
4	719	10.5	10.7	11.0	11.2	11.4	11.5	11.7	12.0	12.4	12.8	13.2	13.6	14.1	14.6	15.2	15.8
4	735	9.6	9.7	9.8	9.9	10.0	10.1	10.2	10.5	10.8	11.1	11.2	11.3	11.4	11.4	11.4	11.4
5	713	9.7	10.0	10.3	10.6	10.9	11.2	11.5	12.0	12.5	13.0	13.4	13.9	14.3	15.0	15.7	16.4
5	714	11.7	11.9	12.1	12.3	12.5	12.8	13.0	13.4	13.8	14.2	14.6	14.9	15.3	15.9	16.6	17.3
5	715	10.2	10.3	10.5	10.6	10.9	11.2	11.5	11.7	11.9	12.1	12.5	13.0	13.4	14.1	14.8	15.5
5	731	9.6	9.8	9.9	10.1	10.5	10.8	11.2	11.5	11.9	12.2	12.6	13.0	13.5	14.1	14.7	15.3
5	750	9.8	10.1	10.3	10.6	10.8	11.0	11.2	11.6	12.1	12.6	13.1	13.6	14.2	14.7	15.3	15.9
6	723	10.3	10.6	10.9	11.2	11.5	11.8	12.1	12.5	12.9	13.3	13.7	14.1	14.5	15.2	15.9	16.6
6	738	10.3	10.5	10.8	11.1	11.4	11.8	12.1	12.5	12.9	13.4	13.7	14.1	14.5	15.2	15.9	16.7
6	739	10.2	10.3	10.5	10.6	10.8	11.1	11.3	11.6	11.9	12.3	12.6	12.9	13.3	13.7	14.2	14.7
6	747	10.5	10.8	11.1	11.4	11.8	12.2	12.6	12.9	13.3	13.7	14.1	14.5	14.9	15.5	16.1	16.8
6	752	10.7	11.0	11.4	11.7	12.0	12.2	12.5	12.9	13.3	13.8	14.2	14.7	15.2	15.8	16.5	17.1
7	703	9.7	10.0	10.3	10.6	10.8	11.0	11.2	11.6	12.0	12.4	12.8	13.2	13.6	14.1	14.6	15.1
7	710	10.0	10.1	10.2	10.4	10.5	10.7	10.9	11.3	11.6	12.0	12.5	13.0	13.5	14.2	14.9	15.6
7	717	11.3	11.6	11.9	12.3	12.6	12.9	13.2	13.3	13.5	13.6	14.2	14.7	15.3	15.6	16.0	16.4
7	740	11.3	11.3	11.4	11.5	11.6	11.6	11.7	12.0	12.3	12.6	12.6	12.5	12.5	13.0	13.5	14.0
7	746	10.0	10.1	10.2	10.3	10.6	10.8	11.1	11.6	12.0	12.5	12.8	13.1	13.4	13.9	14.5	15.0
8	716	9.5	9.7	9.8	10.0	10.2	10.4	10.7	11.1	11.5	11.9	12.2	12.5	12.8	13.4	13.9	14.5
8	720	10.1	10.3	10.6	10.8	11.0	11.3	11.5	11.9	12.3	12.7	13.1	13.5	13.9	14.4	14.9	15.4
8	736	10.9	11.2	11.5	11.8	12.1	12.5	12.8	13.3	13.7	14.2	14.5	14.8	15.1	15.7	16.3	16.9
8	737	10.7	10.9	11.2	11.4	11.7	12.0	12.4	12.5	12.6	12.7	13.1	13.5	13.9	14.6	15.3	16.0
8	743	9.9	10.1	10.3	10.5	10.7	10.9	11.1	11.5	11.9	12.4	12.5	12.7	12.9	13.5	14.0	14.6
9	702	10.9	11.1	11.4	11.7	12.0	12.3	12.6	13.1	13.7	14.2	14.6	15.1	15.5	16.1	16.6	17.2
9	728	10.4	10.7	11.1	11.4	11.7	11.9	12.2	12.5	12.8	13.1	13.6	14.1	14.6	15.3	15.9	16.6
9	733	10.7	10.9	11.2	11.4	11.7	11.9	12.2	12.5	12.9	13.3	13.7	14.0	14.4	14.8	15.3	15.8
9	744	8.5	8.7	8.9	9.1	9.4	9.7	10.0	10.4	10.8	11.3	11.6	11.9	12.2	12.6	13.1	13.6
9	745	9.5	9.7	9.9	10.1	10.4	10.7	11.0	11.5	11.9	12.4	12.7	13.0	13.3	13.9	14.6	15.3
10	709	11.9	12.3	12.7	13.1	13.4	13.7	14.0	14.5	15.1	15.6	16.1	16.6	17.1	17.9	18.8	19.7
10	711	10.6	10.8	11.0	11.2	11.5	11.7	12.0	12.4	12.8	13.2	13.7	14.1	14.6	15.1	15.7	16.3
10	730	10.9	11.1	11.4	11.7	11.9	12.2	12.4	12.9	13.4	13.9	14.2	14.6	14.9	15.6	16.2	16.9

TABLE A-4 ANIMAL HEALTH

Naxcel Treatment

First Day of Treatment*	Pig	Group	Indications
Day -5 (2/09/05)	749	1	Elevated temperature, diarrhea
	710	7	
	750	5	
Day -1 (2/13/05)	719	4	Elevated temperature, anorexia
Day 1 (2/15/05)	735	4	Elevated temperature, diarrhea
	705	1	
Day 5 (2/19/05)	737	8	Elevated temperature, anorexia
Day 13 (2/27/05)	735	4	Diarrhea
	705	1	

*Treatment duration: 3 days

Necropsy

Pig 737 (group 8) had one testicle retained in abdomen.
Kidneys appeared small in $VOSO_4$ groups; however, organs were not weighed so this observation could not be verified statistically.

TABLE A-5 DOSE PREPARATION AND ADMINISTRATION

Quantifiable missed doses are noted at the bottom of Tables A-6 and A-7.

There were two major difficulties in dose preparation: 1) this batch of special feed became very sticky when mixed with water and 2) a large amount of soil was necessary for the soil groups. Details are provided below.

Day -1 (2/13/05): **Dose preparation:** All doses were made by adding the dose material to doughballs, which consisted of special feed mixed with water. Reference material doses were made by pipeting the stock solution into a small hole in the doughball made with a flask stopper, allowed to soak in, and then squeezed shut. Soil doses were made by first mixing soil with an equal amount of special feed, wetting this mixture and rolling it into small logs, and allowing it to dry for a few hours; these logs were then broken into pieces and placed in the center of doughballs in an attempt to reduce the number of soil doughballs and still prevent the soil from falling out. Upon storing, all doughballs became very wet and sticky in the storage bags and were difficult to get out; the soil stayed as a hard lump in the center and the dough did not cling to them well.

Day 2 (2/16/05): **Dose preparation:** Doughballs were made from a mixture of 3/4 cup vegetable shortening, 1 cup powdered sugar, 1 pound cornstarch, an equal amount of special feed, and enough water to make the mixture malleable. This dough was non-sticky and did not become wet over time. Reference material doses were prepared the same way as on Day -1. Soil doses were prepared as follows: 1) a log of dough about 3 inches long was flattened on cornstarch-dusted bench paper to approximately 3" by 4"; 2) this was brushed with a mixture of equal amounts of powdered sugar and water to dampen the surface; 3) the weighed soil was sprinkled over the dough, staying back from the edge; 4) the soil-covered dough was rolled up cinnamon-roll style and placed in a dosing bag. Soil for groups 4 and 5 were able to be placed in just one doughball, while Group 6 required 2, and then 3, later on. The soil wetted into the doughball, so they were easily broken into bite-size pieces at dosing without the soil falling out. Group 4 doughballs had some flour in them instead of cornstarch.

Day 5 (2/19/05): **Dose preparation:** Doughballs were made from a mixture of 3/4 cup vegetable shortening, 1 pound flour, an equal amount of special feed, and enough water to make the mixture malleable. It became apparent that there was insufficient soil to last through the end of the study. In order to extend the soil supply, doses for the soil groups (groups 4-6) consisted of the archived soil doughballs from the previous two dose preparations (Day -1 and Day 2), which had been stored in the freezer, in addition to a new doughball made with an amount of soil calculated to supplement the amount in the archived sample to make the dose necessary for this preparation. No archives were made at this dose preparation or in further dose preparations.

Day 6 (2/20/05): **Dosing:** Pig 713 (Group 5) drinks excessively; lots of soil in urine bucket (morning and afternoon doses). Loss of dose not quantified, so actual dose not adjusted.

Day 8 (2/22/05): **Dosing:** At the afternoon dosing, there was uncertainty regarding the prepared doughballs for Group 4, so new doughballs were made; animals were dosed 20 minutes late.
Dose preparation: Doughballs were made using the same recipe as Day 5. Only 200g of soil remained after this preparation.

Day 11 (2/25/05): **Dose preparation:** Doughballs were made using the same recipe as Day 5. The supplier sent more soil, which was mixed with the remaing 200 g, rolled, and used. A sample of the new mixed soil was taken for analysis. There still was insufficient soil to make the afternoon dose for all three soil groups on Day 14 (the last dosing day), so no doughballs for any groups were prepared for the Day 14 afternoon dose.

Day 14 (2/28/05): **Dosing:** No animals received the afternoon dose; dosing ended with the morning dose of Day 14.

TABLE A-6 ACTUAL ADMINISTERED ARSENIC DOSES

Doses shown have been adjusted for individual body weights (see Table A-3); units are µg/kg-d.

Group	Pig #	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 Dose (Days 0-14)
1	705	34.4	34.3	34.2	35.7	35.2	34.8	35.5	34.2	32.9	35.5	34.7	34.0	35.8	35.1	25.8	34.1
1	727	34.1	33.1	32.1	33.0	32.0	31.1	31.8	30.7	29.7	31.8	30.9	30.1	31.0	29.8	21.5	30.8
1	732	30.7	30.1	29.5	30.5	29.9	29.2	30.1	29.2	28.4	30.4	29.5	28.7	29.8	28.8	20.9	29.0
1	742	32.5	31.7	30.9	32.0	31.3	30.7	31.5	30.4	29.3	31.3	30.4	29.5	30.3	29.1	21.0	30.1
1	749	29.7	29.0	28.5	29.3	28.6	27.9	28.6	27.6	26.6	28.9	28.4	27.9	28.9	27.8	20.1	27.9
2	718	58.4	57.4	56.5	59.0	57.6	56.3	58.9	57.3	55.7	58.1	55.8	53.6	57.3	55.6	40.5	55.9
2	721	65.7	63.9	62.2	64.5	62.4	60.5	63.5	61.8	60.2	63.3	61.2	59.2	62.9	60.8	44.1	61.1
2	722	62.1	60.4	58.7	61.6	60.4	59.3	61.4	59.1	56.9	60.0	58.2	56.4	60.1	58.2	42.3	58.3
2	726	67.7	66.1	64.6	67.1	65.1	63.2	65.9	63.9	61.9	64.9	62.5	60.3	63.9	61.7	44.7	62.9
2	751	68.2	66.2	64.3	66.7	64.6	62.6	65.7	63.9	62.2	65.9	64.1	62.5	65.5	62.5	44.8	63.3
3	701	134.9	132.6	130.4	134.8	129.8	125.2	130.7	128.4	126.3	130.7	125.5	120.6	126.9	121.2	87.0	125.7
3	707	131.9	127.6	123.6	131.3	129.6	128.1	131.8	127.9	124.2	129.1	124.5	120.2	128.8	125.0	91.1	125.0
3	724	129.3	126.4	123.6	129.2	125.8	122.5	126.1	122.3	118.8	123.5	119.1	115.0	122.7	118.6	86.1	120.6
3	734	121.0	117.3	113.9	120.1	117.7	115.5	118.8	115.1	111.7	117.4	114.4	111.5	117.9	113.0	81.4	113.8
3	748	126.6	123.0	119.7	126.9	125.2	123.6	127.4	123.7	120.2	124.7	119.9	115.5	122.9	118.5	85.8	120.2
4	704	45.3	44.1	42.9	44.8	44.1	43.4	43.5	41.8	40.2	42.8	41.8	40.8	55.4	53.3	38.5	44.2
4	708	39.8	38.8	37.9	39.5	38.8	38.1	38.6	37.5	36.4	38.6	37.5	36.5	49.4	47.4	34.2	39.3
4	712	40.9	40.1	39.4	40.8	39.9	39.0	39.5	38.4	37.4	39.7	38.7	37.7	51.0	49.0	35.3	40.5
4	719	42.4	41.5	40.6	42.5	41.9	41.4	41.8	40.5	39.3	41.5	40.3	39.1	53.0	50.9	36.8	42.2
4	735	47.1	46.5	26.4	48.3	47.9	47.5	48.1	46.8	45.5	49.2	48.8	48.4	68.3	68.3	17.1	46.9
5	713	89.7	87.3	84.9	87.1	84.7	82.5	42.4	81.3	78.2	82.4	79.7	77.2	106.4	101.8	73.1	82.6
5	714	75.4	74.2	73.1	75.6	74.1	72.7	75.6	73.4	71.3	75.9	74.1	72.4	100.1	96.0	69.1	76.9
5	715	86.7	85.6	84.5	86.7	84.3	82.1	86.5	85.1	83.7	88.1	85.2	82.4	113.2	108.0	77.4	88.0
5	731	91.7	90.2	88.7	90.4	87.5	84.7	88.0	85.4	83.0	87.5	84.7	82.1	113.4	108.6	78.2	89.6
5	750	89.1	87.0	84.9	87.9	86.3	84.7	87.0	83.6	80.3	84.2	81.0	78.1	108.4	104.3	75.5	86.8
6	723	172.6	167.8	163.3	170.3	166.0	161.9	168.4	163.1	158.2	166.9	161.9	157.3	214.8	205.3	147.5	169.7
6	738	64.8	168.3	86.1	170.6	165.8	161.2	167.5	162.1	157.0	166.1	161.5	157.3	214.6	204.9	147.1	157.0
6	739	176.2	174.0	171.8	180.0	176.3	172.6	180.4	175.7	171.1	181.2	176.6	172.1	237.7	229.7	166.6	182.8
6	747	168.6	102.5	159.7	165.5	160.3	155.4	162.3	157.8	153.6	162.1	157.5	153.0	210.4	202.4	146.2	161.2
6	752	165.0	88.1	155.6	163.0	159.4	156.0	162.3	157.2	152.5	160.2	155.0	150.0	206.2	198.3	143.2	158.1
10	709	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	711	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	730	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Dosing Anomalies:

Day 1 - Pig 738 did not eat entire AM or PM dose (ate approximately 50% and 25%, respectively). Daily dose adjusted to 37.5%.
Day 1 - Pig 747 did not eat entire PM dose (ate approximately 25%). Daily dose adjusted to 62.5%.
Day 1 - Pig 752 did not eat entire PM dose (ate approximately 10%). Daily dose adjusted to 55%.
Day 2 - Pig 735 did not eat entire AM dose (ate approximately 15%). Daily dose adjusted to 57.5%.
Day 2 - Pig 738 did not eat entire AM dose (ate approximately 5%). Daily dose adjusted to 52.5%.
Day 6 - Pig 713 was drinking excessively and a large amount of dosing material was found in the urine bucket; however, a reliable estimate of the amount of dose lost could not be made. Therefore, for the purposes of these calculations, a value of 50% was assumed to minimized bias.
Day 14 - Pig 735 did not eat entire AM dose (ate approximately 50%) and did not receive PM dose (see note below). Daily dose adjusted to 25%.
Day 14 - There was insufficient soil to prepare the PM doses for Groups 4, 5, and 6. As a result, no groups received PM doses.

TABLE A-7 ACTUAL ADMINISTERED VANADIUM DOSES

Doses shown have been adjusted for individual body weights (see Table A-3); units are µg/kg-d.

Group	Pig #	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 Dose (Days 0-14)
4	704	116.7	113.5	110.5	115.3	113.5	111.8	111.9	107.6	103.6	110.3	107.6	105.1	131.3	126.3	91.3	111.8
4	708	102.4	100.0	97.6	101.6	99.8	98.1	99.3	96.5	93.8	99.4	96.6	93.9	117.0	112.4	81.1	99.3
4	712	105.4	103.3	101.4	105.1	102.7	100.5	101.8	99.0	96.3	102.3	99.6	97.1	121.0	116.2	83.8	102.4
4	719	109.1	106.8	104.6	109.4	108.0	106.6	107.6	104.3	101.1	106.9	103.7	100.6	125.6	120.8	87.2	106.8
4	735	121.2	119.7	68.0	124.4	123.3	122.3	123.9	120.4	117.2	126.8	125.6	124.5	161.9	161.9	40.5	118.8
5	713	231.0	224.6	218.6	224.2	218.1	212.4	109.1	209.3	201.3	212.2	205.3	198.8	252.3	241.3	173.4	208.8
5	714	194.1	191.1	188.3	194.6	190.8	187.1	194.5	188.9	183.5	195.4	190.8	186.5	237.3	227.5	163.9	194.3
5	715	223.2	220.3	217.6	223.1	217.2	211.5	222.8	219.0	215.4	226.9	219.3	212.2	268.5	256.0	183.5	222.4
5	731	236.1	232.2	228.3	232.7	225.2	218.1	226.6	219.9	213.6	225.4	218.2	211.4	268.8	257.5	185.3	226.6
5	750	229.5	223.9	218.6	226.2	222.1	218.1	224.0	215.1	206.9	216.8	208.6	201.0	256.9	247.4	178.9	219.6
6	723	444.3	432.0	420.4	438.6	427.4	416.7	433.5	420.0	407.3	429.6	416.9	404.9	509.3	486.9	349.7	429.2
6	738	166.9	433.4	221.7	439.2	426.8	415.0	431.2	417.3	404.2	427.5	415.9	404.9	508.7	485.8	348.7	396.5
6	739	453.6	447.9	442.2	463.5	453.8	444.4	464.6	452.2	440.5	466.6	454.5	443.1	563.7	544.5	395.0	462.0
6	747	434.0	263.9	411.2	426.2	412.7	400.1	417.8	406.3	395.4	417.4	405.4	394.0	498.9	479.8	346.6	407.3
6	752	424.9	226.8	400.6	419.6	410.5	401.7	417.8	404.7	392.5	412.5	398.9	386.2	488.9	470.1	339.5	399.7
7	703	91.6	89.1	86.7	89.2	87.6	86.0	87.1	214.2	81.3	85.0	82.4	80.0	83.0	80.2	58.1	92.1
7	710	90.4	89.4	88.3	91.1	89.5	88.0	89.6	221.3	84.4	87.4	84.0	80.9	82.7	78.8	56.4	93.5
7	717	78.9	76.7	74.6	76.4	74.6	72.9	75.8	190.9	74.1	76.9	74.0	71.4	74.9	73.1	53.5	81.3
7	740	80.7	80.1	79.5	82.9	82.5	82.0	84.0	208.7	80.0	86.6	86.8	87.0	90.0	86.7	62.7	90.7
7	746	90.8	89.8	88.8	90.8	88.5	86.4	87.3	84.0	81.0	85.3	83.4	81.5	84.2	81.0	58.5	84.1
8	716	185.7	182.9	180.1	186.6	182.5	178.5	183.5	177.0	171.1	180.8	176.2	171.9	176.2	169.2	122.1	174.9
8	720	174.0	169.9	165.9	172.3	168.7	165.3	170.4	164.8	159.6	168.2	163.4	158.9	163.7	158.0	114.5	162.5
8	736	160.5	156.0	151.9	156.7	152.5	148.5	153.0	148.0	143.3	152.1	148.8	145.7	150.0	144.6	104.7	147.7
8	737	163.9	160.5	157.2	162.2	158.0	153.9	162.6	161.1	159.6	168.2	163.4	158.9	161.7	154.2	110.6	157.1
8	743	177.7	174.5	171.5	178.5	175.2	172.0	176.5	170.1	164.1	175.5	173.0	170.6	174.7	167.6	120.8	169.5
9	702	316.1	308.7	301.6	313.7	305.6	297.9	306.7	294.8	283.7	302.7	293.9	285.7	298.5	288.6	209.5	293.9
9	728	328.4	318.0	308.2	321.8	173.0	307.7	322.3	314.7	307.5	325.7	314.1	303.3	313.8	300.7	216.5	298.4
9	733	321.4	314.7	308.2	322.2	315.5	309.0	321.4	311.9	302.9	324.5	316.3	308.6	323.0	312.8	227.4	309.3
9	744	403.9	394.8	386.1	399.4	387.0	375.4	386.7	371.9	358.1	383.4	373.7	364.5	379.2	365.2	264.2	372.9
9	745	362.3	354.9	347.9	361.0	350.8	341.3	351.8	338.5	326.2	350.1	342.0	334.3	343.8	327.8	234.8	337.8
10	709	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	711	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	730	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Dosing Anomalies:

Day 1 - Pig738 did not eat entire AM or PM dose (ate approximately 50% and 25%, respectively). Daily dose adjusted to 37.5%.
Day 1 - Pig 747 did not eat entire PM dose (ate approximately 25%). Daily dose adjusted to 62.5%.
Day 1 - Pig 752 did not eat entire PM dose (ate approximately 10%). Daily dose adjusted to 55%.
Day 2 - Pig 735 did not eat entire AM dose (ate approximately 15%). Daily dose adjusted to 57.5%.
Day 2 - Pig 738 did not eat entire AM dose (ate approximately 5%). Daily dose adjusted to 52.5%.
Day 4 - Pig 728 did not eat entire AM dose (ate approximately 10%). Daily dose adjusted to 55%.
Day 6 - Pig 713 was drinking excessively and a large amount of dosing material was found in the urine bucket; however, a reliable estimate of the amount of dose lost could not be made. Therefore, for the purposes of these calculations, a value of 50% was assumed to minimized bias.
Day 7 - Pigs 703, 710, 717, and 740 received Group 9's AM dose. Daily dose adjusted upward accordingly, to 255%.
Day 14 - Pig 735 did not eat entire AM dose (ate approximately 50%) and did not receive PM dose (see note below). Daily dose adjusted to 25%.
Day 14 - There was insufficient soil to prepare the PM doses for Groups 4, 5, and 6. As a result, no groups received PM doses.

TABLE A-8 URINE VOLUMES - 48 HOUR COLLECTIONS

Units of Volume: mls

Group	Pig ID	Urine Collection		
		U-1 Days 6-7 2/20-2/21/05	**U-2** Days 9-10 2/23-2/24/05	**U-3** Days 12-13 2/26-2/27/05
1	705	4590	7680	5060
	727	5620	8680	7820
	732	7790	6780	6480
	742	2900	2920	3520
	749	4280	5200	4040
2	718	6075	10220	9580
	721	7980	7100	11020
	722	7480	7880	8420
	726	8220	6400	5580
	751	17900	15720	12500
3	701	7440	5060	4000
	707	18150	15200	24820
	724	7340	9280	8020
	734	6590	4820	8060
	748	2410	4960	3040
4	704	8200	6540	16840
	708	7570	9660	10220
	712	2770	4920	2980
	719	4440	8780	11300
	735	2270	3140	2440
5	713	12600	17460	42520
	714	8380	9240	10280
	715	8600	5440	10400
	731	11740	6520	6220
	750	3020	2020	2300
6	723	5400	3720	5180
	738	11620	8420	6000
	739	4560	5920	3720
	747	13740	8600	12960
	752	14060	9620	10980
7	703	URINE SAMPLES NOT COLLECTED FOR $VOSO_4$ GROUPS		
	710			
	717			
	740			
	746			
8	716			
	720			
	736			
	737			
	743			
9	702			
	728			
	733			
	744			
	745			
10	709	5200	10860	10020
	711	2880	4400	4540
	730	2080	2050	2340
Volume measured by:		AA,JB	AA	AA
Date:		2/22/05	2/24/05	2/28/05

TABLE A-9 URINARY ARSENIC ANALYTICAL RESULTS FOR STUDY SAMPLES

Sample Number	Tag Number	Pig Number	Group	Material Administered	Urine Collection Days	48-hr As Dose (ug/48hr)	Q	Reported Conc (ng/mL)	AdjConc* (ng/mL)	Urine Volume (mL)	Total Excreted (ug/48hrs)
PTX-705-U1	PTX-115	705	1	$NaHAsO_4$	6/7	780		130	130	4590	596.7
PTX-727-U1	PTX-111	727	1	$NaHAsO_4$	6/7	780		100	100	5620	562
PTX-732-U1	PTX-113	732	1	$NaHAsO_4$	6/7	780		91	91	7790	708.89
PTX-742-U1	PTX-117	742	1	$NaHAsO_4$	6/7	780		190	190	2900	551
PTX-749-U1	PTX-122	749	1	$NaHAsO_4$	6/7	780		140	140	4280	599.2
PTX-718-U1	PTX-134	718	2	$NaHAsO_4$	6/7	1554		180	180	6075	1093.5
PTX-721-U1	PTX-135	721	2	$NaHAsO_4$	6/7	1554		150	150	7980	1197
PTX-722-U1	PTX-102	722	2	$NaHAsO_4$	6/7	1554		160	160	7480	1196.8
PTX-726-U1	PTX-104	726	2	$NaHAsO_4$	6/7	1554		160	160	8220	1315.2
PTX-751-U1	PTX-130	751	2	$NaHAsO_4$	6/7	1554		68	68	17900	1217.2
PTX-701-U1	PTX-118	701	3	$NaHAsO_4$	6/7	2992.8		320	320	7440	2380.8
PTX-707-U1	PTX-132	707	3	$NaHAsO_4$	6/7	2992.8		140	140	18150	2541
PTX-724-U1	PTX-106	724	3	$NaHAsO_4$	6/7	2992.8		340	340	7340	2495.6
PTX-734-U1	PTX-129	734	3	$NaHAsO_4$	6/7	2992.8		280	280	6590	1845.2
PTX-748-U1	PTX-114	748	3	$NaHAsO_4$	6/7	2992.8		820	820	2410	1976.2
PTX-704-U1	PTX-119	704	4	Soil	6/7	1005.8		21	21	8200	172.2
PTX-708-U1	PTX-116	708	4	Soil	6/7	1005.8		23	23	7570	174.11
PTX-712-U1	PTX-101	712	4	Soil	6/7	1005.8		58	58	2770	160.66
PTX-719-U1	PTX-128	719	4	Soil	6/7	1005.8		31	31	4440	137.64
PTX-735-U1	PTX-125	735	4	Soil	6/7	1005.8		53	53	2270	120.31
PTX-713-U1	PTX-107	713	5	Soil	6/7	1518.57		60	60	12600	756
PTX-714-U1	PTX-112	714	5	Soil	6/7	2024.76		130	130	8380	1089.4
PTX-715-U1	PTX-131	715	5	Soil	6/7	2024.76		31	31	8600	266.6
PTX-731-U1	PTX-127	731	5	Soil	6/7	2024.76		26	26	11740	305.24
PTX-750-U1	PTX-105	750	5	Soil	6/7	2024.76		110	110	3020	332.2
PTX-723-U1	PTX-108	723	6	Soil	6/7	4192.4		110	110	5400	594
PTX-738-U1	PTX-133	738	6	Soil	6/7	4192.4		44	44	11620	511.28
PTX-739-U1	PTX-123	739	6	Soil	6/7	4192.4		100	100	4560	456
PTX-747-U1	PTX-103	747	6	Soil	6/7	4192.4		58	58	13740	796.92
PTX-752-U1	PTX-110	752	6	Soil	6/7	4192.4		49	49	14060	688.94
PTX-709-U1	PTX-120	709	10	Control	6/7	0		1	1	5200	5.2
PTX-711-U1	PTX-124	711	10	Control	6/7	0		1	1	2880	2.88
PTX-730-U1	PTX-136	730	10	Control	6/7	0		3.1	3.1	2080	6.448
PTX-705-U2	PTX-142	705	1	$NaHAsO_4$	9/10	860.4		100	100	7680	768
PTX-727-U2	PTX-161	727	1	$NaHAsO_4$	9/10	860.4		78	78	8680	677.04
PTX-732-U2	PTX-165	732	1	$NaHAsO_4$	9/10	860.4		120	120	6780	813.6
PTX-742-U2	PTX-143	742	1	$NaHAsO_4$	9/10	860.4		280	280	2920	817.6
PTX-749-U2	PTX-145	749	1	$NaHAsO_4$	9/10	860.4		150	150	5200	780
PTX-718-U2	PTX-172	718	2	$NaHAsO_4$	9/10	1693.2		140	140	10220	1430.8
PTX-721-U2	PTX-153	721	2	$NaHAsO_4$	9/10	1693.2		220	220	7100	1562
PTX-722-U2	PTX-154	722	2	$NaHAsO_4$	9/10	1693.2		230	230	7880	1812.4
PTX-726-U2	PTX-163	726	2	$NaHAsO_4$	9/10	1693.2		210	210	6400	1344
PTX-751-U2	PTX-139	751	2	$NaHAsO_4$	9/10	1693.2		110	110	15720	1729.2
PTX-701-U2	PTX-155	701	3	$NaHAsO_4$	9/10	3232.8		580	580	5060	2934.8
PTX-707-U2	PTX-166	707	3	$NaHAsO_4$	9/10	3232.8		200	200	15200	3040
PTX-724-U2	PTX-170	724	3	$NaHAsO_4$	9/10	3232.8		810	810	9280	7516.8
PTX-734-U2	PTX-158	734	3	$NaHAsO_4$	9/10	3232.8		610	610	4820	2940.2
PTX-748-U2	PTX-171	748	3	$NaHAsO_4$	9/10	3232.8		300	300	4960	1488
PTX-704-U2	PTX-146	704	4	Soil	9/10	1097.92		22	22	6540	143.88
PTX-708-U2	PTX-167	708	4	Soil	9/10	1097.92		21	21	9660	202.86
PTX-712-U2	PTX-168	712	4	Soil	9/10	1097.92		33	33	4920	162.36
PTX-719-U2	PTX-138	719	4	Soil	9/10	1097.92		21	21	8780	184.38
PTX-735-U2	PTX-151	735	4	Soil	9/10	1097.92		36	36	3140	113.04
PTX-713-U2	PTX-162	713	5	Soil	9/10	2209		23	23	17460	401.58
PTX-714-U2	PTX-140	714	5	Soil	9/10	2209		40	40	9240	369.6

TABLE A-9, CONTINUED: URINARY ARSENIC ANALYTICAL RESULTS FOR STUDY SAMPLES

Sample Number	Tag Number	Pig Number	Group	Material Administered	Urine Collection Days	48-hr As Dose (ug/48hr)	Q	Reported Conc (ng/mL)	AdjConc* (ng/mL)	Urine Volume (mL)	Total Excreted (ug/48hrs)
PTX-715-U2	PTX-144	715	5	Soil	9/10	2209		63	63	5440	342.72
PTX-731-U2	PTX-141	731	5	Soil	9/10	2209		54	54	6520	352.08
PTX-750-U2	PTX-157	750	5	Soil	9/10	2209		190	190	2020	383.8
PTX-723-U2	PTX-149	723	6	Soil	9/10	4560.88		150	150	3720	558
PTX-738-U2	PTX-164	738	6	Soil	9/10	4560.88		70	70	8420	589.4
PTX-739-U2	PTX-148	739	6	Soil	9/10	4560.88		97	97	5920	574.24
PTX-747-U2	PTX-160	747	6	Soil	9/10	4560.88		80	80	8600	688
PTX-752-U2	PTX-147	752	6	Soil	9/10	4560.88		58	58	9620	557.96
PTX-709-U2	PTX-169	709	10	Control	9/10	0		1	1	10860	10.86
PTX-711-U2	PTX-159	711	10	Control	9/10	0	<	1	0.5	4400	2.2
PTX-730-U2	PTX-137	730	10	Control	9/10	0		2	2	2050	4.1
PTX-705-U3	PTX-175	705	1	$NaHAsO_4$	12/13	923.6		80	80	5060	404.8
PTX-727-U3	PTX-206	727	1	$NaHAsO_4$	12/13	923.6		120	120	7820	938.4
PTX-732-U3	PTX-195	732	1	$NaHAsO_4$	12/13	923.6		130	130	6480	842.4
PTX-742-U3	PTX-198	742	1	$NaHAsO_4$	12/13	923.6		230	230	3520	809.6
PTX-749-U3	PTX-174	749	1	$NaHAsO_4$	12/13	923.6		180	180	4040	727.2
PTX-718-U3	PTX-194	718	2	$NaHAsO_4$	12/13	1864.8		160	160	9580	1532.8
PTX-721-U3	PTX-197	721	2	$NaHAsO_4$	12/13	1864.8		180	180	11020	1983.6
PTX-722-U3	PTX-187	722	2	$NaHAsO_4$	12/13	1864.8		170	170	8420	1431.4
PTX-726-U3	PTX-173	726	2	$NaHAsO_4$	12/13	1864.8		460	460	5580	2566.8
PTX-751-U3	PTX-183	751	2	$NaHAsO_4$	12/13	1864.8		130	130	12500	1625
PTX-701-U3	PTX-193	701	3	$NaHAsO_4$	12/13	3571.2		680	680	4000	2720
PTX-707-U3	PTX-200	707	3	$NaHAsO_4$	12/13	3571.2		160	160	24820	3971.2
PTX-724-U3	PTX-203	724	3	$NaHAsO_4$	12/13	3571.2		380	380	8020	3047.6
PTX-734-U3	PTX-207	734	3	$NaHAsO_4$	12/13	3571.2		400	400	8060	3224
PTX-748-U3	PTX-191	748	3	$NaHAsO_4$	12/13	3571.2		870	870	3040	2644.8
PTX-704-U3	PTX-185	704	4	Soil	12/13	1550		14	14	16840	235.76
PTX-708-U3	PTX-188	708	4	Soil	12/13	1550		25	25	10220	255.5
PTX-712-U3	PTX-181	712	4	Soil	12/13	1550		60	60	2980	178.8
PTX-719-U3	PTX-199	719	4	Soil	12/13	1550		22	22	11300	248.6
PTX-735-U3	PTX-202	735	4	Soil	12/13	1550		19	19	2440	46.36
PTX-713-U3	PTX-201	713	5	Soil	12/13	3189.28		7.2	7.2	42520	306.144
PTX-714-U3	PTX-196	714	5	Soil	12/13	3189.28		38	38	10280	390.64
PTX-715-U3	PTX-179	715	5	Soil	12/13	3189.28		39	39	10400	405.6
PTX-731-U3	PTX-182	731	5	Soil	12/13	3189.28		62	62	6220	385.64
PTX-750-U3	PTX-190	750	5	Soil	12/13	3189.28		180	180	2300	414
PTX-723-U3	PTX-208	723	6	Soil	12/13	6529.84		140	140	5180	725.2
PTX-738-U3	PTX-192	738	6	Soil	12/13	6529.84		110	110	6000	660
PTX-739-U3	PTX-184	739	6	Soil	12/13	6529.84		160	160	3720	595.2
PTX-747-U3	PTX-178	747	6	Soil	12/13	6529.84		77	77	12960	997.92
PTX-752-U3	PTX-186	752	6	Soil	12/13	6529.84		63	63	10980	691.74
PTX-709-U3	PTX-180	709	10	Control	12/13	0	<	1	0.5	10020	5.01
PTX-711-U3	PTX-189	711	10	Control	12/13	0		3	3	4540	13.62
PTX-730-U3	PTX-205	730	10	Control	12/13	0		2	2	2340	4.68

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

TABLE A-10 VANDIUM ANALYTICAL RESULTS FOR STUDY SAMPLES

Sample Number	Tag Number	Pig Number	Group	Material Administered	Event/Day	Actual V BWAdj Dose (ug/kg-d)	Q	Reported Conc (ug/g)	AdjConc* (ug/g)
PTX-704-L	PTX-209	704	4	Soil	15	111.76		0.058	0.058
PTX-708-L	PTX-241	708	4	Soil	15	99.31		0.032	0.032
PTX-712-L	PTX-253	712	4	Soil	15	102.37		0.02	0.02
PTX-719-L	PTX-235	719	4	Soil	15	106.81		0.02	0.02
PTX-735-L	PTX-234	735	4	Soil	15	118.77		0.02	0.02
PTX-713-L	PTX-222	713	5	Soil	15	208.8		0.046	0.046
PTX-714-L	PTX-232	714	5	Soil	15	194.28		0.036	0.036
PTX-715-L	PTX-247	715	5	Soil	15	222.43		0.033	0.033
PTX-731-L	PTX-223	731	5	Soil	15	226.63		0.046	0.046
PTX-750-L	PTX-218	750	5	Soil	15	219.6		0.059	0.059
PTX-723-L	PTX-244	723	6	Soil	15	429.16		0.05	0.05
PTX-738-L	PTX-238	738	6	Soil	15	396.47		0.062	0.062
PTX-739-L	PTX-228	739	6	Soil	15	462		0.05	0.05
PTX-747-L	PTX-243	747	6	Soil	15	407.31		0.045	0.045
PTX-752-L	PTX-233	752	6	Soil	15	399.69		0.045	0.045
PTX-703-L	PTX-215	703	7	$VOSO_4$	15	92.11		0.12	0.12
PTX-710-L	PTX-213	710	7	$VOSO_4$	15	93.48		0.077	0.077
PTX-717-L	PTX-212	717	7	$VOSO_4$	15	81.26		0.11	0.11
PTX-740-L	PTX-239	740	7	$VOSO_4$	15	90.68		0.17	0.17
PTX-746-L	PTX-214	746	7	$VOSO_4$	15	84.09		0.14	0.14
PTX-716-L	PTX-246	716	8	$VOSO_4$	15	174.95		0.15	0.15
PTX-720-L	PTX-219	720	8	$VOSO_4$	15	162.5		0.16	0.16
PTX-736-L	PTX-225	736	8	$VOSO_4$	15	147.74		0.2	0.2
PTX-737-L	PTX-221	737	8	$VOSO_4$	15	157.06		0.16	0.16
PTX-743-L	PTX-259	743	8	$VOSO_4$	15	169.49		0.18	0.18
PTX-702-L	PTX-226	702	9	$VOSO_4$	15	293.85		0.31	0.31
PTX-728-L	PTX-240	728	9	$VOSO_4$	15	298.38		0.19	0.19
PTX-733-L	PTX-255	733	9	$VOSO_4$	15	309.32		0.25	0.25
PTX-744-L	PTX-220	744	9	$VOSO_4$	15	372.91		0.41	0.41
PTX-745-L	PTX-248	745	9	$VOSO_4$	15	337.83		0.26	0.26
PTX-709-L	PTX-250	709	10	Control	15	0		0.02	0.02
PTX-711-L	PTX-254	711	10	Control	15	0		0.01	0.01
PTX-730-L	PTX-256	730	10	Control	15	0		0.02	0.02
PTX-704-K	PTX-283	704	4	Soil	15	111.76		0.066	0.066
PTX-708-K	PTX-295	708	4	Soil	15	99.31		0.067	0.067
PTX-712-K	PTX-301	712	4	Soil	15	102.37		0.041	0.041
PTX-719-K	PTX-261	719	4	Soil	15	106.81		0.032	0.032
PTX-735-K	PTX-275	735	4	Soil	15	118.77		0.034	0.034
PTX-713-K	PTX-271	713	5	Soil	15	208.8		0.063	0.063
PTX-714-K	PTX-276	714	5	Soil	15	194.28		0.082	0.082
PTX-715-K	PTX-269	715	5	Soil	15	222.43		0.097	0.097
PTX-731-K	PTX-278	731	5	Soil	15	226.63		0.096	0.096
PTX-750-K	PTX-284	750	5	Soil	15	219.6		0.091	0.091
PTX-723-K	PTX-298	723	6	Soil	15	429.16		0.087	0.087
PTX-738-K	PTX-300	738	6	Soil	15	396.47		0.088	0.088
PTX-739-K	PTX-306	739	6	Soil	15	462		0.088	0.088
PTX-747-K	PTX-265	747	6	Soil	15	407.31		0.092	0.092
PTX-752-K	PTX-260	752	6	Soil	15	399.69		0.076	0.076
PTX-703-K	PTX-302	703	7	$VOSO_4$	15	92.11		0.25	0.25
PTX-710-K	PTX-277	710	7	$VOSO_4$	15	93.48		0.15	0.15
PTX-717-K	PTX-290	717	7	$VOSO_4$	15	81.26		0.31	0.31
PTX-740-K	PTX-294	740	7	$VOSO_4$	15	90.68		0.33	0.33

TABLE A-10, CONTINUED: VANDIUM ANALYTICAL RESULTS FOR STUDY SAMPLES

Sample Number	Tag Number	Pig Number	Group	Material Administered	Event/Day	Actual V BWAdj Dose (ug/kg-d)	Q	Reported Conc (ug/g)	AdjConc* (ug/g)
PTX-746-K	PTX-279	746	7	$VOSO_4$	15	84.09		0.44	0.44
PTX-716-K	PTX-287	716	8	$VOSO_4$	15	174.95		0.36	0.36
PTX-720-K	PTX-280	720	8	$VOSO_4$	15	162.5		0.34	0.34
PTX-736-K	PTX-291	736	8	$VOSO_4$	15	147.74		0.45	0.45
PTX-737-K	PTX-307	737	8	$VOSO_4$	15	157.06		0.39	0.39
PTX-743-K	PTX-289	743	8	$VOSO_4$	15	169.49		0.4	0.4
PTX-702-K	PTX-281	702	9	$VOSO_4$	15	293.85		0.84	0.84
PTX-728-K	PTX-304	728	9	$VOSO_4$	15	298.38		0.55	0.55
PTX-733-K	PTX-282	733	9	$VOSO_4$	15	309.32		0.55	0.55
PTX-744-K	PTX-272	744	9	$VOSO_4$	15	372.91		0.93	0.93
PTX-745-K	PTX-286	745	9	$VOSO_4$	15	337.83		0.84	0.84
PTX-709-K	PTX-293	709	10	Control	15	0		0.02	0.02
PTX-711-K	PTX-262	711	10	Control	15	0		0.03	0.03
PTX-730-K	PTX-267	730	10	Control	15	0		0.02	0.02
PTX-704-F	PTX-342	704	4	Soil	15	111.76		0.9	0.9
PTX-708-F	PTX-321	708	4	Soil	15	99.31		1.1	1.1
PTX-712-F	PTX-341	712	4	Soil	15	102.37		0.6	0.6
PTX-719-F	PTX-345	719	4	Soil	15	106.81		0.8	0.8
PTX-735-F	PTX-311	735	4	Soil	15	118.77		0.4	0.4
PTX-713-F	PTX-337	713	5	Soil	15	208.8		1.2	1.2
PTX-714-F	PTX-332	714	5	Soil	15	194.28		1.1	1.1
PTX-715-F	PTX-314	715	5	Soil	15	222.43		1.3	1.3
PTX-731-F	PTX-318	731	5	Soil	15	226.63		0.9	0.9
PTX-750-F	PTX-346	750	5	Soil	15	219.6		1.6	1.6
PTX-723-F	PTX-327	723	6	Soil	15	429.16		2.2	2.2
PTX-738-F	PTX-331	738	6	Soil	15	396.47		1.2	1.2
PTX-739-F	PTX-317	739	6	Soil	15	462		1.1	1.1
PTX-747-F	PTX-320	747	6	Soil	15	407.31		1.5	1.5
PTX-752-F	PTX-334	752	6	Soil	15	399.69		1.2	1.2
PTX-703-F	PTX-313	703	7	$VOSO_4$	15	92.11		5.3	5.3
PTX-710-F	PTX-326	710	7	$VOSO_4$	15	93.48		2.5	2.5
PTX-717-F	PTX-344	717	7	$VOSO_4$	15	81.26		4.4	4.4
PTX-740-F	PTX-335	740	7	$VOSO_4$	15	90.68		3.7	3.7
PTX-746-F	PTX-330	746	7	$VOSO_4$	15	84.09		5.1	5.1
PTX-716-F	PTX-343	716	8	$VOSO_4$	15	174.95		5.2	5.2
PTX-720-F	PTX-316	720	8	$VOSO_4$	15	162.5		3.7	3.7
PTX-736-F	PTX-319	736	8	$VOSO_4$	15	147.74		6.5	6.5
PTX-737-F	PTX-323	737	8	$VOSO_4$	15	157.06		5.6	5.6
PTX-743-F	PTX-325	743	8	$VOSO_4$	15	169.49		6.1	6.1
PTX-702-F	PTX-312	702	9	$VOSO_4$	15	293.85		12	12
PTX-728-F	PTX-322	728	9	$VOSO_4$	15	298.38		7.4	7.4
PTX-733-F	PTX-324	733	9	$VOSO_4$	15	309.32		8.7	8.7
PTX-744-F	PTX-328	744	9	$VOSO_4$	15	372.91		14	14
PTX-745-F	PTX-339	745	9	$VOSO_4$	15	337.83		9.8	9.8
PTX-709-F	PTX-333	709	10	Control	15	0		0.6	0.6
PTX-711-F	PTX-340	711	10	Control	15	0		0.6	0.6
PTX-730-F	PTX-336	730	10	Control	15	0		0.5	0.5

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

TABLE A-11 ANALYTICAL RESULTS FOR QUALITY CONTROL SAMPLES

Sample Type	Sample Number	Tag Number	Pig Number	Analyte	Matrix	Original Pig #	Group	Material Administered	Urine Collection	Q	Conc (ng/mL)	DL	AdjConc* (ng/mL)	Original Result* (ng/mL)
Blind Dup	PTX-2734-U1	PTX-121	2734	As	urine	734	3	$NaHAsO_4$	U1		290	5	290	280
Blind Dup	PTX-2704-U1	PTX-126	2704	As	urine	704	4	Soil	U1		21	1	21	21
Blind Dup	PTX-2721-U1	PTX-109	2721	As	urine	721	2	$NaHAsO_4$	U1		160	5	160	150
Blind Dup	PTX-2749-U2	PTX-156	2749	As	urine	749	1	$NaHAsO_4$	U2		140	5	140	150
Blind Dup	PTX-2708-U2	PTX-150	2708	As	urine	708	4	Soil	U2		21	1	21	21
Blind Dup	PTX-2709-U2	PTX-152	2709	As	urine	709	10	Control	U2	<	1	1	0.5	1
Blind Dup	PTX-2751-U3	PTX-176	2751	As	urine	751	2	$NaHAsO_4$	U3		130	5	130	130
Blind Dup	PTX-2714-U3	PTX-177	2714	As	urine	714	5	Soil	U3		38	1	38	38
Blind Dup	PTX-2712-U3	PTX-204	2712	As	urine	712	4	Soil	U3		62	1	62	60

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

Lab QC Type	Submitter I.D.	Certified Mean	+/- SD	Analyte	DL	Q	Conc	Units	Orig Q	Orig Sample Conc	Lab QC Evaluation	
Lab Dup	PTX-102			As	5		160	ng/mL		160	0	% Deviation
Lab Dup	PTX-114			As	10		810	ng/mL		820	1.2	% Deviation
Lab Dup	PTX-123			As	5		98	ng/mL		100	2	% Deviation
Lab Dup	PTX-132			As	5		130	ng/mL		140	7.4	% Deviation
Lab Dup	PTX-143			As	5		290	ng/mL		280	3.5	% Deviation
Lab Dup	PTX-152			As	1	<	1	ng/mL	<	1	0	% Deviation
Lab Dup	PTX-163			As	5		210	ng/mL		210	0	% Deviation
Lab Dup	PTX-172			As	5		140	ng/mL		140	0	% Deviation
Lab Dup	PTX-183			As	5		130	ng/mL		130	0	% Deviation
Lab Dup	PTX-192			As	5		100	ng/mL		110	9.5	% Deviation
Lab Dup	PTX-202			As	1		19	ng/mL		19	0	% Deviation
Lab Dup	PTX-242			V	0.01		0.12	mcg/g		0.11	8.3	% Deviation
Lab Dup	PTX-258			V	0.01		0.19	mcg/g		0.19	0	% Deviation
Lab Dup	PTX-231			V	0.01		0.034	mcg/g		0.033	2.9	% Deviation
Lab Dup	PTX-309			V	0.01		0.038	mcg/g		0.032	17.1	% Deviation
Lab Dup	PTX-310			V	0.01		1.06	mcg/g		0.93	13	% Deviation
Lab Dup	PTX-308			V	0.01		0.27	mcg/g		0.25	7.7	% Deviation

TABLE A-11, CONTINUED: ANALYTICAL RESULTS FOR QUALITY CONTROL SAMPLES

Lab QC Type	Submitter I.D.	Certified Mean	+/- SD	Analyte	DL	Q	Conc	Units	Orig Q	Orig Sample Conc	Lab QC Evaluation	
Lab Dup	PTX-338			V	0.3		7.8	mcg/g		7.4	5.3	% Deviation
Lab Dup	PTX-315			V	0.3		3.4	mcg/g		3.7	8.3	% Deviation
Lab Dup	PTX-329			V	0.3		1.8	mcg/g		1.6	11.8	% Deviation
Spike	PTX-106			As	5		540	ng/mL		340	**	% Recovery
Spike	PTX-118			As	5		520	ng/mL		320	**	% Recovery
Spike	PTX-127			As	1		230	ng/mL		26	102	% Recovery
Spike	PTX-137			As	1		210	ng/mL		2	104	% Recovery
Spike	PTX-146			As	1		240	ng/mL		22	109	% Recovery
Spike	PTX-157			As	5		400	ng/mL		190	105	% Recovery
Spike	PTX-168			As	1		240	ng/mL		33	104	% Recovery
Spike	PTX-177			As	1		240	ng/mL		38	101	% Recovery
Spike	PTX-188			As	1		250	ng/mL		25	113	% Recovery
Spike	PTX-196			As	1		260	ng/mL		38	111	% Recovery
Spike	PTX-206			As	5		330	ng/mL		120	105	% Recovery
Spike	PTX-226-SPK-M			V	0.01		0.39	Mcg/g		0.31	**	% Recovery
Spike	PTX-254-SPK-H			V	0.01		0.18	Mcg/g		0.01	113	% Recovery
Spike	PTX-256-SPK-L			V	0.01		0.087	Mcg/g		0.02	134	% Recovery
Spike	PTX-277-SPK-H			V	0.01		0.49	Mcg/g		0.15	113	% Recovery
Spike	PTX-281-SPK-L			V	0.01		0.96	Mcg/g		0.84	**	% Recovery
Spike	PTX-304-SPK-M			V	0.01		0.71	Mcg/g		0.55	**	% Recovery
Spike	PTX-317-SPK-L			V	0.3		4.2	Mcg/g		1.1	124	% Recovery
Spike	PTX-326-SPK-M			V	0.3		8.3	Mcg/g		2.5	116	% Recovery
Spike	PTX-345-SPK-H			V	0.3		9.4	Mcg/g		0.8	115	% Recovery
Ref Mat	NIST 1640	0.0267	0.0004	As	0.003		0.03	mcg/mL		0		
Ref Mat	NRCC TORT-2	21.6	1.8	As	0.5		21	mcg/mL		0		
Ref Mat	NIST 1566b	7.65	0.65	As	0.2		7.9	mcg/mL		0		
Ref Mat	NRCC TORT-2	21.6	1.8	As	0.5		21	mcg/mL		0		
Ref Mat	NIST 1566b	7.65	0.65	As	0.2		7.8	mcg/mL		0		
Ref Mat	NIST 1640	0.0267	0.0004	As	0.003		0.029	mcg/mL		0		
Ref Mat	NIST 1640	0.01299	0.0004	V	0.001		0.013	mcg/g		0		
Ref Mat	NRCC TORT-2	1.64	0.19	V	0.1		1.8	mcg/g		0		
Ref Mat	NRCC TORT-2	1.64	0.19	V	0.05		1.7	mcg/g		0		
Ref Mat	NIST 1640	0.01299	0.0004	V	0.001		0.013	mcg/g		0		
Ref Mat	NRCC TORT-2	1.64	0.19	V	0.05		1.7	mcg/g		0		

TABLE A-11, CONTINUED: ANALYTICAL RESULTS FOR QUALITY CONTROL SAMPLES

Lab QC Type	Submitter I.D.	Certified Mean	+/- SD	Analyte	DL	Q	Conc	Units	Orig Q	Orig Sample Conc	Lab QC Evaluation
Ref Mat	NIST 1640	0.01299	0.0004	V	0.001		0.013	mcg/g		0	
Ref Mat	NRCC TORT-2	1.64	0.19	V	0.02		1.6	mcg/g		0	
Ref Mat	NIST 1640	0.01299	0.0004	V	0.001		0.013	mcg/g		0	
Ref Mat	NIST 1640	0.01299	0.0004	V	0.001		0.014	mcg/g		0	
Ref Mat	NIST 1640	0.01299	0.0004	V	0.001		0.012	mcg/g		0	
Blank	Blank-1			As	1	<	1	ng/mL		0	
Blank	Blank-2			As	1	<	1	ng/mL		0	
Blank	Blank-3			As	1	<	1	ng/mL		0	
Blank	Blank-4			As	1	<	1	ng/mL		0	
Blank	Blank-5			As	1	<	1	ng/mL		0	
Blank	Blank-6			As	1	<	1	ng/mL		0	
Blank	Blank-1			V	0.01	<	0.01	mcg/g		0	
Blank	Blank-2			V	0.01	<	0.01	mcg/g		0	
Blank	Blank-3			V	0.01	<	0.01	mcg/g		0	
Blank	Blank-4			V	0.01	<	0.01	mcg/g		0	
Blank	Blank-5			V	0.3	<	0.3	mcg/g		0	
Blank	Blank-6			V	0.3	<	0.3	mcg/g		0	
Blank	Blank-7			V	0.001	<	0.001	mcg/g		0	
Blank	PTX-Blank-Liver			V	0.01	<	0.01	mcg/g		0	
Blank	PTX-BLANK-KIDNEY			V	0.01	<	0.01	mcg/g		0	
Blank	PTX-Blank-Femur			V	0.3	<	0.3	mcg/g		0	

** indicates spike too low