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RELATIVE BIOAVAILABILITY OF ARSENIC IN A MOHR ORCHARD SOIL

Prepared for:

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EXECUTIVE SUMMARY

A study using juvenile swine as test animals was performed to measure the gastrointestinal absorption of arsenic from a Mohr Orchard soil sample. The soil sample was collected from the Mohr Orchard site located in Lehigh County, Pennsylvania. The property was historically largely utilized as orchards and currently consists of farmland, woodland, residential, commercial, and industrial properties. The arsenic concentration of the Mohr Orchard soil sample is 340±4.5 mg/kg (mean±SD).

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

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

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

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

Estimated RBA for Mohr Orchard Soil

Measurement Interval	Estimated RBA (90% Confidence Interval)
Days 6/7	0.50 (0.46–0.55)
Days 9/10	0.54 (0.49–0.59)
Days 12/13	0.56 (0.50–0.63)
All Days	0.53 (0.51–0.57)

The best fit point estimate RBA for the Mohr Orchard soil sample is 53%.

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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
°C	Degrees Celsius
D	Ingested dose
DMA	Dimethyl arsenic
g	Gram
GLP	Good Laboratory Practices
kg	Kilogram
K_u	Fraction of absorbed arsenic which is excreted in urine
mL	Milliliter
MMA	Monomethyl arsenic
N	Number of data points
NAA	Neutron activation analysis
NaAs	Sodium arsenate
NERL	National Exposure Research Laboratory
NIST	National Institute of Standards and Technology
NRCC	National Research Council of Canada
QC	Quality control
RBA	Relative bioavailability
ref	Reference material
RfD	Reference dose
RPD	Relative percent difference
SD	Standard deviation
SF	Slope factor
SRM	Standard reference material
TM	Test material
UEF	Urinary excretion fraction
µg	Microgram
µm	Micrometer
USEPA	United States Environmental Protection Agency
XRF	X-ray fluorescence

1.0 INTRODUCTION

1.1 Overview of Bioavailability

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

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

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

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

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

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

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

For example, if 100 micrograms (μg) of a chemical (*e.g.*, arsenic) dissolved in drinking water were ingested and a total of 50 μg were absorbed into the body, the AF_o would be 50/100,

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

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

1.2 Using RBA Data to Improve Risk Calculations

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

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

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

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

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

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

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

1.3 Purpose of this Study

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

2.0 STUDY DESIGN

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

Table 2-1. Study Design and Dosing Information

Group	Group name abbreviation	Dose material administered	As concentration of material (µg/g or µg/µL)	Number swine in group	Arsenic Dose		
					Target (µg/kg BW-day)	Actual [a] (µg/kg BW-day)	Actual [b] (µg-day)
1	NaAs	Sodium Arsenate	2	4	25	29	308
2	NaAs	Sodium Arsenate	10	4	50	62	620
3	NaAs	Sodium Arsenate	10	4	100	130	1240
4	TM1	Mohr Orchard Soil	340	4	40	52	493
5	TM1	Mohr Orchard Soil	340	4	60	72	738
6	TM1	Mohr Orchard Soil	340	4	120	153	1476
7	Control	None (negative control)	0	3	0	0	0

[a] Calculated as the administered daily dose divided by the measured or extrapolated daily body weight, averaged over days 0–14 for each animal and each group.
[b] Calculated as the mass of soil or sodium arsenate solution administered times the concentration of the soil or sodium arsenate solution.

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

2.1 Test Materials

2.1.1 Sample Description

The former Mohr Orchard site is located in Lehigh County, Pennsylvania and consists of farmland, woodland, residential, commercial, and industrial properties. Historically, large portions of the site were utilized as orchards and arsenical pesticides were commonly used to control pests.

2.1.2 Sample Preparation and Analysis

Soil was collected from two, 200-square foot grids that were located next to one another on county property. These areas had arsenic concentrations >100 ppm (as identified *in situ* using X-ray fluorescence [XRF] technology). The soil material was collected into 2-gallon buckets, homogenized, and placed into large plastic bags for storage. Upon receipt of soil at EPA's Office of Research and Development, National Exposure Research Laboratory (NERL), soil was air-dried on drying trays for 4 days at 40°C. Soil was then sieved to remove plant material, rocks and large chunks of aggregated soil, and finally screened to <250 µm. Soil was then passed through a riffler 5 times and 200 gram aliquots were collected in pre-cleaned 250 mL high-density polyethylene bottles for the study.

Soil metal concentrations were determined by neutron activation analysis (NAA). Two subsamples of the Mohr Orchard soil were analyzed in duplicate. The arsenic concentration of the Mohr Orchard soil sample is 340±4.5 mg/kg (mean±SD).

X-ray absorption spectroscopy was conducted on the test material to characterize the arsenic mineralogy (Miller and Scheckel, 2012).

2.2 Experimental Animals

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

The number of animals purchased for the study was several more than required by the protocol. These animals were purchased at an age of about 5–6 weeks (weaning occurs at age

3 weeks) and housed in individual stainless steel cages. The animals were then held under quarantine for one week to observe their health before beginning exposure to dosing materials. Each animal was examined by a certified veterinary clinician (swine specialist) and any animals that appeared to be in poor health during this quarantine period were excluded from the study. To minimize weight variations among animals and groups, extra animals most different in body weight (either heavier or lighter) five days prior to exposure (day 5) were also excluded from the study. The remaining animals were assigned to dose groups at random (group assignments are presented in Appendix A).

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

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

2.3 Diet

Animals were weaned onto standard swine chow (made at the University of Missouri Animal Science Feed Mill). The feed was nutritionally complete (NRC, 1988). The ingredients of the feed are presented in Appendix C. Arsenic concentration in a randomly selected feed sample measured 0.1 μg/g.

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

Drinking water was provided *ad libitum* via self-activated watering nozzles within each cage. Arsenic concentration of 5 water samples from randomly selected drinking water nozzles were <1 μg/L.

2.4 Dosing

Animals were exposed to dosing materials (sodium arsenate or sieved test material) for 14 days, with the dose for each day being administered in two equal portions beginning at 9:00 AM and 3:00 PM (two hours before feeding). Swine were dosed two hours before feeding to ensure that they were in a semi-fasted state. To facilitate dose administration, dosing materials were placed in a small depression in a ball of dough consisting of moistened feed (typically about 5 g) and the dough was pinched shut. This was then placed in the feeder at dosing time.

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

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

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

2.5 Collection and Preservation of Urine Samples

Samples of urine were collected from each animal for 48-hour periods on days 6 to 7 (U-1), 9 to 10 (U-2), and 12 to 13 (U-3) of the study. Collection began at 8:00 AM and ended 48 hours later. The urine was collected in a plastic bucket placed beneath each cage, which was emptied into a plastic storage bottle. Aluminum screens were placed under the cages to minimize contamination with feces or spilled food. Due to the length of the collection period, collection containers were emptied periodically (typically twice daily) into a separate plastic bottles to ensure that there was no loss of sample due to overflow.

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

2.6 Arsenic Analysis

Urine samples were assigned random chain-of-custody tag numbers and submitted to the analytical laboratory for analysis in a blind fashion. The samples were analyzed for arsenic by L. E. T., Inc., (Columbia, Missouri). In brief, 25-mL samples of urine were digested by refluxing and then heating to dryness in the presence of magnesium nitrate and concentrated nitric acid. Following magnesium nitrate digestion, samples were transferred to a muffle furnace and ashed at 500°C. The digested and ashed residue was dissolved in hydrochloric acid and analyzed by the hydride generation technique using a PerkinElmer 3100 atomic absorption spectrometer. Previous tests of this method established that each of the different forms of arsenic that may occur in urine, including trivalent inorganic arsenic (As^{+3}), pentavalent inorganic arsenic (As^{+5}), monomethyl arsenic (MMA), and dimethyl arsenic (DMA) are all recovered with high efficiency.

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

2.7 Quality Control

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

Blind Duplicates (Sample Preparation Replicates)

A random selection of about 10% of all urine samples generated during the study were prepared for laboratory analysis in duplicate (*i.e.*, two separate subsamples of urine were digested) and submitted to the laboratory in a blind fashion. Results are shown in Appendix E (see Table E-1 and Figure E-1). There was generally good agreement between results for the duplicate pairs.

Spike Recovery

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

Laboratory Duplicates

During arsenic analysis, every tenth sample was analyzed in duplicate. Duplicate results for urine samples typically agreed within 10% relative percent difference (RPD) (see Appendix E, Table E-3). The duplicate water sample was below the detection limit. A duplicate analysis of a feed sample matched the original feed sample concentration (0.1 µg/g).

Laboratory Control Standards

National Institute of Standards and Technology (NIST) Standard Reference Materials® (SRM), for which a certified concentration of specific analytes has been established, were tested periodically during sample analysis (NIST, 2003). Recovery of arsenic from these standards was generally good and within the acceptable range (see Appendix E, Table E-4 and Figure E-2).

Blanks

Blank samples run along with each batch of samples (n=8). Blanks never yielded a measurable level of arsenic (see Appendix E, Table E-5).

Summary of QC Results

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

3.0 DATA ANALYSIS

3.1 Overview

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

- In most animals (including humans), absorbed arsenic is excreted mainly in the urine over the course of several days. Thus, the urinary excretion fraction (UEF), defined as

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

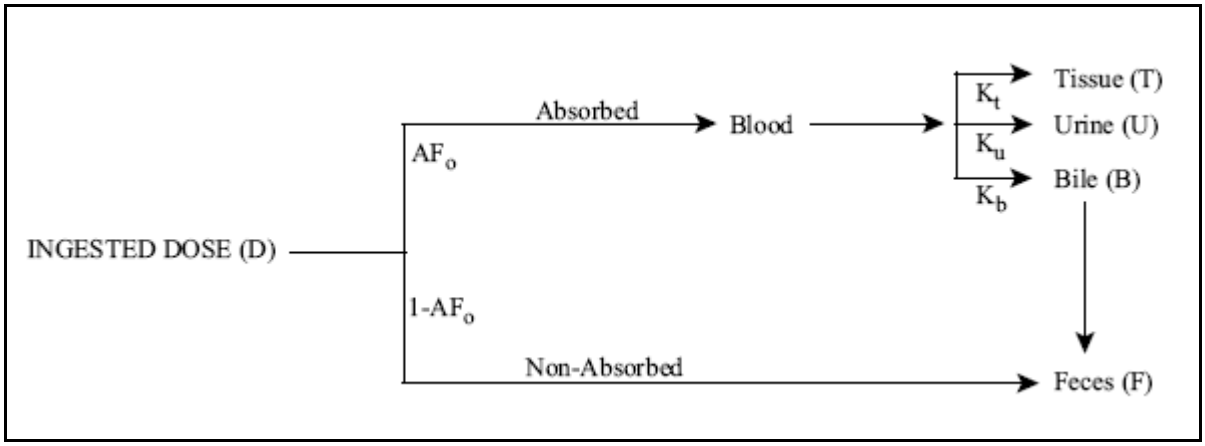
- The RBA of two orally administered materials (*i.e.*, a test material and reference material) can be calculated from the ratio of the urinary excretion fraction of the two materials. This calculation is independent of the extent of tissue binding and of biliary excretion:

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

where:

D = ingested dose (μg)
K_u = fraction of absorbed arsenic that is excreted in the urine

Figure 3-1. Conceptual Model for Arsenic Toxicokinetics



where:

D = ingested dose
AF_o = oral absorption fraction
K_t = fraction of absorbed arsenic that is retained in tissues
K_u = fraction of absorbed arsenic that is excreted in urine
K_b = fraction of absorbed arsenic that is excreted in bile

Basic Equations

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

Amount excreted in urine (μg) $= Amount\ absorbed \times K_u$
$= D \times AF_o \times K_u$

Urinary excretion fraction (UEF) $= Amount\ excreted\ /\ Amount\ ingested$
$= (D \times AF_o \times K_u) / D$
$= AF_o \times K_u$

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

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

1. Plot the amount of arsenic excreted in the urine (μg per 48 hours) as a function of the administered amount of arsenic (μg per 48 hours), both for reference material and for test material.
2. Find the best fit linear regression line through the each data set. The slope of each line (μg per 48 hours excreted per μg per 48 hours ingested) is the best estimate of the UEF for each material.
3. Calculate RBA for each test material as the ratio of the UEF for test material compared to UEF for reference material:

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

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

3.2 Dose-Response Model

Simultaneous Regression

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

Separate Models:
$$\mu_r(i) = a + b_r \cdot x_r(i)$$
$$\mu_t(i) = a + b_t \cdot x_t(i)$$

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

where: $\mu(i)$ indicates the expected mean response of animals exposed at dose $x(i)$, and the subscripts r and t refer to reference and test material, respectively. The coefficients of this combined model are derived using multivariate regression, with the understanding that the combined data set is restricted to cases in which one (or both) of x_r and x_t are zero (Finney, 1978).

Weighted Regression

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

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

where:

w_i = weight assigned to all data points in dose group *i*
σ_i^2 = variance of responses in animals in dose group *i*

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

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

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

where:

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

Based on these data, values of *k*1 and *k*2 were derived using ordinary least squares minimization. The resulting values were -1.10 for *k*1 and 1.64 for *k*2.

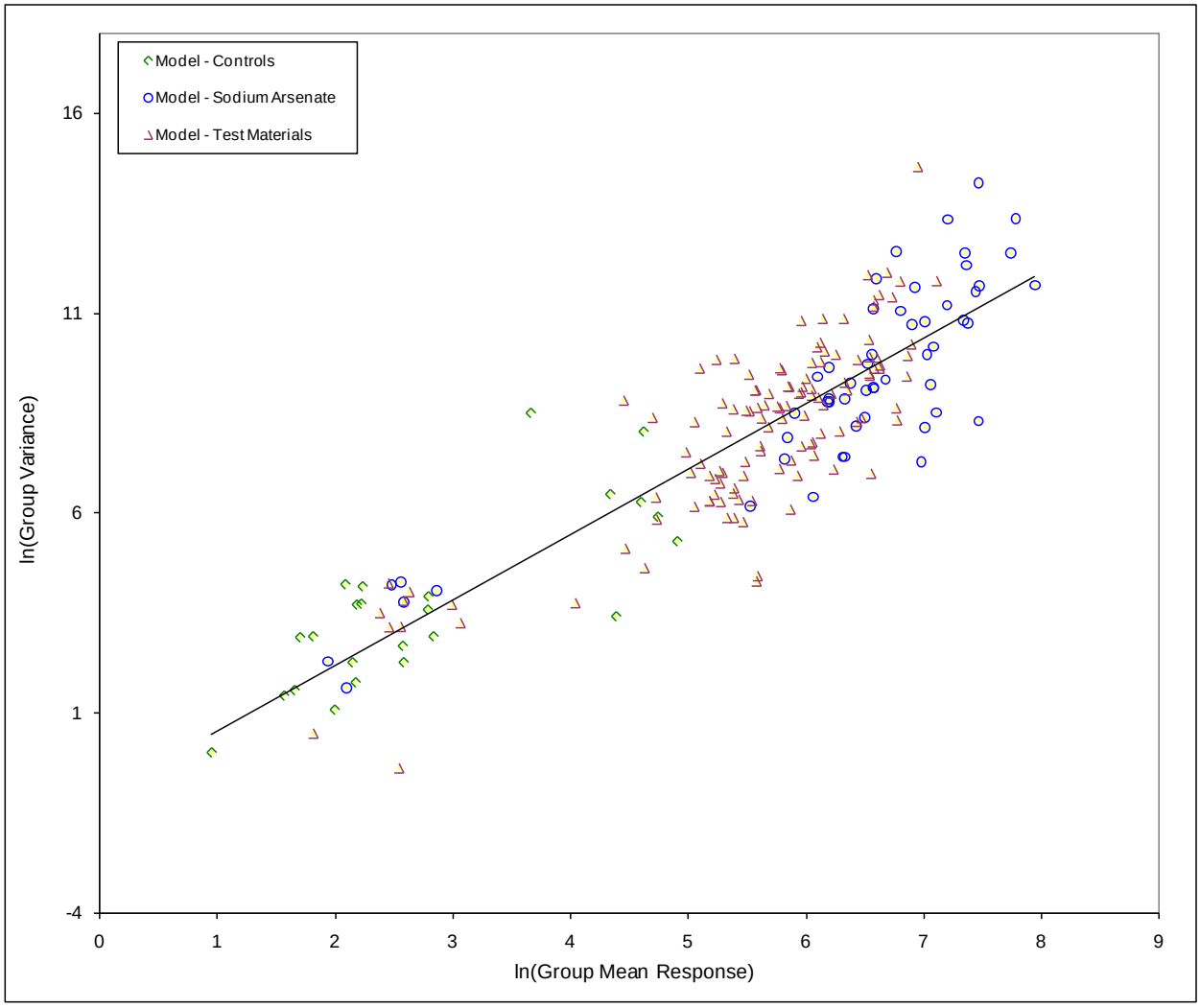
Goodness-of-Fit

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

Assessment of Outliers

In biological assays, it is not uncommon to note the occurrence of individual measured responses that appear atypical compared to the responses from other animals in the same dose group. In this study, responses that yielded standardized weighted residuals greater than 3.5 or less than -3.5 were considered to be potential outliers (Canavos, 1984). Such a data point was encountered in the data set for this study. Therefore, RBA values were calculated both for all the data (outliers included) and without the outlier, and the result with the outlier excluded was used as the preferred estimate.

Figure 3-2. Urinary Arsenic Variance Model



3.3 Calculation of RBA Estimates

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

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

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

4.0 RESULTS

4.1 Clinical Signs

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

4.2 Dosing Deviations

There were no missed doses during this study. Swine 565 was slow to consume his dough balls on days 2, 3, and 4. This was noted during the study but the final dose amount was not affected by the late consumption.

4.3 Background Arsenic Excretion

Measured values for urinary arsenic excretion (mean and standard deviation) for control animals from days 6 to 13 are shown in Table 4-1.

Table 4-1. Background Urinary Arsenic

Sample ID	Swine Number	Collection Period (days)	Arsenic concentration in urine (µg/L)	Arsenic mass in urine (µg/48 hours)
MO-235	564	6/7	35	51.1
MO-155	564	9/10	46	68.1
MO-187	564	12/13	41	59
MO-227	570	6/7	19	35.3
MO-154	570	9/10	21	50.4
MO-204	570	12/13	26	60.3
MO-236	571	6/7	38	54
MO-149	571	9/10	23	61.4
MO-188	571	12/13	45	84.6

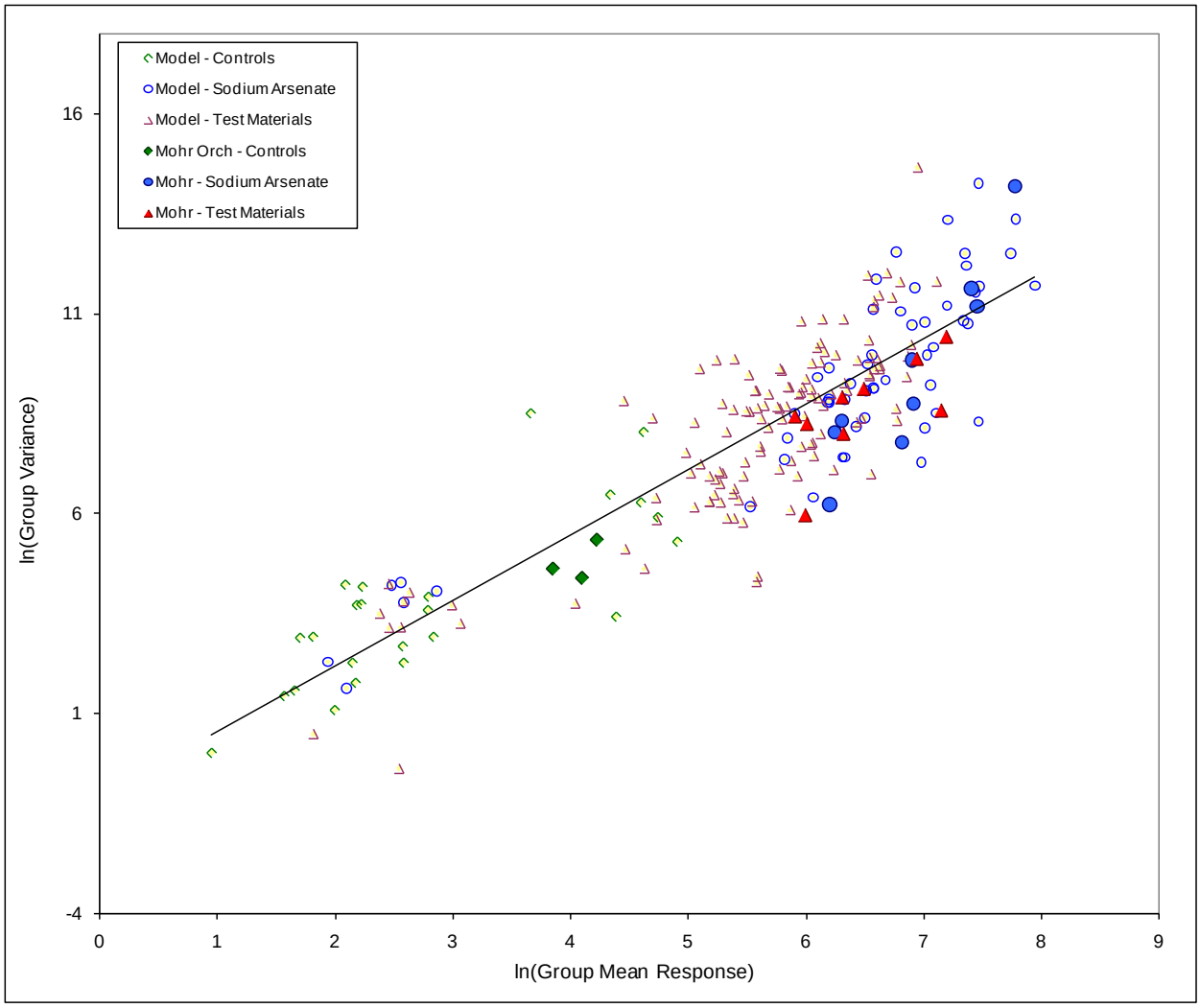
Mean urinary arsenic concentration was 32.6±10.6 µg/L. The values shown are representative of endogenous background levels in food and water and support the view that the animals were not exposed to any significant exogenous sources of arsenic throughout the study.

4.4 Urinary Arsenic Variance

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

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

Figure 4-1. Mohr Orchard Data Compared to Urinary Arsenic Variance Model

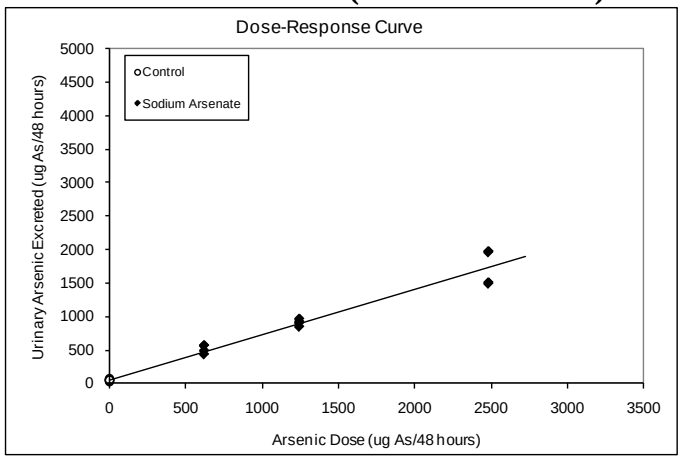


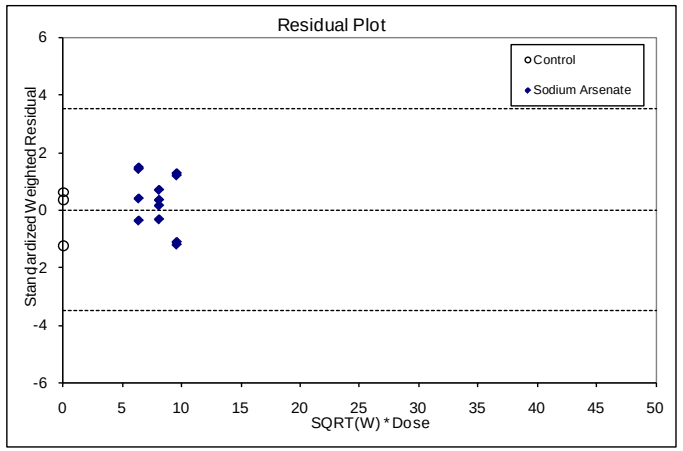
4.5 Dose-Response Modeling

The dose-response data for arsenic in urine were initially modeled using all of the data, and an outlier was identified as discussed in Section 3.2. Initial modeling results are shown in Figures 4-2 through 4-5. Based on this analysis, data for swine 574 on day 9/10 were excluded from the final evaluation for arsenic RBA. Final regression fittings are shown in Figures 4-6 through 4-9.

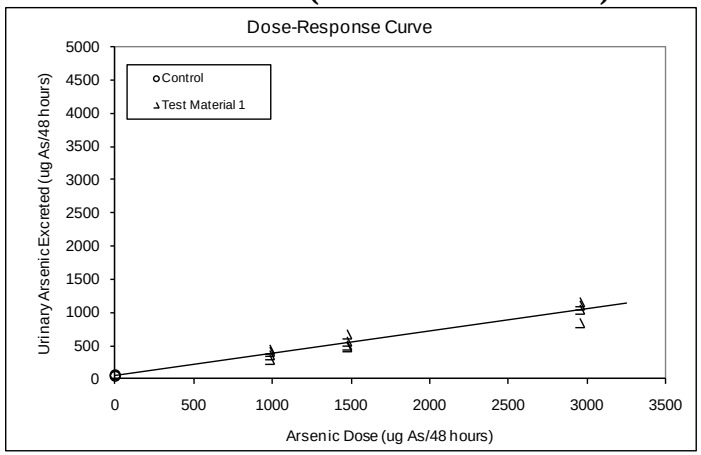
Figure 4-2. Mohr Orchard Urinary Excretion of Arsenic: Days 6/7 (All Data)

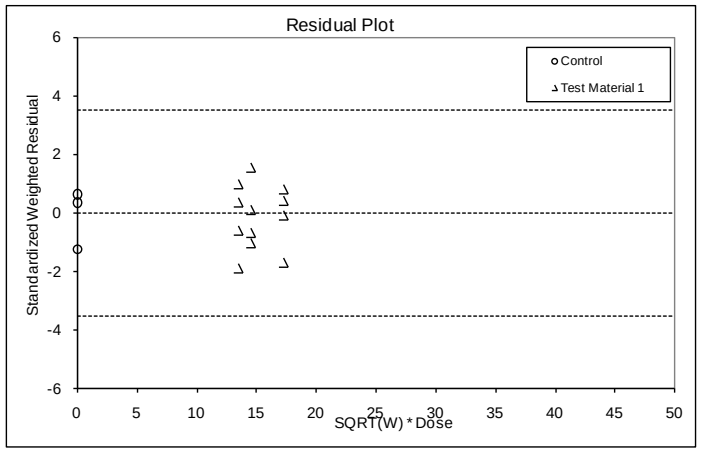
Reference Material (Sodium Arsenate)





Test Material 1 (Mohr Orchard TM1)





Summary of Fitting [a]

Parameter	Estimate	Standard Error
a	47.7	18.8
b_r	0.67	0.03
b_{t1}	0.34	0.02
Covariance (b_r,b_{t1})	0.3723	–
Degrees of Freedom	25	–

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

ANOVA

Source	SSE	DF	MSE
Fit	623.58	2	311.79
Error	14.21	24	0.59
Total	637.79	26	24.53

Statistic	Estimate
F	526.616

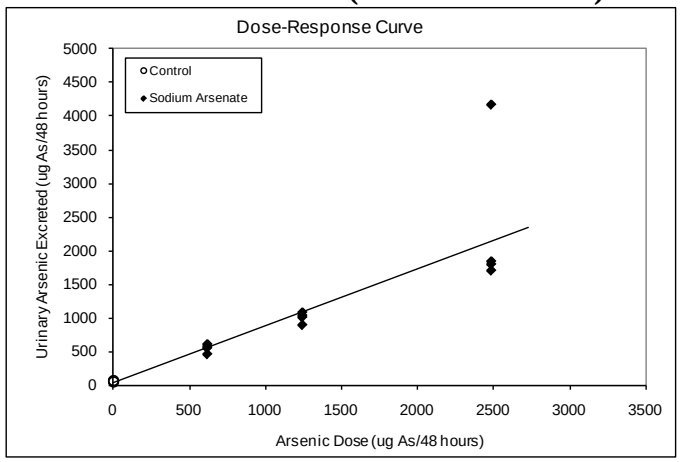
RBA and Uncertainty

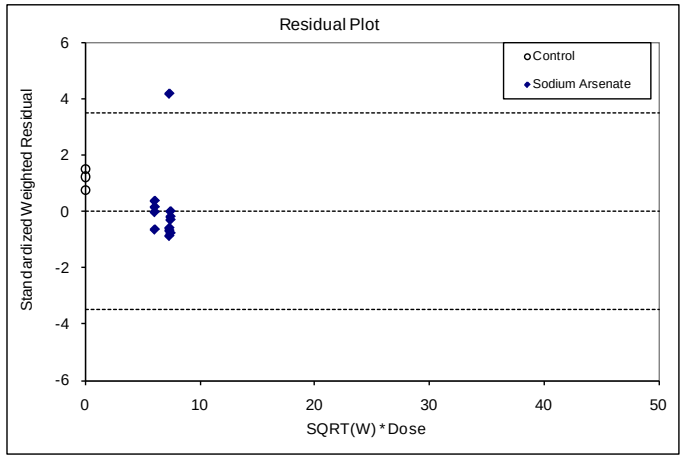
	Test Material 1
RBA	0.50
Lower bound [c]	0.46
Upper bound [c]	0.55
Standard Error [c]	0.027

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

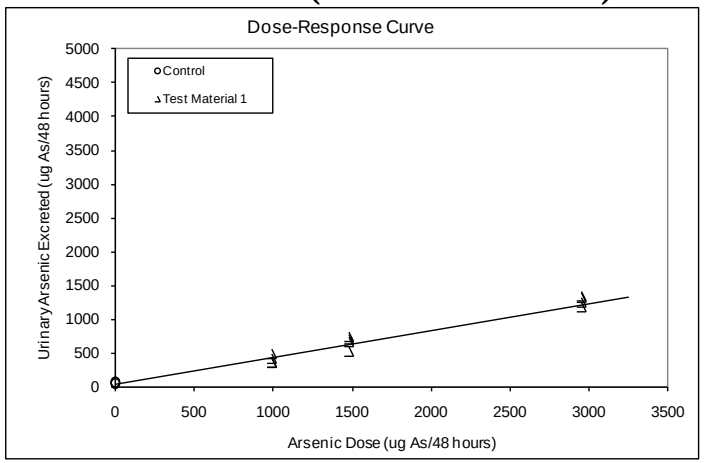
Figure 4-3. Mohr Orchard Urinary Excretion of Arsenic: Days 9/10 (All Data)

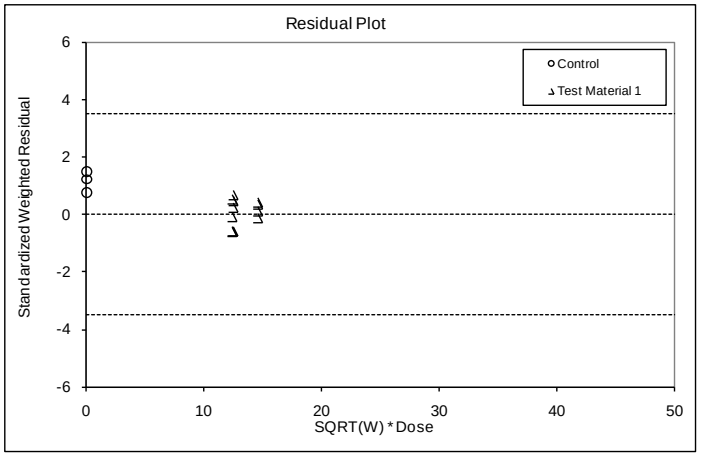
Reference Material (Sodium Arsenate)





Test Material 1 (Mohr Orchard TM1)





Summary of Fitting [a]

Parameter	Estimate	SE
a	32.0	38.9
b_r	0.84	0.07
b_{t1}	0.40	0.04
Covariance (b_r,b_{t1})	0.2500	–
Degrees of Freedom	25	–

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

ANOVA

Source	SSE	DF	MSE
Fit	683.86	2	341.93
Error	56.92	24	2.37
Total	740.78	26	28.49

Statistic	Estimate
F	144.179
p	<0.001
Adjusted R^2	0.9168

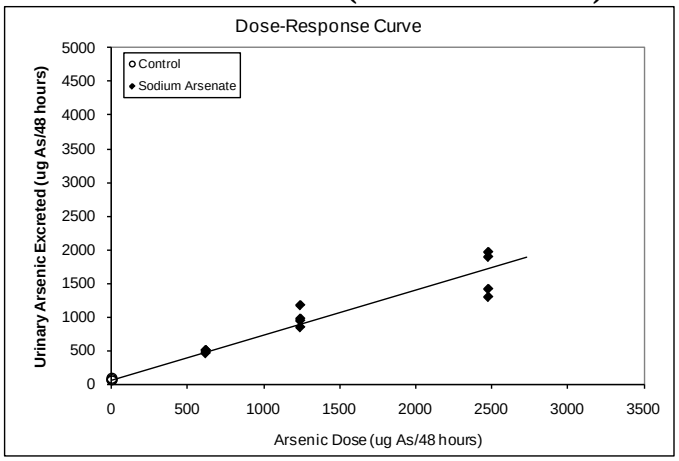
RBA and Uncertainty

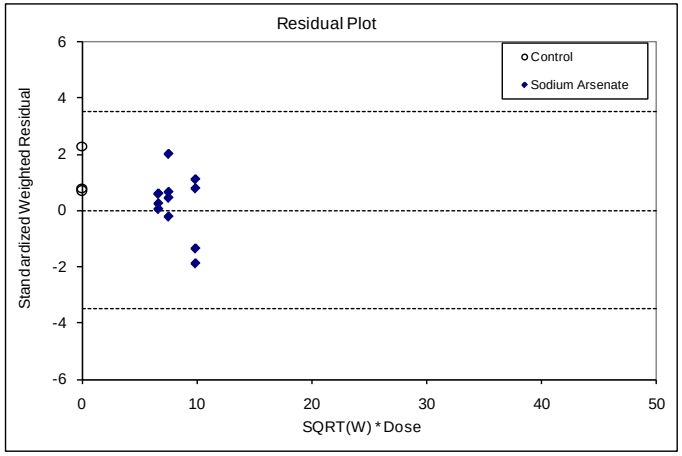
	Test Material 1
RBA	0.47
Lower bound [c]	0.39
Upper bound [c]	0.57
Standard Error [c]	0.053

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

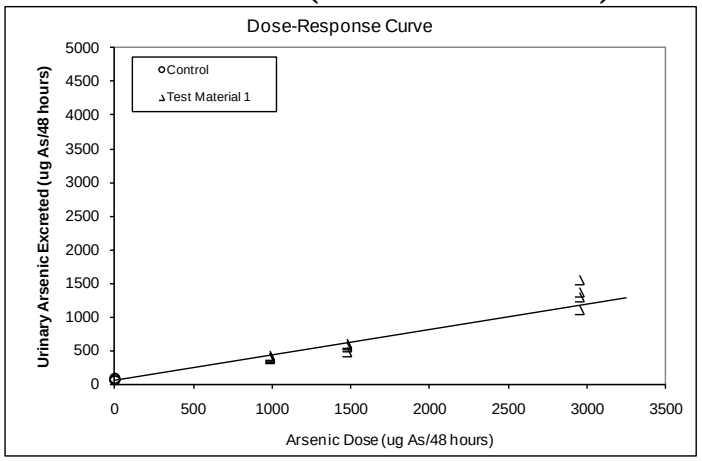
Figure 4-4. Mohr Orchard Urinary Excretion of Arsenic: Days 12/13 (All Data)

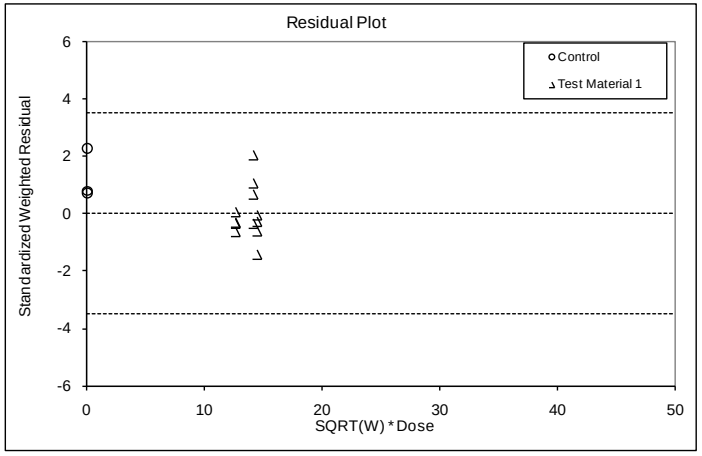
Reference Material (Sodium Arsenate)





Test Material 1 (Mohr Orchard TM1)





Summary of Fitting [a]

Parameter	Estimate	SE
a	47.4	22.8
b_r	0.68	0.03
b_{t1}	0.38	0.02
Covariance (b_r,b_{t1})	0.2729	–
Degrees of Freedom	25	–

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

ANOVA

Source	SSE	DF	MSE
Fit	600.95	2	300.48
Error	22.09	24	0.92
Total	623.04	26	23.96

Statistic	Estimate
F	326.507
p	<0.001
Adjusted R^2	0.9616

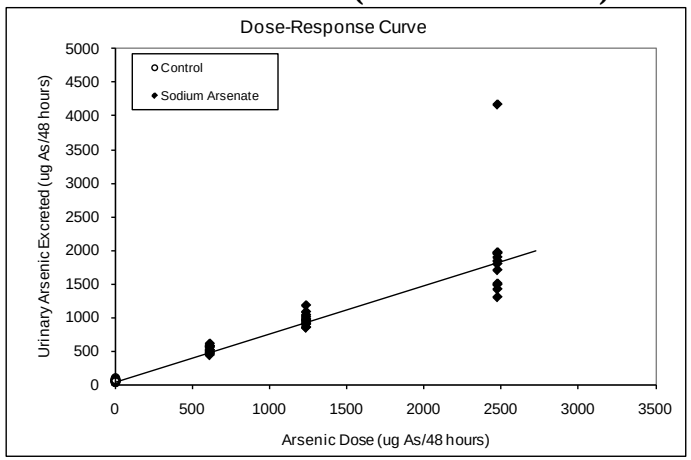
RBA and Uncertainty

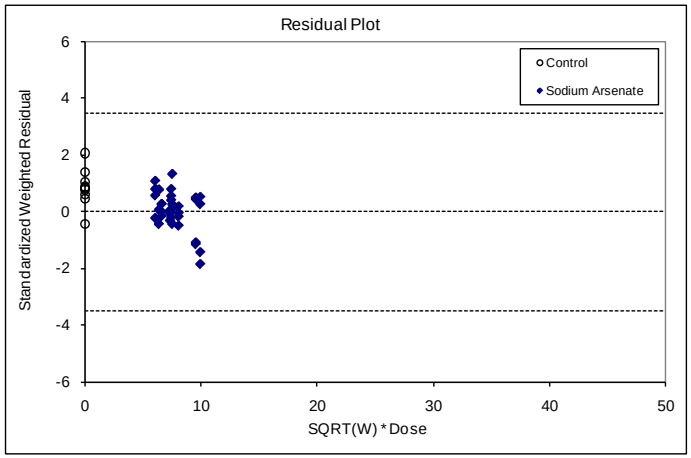
	Test Material 1
RBA	0.56
Lower bound [c]	0.50
Upper bound [c]	0.63
Standard Error [c]	0.037

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

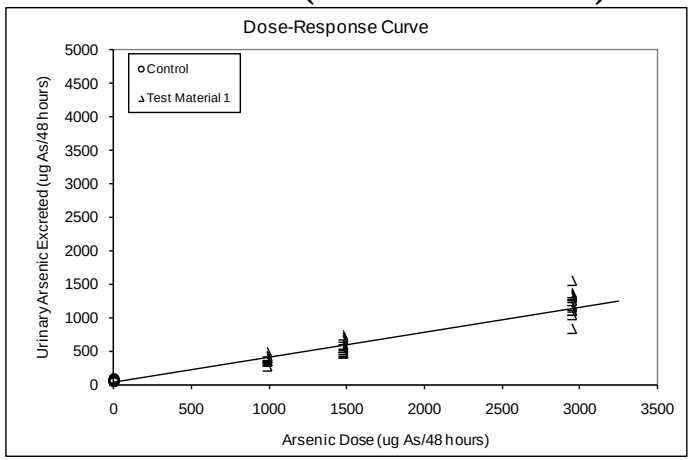
Figure 4-5. Mohr Orchard Urinary Excretion of Arsenic: All Days (Outlier Excluded)

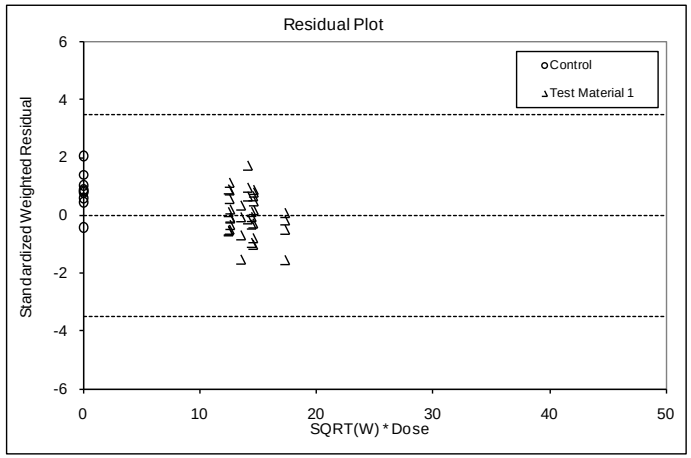
Reference Material (Sodium Arsenate)





Test Material 1 (Mohr Orchard TM1)





Summary of Fitting [a]

Parameter	Estimate	SE
a	41.9	16.9
b_r	0.72	0.03
b_{t1}	0.37	0.02
Covariance (b_r,b_{t1})	0.3052	–
Degrees of Freedom	79	–

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

ANOVA

Source	SSE	DF	MSE
Fit	1894.87	2	947.44
Error	106.46	78	1.36
Total	2001.33	80	25.02

Statistic	Estimate
F	694.188
p	<0.001
Adjusted R^2	0.9454

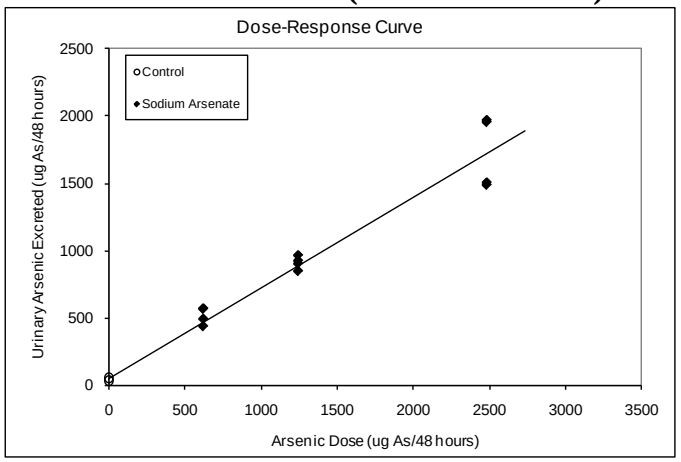
RBA and Uncertainty

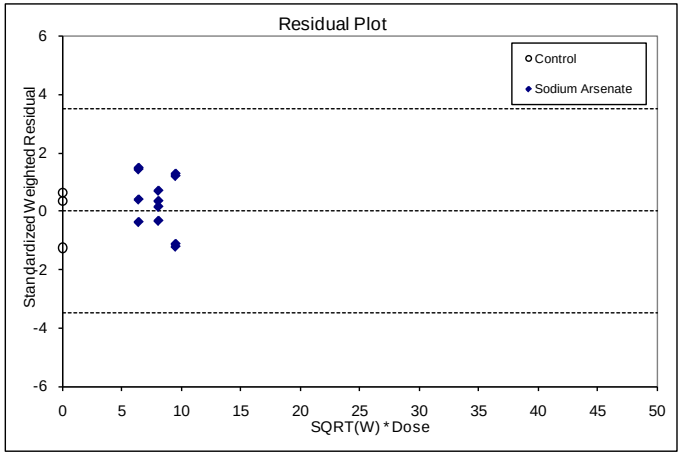
	Test Material 1
RBA	0.52
Lower bound [c]	0.48
Upper bound [c]	0.56
Standard Error [c]	0.025

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

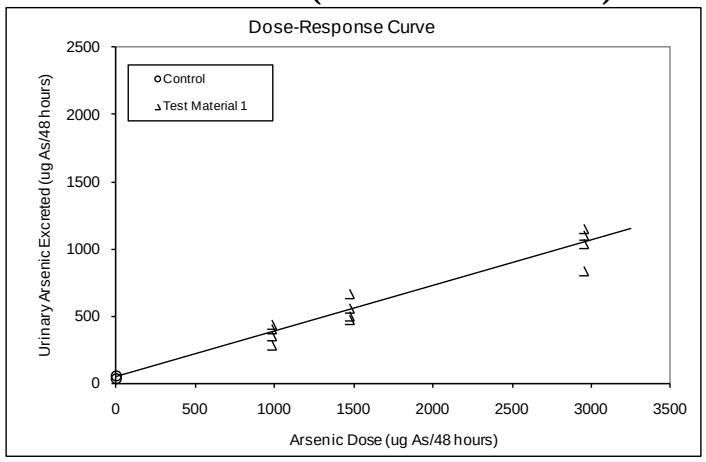
Figure 4-6. Mohr Orchard Urinary Excretion of Arsenic: Days 6/7 (Outlier Excluded)

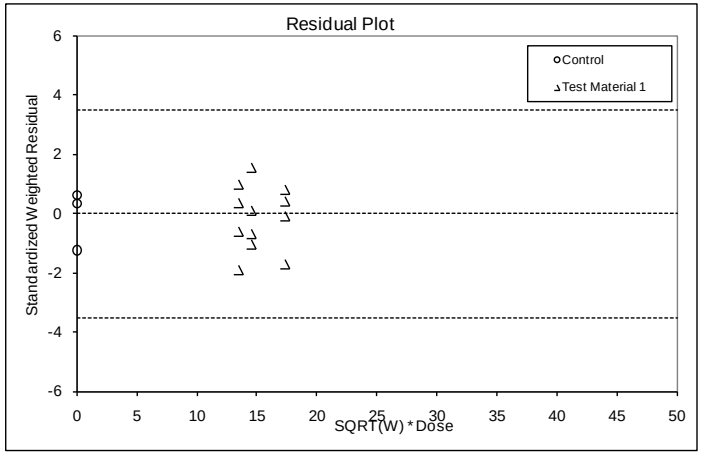
Reference Material (Sodium Arsenate)





Test Material 1 (Mohr Orchard TM1)





Summary of Fitting [a]

Parameter	Estimate	Standard Error
a	47.7	18.8
b_r	0.67	0.03
b_{t1}	0.34	0.02
Covariance (b_r,b_{t1})	0.3723	–
Degrees of Freedom	25	–

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

ANOVA

Source	SSE	DF	MSE
Fit	623.58	2	311.79
Error	14.21	24	0.59
Total	637.79	26	24.53

Statistic	Estimate
F	526.616
p	<0.001
Adjusted R^2	0.9759

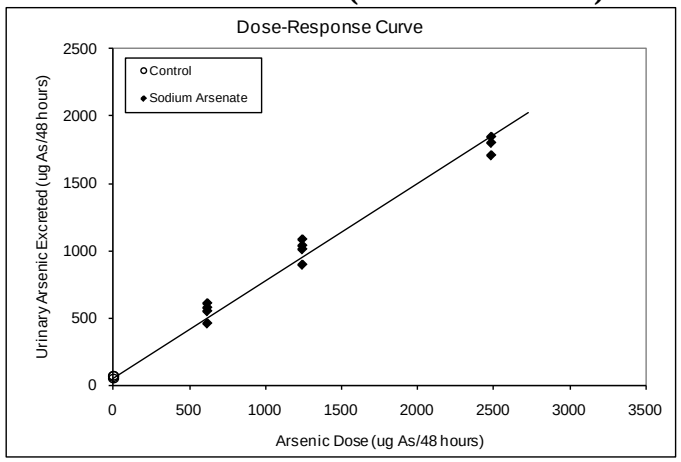
RBA and Uncertainty

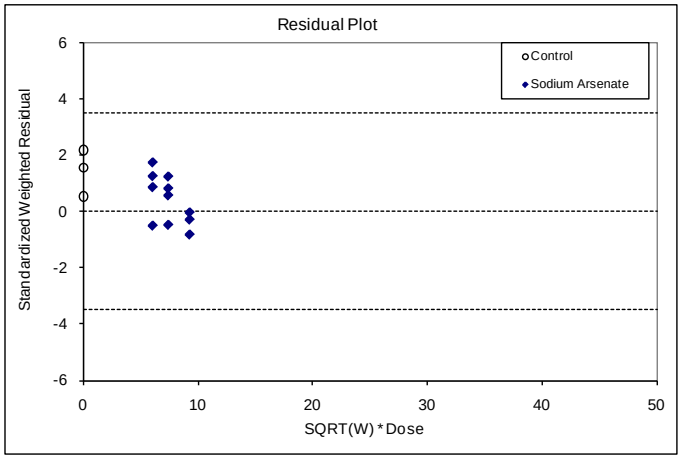
	Test Material 1
RBA	0.50
Lower bound [c]	0.46
Upper bound [c]	0.55
Standard Error [c]	0.027

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

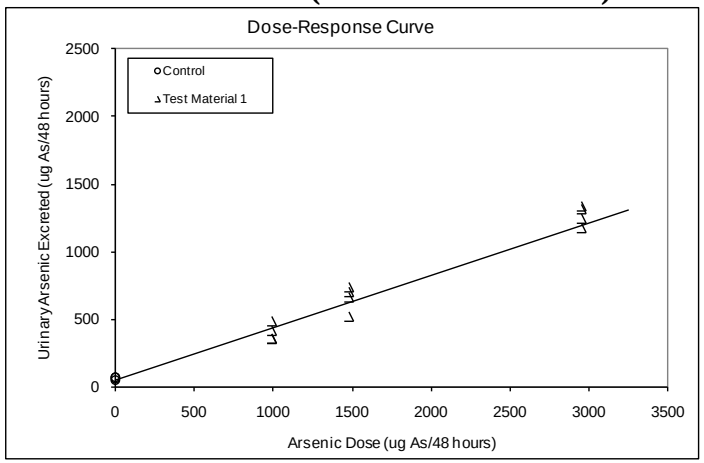
Figure 4-7. Mohr Orchard Urinary Excretion of Arsenic: Days 9/10 (Outlier Excluded)

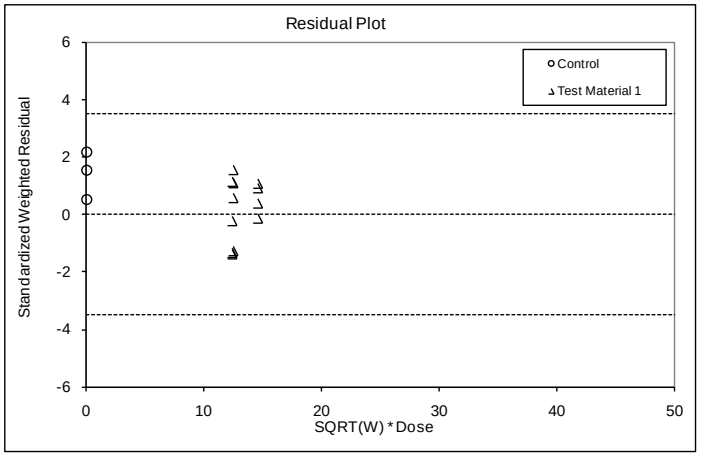
Reference Material (Sodium Arsenate)





Test Material 1 (Mohr Orchard TM1)





Summary of Fitting [a]

Parameter	Estimate	SE
a	44.6	16.8
b_r	0.73	0.03
b_{t1}	0.39	0.02
Covariance (b_r,b_{t1})	0.2503	–
Degrees of Freedom	24	–

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

ANOVA

Source	SSE	DF	MSE
Fit	590.41	2	295.20
Error	12.51	23	0.54
Total	602.92	25	24.12

Statistic	Estimate
F	542.559
p	<0.001
Adjusted R^2	0.9774

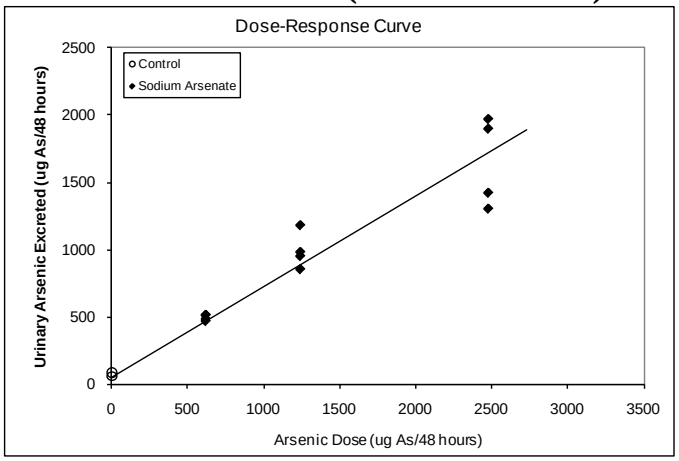
RBA and Uncertainty

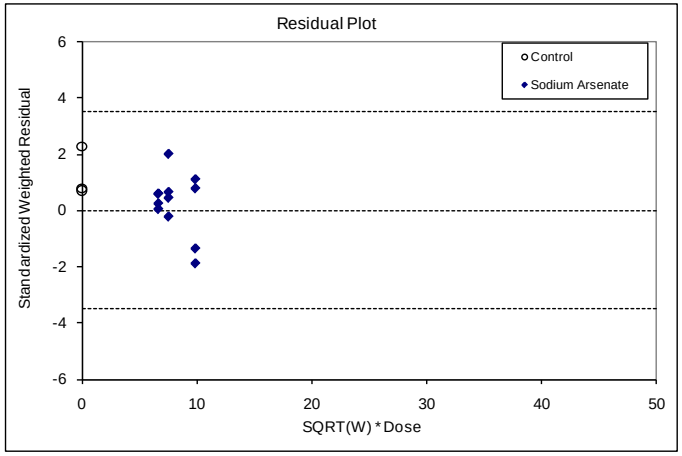
	Test Material 1
RBA	0.54
Lower bound [c]	0.49
Upper bound [c]	0.59
Standard Error [c]	0.027

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

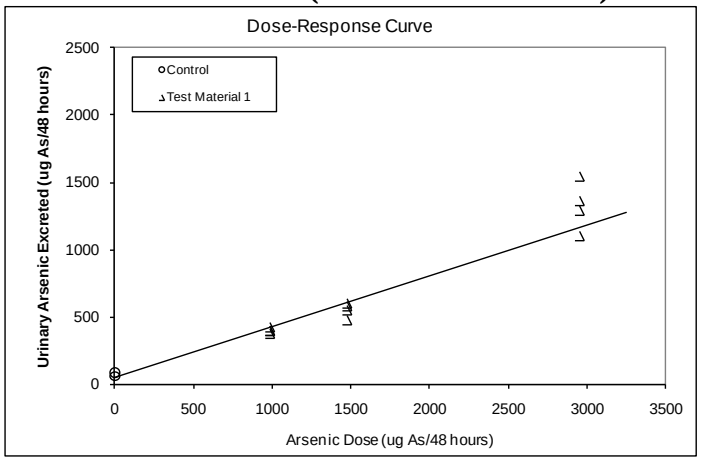
Figure 4-8. Mohr Orchard Urinary Excretion of Arsenic: Days 12/13 (Outlier Excluded)

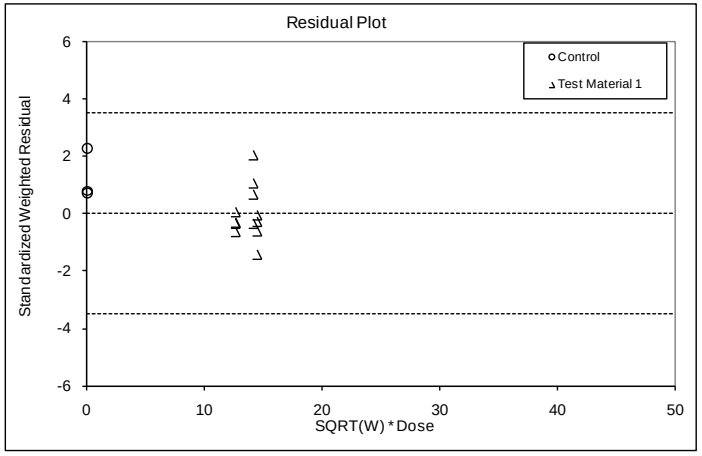
Reference Material (Sodium Arsenate)





Test Material 1 (Mohr Orchard TM1)





Summary of Fitting [a]

Parameter	Estimate	SE
a	47.4	22.8
b_r	0.68	0.03
b_{t1}	0.38	0.02
Covariance (b_r,b_{t1})	0.2729	–
Degrees of Freedom	25	–

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

ANOVA

Source	SSE	DF	MSE
Fit	600.95	2	300.48
Error	22.09	24	0.92
Total	623.04	26	23.96

Statistic	Estimate
F	326.507
p	<0.001
Adjusted R^2	0.9616

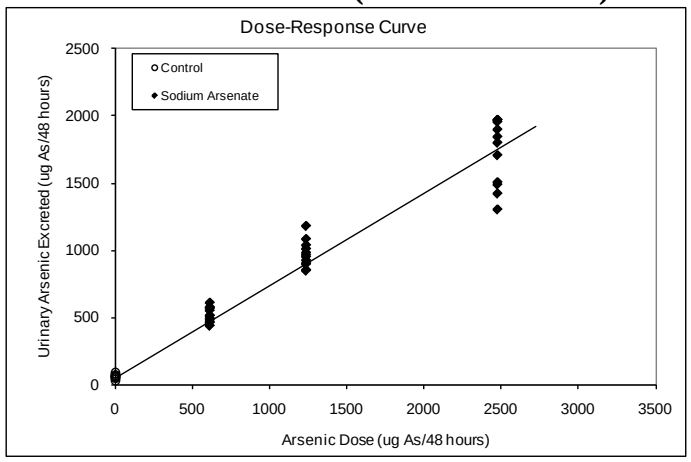
RBA and Uncertainty

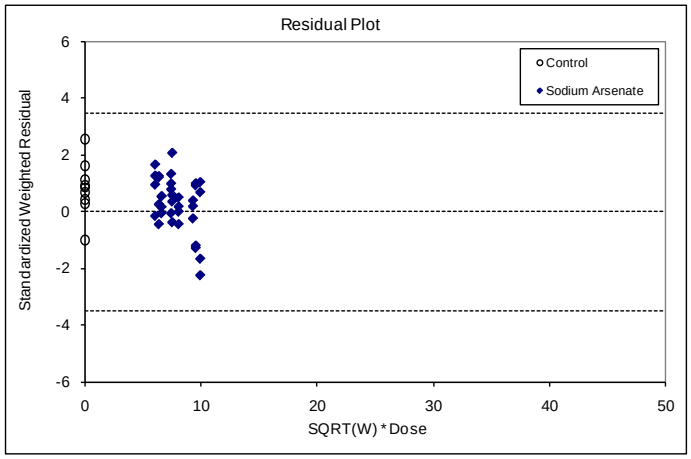
	Test Material 1
RBA	0.56
Lower bound [c]	0.50
Upper bound [c]	0.63
Standard Error [c]	0.037

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

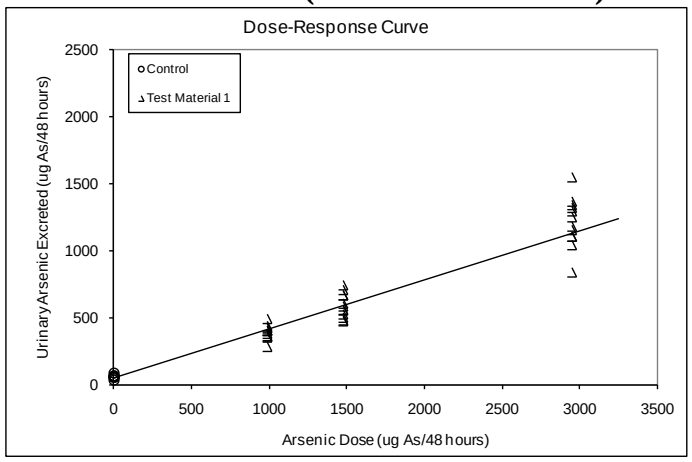
Figure 4-9. Mohr Orchard Urinary Excretion of Arsenic: All Days (Outlier Excluded)

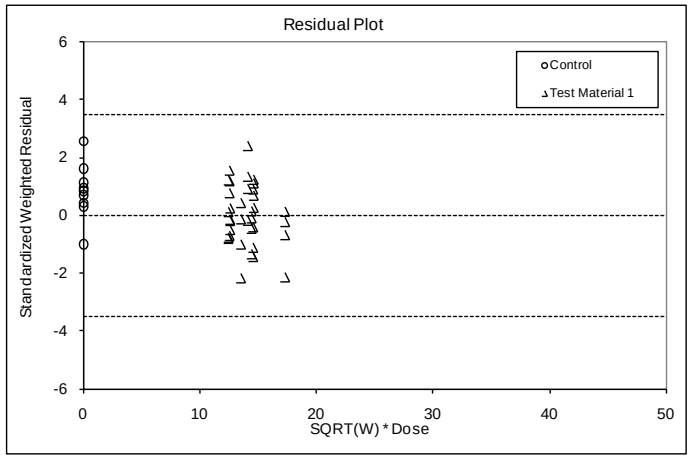
Reference Material (Sodium Arsenate)





Test Material 1 (Mohr Orchard TM1)





Summary of Fitting [a]

Parameter	Estimate	SE
a	46.4	11.4
b_r	0.69	0.02
b_{t1}	0.37	0.01
Covariance (b_r,b_{t1})	0.3045	–
Degrees of Freedom	78	–

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

ANOVA

Source	SSE	DF	MSE
Fit	1819.76	2	909.88
Error	55.41	77	0.72
Total	1875.17	79	23.74

Statistic	Estimate
F	1264.308
p	<0.001
Adjusted R^2	0.9697

RBA and Uncertainty

	Test Material 1
RBA	0.53
Lower bound [c]	0.51
Upper bound [c]	0.57
Standard Error [c]	0.018

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

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

Table 4-2. UEF Estimates

Measurement Interval	Outliers Excluded	Slopes (UEF Estimates)	
		b_r	b_{t1}
Days 6/7		0.67	0.34
Days 9/10	0	0.73	0.39
Days 12/13	1	0.68	0.38
All Days	0	0.69	0.37

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

4.6 Calculated RBA Values

Estimated RBA values (mean and 90% confidence interval) are shown below in Table 4-3:

Table 4-3. Estimated RBA for Mohr Orchard Soil

Measurement Interval	Estimated RBA (90% Confidence Interval)
Days 6/7	0.50 (0.46–0.55)
Days 9/10	0.54 (0.49–0.59)
Days 12/13	0.56 (0.50–0.63)
All Days	0.53 (0.51–0.57)

The best fit point estimate RBA for the Mohr Orchard soil sample is 53%.

4.7 Uncertainty

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

Such statistical uncertainty is accounted for by the statistical models used above and is characterized by the uncertainty range around the RBA estimates.

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

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APPENDIX A: GROUP ASSIGNMENTS

Table A-1. Group Assignments for the Mohr Orchard Arsenic Study

Swine number	Group	Treatment	Target arsenic dose µg/kg-day
552 554 561 572	1	NaAs	25
551 553 566 573	2	NaAs	50
555 560 563 574	3	NaAs	100
557 575 576 579	4	TM1	40
559 565 568 578	5	TM1	60
556 562 569 577	6	TM1	120
564 570 571	7	Control	0

APPENDIX B: BODY WEIGHTS

Table B-1. Body Weights

Group	Swine number	Weight (kg)													
		Day 5 8/12/09	Group MBW	Day 1 8/16/09	Group MBW	Day 2 8/19/09	Group MBW	Day 5 8/22/09	Group MBW	Day 8 8/25/09	Group MBW	Day 11 8/28/09	Group MBW	Day 14 8/31/09	Group MBW
1 NaAs 25	552	8.9		9.2		10		10.3		10.8		11.4		12.2	
	554	9.7		10		10.4		10.9		11.7		12.3		12.8	
	561	7.8		8		8.7		9.3		9.7		10.4		11	
	572	9	8.85	9.1	9.08	9.8	9.73	10.3	10.20	11.1	10.83	11.8	11.48	12.4	12.10
2 NaAs 50	551	9.3		9.6		10.2		10.6		11.2		11.8		12.5	
	553	7.6		7.9		8.2		8.5		9.1		9.7		10.2	
	566	7.8		8.4		8.6		9.2		9.9		10.5		11.2	
	573	8.7	8.35	9.3	8.80	9.6	9.15	10.1	9.60	10.8	10.25	11.4	10.85	12	11.48
3 NaAs 100	555	7.5		7.9		8.3		8.7		9.1		9.8		10.6	
	560	8.2		8.4		8.9		9.3		10.1		10.6		11.3	
	563	7.5		7.9		8.4		9		9.3		10		10.8	
	574	8.1	7.83	8.8	8.25	9.2	8.70	9.6	9.15	10.6	9.78	11.2	10.40	11.9	11.15
4 TM1 40	557	8.2		8.4		9		9.5		10.1		10.9		11.7	
	575	7.6		8.2		8.5		8.8		9.5		10.2		11	
	576	6.6		7.2		7.5		8		8.8		9.5		10.2	
	579	8.1	7.63	8.6	8.10	9	8.50	9.2	8.88	9.9	9.58	10.5	10.28	11.2	11.03
5 TM1 60	559	8		9.2		9.8		10.3		10.8		11.5		12.2	
	565	8.1		8.5		9		9.2		10.1		10.6		11.2	
	568	7.7		8.2		8.7		9.2		9.8		10.4		11.2	
	578	9.3	8.28	9.6	8.88	10.3	9.45	10.8	9.88	11.3	10.50	12.1	11.15	12.8	11.85
6 TM1 120	556	8.5		8.9		9.7		10.2		10.9		11.7		12.6	
	562	6.7		7.2		7.6		7.9		8.4		9.2		10	
	569	7.9		8.6		9.2		9.6		10.4		11.1		11.9	
	577	7.5	7.65	7.8	8.13	8.5	8.75	9	9.18	9.6	9.83	10.4	10.60	11.2	11.43
7 Control 0	564	7.9		8.3		8.2		8.7		9.5		10.2		10.7	
	570	7.7		8.5		8.9		9.5		10.2		10.8		11.2	
	571	8.7	8.10	9.6	8.80	9.9	9.00	10.3	9.50	11	10.23	11.8	10.93	12.6	11.50

APPENDIX C: URINE VOLUMES AND URINARY ARSENIC ANALYTICAL RESULTS FOR STUDY SAMPLES

Table C-1. Typical Feed Composition: Purina TestDiet® 5TXP: Porcine Grower Purified Diet with Low Lead [1]

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

NUTRITIONAL PROFILE [2]				
Protein, %	**21**	**Fat, %**		**3.5**
Arginine, %	1.42	Cholesterol, ppm		0
Histidine, %	0.61	Linoleic Acid, %		1.95
Isoleucine, %	1.14	Linolenic Acid, %		0.03
Leucine, %	1.95	Arachidonic Acid, %		0
Lysine, %	1.56	Omega-3 Fatty Acids, %		0.03
Methionine, %	0.49	Total Saturated Fatty Acids, %		0.43
Cystine, %	0.23	Total Monounsaturated Fatty Acids, %		0.82
Phenylalanine, %	1.22	Polyunsaturated Fatty Acids, %		1.98
Tyrosine, %	1.03			
Threonine, %	0.88			
Tryptophan, %	0.32	**Fiber (max), %**		**6.8**
Valine, %	1.16			
Alanine, %	0.95	**Carbohydrates, %**		**62.2**
Aspartic Acid, %	2.33			
Glutamic Acid, %	4.96	**Energy (kcal/g) [3]**		**3.62**
Glycine, %	0.79	*From:*	*kcal*	*%*
Proline, %	1.83	Protein	0.84	23.1
Serine, %	1.25	Fat (ether extract)	0.315	8.7
Taurine, %	0	Carbohydrates	2.487	68.3
Minerals		**Vitamins**		
Calcium, %	0.8	Vitamin A, IU/g		1.7
Phosphorus, %	0.72	Vitamin 0-3 (added), IU/g		0.2
Phosphorus (available), %	0.4	Vitamin E, IU/kg		11
Potassium, %	0.27	Vitamin K (as menadione), ppm		0.52
Magnesium, %	0.04	Thiamin Hydrochloride, ppm		1
Sodium, %	0.3	Ribonavin, ppm		3.1
Chlorine, %	0.31	Niacin, ppm		13
Fluorine, ppm	0	Pantothenic Acid, ppm		9
Iron, ppm	82	Folic Acid, ppm		0.3
Zinc, ppm	84	Pyridoxine, ppm		1.7
Manganese, ppm	3	Biotin, ppm		0.1
Copper, ppm	4.9	Vitamin B-12, mcg/kg		15
Cobalt, ppm	0.1	Choline Chloride, ppm		410
Iodine, ppm	0.15	Ascorbic Acid, ppm		0
Chromium, ppm	0			
Molybdenum, ppm	0.01			
Selenium, ppm	0.26			

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

APPENDIX D: ANALYTICAL RESULTS FOR QUALITY CONTROL SAMPLES

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

Group	Material	Collection period (days)	Sample ID	Swine number	Urine_As (µg/L)	Urine volume (µL)
1	NaAs	06/07	MO-126	561	69	8200
1	NaAs	06/07	MO-128	552	29	19640
1	NaAs	06/07	MO-130	554	400	1230
1	NaAs	06/07	MO-135	572	560	780
1	NaAs	12/13	MO-171	561	79	6120
1	NaAs	12/13	MO-182	572	470	1090
1	NaAs	12/13	MO-186	554	270	1730
1	NaAs	12/13	MO-192	552	53	9670
1	NaAs	09/10	MO-146	572	550	1000
1	NaAs	09/10	MO-148	552	53	11480
1	NaAs	09/10	MO-150	561	76	7580
1	NaAs	09/10	MO-168	554	280	1640
2	NaAs	06/07	MO-105	566	140	6440
2	NaAs	06/07	MO-106	551	280	3300
2	NaAs	06/07	MO-109	553	206	4680
2	NaAs	06/07	MO-113	573	730	1160
2	NaAs	12/13	MO-174	553	190	5000
2	NaAs	12/13	MO-183	551	440	2680
2	NaAs	12/13	MO-191	573	300	2840
2	NaAs	12/13	MO-195	566	190	5160
2	NaAs	09/10	MO-137	573	710	1260
2	NaAs	09/10	MO-144	551	370	2800
2	NaAs	09/10	MO-147	553	200	5410
2	NaAs	09/10	MO-151	566	130	7760
3	NaAs	06/07	MO-108	574	1600	1230
3	NaAs	06/07	MO-110	560	590	2550
3	NaAs	06/07	MO-125	555	630	2360
3	NaAs	06/07	MO-132	563	760	2570
3	NaAs	12/13	MO-172	574	1200	1640
3	NaAs	12/13	MO-176	560	600	3160
3	NaAs	12/13	MO-177	555	710	2000
3	NaAs	12/13	MO-193	563	470	2770
3	NaAs	09/10	MO-140	560	620	2900
3	NaAs	09/10	MO-156	555	690	2670
3	NaAs	09/10	MO-162	574	1200	3480
3	NaAs	09/10	MO-164	563	580	2940
4	TM1	06/07	MO-111	579	81	3460
4	TM1	06/07	MO-119	557	150	2680
4	TM1	06/07	MO-120	576	140	2500
4	TM1	06/07	MO-122	575	45	9680
4	TM1	12/13	MO-199	576	130	3060
4	TM1	12/13	MO-200	575	55	7740
4	TM1	12/13	MO-201	557	140	2860
4	TM1	12/13	MO-202	579	76	4970
4	TM1	09/10	MO-142	579	83	4340
4	TM1	09/10	MO-157	575	51	9580
4	TM1	09/10	MO-163	557	160	2610
4	TM1	09/10	MO-165	576	120	2980

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

Group	Material	Collection period (days)	Sample ID	Swine number	Urine_As (µg/L)	Urine volume (µL)
5	TM1	06/07	MO-107	565	140	4750
5	TM1	06/07	MO-115	578	230	2420
5	TM1	06/07	MO-123	559	48	9820
5	TM1	06/07	MO-131	568	190	2620
5	TM1	12/13	MO-170	565	66	8820
5	TM1	12/13	MO-179	559	44	10870
5	TM1	12/13	MO-180	578	230	2620
5	TM1	12/13	MO-190	568	100	5520
5	TM1	09/10	MO-141	559	49	10660
5	TM1	09/10	MO-152	568	120	5540
5	TM1	09/10	MO-158	578	250	2960
5	TM1	09/10	MO-161	565	81	8700
6	TM1	06/07	MO-103	562	370	2980
6	TM1	06/07	MO-114	569	73	11450
6	TM1	06/07	MO-118	556	210	4950
6	TM1	06/07	MO-228	577	300	3840
6	TM1	12/13	MO-181	569	86	15020
6	TM1	12/13	MO-189	556	420	3680
6	TM1	12/13	MO-197	562	310	4400
6	TM1	12/13	MO-198	577	280	3940
6	TM1	09/10	MO-139	562	380	3100
6	TM1	09/10	MO-145	556	540	2440
6	TM1	09/10	MO-166	577	280	4780
6	TM1	09/10	MO-167	569	110	11340
7	Control	06/07	MO-227	570	19	1860
7	Control	06/07	MO-235	564	35	1460
7	Control	06/07	MO-236	571	38	1420
7	Control	12/13	MO-187	564	41	1440
7	Control	12/13	MO-188	571	45	1880
7	Control	12/13	MO-204	570	26	2320
7	Control	09/10	MO-149	571	23	2670
7	Control	09/10	MO-154	570	21	2400
7	Control	09/10	MO-155	564	46	1480

APPENDIX E: ANALYTICAL RESULTS FOR QUALITY CONTROL SAMPLES

Table E-1. Blind Duplicate Samples

Blind duplicate sample ID	Sample type	Swine number	Urine collection days	Original sample concentration (µg/L)	Duplicate concentration (µg/L)	RPD (%)
MO-175	Urine	551	12/13	440	390	12
MO-223	Urine	556	06/07	210	217	3
MO-138	Urine	560	09/10	620	610	2
MO-153	Urine	571	09/10	23	21	9
MO-136	Urine	572	09/10	550	570	4
MO-231	Urine	573	06/07	730	780	7
MO-194	Urine	576	12/13	130	130	0
MO-173	Urine	577	12/13	280	290	4
MO-224	Urine	578	06/07	230	228	4

RPD = relative percent difference

Table E-2. Laboratory Spikes

Spike sample ID	Sample type	Original sample concentration (ppb)	Added spike concentration (ppb)	Measured sample concentration (ppb)	Recovered spike (ppb)	Recovery (%)
MO-114	Urine	73	200	280	207	104
MO-128	Urine	29	200	240	211	106
MO-140	Urine	620	200	790	170	85
MO-150	Urine	76	200	290	214	107
MO-160	Urine	110	200	310	200	100
MO-170	Urine	66	200	270	204	102
MO-180	Urine	230	200	424	194	97
MO-190	Urine	100	200	300	200	100
MO-200	Urine	55	200	280	225	113
MO-204	Urine	26	200	240	214	107
MO-227	Urine	19	200	220	201	101
MO-273	Feed	<1	100	100	100	100

Table E-3. Laboratory Duplicates

Duplicate sample ID	Sample type	Original sample concentration (ppb)	Duplicate concentration (ppb)	RPD (%)	Absolute difference
MO-108	Urine	1600	1600	0	0
MO-120	Urine	140	150	7	10
MO-133	PE Sample	130	120	8	10
MO-145	Urine	540	580	7	40
MO-155	Urine	46	41	11	5
MO-165	Urine	120	120	0	0
MO-175	Urine	390	390	0	0
MO-185	PE Sample	55	54	2	1
MO-195	Urine	190	180	5	10
MO-202	Urine	76	78	3	2
MO-236	Urine	38	39	3	1
MO-269	Feed	0.1	0.1	0	0
MO-271	Water	<1	<1	0	0

RPD = relative percent difference; PE = performance evaluation

Table E-4. Laboratory Quality Control Standards

Sample ID	Measured arsenic concentration (ppb)	Detection limit (ppb)	Reference material ID	Certified mean[a]	Recovery (%)
QC-1	3	3	NIST 2670a-L	3	100
QC-2	240	10	NIST 2670a-H	220 ± 10	109
QC-3	230	10	NIST 2670a-H	220 ± 10	105
QC-4	5	3	NIST 2670a-L	3	167
QC-5	220	10	NIST 2670a-H	220 ± 10	100
QC-6	250	10	NIST 2670a-H	220 ± 10	114
QC-7	60	1	NIST 1643e	58.98 ±0.7	102
QC-8	7.4	0.1	NIST 1566b	7.65 ± 0.65	97

[a]mean or mean ± SD

Table E-5. Blanks

Sample ID	Measured arsenic concentration (ppb)	Detection limit (ppb)
Blank-1	<1	1
Blank-2	<1	1
Blank-3	<1	1
Blank-4	<1	1
Blank-5	<1	1
Blank-6	<1	1
Blank-7	<1	1
Blank-8	<0.1	0.1

Figure E-1. Urinary Arsenic Blind Duplicates

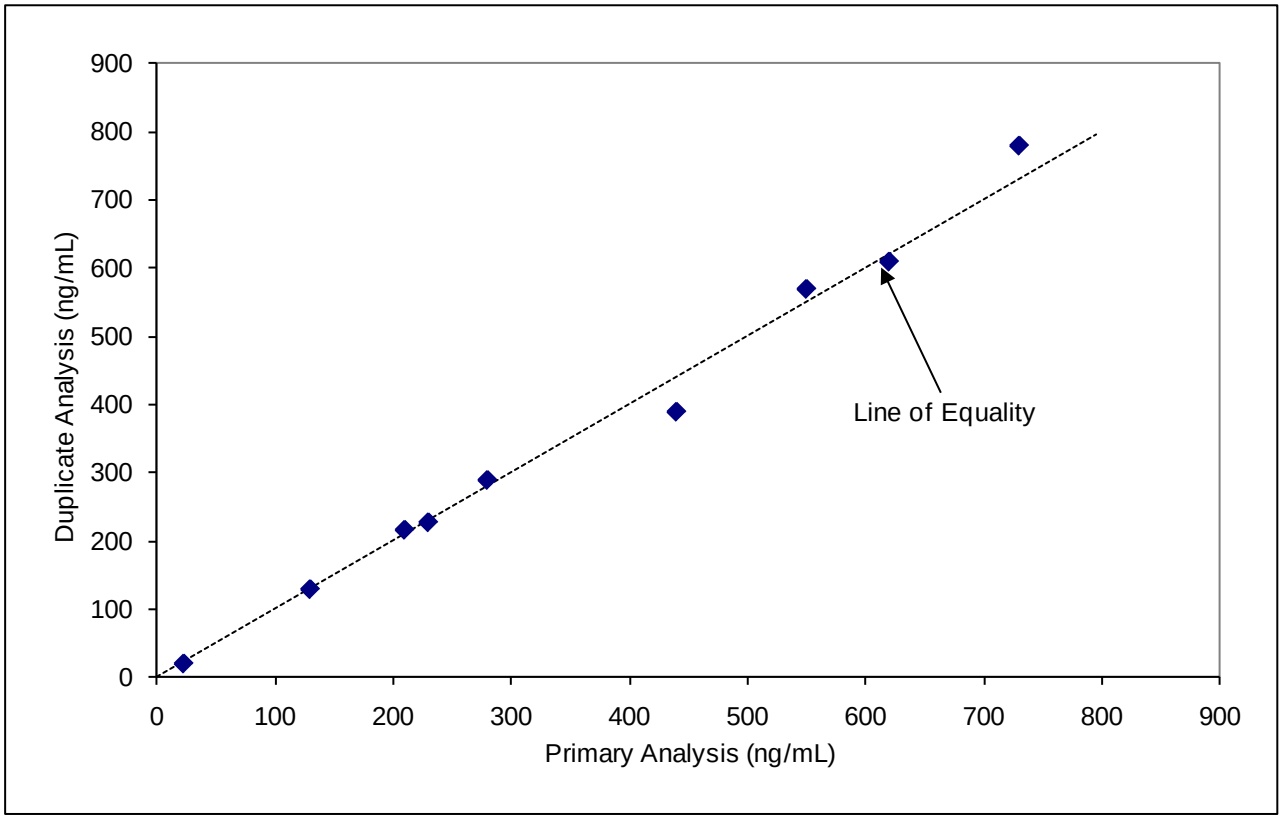

Figure E-2. Performance Evaluation Samples