RELATIVE BIOAVAILABILITY OF DISLODGEABLE ARSENIC FROM CCA-TREATED WOOD

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 in dislodgeable material obtained from the surface of chromated copper arsenate (CCA)-treated wood. The relative bioavailability of arsenic was assessed by comparing the absorption of arsenic from the dislodgeable arsenic material to that of a reference material (sodium arsenate). The study was performed as nearly as possible within the spirit and guidelines of Good Laboratory Practices and followed a protocol that was submitted to and reviewed by USEPA.

The arsenic concentration in the dislodgeable arsenic material was 3500 µg/g. Groups of five swine were given oral doses of sodium arsenate or dislodgeable arsenic twice a day for 12 days. The amount of arsenic absorbed by each animal was evaluated by measuring the amount of arsenic excreted in the urine (as measured on days 6 to 7, 8 to 9, and 10 to 11). 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 sodium arsenate and the dislodgeable arsenic using linear regression analysis. The relative bioavailability (RBA) of arsenic in the dislodgeable arsenic compared to that in sodium arsenate was calculated as:

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

The results are summarized below:

Material Administered	UEF ± SEM (N)	RBA (90% CI)
Sodium Arsenate (reference material)	0.81 ± 0.034 (45)	[1.00]
Dislodgeable Arsenic (test material)	0.233 ± 0.009 (60)	0.29 (0.26-0.32)

SEM = Standard error of the mean (standard deviation)
N = Number of data points used in curve fitting
CI = Confidence interval

Using sodium arsenate as a relative frame of reference, the RBA estimate for the test material is 29% (90th % CI = 26% - 32%). This value is significantly lower than the default value of 80%-100% that is usually employed when reliable site-specific data are lacking. This indicates that the arsenic in the dislodgeable arsenic material is not as well absorbed as soluble arsenic. Use of these data is likely to improve the accuracy of risk estimates for humans who may incidentally ingest dislodgeable arsenic from CCA-treated wood.

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ABBREVIATIONS

ABA	Absolute bioavailability
AF_o	Oral absorption fraction
As+3	Trivalent inorganic arsenic
As+5	Pentavalent inorganic arsenic
CCA	Chromated copper arsenate
CI	Confidence interval
DMA	Dimethyl arsenic
$Dose_{adj}$	Adjusted dose based on site-specific data
g	Gram
GLP	Good Laboratory Practices
Hg	Mercury
ICP-AES	Inductively coupled plasma atomic emission spectroscopy
IRIS	USEPA's Integrated Risk Information System
kg	Kilogram
K_u	Fraction of absorbed arsenic which is excreted in urine
L	Liter
mL	Milliliter
MMA	Monomethyl arsenic
N	Number of data points
NaAs	Sodium arsenate
Ng	Nanogram
PDF	Probability density function
PE	Performance Evaluation
PIC	Pig Improvement Corporation
QA	Quality assurance
RBA	Relative bioavailability
RBA(test vs. NaAs)	RBA of the test material compared to sodium arsenate
RBA(test vs. ref)	RBA of the test material compared to the reference material
RFD_{adj}	Adjusted reference dose (RfD) value for non-cancer risk based on site-specific data
RFD_{IRIS}	Default reference dose (RfD) value for non-cancer risk from IRIS
RPD	Relative percent difference
SEM	Standard error of the mean (standard deviation)
SF_{adj}	Adjusted slope factor (SF) value for cancer risk based on site-specific data
SF_{IRIS}	Default slope factor (SF) value for cancer risk from IRIS
UEF	Urinary excretion fraction
UEF(NaAs)	UEF of sodium arsenate
UEF(ref)	UEF of the reference material
UEF(test)	UEF of the test material
USEPA	United States Environmental Protection Agency
wt	Weight
µg	Microgram
γ	Gamma

RELATIVE BIOAVAILABILITY OF DISLODGEABLE ARSENIC FROM CCA-TREATED WOOD

1.0 INTRODUCTION

Accurate assessment of the health risks resulting from oral exposure to arsenic requires knowledge of the amount of arsenic absorbed from the gastrointestinal tract into the body. This information on absorption may be described either in absolute or relative terms:

Absolute Bioavailability (ABA) is the ratio of the amount of arsenic 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 absolute bioavailability of arsenic present in some test material to the absolute bioavailability of arsenic in some appropriate reference material:

$$RBA = \frac{ABA\ (test\ material)}{ABA\ (reference\ material)}$$

Usually the form of arsenic used as the reference material is an arsenic compound dissolved in water or some readily soluble form (e.g., sodium arsenate) that is expected to completely dissolve when ingested.

For example, if 100 µg of arsenic dissolved in drinking water were ingested and a total of 90 µg were absorbed into the body, the ABA would be 0.90 (90%). Likewise, if 100 µg of arsenic contained in soil were ingested and 30 µg were absorbed into the body, the ABA for soil would be 0.30 (30%). If the arsenic dissolved in water was used as the frame of reference for describing the relative amount of arsenic absorbed from soil, the RBA would be 0.30/0.90, or 0.33 (33%).

Using Relative Bioavailability Data to Improve Risk Calculations for Arsenic

When reliable data are available on the relative bioavailability of arsenic in a site medium (e.g., soil), this information can be used to adjust the default toxicity values (RfD_{IRIS}, SF_{IRIS}) for arsenic to account for differences in absorption between arsenic ingested in water (RBA) and arsenic ingested in site media, as follows:

$$RfD_{adj} = \frac{RfD_{IRIS}}{RBA}$$

$$SF_{adj} = SF_{IRIS} \cdot RBA$$

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

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

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

Purpose of This Study

The objective of this study, sponsored by the American Chemistry Council, was to use juvenile swine as a test system in order to determine the oral bioavailability of arsenic in dislodgeable material obtained from the surface of chromated copper arsenate (CCA)-treated wood[1] relative to the bioavailability of a soluble form of arsenic. The relative bioavailability estimates will be used to improve accuracy and decrease uncertainty in estimating exposures to ingested dislodgeable arsenic in human health risk assessments for CCA-treated wood.

[1] Dislodgeable arsenic refers to arsenic that can be removed from the surface of CCA-treated wood by hand contact or an equivalent means (e.g., brushing with a soft bristle brush as described in Section 2.1.2).

2.0 STUDY DESIGN

This investigation of arsenic relative bioavailability was performed according to the basic design presented in Table 2-1. As shown, the study investigated arsenic absorption from sodium arsenate (the reference material) and dislodgeable arsenic (the test material), each administered to groups of five animals at several different dose levels for 12 days (a detailed schedule is presented in Appendix A, Table A-1). Additionally, the study included a non-treated group to serve as a control for determining background arsenic levels. All doses were administered orally.

This study was performed as nearly as possible within the spirit and guidelines of Good Laboratory Practices (GLP: 40 CFR 792). The study followed a protocol that was submitted to and reviewed by the United States Environmental Protection Agency (USEPA). USEPA provided comments on the draft protocol and the protocol was revised to reflect USEPA comments and concerns.

2.1 Test Material

2.1.1 Sample Description

The test material for this study consisted of samples of dislodgeable material collected from the surface of CCA-treated wood. The wood used to obtain the samples consisted of CCA type C-treated southern yellow pine boards of various commercial dimensions. The boards were obtained from in-service residential decks aged outdoors for approximately one or three years. Both the weathered surface and the underside of the deck boards were used to collect the dislodgeable material.

2.1.2 Sample Preparation

For ease of handling, the boards were cut into approximately 24-inch lengths. Any sawdust remaining on the board after cutting was removed using an air gun. To obtain the samples, board surfaces were wetted and then brushed five times with moderate pressure using a laboratory test tube brush. All brushings followed the same direction from top to bottom along the long axis of the boards. Remaining residues were then rinsed from the wood surface and brush. All materials from brushing and rinsing were collected and filtered with glass wool to remove wood splinters and other large debris. The filtered mixture was concentrated by rotary evaporation under 27" Hg vacuum at 50°C to remove nearly all of the water. This concentrated mixture was air-dried at 22±1°C and 65±5% humidity until two consecutive weight measurements taken two hours apart were within ± 1.0% of each other. A homogenous sample of the air-dried material was collected for total arsenic analysis. The remaining material was transferred to a glass vial and γ-irradiated using a Cobalt-60 Irradiator with an activity of 250 curies for three hours to prevent microbial contamination of the sample.

2.1.3 Arsenic Concentration

The concentration of arsenic in the dried and homogenized dislodgeable arsenic material was measured in duplicate by inductively coupled plasma atomic emission spectroscopy (ICP-AES) by L. E. T., Inc., (Columbia, Missouri). The resulting value was 3500 µg/g.

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). The swine model has been used previously by USEPA to evaluate the bioavailability of arsenic in soil.

The animals were intact males of the Pig Improvement Corporation (PIC) genetically defined Line 26, and were purchased from Chinn Farms, Clarence, Missouri. The number of animals purchased for the study was five more than required by the protocol (35 total). These animals were purchased at an age of about 4-5 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 study materials. Each animal was examined by a certified veterinary clinician (swine specialist); no animals appeared to be in poor health during this quarantine period. To minimize weight variations between animals and groups, the five extra animals most different in body weight (either heavier or lighter) four days prior to exposure (day -4) were 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 5-6 weeks old and weighed an average of about 8.8 kg. The animals were weighed every three days during the course of the study. On average, animals gained about 0.29 kg/day and the rate of weight gain was comparable in all groups, ranging from 0.26 to 0.34 kg/day. These body weight data are summarized in Figure 2-1 and are presented in detail in Appendix A, Table A-3. The study followed an approved animal care protocol and all animals were examined daily by an attending veterinarian while on study.

2.3 Diet

Animals were weaned onto standard pig chow (purchased from MFA Inc., Columbia, Missouri) by the supplier. In order to minimize arsenic exposure from the diet, the animals were gradually transitioned from the MFA feed to a special feed (Zeigler Brothers, Inc., Gardners, Pennsylvania) over the time interval from day -6 to day -3, and this feed was then 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 is 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. Feed amounts were adjusted every three days, when pigs were weighed. Feed was administered in two equal portions of 2% of the mean body weight at 11:00 AM and 5:00 PM daily. Analysis of a single feed sample from this study indicated that the arsenic level was 0.06 µg/g (corresponding to a dose contribution from food of 3 µg/kg-day). Previous analysis of feed samples indicated that the arsenic level was consistently below 0.1 µg/g.

Drinking water was provided *ad libitum* via self-activated watering nozzles within each cage. Analysis of samples from randomly selected drinking water nozzles (N=4) indicated that the arsenic concentration was consistently less than the quantitation limit (about 1 µg/L). Assuming water intake of about 0.1 L/kg-day, this corresponds to a dose contribution from water of less than 0.1 μg/kg-day.

2.4 Dosing

Animals were exposed to sodium arsenate (abbreviated in this report as "NaAs") or the test material (dislodgeable arsenic) for 12 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. Dose material was placed in the center of a small portion (about 5 grams) of moistened feed (this is referred to as a "doughball"), and this was administered to the animals by hand. If uneaten portions of doughballs were discovered, these were retrieved and offered again for consumption. All uneaten portions of doughballs were consumed before the next dosing period, so no dose adjustments were necessary.

The dose levels administered were calculated based on the arsenic content of the test material and the measured group mean body weights. Specifically, arsenic doses for the three days following each weighing were based on the group mean body weight adjusted by the addition of 1 kg to account for the expected weight gain over the 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 administered doses for each dosing group are included in Table 2-1; the actual arsenic doses administered to each individual pig are presented in Appendix A, Table A-3, and were used for all RBA calculations.

2.5 Collection and Preservation of Urine Samples

Samples of urine were collected from each animal for 48-hour periods on days -2 to -1, 6 to 7, 8 to 9, and 10 to 11 of the study. Each 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. Due to the length of the collection period, collection containers were checked throughout the day (e.g., at feeding, dosing, and in the evening) and emptied periodically into a separate holding container to ensure that there was no loss of sample due to overflow. Collection containers typically were emptied twice a day.

At the end of each collection period, the total urine volume for each animal was measured (see Appendix A, Table A-4) and three 60-mL portions were removed and acidified with 0.6 mL concentrated nitric acid. Two of the aliquots of each sample were archived in the refrigerator and one aliquot was sent for arsenic analysis. All samples were refrigerated until analyzed for total arsenic.

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. Details of urine sample preparation and analysis are provided in USEPA (1999). 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 Perkin-Elmer 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-5.

2.7 Quality Assurance

A number of quality assurance (QA) steps were taken during this project to evaluate the accuracy of the analytical procedures.

2.7.1 Laboratory Quality Assurance

Steps performed by the analytical laboratory included:

<u>Spike Recovery</u>

Randomly selected urine samples were spiked with known amounts of arsenic (approximately 400 ng/mL as sodium arsenate) and the recovery of the added arsenic was measured. Recovery for individual samples ranged from 95% to 112%, with an average across all analyses of 102 ± 5% (N=16).

<u>Duplicate Analysis</u>

The laboratory analyst selected random urine samples for duplicate analysis. Duplicate results had a relative percent difference (RPD) of 0% to 11.8%, with an average of 3.4% ± 4.2% (N=9).

<u>Laboratory Control Standards</u>

Four different types of laboratory control standards were tested periodically during the analysis. These are samples for which a certified concentration of arsenic has been established. Results for these standards are summarized below:

Laboratory Control Standards	Certified Value	Average Recovery	SEM	N
E.R.A. P081 - Metals WasteWatR	366 ng/mL (accepted range: 305-430)	99%	2.0%	57
N.I.S.T. Oyster 1566b	7.65 +/- 0.65 μg/g dry wt	99%	7.6%	4
N.R.C.C. Tort-2 Lobster	21.6 +/- 1.8 μg/g dry wt	100%	5.3%	3
N.R.C.C. Dolt-2 Dogfish Liver	16.6 +/- 1.1 μg/g dry wt	87%	4.3%	2

SEM = Standard error of the mean (standard deviation)
N = Number of data points used in curve fitting

As seen, recovery of arsenic from these standards was generally good.

Blanks

All blank samples run along with each batch of samples were at or below the detection limit for arsenic (1 ng/mL) (N=9).

2.7.2 Blind Quality Assurance Samples

In addition to these laboratory-sponsored QA samples, an additional series of QA samples were submitted to the laboratory in a blind fashion. This included a number of Performance Evaluation (PE) samples (control urine spiked with a known amount of arsenic in the form of As+3, As+5, MMA, or DMA) and blind duplicates. Detailed results for blind QA samples are presented in Appendix A, Table A-6.

The results for the PE samples are shown in Figure 2-2. As seen, all sample results were close to the expected values, indicating that there was good recovery of the arsenic in all cases.

The results for blind duplicates are shown in Figure 2-3. As seen, there was good agreement between the results for the duplicate pairs.

2.7.3 Dose Verification

At each dose preparation, extra doughballs for each dose group were prepared and archived in the freezer for dose verification. Three of these dose verification samples were randomly selected for analysis; detailed results for these samples are presented in the lower portion of Table A-6 in Appendix A. The absolute difference between the nominal and the measured arsenic concentration ranged from 0.1 to 6.6 μg/doughball, which corresponds to less than a 4% difference from the nominal arsenic concentration in all cases. These data support the conclusion that the prepared doses were sufficiently close to the target doses.

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

3.0 DATA ANALYSIS

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 oral absorption fraction 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 relative bioavailability (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:

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

  where K_u represents the fraction of absorbed arsenic that is excreted in the urine.

Based on the conceptual model above, raw data from this study were reduced and analyzed as follows:

- The amount of arsenic excreted in urine by each animal over each collection period was calculated by multiplying the urine volume by the urine concentration:

  Excreted (µg/48 hours) = Concentration (µg/L) · Volume (L/48hours)

- For each test material, the amount of arsenic excreted by each animal was plotted as a function of the amount administered (µg/48 hours), and the best fit straight line (calculated by linear regression) through the data (µg excreted per µg administered) was used as the best estimate of the UEF.

- The relative bioavailability of arsenic in a test material was calculated as:

  RBA = UEF(test) / UEF(NaAs)

  where sodium arsenate (NaAs) is used as the frame of reference.

- As noted above, each RBA value is calculated as the ratio of two slopes (UEFs), each of which is estimated by linear regression through a set of data points. Because of the variability in the data, there is uncertainty in the estimated slope (UEF) for each material. This uncertainty in the slope is described by the standard error of the mean (SEM) for the slope parameter. Given the best estimate and the SEM for each slope, the uncertainty in the ratio may be calculated using Monte Carlo simulation. The probability density function (PDF) describing the confidence around each slope term (UEF) was assumed to be characterized by a t-distribution with n-2 degrees of freedom :

$$\frac{UEF(measured) - UEF(true)}{SEM} \sim t_{n-2}$$

For convenience, this PDF is abbreviated T(slope, sem, n), where slope = best estimate of the slope derived by linear regression, sem = standard deviation in the best estimate of the slope, and n = number of data points upon which the regression analysis was performed. Thus, the confidence distribution around each ratio was simulated as:

$$PDF(RBA) = \frac{T(slope, sem, n)_{test}}{T(slope, sem, n)_{ref}}$$

Using this equation, a Monte Carlo simulation was run for the RBA calculation. The 5^{th} and 95^{th} percentile values from the simulated distribution of RBA values were then taken to be the 90% confidence interval (CI) for the RBA.

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, and no clinical signs of arsenic-induced toxicity were noted in any of the animals used in the study. The animals remained healthy throughout the study, except for a few cases of transient diarrhea, which is normal in juvenile pigs. The gross findings of the autopsies were negligible.

4.2 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/L) and a total urine volume greater than 5000 mL. When both of these conditions are met, the data are deemed unreliable and excluded from further calculations. In this study, one data point from the day -2 to -1 urine collection (pig #561, group 2) was deemed unreliable for this reason and excluded. No other data were excluded.

4.3 Background Arsenic Excretion

The urinary excretion results for days -2 to -1 (prior to dosing) ranged from 2.1 to 26.3 μg/48 hours across all animals, with a mean of 10.0 μg/48 hours. These values are representative of endogenous background levels in food and water. The urinary excretion results for control animals during dosing (from days 6-11) were very similar to the pre-dosing levels, ranging from 2.6 to 19.0 μg/48 hours with a mean of 7.5. These results support the view that the animals were not exposed to any significant exogenous sources of arsenic throughout the study. Detailed data are included in Appendix A.

4.4 Urinary Arsenic Excretion Over Time

Previous swine arsenic bioavailability studies have shown that urinary arsenic excretion patterns are stable after five days of dosing (USEPA, 1997). To verify that this study's RBA estimates were made during steady-state elimination conditions, the pattern of urinary arsenic excretion over time was examined. Figures 4-1 (sodium arsenate) and 4-2 (dislodgeable arsenic) show the urinary arsenic excretion values as a function of time, as well as the expected conceptual dose-response models. As seen, there appears to be a slight trend to drift upwards with time; however, this generally is not significantly different from equilibrium. Moreover, the slopes for each time period are generally similar to one another for animals receiving the same dosing material (see Figures 4-3 and 4-4), suggesting that a steady-state elimination stage had been reached. Therefore, the data for the three time periods were combined to obtain the RBA.

4.5 Urinary Excretion Fractions and Relative Bioavailability

Detailed results from the study are presented in Appendix A. The urinary excretion results for NaAs and dislodgeable arsenic are summarized in Figures 4-3 and 4-4, respectively. In general, the absolute variability of the data tends to increase somewhat with dose. The reason for this is unknown; however, increased variability with dose is not uncommon in swine arsenic bioavailability studies (data not presented). Although there is variability in the data, all of the dose-response curves are approximately linear, with the slope of the best-fit straight line being equal to the best estimate of the urinary excretion fraction (UEF).

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

RBA(test *vs.* NaAs) = UEF(test) / UEF(NaAs)

The following table summarizes the best fit slopes (urinary excretion fractions) for sodium arsenate and the dislodgeable arsenic material, as well as the estimated RBA:

Material Administered	UEF ± SEM (N)	RBA (90% CI)
Sodium Arsenate (reference material)	0.81 ± 0.034 (45)	[1.00]
Dislodgeable Arsenic (test material)	0.233 ± 0.009 (60)	0.29 (0.26-0.32)

SEM = Standard error of the mean (standard deviation)
N = Number of data points used in curve fitting
CI = Confidence interval

As seen, using sodium arsenate as a relative frame of reference, the RBA estimate for the test material is 29%. The RBA estimate for the dislodgeable arsenic material is significantly lower than the default value of 80%-100% that is usually employed for arsenic in soil when reliable site-specific data are lacking. This indicates that the arsenic in this material is not as well absorbed as soluble arsenic, and it is appropriate to take this into account when evaluating potential risks to humans from incidental ingestion of dislodgeable arsenic from CCA-treated wood.

5.0 DEVIATIONS FROM PROTOCOL

During the course of this study, there were several deviations from the protocol. These deviations and their potential implications on the study are discussed below.

Dislodgeable Arsenic Analysis

The dislodgeable arsenic material was analyzed for arsenic in duplicate, not triplicate as called for in the protocol. Because there was 100% agreement between the duplicate analyses (3500 µg/g), the omission of a third analysis results in little additional uncertainty around the mean arsenic concentration.

Urine Volumes

Urine volumes were measured to the nearest 10 mL rather than the nearest 5 mL as specified in the protocol. Because the urine volumes range from 900 mL to 21100 mL, the additional uncertainty from measuring only to the nearest 10 mL is negligible compared to the uncertainty among the animals' urine volumes. Thus, this deviation is a very small source of uncertainty and has no significant impact on the calculations.

Creatinine Samples

Because drinking water was often spilled by animals from the watering nozzles into the urine collection pans, many urine samples were diluted with water and, as a result, the creatinine data cannot be meaningfully evaluated. However, this study compares the total mass of arsenic excreted in urine (i.e., concentration of arsenic in the analyzed aliquot multiplied by the total volume of urine collected) to the total mass of arsenic administered in the dose. Because we are dealing only with the total mass of arsenic rather than urinary arsenic concentrations, urinary creatinine measurements are not necessary.

6.0 REFERENCES

USEPA. 1999. Quality Assurance Project Plan for Vasquez Blvd-I70. Bioavailability of Arsenic in Site Soils Using Juvenile Swine as an Animal Model. Report prepared by ISSI Consulting Group for USEPA Region VIII. United States Environmental Protection Agency. September, 1999.

USEPA. 1997. Relative bioavailability of arsenic in mining wastes. Report prepared by Roy F. Weston, Inc., for USEPA Region VIII. Document Control Number 4500-88-AOLI. United States Environmental Protection Agency. October, 1997.

Weis, C.P., and J. M. LaVelle. 1991. Characteristics to consider when choosing an animal model for the study of lead bioavailability. In: The Proceedings of the International Symposium on the Bioavailability and Dietary Uptake of Lead. Science and Technology Letters 3:113-119.

TABLE 2-1 DOSING PROTOCOL

Group	Number of Animals	Dose Material Administered	Arsenic Dose (ug/kg-day)	
			Target	Actual [a]
1	5	Control	0	0.0
2	5	Sodium Arsenate	30	31.1
3	5	Sodium Arsenate	60	63.2
4	5	Dislodgeable Arsenic	30	31.4
5	5	Dislodgeable Arsenic	60	62.7
6	5	Dislodgeable Arsenic	120	125.4

[a] Calculated as the administered daily dose divided by the measured or extrapolated daily body weight, averaged over days 0-11 for each animal and each group.

Table 2-2 Typical Feed Composition

Nutrient Name	Amount	Nutrient Name	Amount
Protein	20.1021%	Chlorine	0.1911%
Arginine	1.2070%	Magnesium	0.0533%
Lysine	1.4690%	Sulfur	0.0339%
Methionine	0.8370%	Manganese	20.4719 µg/g
Met+Cys	0.5876%	Zinc	118.0608 µg/g
Tryptophan	0.2770%	Iron	135.3710 µg/g
Histidine	0.5580%	Copper	8.1062 µg/g
Leucine	1.8160%	Cobalt	0.0110 µg/g
Isoleucine	1.1310%	Iodine	0.2075 µg/g
Phenylalanine	1.1050%	Selenium	0.3196 µg/g
Phe+Tyr	2.0500%	Nitrogen Free Extract	60.2340%
Threonine	0.8200%	Vitamin A	5.1892 kIU/kg
Valine	1.1910%	Vitamin D3	0.6486 kIU/kg
Fat	4.4440%	Vitamin E	87.2080 IU/kg
Saturated Fat	0.5590%	Vitamin K	0.9089 µg/g
Unsaturated Fat	3.7410%	Thiamine	9.1681 µg/g
Linoleic 18:2:6	1.9350%	Riboflavin	10.2290 µg/g
Linoleic 18:3:3	0.0430%	Niacin	30.1147 µg/g
Crude Fiber	3.8035%	Pantothenic Acid	19.1250 µg/g
Ash	4.3347%	Choline	1019.8600 µg/g
Calcium	0.8675%	Pyridoxine	8.2302 µg/g
Phos Total	0.7736%	Folacin	2.0476 µg/g
Available Phosphorous	0.7005%	Biotin	0.2038 µg/g
Sodium	0.2448%	Vitamin B12	23.4416 µg/g
Potassium	0.3733%		

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

FIGURE 2-1 BODY WEIGHT GAIN

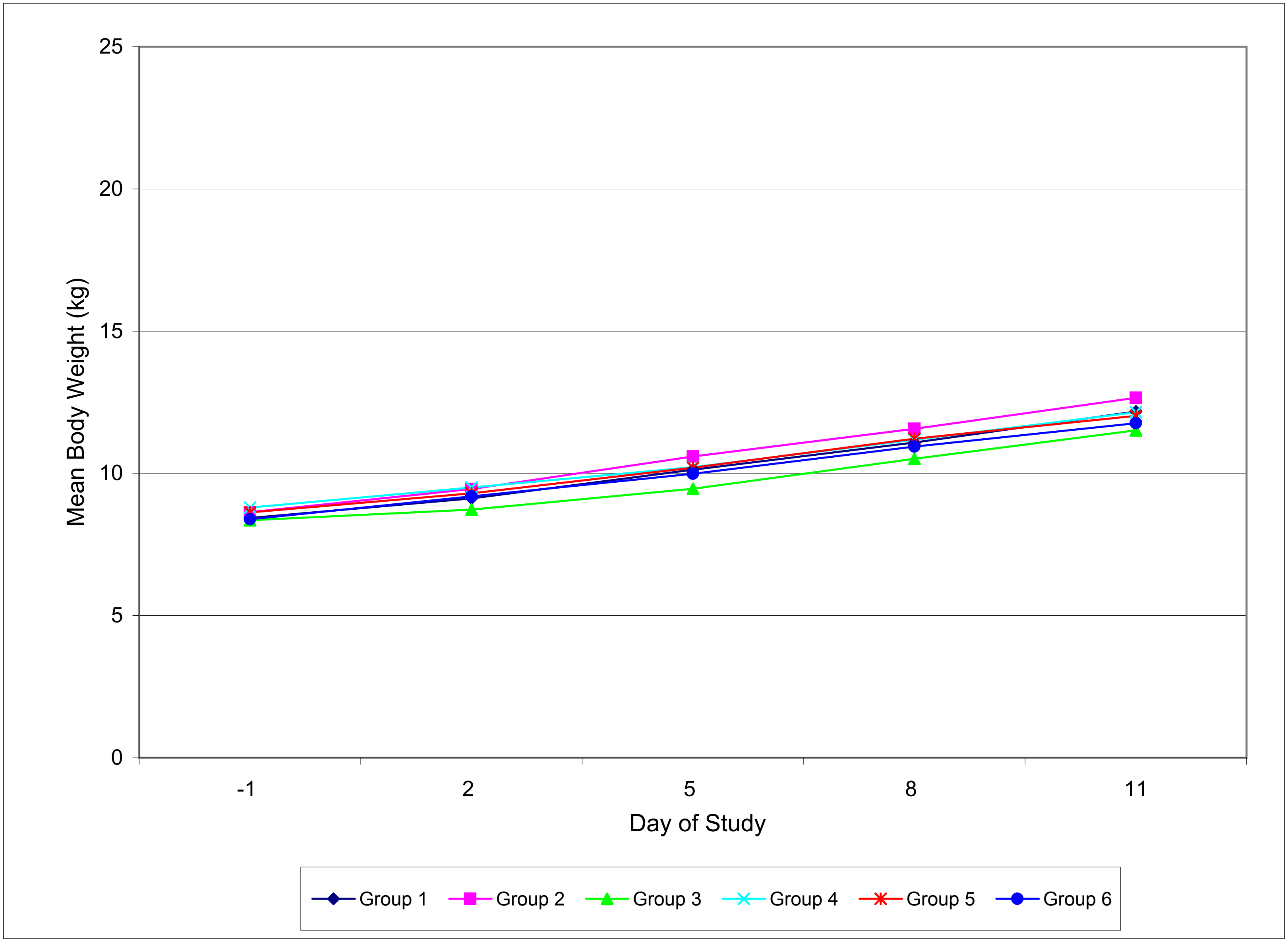


FIGURE 2-2 PERFORMANCE EVALUATION SAMPLES

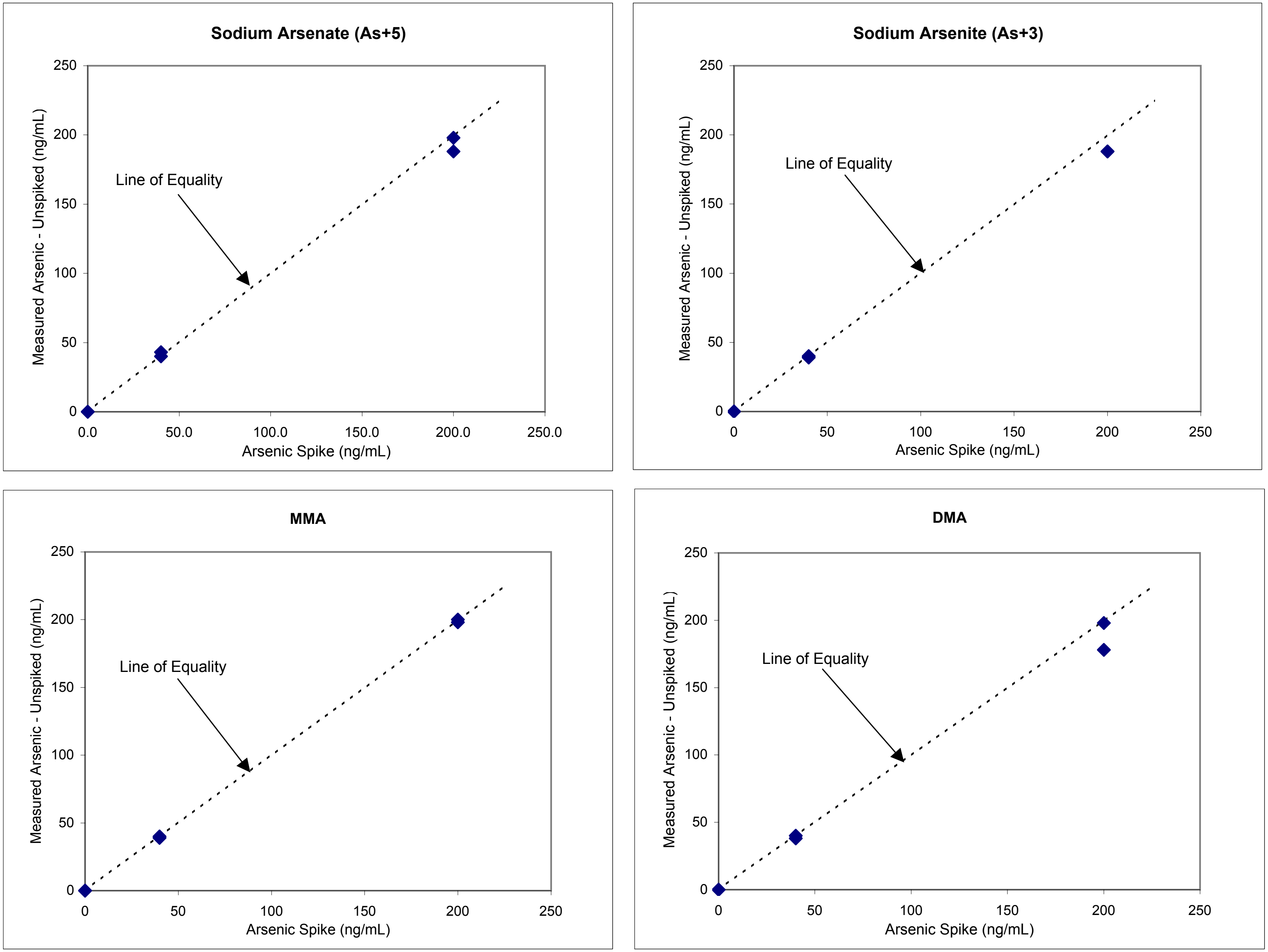
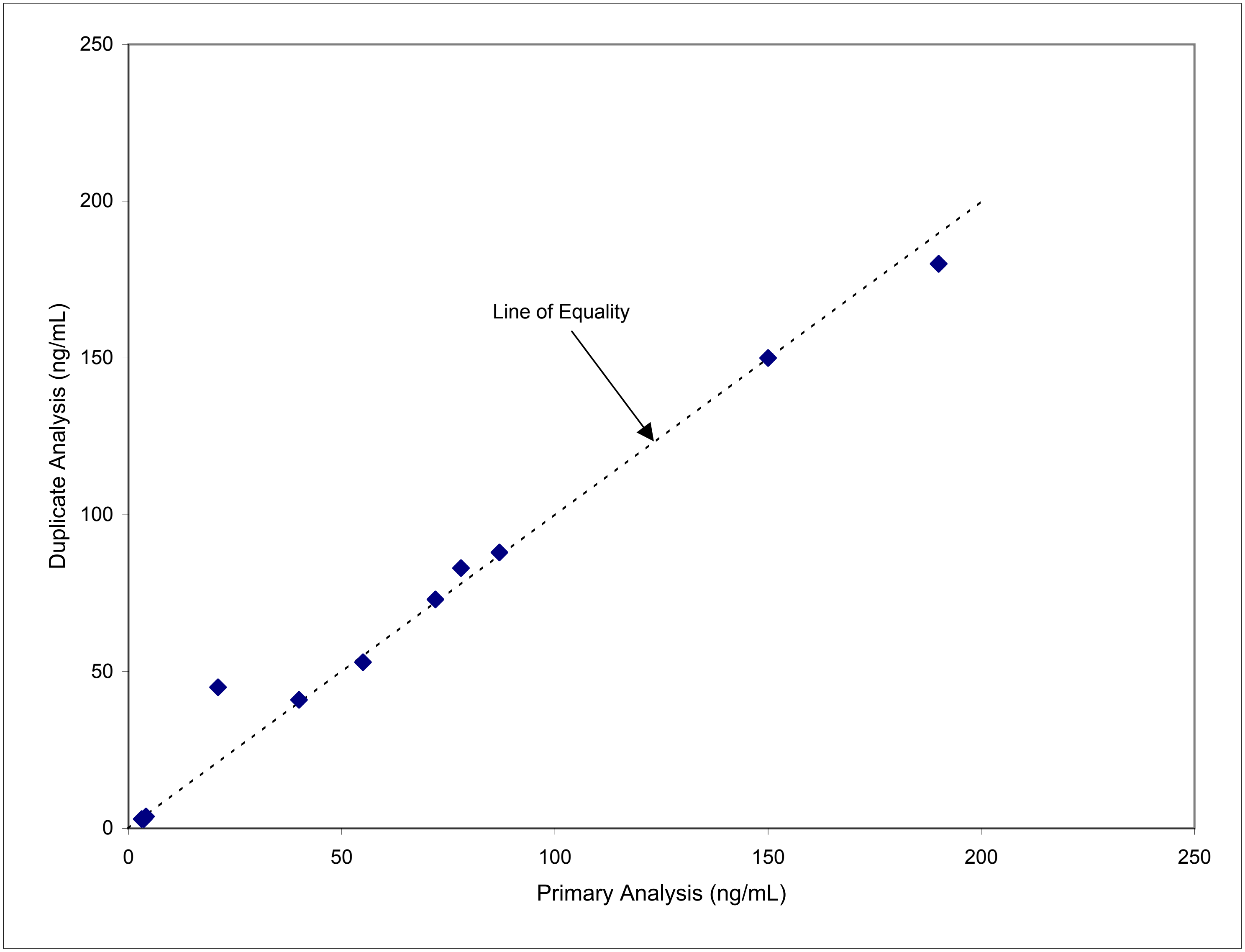
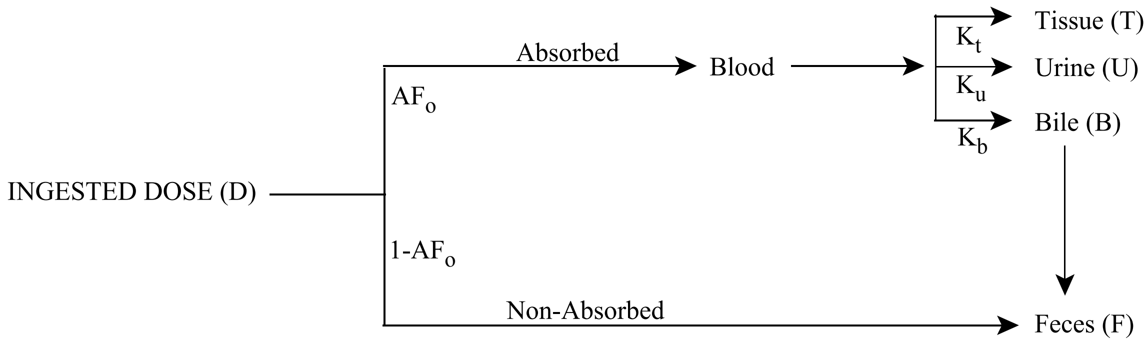


FIGURE 2-3 BLIND DUPLICATE SAMPLES



[ACC-2 Blind QC Analysis.xls (Fig2-3_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) $= D \cdot AF_o$

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

$= D \cdot AF_o \cdot K_u$

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

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

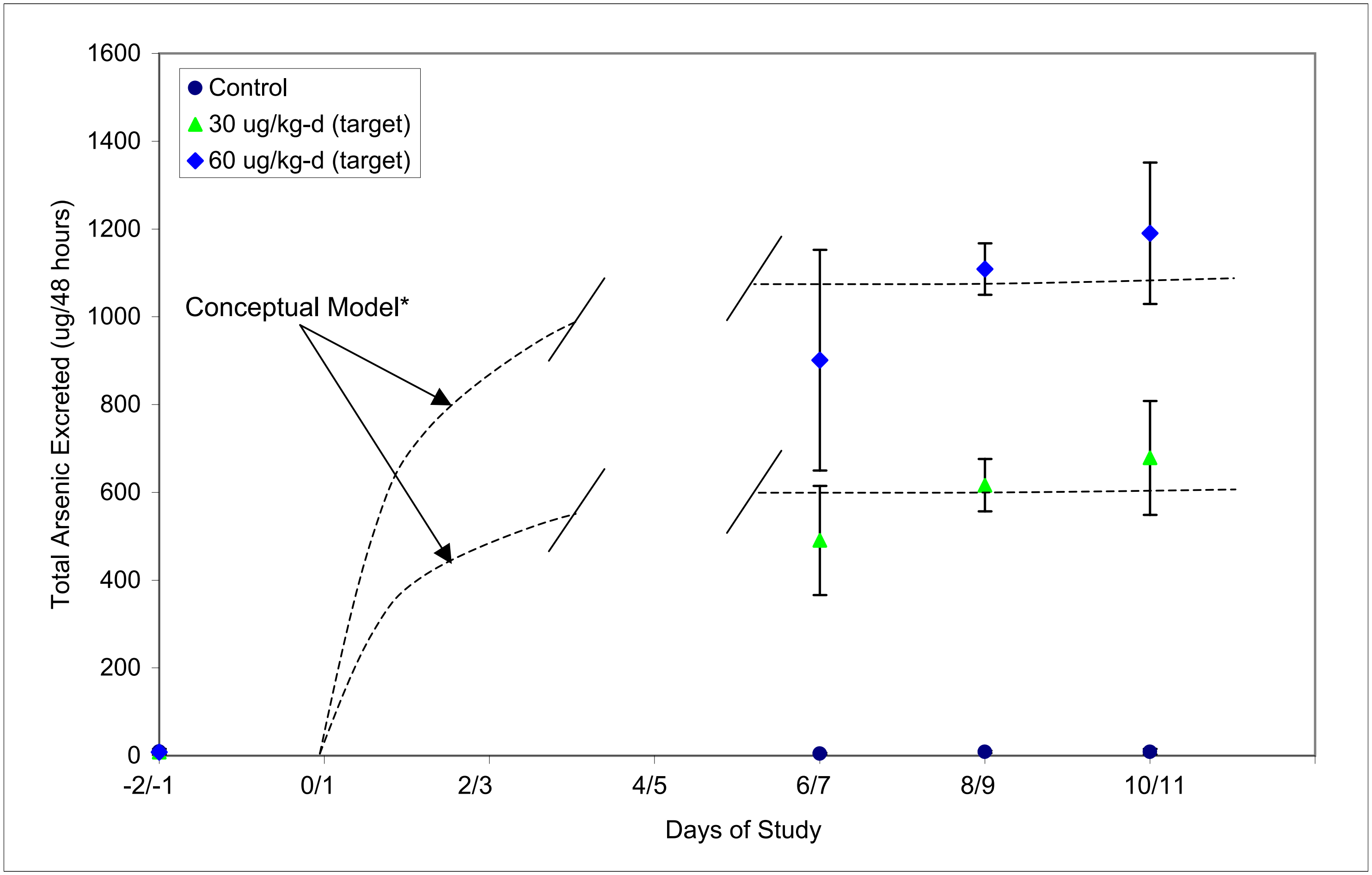
$= AF_o \cdot K_u$

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

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

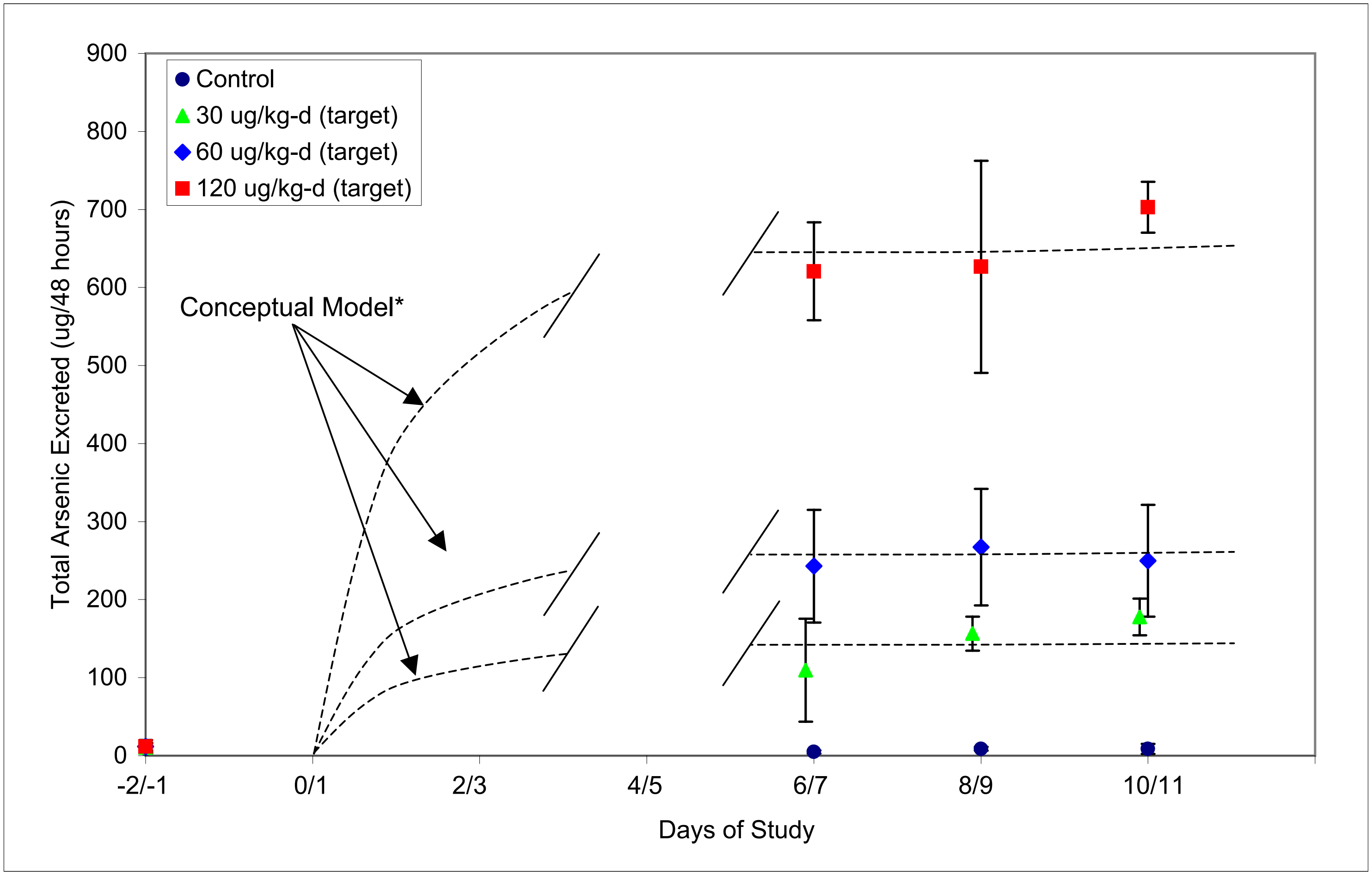
$= AF_o(x) / AF_o(y)$

FIGURE 4-1 URINARY ARSENIC EXCRETION OVER TIME - SODIUM ARSENATE



*The conceptual models shown in the figure are illustrative of the relationship that is expected to apply; they are not based on any statistical analysis of the study data.

FIGURE 4-2 URINARY ARSENIC EXCRETION OVER TIME - DISLODGEABLE ARSENIC



*The conceptual models shown in the figure are illustrative of the relationship that is expected to apply; they are not based on any statistical analysis of the study data.

FIGURE 4-3 URINARY EXCRETION OF ARSENIC FROM SODIUM ARSENATE

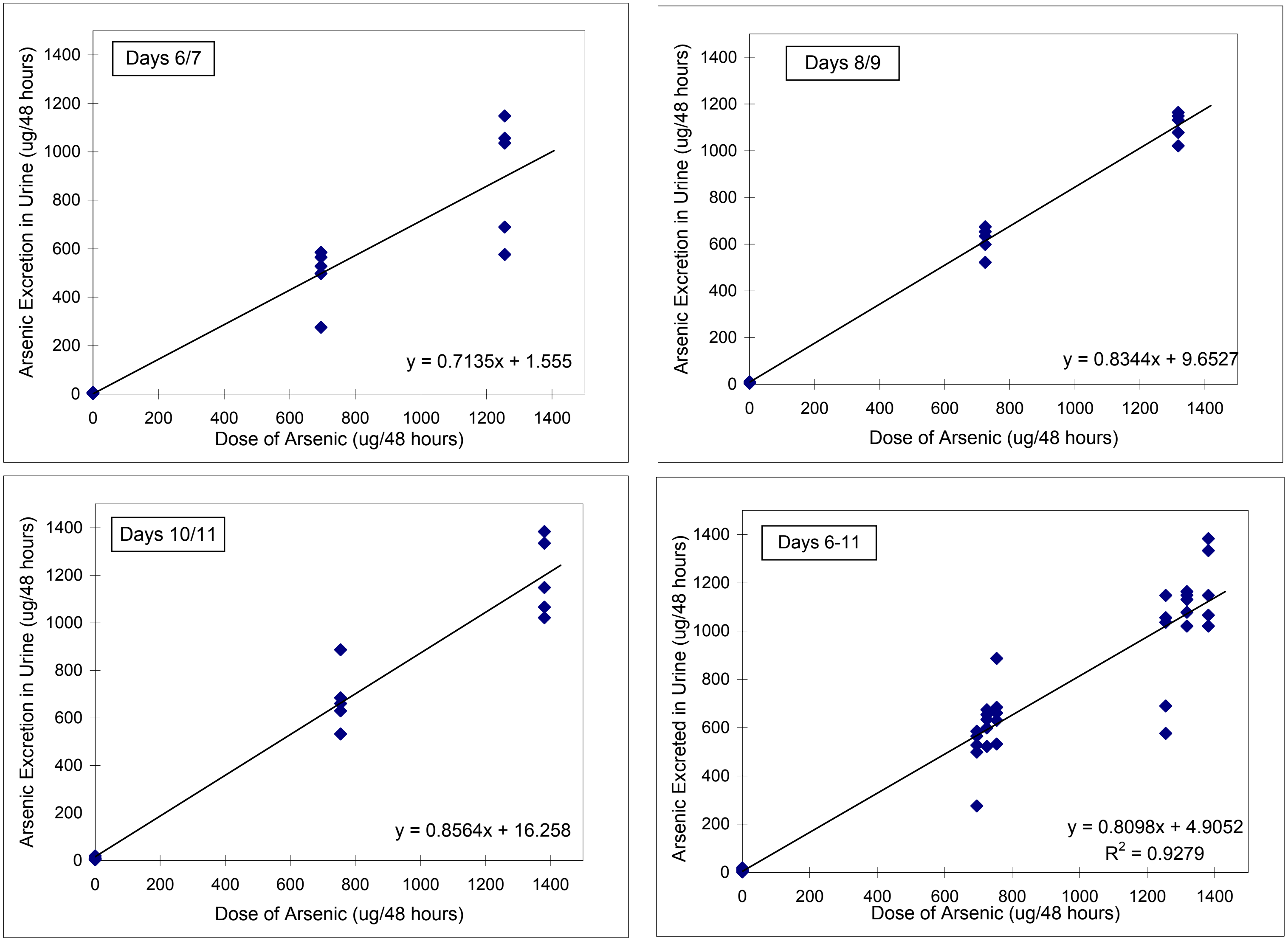
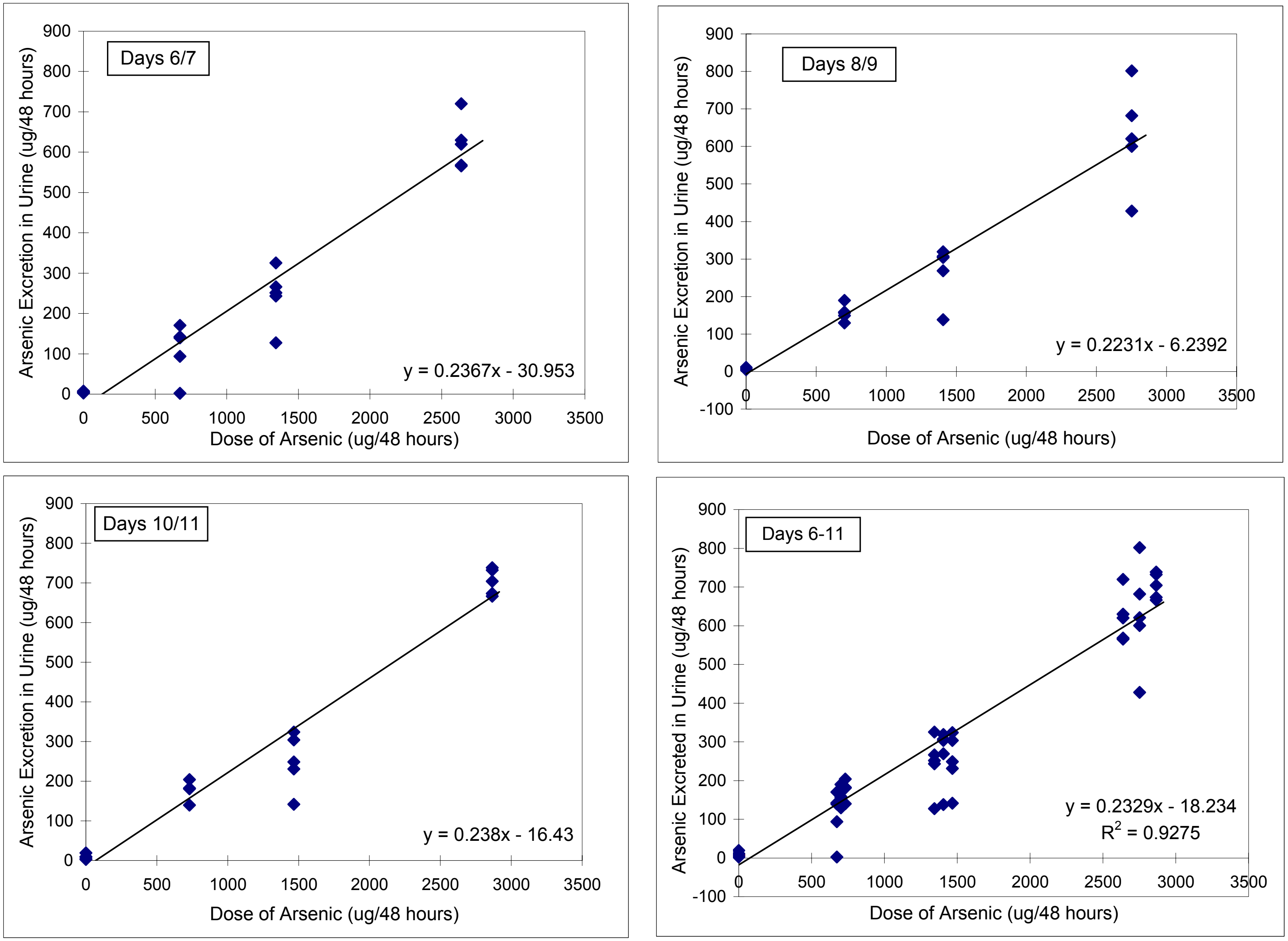


FIGURE 4-4 URINARY EXCRETION OF ARSENIC FROM DISLODGEABLE ARSENIC



APPENDIX A

DETAILED RESULTS

TABLE A-1 SCHEDULE

Study Day	Day	Date	Dose Administration	Feed Special Diet	Weigh	Dose Prep	Cull Pigs/ Assign Dose Group	48-hour Urine Collection	Sacrifice/ Necropsy
-6	Monday	1/27/03		MFA					
-5	Tuesday	1/28/03		75% / 25%					
-4	Wednesday	1/29/03		50% / 50%	X		X		
-3	Thursday	1/30/03		25% / 75%					
-2	Friday	1/31/03		100% Low Pb				↕	
-1	Saturday	2/1/03		X	X	X			
0	Sunday	2/2/03	X	X					
1	Monday	2/3/03	X	X					
2	Tuesday	2/4/03	X	X	X	X			
3	Wednesday	2/5/03	X	X					
4	Thursday	2/6/03	X	X					
5	Friday	2/7/03	X	X	X	X			
6	Saturday	2/8/03	X	X				↕	
7	Sunday	2/9/03	X	X					
8	Monday	2/10/03	X	X	X	X		↕	
9	Tuesday	2/11/03	X	X					
10	Wednesday	2/12/03	X	X				↕	
11	Thursday	2/13/03	X	X	X				
12	Friday	2/14/03							X

TABLE A-2 GROUP ASSIGNMENTS

Pig Number	Dose Group	Material Administered	Target Dose of Arsenic (ug/kg-day)
260 262 264 274 970	1	Control	0
265 268 561 564 572	2	NaAs	30
254 275 563 569 962	3	NaAs	60
263 273 557 566 964	4	Dislodgeable Arsenic	30
261 959 965 969 971	5	Dislodgeable Arsenic	60
271 565 567 960 967	6	Dislodgeable Arsenic	120

TABLE A-3 BODY WEIGHTS AND ACTUAL ADMINISTERED DOSES, BY DAY

Body weights were measured on days -1, 2, 5, 8, and 11. Weights for other days are estimated, based on linear interpolation between measured values.
All doses were fully consumed, so no dose adjustments were necessary.

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	
		BW (kg)	As Dose (ug/kg-d)	BW (kg)	As Dose (ug/kg-d)	BW (kg)	As Dose (ug/kg-d)	BW (kg)	As Dose (ug/kg-d)	BW (kg)	As Dose (ug/kg-d)	BW (kg)	As Dose (ug/kg-d)	BW (kg)	As Dose (ug/kg-d)	BW (kg)	As Dose (ug/kg-d)	BW (kg)	As Dose (ug/kg-d)	BW (kg)	As Dose (ug/kg-d)	BW (kg)	As Dose (ug/kg-d)	BW (kg)	As Dose (ug/kg-d)
1	260	7.9	0.00	8.0	0.00	8.2	0.00	8.4	0.00	8.8	0.00	9.1	0.00	9.5	0.00	9.8	0.00	10.1	0.00	10.4	0.00	10.8	0.00	11.1	0.00
1	262	8.7	0.00	9.0	0.00	9.2	0.00	9.5	0.00	9.8	0.00	10.1	0.00	10.4	0.00	10.7	0.00	11.1	0.00	11.5	0.00	11.8	0.00	12.2	0.00
1	264	8.6	0.00	8.9	0.00	9.1	0.00	9.4	0.00	9.8	0.00	10.1	0.00	10.5	0.00	10.9	0.00	11.3	0.00	11.7	0.00	12.0	0.00	12.4	0.00
1	274	8.7	0.00	8.8	0.00	8.9	0.00	9.1	0.00	9.3	0.00	9.6	0.00	9.9	0.00	10.2	0.00	10.5	0.00	10.8	0.00	11.1	0.00	11.5	0.00
1	970	8.3	0.00	8.6	0.00	9.0	0.00	9.3	0.00	9.7	0.00	10.1	0.00	10.5	0.00	10.7	0.00	10.9	0.00	11.2	0.00	11.6	0.00	12.0	0.00
2	265	8.7	0.00	8.8	32.80	9.0	32.19	9.2	31.61	9.4	33.23	9.7	32.26	10.0	31.35	10.3	33.65	10.7	32.60	11.0	31.61	11.5	32.89	11.9	31.60
2	268	9.9	0.00	10.3	28.12	10.7	27.11	11.1	26.17	11.4	27.50	11.8	26.68	12.1	25.91	12.5	27.93	12.8	27.16	13.2	26.44	13.5	28.04	13.8	27.43
2	561	8.1	0.00	8.3	34.77	8.6	33.69	8.9	32.68	9.2	33.95	9.6	32.60	10.0	31.35	10.3	33.65	10.7	32.60	11.0	31.61	11.4	33.13	11.8	32.05
2	564	8.4	0.00	8.7	33.43	8.9	32.49	9.2	31.61	9.6	32.54	10.1	30.99	10.6	29.58	10.9	31.90	11.2	31.04	11.5	30.23	11.8	32.00	12.1	31.25
2	572	8.2	0.00	8.5	34.09	8.8	32.99	9.1	31.96	9.5	33.17	9.9	31.83	10.3	30.59	10.6	32.91	10.9	31.95	11.2	31.04	11.6	32.56	12.0	31.51
3	254	8.7	0.00	8.9	63.15	9.1	61.54	9.4	60.00	9.6	60.81	9.9	59.27	10.1	57.80	10.4	60.64	10.6	59.21	10.9	57.84	11.2	61.57	11.6	59.62
3	275	8.4	0.00	8.3	67.32	8.3	67.86	8.2	68.41	8.4	69.36	8.6	67.62	8.9	65.97	9.2	68.59	9.5	66.41	9.8	64.37	10.1	68.15	10.5	65.67
3	563	8.5	0.00	8.7	64.73	8.9	63.15	9.1	61.65	9.3	62.66	9.5	61.24	9.8	59.88	10.1	62.04	10.5	59.87	10.9	57.84	11.1	62.12	11.4	60.67
3	569	8.0	0.00	8.0	69.83	8.1	69.12	8.2	68.41	8.5	68.41	8.9	65.84	9.2	63.46	9.6	65.38	10.0	62.76	10.4	60.35	10.7	64.34	11.1	62.40
3	962	8.3	0.00	8.5	66.26	8.6	64.98	8.8	63.75	9.0	64.87	9.2	63.46	9.4	62.11	9.8	63.82	10.3	61.13	10.7	58.65	11.0	62.69	11.3	60.94
4	263	8.3	0.00	8.5	34.72	8.6	34.05	8.8	33.41	9.1	34.74	9.3	33.75	9.6	32.81	9.9	33.89	10.3	32.79	10.6	31.75	11.0	33.22	11.4	32.05
4	273	8.6	0.00	8.9	33.16	9.1	32.19	9.4	31.28	9.5	33.04	9.7	32.59	9.8	32.14	10.1	33.27	10.4	32.26	10.8	31.31	11.0	33.17	11.3	32.38
4	557	8.2	0.00	8.4	35.14	8.6	34.25	8.8	33.41	9.1	34.74	9.3	33.75	9.6	32.81	10.0	33.77	10.3	32.57	10.7	31.46	11.0	33.37	11.2	32.63
4	566	9.1	0.00	9.3	31.61	9.6	30.79	9.8	30.00	10.1	31.09	10.5	30.10	10.8	29.17	11.1	30.32	11.4	29.53	11.7	28.77	12.0	30.41	12.3	29.63
4	964	9.9	0.00	10.2	28.92	10.4	28.18	10.7	27.48	10.9	28.90	11.1	28.38	11.3	27.88	11.6	29.06	11.9	28.37	12.2	27.70	12.5	29.15	12.9	28.29
5	261	8.2	0.00	8.3	69.61	8.4	68.79	8.5	67.98	8.9	69.70	9.2	66.93	9.6	64.38	10.0	67.54	10.3	65.24	10.7	63.10	11.0	66.76	11.3	64.79
5	959	8.7	0.00	9.0	64.32	9.3	62.35	9.6	60.50	9.8	62.85	10.1	61.09	10.4	59.42	10.8	62.51	11.1	60.54	11.5	58.69	11.7	62.76	11.9	61.53
5	965	8.0	0.00	8.2	70.32	8.5	68.11	8.8	66.03	9.1	68.29	9.4	66.10	9.7	64.04	9.9	67.65	10.2	65.77	10.5	64.00	10.8	67.78	11.1	65.86
5	969	9.1	0.00	9.2	62.58	9.4	61.36	9.6	60.19	9.9	62.74	10.1	61.19	10.4	59.71	10.7	62.71	11.1	60.63	11.5	58.69	11.7	62.67	12.0	61.36
5	971	9.3	0.00	9.5	60.61	9.8	58.86	10.1	57.21	10.4	59.42	10.7	57.76	11.0	56.18	11.4	59.21	11.7	57.44	12.1	55.77	12.3	59.53	12.6	58.27
6	271	8.9	0.00	9.2	122.26	9.5	118.20	9.9	114.40	10.1	121.67	10.3	119.30	10.5	117.01	10.8	122.11	11.2	118.28	11.5	114.68	11.8	121.77	12.0	119.07
6	565	8.2	0.00	8.4	134.68	8.6	131.28	8.8	128.05	9.2	133.64	9.5	128.72	9.9	124.14	10.2	129.72	10.5	125.80	10.8	122.11	11.1	129.28	11.4	126.05
6	567	8.1	0.00	8.4	134.41	8.7	129.27	9.1	124.51	9.4	130.55	9.7	126.28	10.0	122.28	10.4	126.81	10.8	122.11	11.2	117.75	11.5	125.14	11.7	122.46
6	960	8.6	0.00	8.8	128.53	8.9	126.13	9.1	123.82	9.3	131.48	9.5	128.72	9.7	126.06	10.0	132.54	10.2	129.29	10.5	126.20	10.7	133.70	11.0	130.45
6	967	8.3	0.00	8.6	131.79	8.9	127.32	9.2	123.15	9.4	129.85	9.7	126.28	10.0	122.89	10.2	129.08	10.5	125.80	10.8	122.68	11.1	129.67	11.4	126.24

TABLE A-4 URINE VOLUMES - 48 HOUR COLLECTIONS

Units of Volume: mL

Group	Pig ID	Urine Collection			
		Days -2/-1 1/31-2/1/03	Days 6/7 2/8-2/9/03	Days 8/9 2/10-2/11/03	Days 10/11 2/12-2/13/03
1	260	4600	3320	4120	9500
	262	5520	4100	4900	4400
	264	2240	2620	3620	4920
	274	8980	3600	4620	2860
	970	4150	3360	5340	7760
2	265	3200	5140	5940	5600
	268	2540	3440	2900	4200
	561	13040	15340	13220	21100
	564	1200	1660	1760	1520
	572	1480	1200	3520	4400
3	254	5420	6600	7540	8200
	275	1990	1380	1620	2320
	563	2870	2400	6740	4940
	569	5280	3140	5220	7280
	962	3150	6040	5060	6040
4	263	5200	7880	7200	8620
	273	3380	3100	2400	2800
	557	6800	17640	13180	10760
	566	2400	1540	2000	1820
	964	3000	4580	3720	4860
5	261	1700	900	980	980
	959	5160	7500	5600	7780
	965	5020	3700	3890	3000
	969	6160	7400	7780	7080
	971	2440	1480	920	1620
6	271	2860	3000	2140	3660
	565	6920	5600	5640	6060
	567	1420	2840	5360	5680
	960	5820	6200	8120	7740
	967	4540	4200	4220	4400

TABLE A-5 ARSENIC ANALYTICAL RESULTS FOR URINE SAMPLES

Tag Number	Group	Material Administered	Target Dose (ug/kg-d)	Pig Number	Urine Collection Days	48-hour Dose (ug/48 hours)	48-hour Body Weight Adjusted Dose (ug/kg-48 hours)	Urine Volume (mL/48 hours)	Q	Arsenic Conc (ng/mL)	DL	Adjusted Arsenic Conc (ng/mL)
ACC-2-0137	1	Control	0	260	-2/-1	0	0	4600		3	1	3
ACC-2-0105	1	Control	0	262	-2/-1	0	0	5520		1	1	1
ACC-2-0126	1	Control	0	264	-2/-1	0	0	2240		3	1	3
ACC-2-0127	1	Control	0	274	-2/-1	0	0	8980		2	1	2
ACC-2-0131	1	Control	0	970	-2/-1	0	0	4150	<	1	1	0.5
ACC-2-0101	2	NaAs	30	265	-2/-1	0	0	3200		3.6	1	3.6
ACC-2-0129	2	NaAs	30	268	-2/-1	0	0	2540		3.2	1	3.2
ACC-2-0104	2	NaAs	30	561	-2/-1	0	0	13040		2	1	2
ACC-2-0106	2	NaAs	30	564	-2/-1	0	0	1200		5.4	1	5.4
ACC-2-0125	2	NaAs	30	572	-2/-1	0	0	1480		5	1	5
ACC-2-0135	3	NaAs	60	254	-2/-1	0	0	5420		1	1	1
ACC-2-0107	3	NaAs	60	275	-2/-1	0	0	1990		3.1	1	3.1
ACC-2-0103	3	NaAs	60	563	-2/-1	0	0	2870		3.1	1	3.1
ACC-2-0124	3	NaAs	60	569	-2/-1	0	0	5280		2	1	2
ACC-2-0120	3	NaAs	60	962	-2/-1	0	0	3150		3.1	1	3.1
ACC-2-0128	4	Dislodgeable Arsenic	30	263	-2/-1	0	0	5200		2	1	2
ACC-2-0110	4	Dislodgeable Arsenic	30	273	-2/-1	0	0	3380		2	1	2
ACC-2-0130	4	Dislodgeable Arsenic	30	557	-2/-1	0	0	6800		3	1	3
ACC-2-0122	4	Dislodgeable Arsenic	30	566	-2/-1	0	0	2400		4.3	1	4.3
ACC-2-0132	4	Dislodgeable Arsenic	30	964	-2/-1	0	0	3000		2	1	2
ACC-2-0123	5	Dislodgeable Arsenic	60	261	-2/-1	0	0	1700		5.3	1	5.3
ACC-2-0108	5	Dislodgeable Arsenic	60	959	-2/-1	0	0	5160		3.5	1	3.5
ACC-2-0115	5	Dislodgeable Arsenic	60	965	-2/-1	0	0	5020		3	1	3
ACC-2-0113	5	Dislodgeable Arsenic	60	969	-2/-1	0	0	6160		1	1	1
ACC-2-0133	5	Dislodgeable Arsenic	60	971	-2/-1	0	0	2440		4.1	1	4.1
ACC-2-0117	6	Dislodgeable Arsenic	120	271	-2/-1	0	0	2860		3.2	1	3.2
ACC-2-0109	6	Dislodgeable Arsenic	120	565	-2/-1	0	0	6920		3.8	1	3.8
ACC-2-0102	6	Dislodgeable Arsenic	120	567	-2/-1	0	0	1420		5.4	1	5.4
ACC-2-0118	6	Dislodgeable Arsenic	120	960	-2/-1	0	0	5820		1	1	1
ACC-2-0112	6	Dislodgeable Arsenic	120	967	-2/-1	0	0	4540		2	1	2
ACC-2-0160	1	Control	0	260	6/7	0	0	3320		1	1	1
ACC-2-0147	1	Control	0	262	6/7	0	0	4100		1	1	1
ACC-2-0150	1	Control	0	264	6/7	0	0	2620	<	2	2	1
ACC-2-0171	1	Control	0	274	6/7	0	0	3600		2	1	2
ACC-2-0173	1	Control	0	970	6/7	0	0	3360		2	1	2
ACC-2-0165	2	NaAs	30	265	6/7	695.4	66.25	5140		110	2	110
ACC-2-0143	2	NaAs	30	268	6/7	695.4	55.09	3440		170	2	170
ACC-2-0157	2	NaAs	30	561	6/7	695.4	66.25	15340		18	1	18
ACC-2-0152	2	NaAs	30	564	6/7	695.4	62.94	1660		300	4	300
ACC-2-0175	2	NaAs	30	572	6/7	695.4	64.85	1200		440	10	440
ACC-2-0166	3	NaAs	60	254	6/7	1255.2	119.85	6600		160	2	160
ACC-2-0145	3	NaAs	60	275	6/7	1255.2	135	1380		500	10	500
ACC-2-0167	3	NaAs	60	563	6/7	1255.2	121.9	2400		240	4	240
ACC-2-0170	3	NaAs	60	569	6/7	1255.2	128.14	3140		330	4	330
ACC-2-0144	3	NaAs	60	962	6/7	1255.2	124.95	6040		190	2	190
ACC-2-0164	4	Dislodgeable Arsenic	30	263	6/7	673.2	66.67	7880		18	1	18
ACC-2-0139	4	Dislodgeable Arsenic	30	273	6/7	673.2	65.53	3100		55	1	55
ACC-2-0169	4	Dislodgeable Arsenic	30	557	6/7	673.2	66.35	17640		7.9	1	7.9
ACC-2-0141	4	Dislodgeable Arsenic	30	566	6/7	673.2	59.85	1540		61	1	61
ACC-2-0163	4	Dislodgeable Arsenic	30	964	6/7	673.2	57.42	4580	<	1	1	0.5
ACC-2-0155	5	Dislodgeable Arsenic	60	261	6/7	1344	132.78	900		270	4	270
ACC-2-0138	5	Dislodgeable Arsenic	60	959	6/7	1344	123.05	7500		17	1	17
ACC-2-0140	5	Dislodgeable Arsenic	60	965	6/7	1344	133.43	3700		72	1	72
ACC-2-0142	5	Dislodgeable Arsenic	60	969	6/7	1344	123.34	7400		44	1	44
ACC-2-0146	5	Dislodgeable Arsenic	60	971	6/7	1344	116.64	1480		170	2	170
ACC-2-0161	6	Dislodgeable Arsenic	120	271	6/7	2637.6	240.39	3000		240	4	240
ACC-2-0151	6	Dislodgeable Arsenic	120	565	6/7	2637.6	255.52	5600		101	1	101
ACC-2-0149	6	Dislodgeable Arsenic	120	567	6/7	2637.6	248.92	2840		200	2	200
ACC-2-0162	6	Dislodgeable Arsenic	120	960	6/7	2637.6	261.84	6200		100	2	100
ACC-2-0158	6	Dislodgeable Arsenic	120	967	6/7	2637.6	254.88	4200		150	2	150
ACC-2-0199	1	Control	0	260	8/9	0	0	4120		2	1	2
ACC-2-0208	1	Control	0	262	8/9	0	0	4900		1	1	1
ACC-2-0207	1	Control	0	264	8/9	0	0	3620		3	1	3
ACC-2-0183	1	Control	0	274	8/9	0	0	4620		2	1	2
ACC-2-0194	1	Control	0	970	8/9	0	0	5340		2	1	2
ACC-2-0186	2	NaAs	30	265	8/9	724.8	64.5	5940		110	2	110
ACC-2-0198	2	NaAs	30	268	8/9	724.8	54.48	2900		180	2	180
ACC-2-0193	2	NaAs	30	561	8/9	724.8	64.74	13220		51	1	51
ACC-2-0189	2	NaAs	30	564	8/9	724.8	62.24	1760		360	4	360
ACC-2-0202	2	NaAs	30	572	8/9	724.8	63.6	3520		170	2	170
ACC-2-0182	3	NaAs	60	254	8/9	1318.2	119.41	7540		150	2	150
ACC-2-0185	3	NaAs	60	275	8/9	1318.2	132.52	1620		630	10	630
ACC-2-0179	3	NaAs	60	563	8/9	1318.2	119.97	6740		160	2	160
ACC-2-0181	3	NaAs	60	569	8/9	1318.2	124.69	5220		220	4	220

Tag Number	Group	Material Administered	Target Dose (ug/kg-d)	Pig Number	Urine Collection Days	48-hour Dose (ug/48 hours)	48-hour Body Weight Adjusted Dose (ug/kg-48 hours)	Urine Volume (mL/48 hours)	Q	Arsenic Conc (ng/mL)	DL	Adjusted Arsenic Conc (ng/mL)
ACC-2-0176	3	NaAs	60	962	8/9	1318.2	121.34	5060		230	4	230
ACC-2-0201	4	Dislodgeable Arsenic	30	263	8/9	702	64.97	7200		18	1	18
ACC-2-0187	4	Dislodgeable Arsenic	30	273	8/9	702	64.48	2400		79	1	79
ACC-2-0211	4	Dislodgeable Arsenic	30	557	8/9	702	64.83	13180		12	1	12
ACC-2-0203	4	Dislodgeable Arsenic	30	566	8/9	702	59.18	2000		78	2	78
ACC-2-0209	4	Dislodgeable Arsenic	30	964	8/9	702	56.86	3720		40	1	40
ACC-2-0177	5	Dislodgeable Arsenic	60	261	8/9	1405.2	129.85	980		310	4	310
ACC-2-0184	5	Dislodgeable Arsenic	60	959	8/9	1405.2	121.45	5600		48	1	48
ACC-2-0204	5	Dislodgeable Arsenic	60	965	8/9	1405.2	131.78	3890		79	1	79
ACC-2-0206	5	Dislodgeable Arsenic	60	969	8/9	1405.2	121.36	7780		41	1	41
ACC-2-0205	5	Dislodgeable Arsenic	60	971	8/9	1405.2	115.3	920		150	2	150
ACC-2-0196	6	Dislodgeable Arsenic	120	271	8/9	2751.6	236.45	2140		200	4	200
ACC-2-0197	6	Dislodgeable Arsenic	120	565	8/9	2751.6	251.39	5640		110	2	110
ACC-2-0180	6	Dislodgeable Arsenic	120	567	8/9	2751.6	242.89	5360		112	1	112
ACC-2-0191	6	Dislodgeable Arsenic	120	960	8/9	2751.6	259.9	8120		84	2	84
ACC-2-0192	6	Dislodgeable Arsenic	120	967	8/9	2751.6	252.34	4220		190	2	190
ACC-2-0217	1	Control	0	260	10/11	0	0	9500		2	1	2
ACC-2-0243	1	Control	0	262	10/11	0	0	4400		2	1	2
ACC-2-0238	1	Control	0	264	10/11	0	0	4920		2	1	2
ACC-2-0234	1	Control	0	274	10/11	0	0	2860		1	1	1
ACC-2-0245	1	Control	0	970	10/11	0	0	7760	<	1	1	0.5
ACC-2-0213	2	NaAs	30	265	10/11	754.2	62.01	5600		95	2	95
ACC-2-0219	2	NaAs	30	268	10/11	754.2	54.27	4200		150	2	150
ACC-2-0229	2	NaAs	30	561	10/11	754.2	63.09	21100		42	1	42
ACC-2-0246	2	NaAs	30	564	10/11	754.2	61.79	1520		450	10	450
ACC-2-0237	2	NaAs	30	572	10/11	754.2	62.05	4400		150	2	150
ACC-2-0224	3	NaAs	60	254	10/11	1381.2	117.41	8200		130	2	130
ACC-2-0240	3	NaAs	60	275	10/11	1381.2	129.02	2320		440	10	440
ACC-2-0215	3	NaAs	60	563	10/11	1381.2	119.95	4940		270	4	270
ACC-2-0214	3	NaAs	60	569	10/11	1381.2	122.98	7280		190	2	190
ACC-2-0236	3	NaAs	60	962	10/11	1381.2	120.21	6040		190	4	190
ACC-2-0231	4	Dislodgeable Arsenic	30	263	10/11	730.8	63.02	8620		21	1	21
ACC-2-0220	4	Dislodgeable Arsenic	30	273	10/11	730.8	64.02	2800		65	1	65
ACC-2-0244	4	Dislodgeable Arsenic	30	557	10/11	730.8	64.54	10760		13	1	13
ACC-2-0230	4	Dislodgeable Arsenic	30	566	10/11	730.8	58.51	1820		100	2	100
ACC-2-0216	4	Dislodgeable Arsenic	30	964	10/11	730.8	55.76	4860		42	1	42
ACC-2-0235	5	Dislodgeable Arsenic	60	261	10/11	1466.4	127.73	980		310	4	310
ACC-2-0248	5	Dislodgeable Arsenic	60	959	10/11	1466.4	121.87	7780		32	1	32
ACC-2-0218	5	Dislodgeable Arsenic	60	965	10/11	1466.4	129.89	3000		77	2	77
ACC-2-0249	5	Dislodgeable Arsenic	60	969	10/11	1466.4	121.45	7080		20	1	20
ACC-2-0242	5	Dislodgeable Arsenic	60	971	10/11	1466.4	115.33	1620		200	4	200
ACC-2-0221	6	Dislodgeable Arsenic	120	271	10/11	2865.6	235.56	3660		200	4	200
ACC-2-0241	6	Dislodgeable Arsenic	120	565	10/11	2865.6	249.04	6060		110	2	110
ACC-2-0227	6	Dislodgeable Arsenic	120	567	10/11	2865.6	242.36	5680		130	2	130
ACC-2-0239	6	Dislodgeable Arsenic	120	960	10/11	2865.6	257.81	7740		87	2	87
ACC-2-0225	6	Dislodgeable Arsenic	120	967	10/11	2865.6	249.23	4400		160	2	160

TABLE A-6 ARSENIC ANALYTICAL RESULTS FOR BLIND QUALITY ASSURANCE SAMPLES

Tag Number	LET ID	Matrix	QC Type	QC Identifier	Orginal Pig #	Group	Material Administered	Target Dose (ug/kg-d)	Urine Collection	Analyte	Q	As Conc	DL	AdjConc	Units	OrigAdj Conc
ACC-2-0114	L03020016	Urine	Blind Duplicate	2265	265	3	NaAs	60	U-0	As		3	1	3	ng/mL	3.6
ACC-2-0136	L03020042	Urine	Blind Duplicate	2971	971	2	NaAs	30	U-0	As		3.8	1	3.8	ng/mL	4.1
ACC-2-0111	L03020013	Urine	Blind Duplicate	2275	275	4	Dislodgeable Arsenic	30	U-0	As		3	1	3	ng/mL	3.1
ACC-2-0148	L03020058	Urine	Blind Duplicate	2273	273	3	NaAs	60	U-1	As		53	1	53	ng/mL	55
ACC-2-0168	L03020082	Urine	Blind Duplicate	2965	965	5	Dislodgeable Arsenic	60	U-1	As		73	2	73	ng/mL	72
ACC-2-0154	L03020064	Urine	Blind Duplicate	2962	962	2	NaAs	30	U-1	As		180	2	180	ng/mL	190
ACC-2-0178	L03020095	Urine	Blind Duplicate	2964	964	3	NaAs	60	U-2	As		41	1	41	ng/mL	40
ACC-2-0190	L03020108	Urine	Blind Duplicate	2566	566	5	Dislodgeable Arsenic	60	U-2	As		83	2	83	ng/mL	78
ACC-2-0188	L03020106	Urine	Blind Duplicate	2967	967	4	Dislodgeable Arsenic	30	U-2	As		180	2	180	ng/mL	190
ACC-2-0250	L03020180	Urine	Blind Duplicate	2263	263	3	NaAs	60	U-3	As		45	1	45	ng/mL	21
ACC-2-0222	L03020147	Urine	Blind Duplicate	2268	268	5	Dislodgeable Arsenic	60	U-3	As		150	2	150	ng/mL	150
ACC-2-0232	L03020158	Urine	Blind Duplicate	2960	960	4	Dislodgeable Arsenic	30	U-3	As		88	2	88	ng/mL	87

Tag Number	LET ID	Matrix	QC Type	PE Definition	QC Identifier	QC Replicate	PE Concentration	PE Units	Analyte	Q	As Conc	DL	AdjConc	Units
ACC-2-0195	L03020116	Urine	PE Sample	Control Urine	AsCtrl	a	0	ug/L	As		2	1	2	ng/mL
ACC-2-0159	L03020072	Urine	PE Sample	Control Urine	AsCtrl	b	0	ug/L	As		2	1	2	ng/mL
ACC-2-0223	L03020148	Urine	PE Sample	Sodium arsenate	AsIA200	a	200	ug/L	As		190	4	190	ng/mL
ACC-2-0116	L03020018	Urine	PE Sample	Sodium arsenate	AsIA200	b	200	ug/L	As		200	4	200	ng/mL
ACC-2-0174	L03020088	Urine	PE Sample	Sodium arsenate	AsIA40	a	40	ug/L	As		45	1	45	ng/mL
ACC-2-0200	L03020121	Urine	PE Sample	Sodium arsenate	AsIA40	b	40	ug/L	As		42	1	42	ng/mL
ACC-2-0212	L03020134	Urine	PE Sample	Sodium arsenite	AsIB200	a	200	ug/L	As		190	2	190	ng/mL
ACC-2-0153	L03020063	Urine	PE Sample	Sodium arsenite	AsIB200	b	200	ug/L	As		190	2	190	ng/mL
ACC-2-0226	L03020152	Urine	PE Sample	Sodium arsenite	AsIB40	a	40	ug/L	As		41	1	41	ng/mL
ACC-2-0119	L03020021	Urine	PE Sample	Sodium arsenite	AsIB40	b	40	ug/L	As		42	2	42	ng/mL
ACC-2-0233	L03020162	Urine	PE Sample	Disodium methylarsenate	AsOA200	a	200	ug/L	As		200	4	200	ng/mL
ACC-2-0134	L03020040	Urine	PE Sample	Disodium methylarsenate	AsOA200	b	200	ug/L	As		202	2	202	ng/mL
ACC-2-0210	L03020132	Urine	PE Sample	Disodium methylarsenate	AsOA40	a	40	ug/L	As		42	1	42	ng/mL
ACC-2-0156	L03020066	Urine	PE Sample	Disodium methylarsenate	AsOA40	b	40	ug/L	As		41	1	41	ng/mL
ACC-2-0172	L03020086	Urine	PE Sample	Dimethyl arsenic acid	AsOB200	a	200	ug/L	As		200	2	200	ng/mL
ACC-2-0247	L03020177	Urine	PE Sample	Dimethyl arsenic acid	AsOB200	b	200	ug/L	As		180	2	180	ng/mL
ACC-2-0121	L03020026	Urine	PE Sample	Dimethyl arsenic acid	AsOB40	a	40	ug/L	As		40	1	40	ng/mL
ACC-2-0228	L03020154	Urine	PE Sample	Dimethyl arsenic acid	AsOB40	b	40	ug/L	As		42	1	42	ng/mL

Tag Number	LET ID	Matrix	QC Type	QC Identifier	QC Replicate	DV Prep Date	Dose Administration Days	Analyte	Q	As Conc	DL	AdjConc	Units	Nominal As Conc per doughball	
ACC-2-0313	L03030005	Dough	Dose Verification	GP6	a	2/4/03	3-5	As		618	5	618	µg	611.4	µg
ACC-2-0336	L03030006	Dough	Dose Verification	GP2	a	2/7/03	6-8	As		180	5	180	µg	173.9	µg
ACC-2-0345	L03030010	Dough	Dose Verification	GP1	b	2/10/03	9-11	As		0.1	0.05	0.1	µg	0.0	µg