RELATIVE BIOAVAILABILITY OF ARSENIC IN SEDIMENTS FROM THE ABERJONA RIVER

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EXECUTIVE SUMMARY

The gastrointestinal absorption of arsenic from two composite sediment samples collected from the banks of the Aberjona River was measured using young swine. Groups of animals (four animals per dose group) were given oral doses of a reference material (sodium arsenate) or site sediment twice a day for 12 days. Urine excreted by each animal was collected on days 6/7, 8/9 and 10/11. 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 each test material using linear regression analysis. The relative bioavailability (RBA) of arsenic in a test material compared to that in sodium arsenate was calculated as:

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

The results are summarized below:

Test Material	Description	Arsenic Conc. (ppm)	Relative Bioavailability	
			Best Est.	90% CI
TM1	Composite sample of three sediments with arsenic concentrations greater than 500 ppm	676	37%	32% – 41%
TM2	Composite sample of three sediments with arsenic concentrations of 180-460 ppm	313	51%	46% – 56%

These data indicate that arsenic in site sediments is absorbed less extensively than arsenic in drinking water. Use of these site-specific data is likely to improve the accuracy of risk estimates for humans who may be exposed to the sediments.

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1.0 INTRODUCTION

Accurate assessment of the health risks resulting from oral exposure to any chemical frequently requires knowledge of the amount of the chemical 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 chemical absorbed compared to the amount of chemical ingested:

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

This ratio is also referred to as the oral absorption fraction (AF_0).

Relative Bioavailability (RBA) is the ratio of the absolute bioavailability of some test material compared to the absolute bioavailability of some appropriate reference material, usually the chemical dissolved in water or some fully soluble form that completely dissolves when ingested:

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

For example, if 100 ug of arsenic dissolved in drinking water were ingested and a total of 90 ug entered the body, the ABA would be 0.90 (90%). Likewise, if 100 ug of arsenic contained in soil were ingested and 30 ug entered the body, the ABA for soil would be 0.30 (30%). If the arsenic dissolved in water was used as the reference substance for describing the relative amount of arsenic absorbed from soil, the RBA would be 0.30/0.90 = 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, sediment), this information can be used to improve the accuracy of exposure and risk calculations for that medium at that site as follows:

$$RfD(adjusted) = \frac{RfD\ (IRIS)}{RBA}$$

$$SF(adjusted) = SF(IRIS) \cdot RBA$$

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

$$Dose(adjusted) = Dose(default) \cdot RBA$$

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

Purpose of This Study

USEPA Region 1 is currently investigating potential human health risks from arsenic in sediment samples from along the Aberjona River and associated wetlands and floodplain areas. This study was performed to obtain site-specific data on the relative bioavailability of arsenic in sediment samples from the site in order to improve accuracy and decrease uncertainty in human health risk evaluations.

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 from two site-specific sediments, each administered to groups of animals at three different dose levels for 12 days. All doses were administered orally.

2.1 Test Materials

2.1.1 Preliminary Characterization of Site Sediment Samples

Preparation of the two test materials for this study began by collecting 12 sediment samples from multiple locations along the Aberjona River. Each of these samples was characterized in order to support decisions as to which samples should be selected for use as dose material in the animal study, as well as to answer questions about how the dose material should be prepared and administered. Figure 2-1 is a flow chart that summarizes this characterization process.

Sample Description

The sampling locations of the 12 sediment samples span four basic regions of the Aberjona River. Sediment samples 1-3 were collected from the Halls Brook Holding Area, samples 4-6 were collected from the Wells G&H 38-acre Wetland, samples 7-9 were collected from the Cranberry Bog, and samples 10-12 were from Davidson Park. Samples were selected to cover a range of arsenic concentrations in sediments, and were also selected to provide reasonable spatial representativeness across the site.

Sample Preparation

One portion of each of the 12 samples was coarse-sieved through a 1 cm screen to remove large debris (sticks, leaf matter, stones, etc.). This screening was performed on the moist (un-dried) samples. A portion of this coarse-sieved material was removed for arsenic analysis, and a second portion was removed for *in vitro* bioaccessibility analysis (see below). The remaining portion was air dried and fine-sieved (using a 2 mm screen). This step was performed because it is considered probable that the fine-grained portion of the sediment is more likely to adhere to skin and be ingested by humans than the coarse-grained fraction.

Arsenic Concentration

The concentration of arsenic was measured in both the coarse- and fine-sieved samples by inductively coupled plasma atomic absorption spectrometry (ICP-AES). The results from these analyses are shown below:

River Segment	Sample	Arsenic Concentration (ppm)	
		Fine-sieved	Coarse-sieved
Halls Brook Holding Area	1	459	583
	2	527	590
	3	144	269
Wells G&H Wetland	4	145	411
	5	775	605
	6	176	156
Cranberry Bog	7	301	315
	8	832	560
	9	407	388
Davidson Park	10	43.4	37.0
	11	64.0	91.8
	12	67.1	74.9

As seen, the concentration of arsenic in the sediment samples is quite variable, both within a segment of river and between segments. In general, the concentration of arsenic in coarse-sieved and fine-sieved material tends to be similar (Figure 2-2). Thus, RBA results based on tests using fine-sieved material can be extrapolated to samples for which only bulk sample results are available.

In Vitro Bioaccessibility

In vivo absorption of arsenic from a solid medium such as sediment depends on the rate and extent to which arsenic dissolves from the solid medium into the fluids of the gastrointestinal tract. Dr. John Drexler at the University of Colorado has developed a standard procedure to measure the amount of arsenic that dissolves from a test material into a fluid that is similar to the gastric fluid of humans. The amount of arsenic that solubilizes in this test after a specified period of time (usually one hour) is referred to as the *in vitro* bioaccessibility (IVBA), and this value may be used as a preliminary qualitative indicator of potential *in vivo* RBA.

Figure 2-3 shows the IVBA for each of the 12 dried and fine-sieved sediment samples from the site. As seen, there is a range of values, and the IVBA appears to be inversely correlated with concentration (i.e., the most concentrated samples tend to have the lowest *in vitro* bioaccessibility, while the least concentrated samples tend to have the highest *in vitro* bioaccessibility). The basis for this apparent relationship is not known.

Effect of Drying

Each of the sediment samples collected in the field contained considerable moisture content. *A priori*, it was considered possible that drying the samples might alter (increase) the binding of

arsenic to the sediment particles, potentially resulting in a change (decrease) in bioavailability. In order to investigate this possibility, the IVBA of the dried and un-dried samples were compared. Because the moist, un-dried material could not be effectively sieved through the 2mm screen, the moist sample was selected manually to include as few coarse particles as possible. The results are shown in the following table and in Figure 2-4:

River Segment	Sample	*In Vitro* Bioaccessibility of Arsenic (%)	
		Dry	Moist (Un-dried)
Halls Brook Holding Area	1	40	2
	2	31	5
	3	70	5
Wells G&H Wetland	4	40	26
	5	12	16
	6	55	9
Cranberry Bog	7	37	12
	8	13	12
	9	15	13
Davidson Park	10	39	53
	11	49	53
	12	59	9
Average		38	18

As seen, drying the moist material does not appear to significantly influence the IVBA for some samples, and tends to increase rather than decrease the IVBA for other samples. The basis for this apparent change in IVBA is not known, but the results suggest that dried sediment will be as bioavailable or more bioavailable than un-dried sediments. On this basis, it was decided that the *in vivo* test of RBA would be performed using the dried materials.

Evaluation of Methyl Arsenic

Studies at other sites (e.g., Sanders et al. 1994) have revealed that arsenic in sediments may become methylated by microbial action at times when the oxygen tension in the sediments is low. Because methylated forms of arsenic might have different bioavailability (and different toxicity) than the inorganic forms, aliquots of the dried fine-sieved samples were analyzed for organic methyl arsenic. Samples were sent to West Coast Analytical Services, where they were extracted with carbonate buffer and analyzed for As+3, As+5, MMA, and DMA by ion chromatography-ICPMS. The results are summarized below:

Sample	Total Arsenic (ppm)		Extracted Arsenic (WCAS) (ppm)			
	WCAS	Drexler	As+3	DMA	MMA	As+5
1	630	459	ND	ND	ND	20
2	600	527	ND	ND	ND	ND
3	168	144	ND	ND	ND	ND
4	169	145	ND	ND	ND	ND
5	670	775	ND	ND	ND	ND
6	167	176	ND	ND	ND	ND
7	292	301	ND	ND	ND	ND
8	520	832	ND	ND	ND	ND
9	296	407	ND	ND	ND	ND
10	51	43.4	ND	ND	ND	ND
11	87	64	ND	ND	ND	10
12	83	67.1	ND	ND	ND	11
Detection Limit (ppm)	1		5	5	5	5

WCAS = West Coast Analytical Services

As seen, very low levels were observed for each analyte. Recovery of matrix spikes for As+3 and As+5 was poor, suggesting that recoveries of these species may be low. However, recovery of matrix spikes of MMA and DMA were high (89%). These results indicate that if MMA or DMA are present in the samples, they constitute only a very small fraction of the total arsenic.

Mineral Phase Speciation

Each of the 12 dried fine-sieved samples was characterized by electron microprobe analysis (EMPA) in order to provide preliminary data on the identity and relative abundance of the different mineral forms of arsenic present in the samples. The results are summarized in Table 2-2. As seen, these data suggest that arsenic exists mainly in association with particles of iron oxide, iron sulfate, and zinc-iron sulfate. The preliminary data are too limited to draw firm conclusions, but suggest that the presence of iron oxide is associated with higher arsenic concentrations and lower *in vitro* bioaccessibility, and that the presence of the iron-zinc sulfate complexes is associated with lower arsenic concentrations and higher *in vitro* bioaccessibility.

2.1.2 Test Material Selection and Preparation

Test materials for use in the *in vivo* study were selected by considering the results of the preliminary characterization of 12 site sediment samples (Section 2.1.1, above). Specifically, factors that were considered included the concentration level of arsenic in a sample and the degree to which different samples appear to be similar or dissimilar based on speciation and *in vitro* bioaccessibility testing. Based on the conclusion that the only clear pattern of difference among samples is the *in vitro* bioaccessibility (inversely related to concentration), three test materials were prepared by compositing samples with similar arsenic concentrations, as described below.

Test Material 1

Test Material 1 was prepared by compositing equal masses of dried fine-sieved material from samples 2, 5, and 8. These three samples were selected because they have the highest measured arsenic concentration values (all >500 ppm) and they tend to have low bioaccessibility (average = 19%). In addition, the three samples represent each of the three reaches of river (excluding the Davidson Park area), providing good spatial representativeness. These samples tend to be relatively enriched in the iron oxide form of arsenic.

Test Material 2

Test Material 2 was prepared by compositing equal masses of dried fine-sieved material from samples 1, 6, and 7. These three samples were selected because they have intermediate arsenic concentration values (180-460 ppm), intermediate bioaccessibility values (average = 44%), and represent each of the three upstream reaches of the river. These samples tend to be relatively enriched in the zinc-iron sulfate form of arsenic.

Test Material 3

Test Material 3 was prepared by compositing equal masses of all samples with an arsenic concentration less than 150 ppm (samples 3, 4, 10, 11, and 12). These are the samples with the highest apparent bioaccessibility (average = 51%), but the arsenic levels are too low (average = 93 ppm) to permit effective testing in animals. Although Test Material 3 was not used in the *in vivo* portion of the study, it underwent all of the same detailed characterization efforts as Test Materials 1 and 2.

Test Material Preparation

Each test material was prepared by combining equal masses of the appropriate sediment samples, as indicated above. The samples for a given test material were composited using a stainless steel bowl and mixing spoon, and characterized as detailed below.

2.1.3 Detailed Characterization of Test Materials

Arsenic Concentration

After compositing, the concentration of arsenic in each test material was measured by ICP/AES and by ICP/MS. The results are shown below:

Analytical Method	Arsenic Concentration (mg/kg)		
	TM1	TM2	TM3
ICP/MS	590	290	80
ICP/MS	652	318	93.6
ICP/AES	733	319	--
ICP/AES	730	324	--
Average	676.3	312.8	86.8
Standard Deviation	68.6	15.4	9.6

-- = Not measured

Concentration of Other Inorganics, Organic Carbon, and Sulfide

Each sample was analyzed for EPA's Target Analyte List (TAL) of inorganic chemicals, as well as for total organic content (TOC) and total sulfide content. Results are shown in Table 2-3.

Particle Speciation, Size, and Matrix Association

Each test material was characterized by electron microprobe analysis (EMPA) in order to identify the different mineral forms of arsenic that were present in the sample and to estimate how much of the total arsenic was present in each form. In addition, the size distribution of the particles was characterized along with the matrix association of each particle. The detailed data are presented in Appendix A and the results are summarized below.

Arsenic Phases

Speciation of the three test materials indicated that the arsenic in these samples is associated with four different types of mineral phase: iron oxide, iron pyrite, iron sulfate, and zinc sulfate. Estimates of the relative arsenic mass (an approximation of the fraction of the total arsenic present in each phase) are presented below:

Arsenic Speciation Data

Test Material	Number of Particles Counted	Relative Arsenic Mass			
		Iron Oxide	Pyrite	Iron Sulfate	Zinc Sulfate
TM1	186	69%	0%	29%	2%
TM2	123	16%	2%	27%	55%
TM3	57	24%	1%	59%	16%

As seen, arsenic in primarily associated with iron oxide in TM1, with zinc sulfate in TM2, and iron sulfate in TM3. These differences in mineral phase may influence the RBA of the arsenic in the materials.

It is important to note that these quantitative estimates of relative arsenic mass are based on examination of a limited number of arsenic-bearing particles in each sample (N = 57 to 186). Consequently, the quantitative values reported should not be considered to be highly precise, and apparent differences between samples may be partly due to random variation in the analysis rather than authentic differences in composition.

Particle Size Distribution

Particle size is a potentially important contributor to RBA because the fraction of a particle that undergoes dissolution in gastrointestinal fluids is likely related to the surface area to volume ratio (this ratio is larger for small particles than large particles). The distribution of particle sizes for arsenic-bearing grains in these test materials is summarized below:

Particle Size Distribution

Test Material	Percent of Particles by Size Class		
	≤25 um	26-100 um	>100 um
TM1	79%	15%	6%
TM2	85%	14%	2%
TM3	72%	26%	2%

As seen above, in these test materials, a large majority of all arsenic-containing particles are small: an average of 79% of all particles are 25 um or less in size. This predominance of small particles may tend to increase the RBA compared to what would be expected for larger particles of similar composition.

Matrix Association

Arsenic-containing particles may be characterized according to their association with other particles into four types, as follows:

Matrix Association	Description
Liberated	A grain of arsenic-containing material that is not attached to or contained within any other particle
Rimming	Arsenic is present on the outer surface of a particle, usually as a consequence of adsorption or precipitation
Cemented	The arsenic-containing particle is loosely bound to or associated with other particles or phases that do not contain arsenic
Included	The arsenic-containing particle is entirely contained within another particle

In the first three types of matrix association, the arsenic is exposed at the surface of some or all of the particle, and hence the arsenic is available to be dissolved by gastrointestinal fluids. Particles that are fully included in other particles are not exposed to external fluids and are not likely to have high bioavailability. The distribution of matrix associations for arsenic-bearing particles in the test materials from this site is summarized below:

Particle Matrix Associations

Test Material	Percent of Particles by Matrix Class			
	Liberated	Rimming	Cemented	Included
TM1	27%	2%	67%	4%
TM2	22%	0%	78%	0%
TM3	37%	11%	53%	0%

As seen, relative few particles are fully included, and 96-100% of the particles are entirely or partially exposed to external fluids. This suggests that the RBA of the arsenic is likely to be determined primarily by mineral phase and/or particle size rather than by matrix association.

In Vitro Bioaccessibility

The details of the method used to measure the *in vitro* bioaccessibility of arsenic are described in USEPA (1999). In brief, 1.00 g of test substrate is placed into a 125-mL wide-mouth HDPE bottle. To this is added 100 mL of the extraction fluid (0.4 M glycine, pH 1.5). Each bottle is placed into a heated water bath (water temperature = 37°C) and rotated end-over-end. After a specified period of time (1, 2 or 4 hours), the bottles are removed, dried, and placed upright on the bench top to allow the soil to settle to the bottom. A 15-mL sample of supernatant fluid is removed directly from the extraction bottle into a disposable 20-cc syringe. After withdrawal of the sample into the syringe, a Luer-Lok attachment fitted with a 0.45-µm cellulose acetate disk filter (25 mm diameter) is attached, and the 15 mL aliquot of fluid is filtered through the attachment to remove any particulate matter. This filtered sample of extraction fluid is then analyzed for arsenic. The fraction of arsenic originally present in the sample that occurs in the dissolved phase at the end of the extraction procedure is the *in vitro* bioaccessibility (IVBA). IVBA results for the three test materials in this study are summarized below:

Test Material	Concentration (ppm)	IVBA		
		1 hr.	2 hr.	4 hr.
TM1	676	14%	16%	19%
TM2	313	35%	47%	51%
TM3	86.8	49%	57%	66%

As seen, IVBA values tend to increase slowly as a function of extraction time. In all cases, an inverse relationship is observed between IVBA and arsenic concentration in the sediment

sample, similar to the pattern that was observed previously during the preliminary characterization of the 12 site sediments samples (see Section 2.1, above).

2.2 Experimental Animals

Young swine were selected for use in these studies because they are considered to be a good physiological model for gastrointestinal absorption in children (Weis and LaVelle 1991). The animals were intact males of the Pig Improvement Corporation (PIC) genetically defined Line 26, and were purchased from Chinn Farms, Clarence, MO.

The animals were housed in individual stainless steel cages. All animals were held for several days prior to beginning exposure to test materials in order to allow them to adapt to their new environment and to ensure that all of the animals were healthy. Animals were assigned to dose groups at random. When exposure began (day zero), the animals were about 6 weeks old and weighed an average of about 12.1 kg. Animals were weighed every three days during the course of the study. On average, animals gained about 0.4 kg/day and the rate of weight gain was comparable in all groups, ranging from 0.38 to 0.46 kg/day. These body weight data are summarized in Figure 2-5.

2.3 Diet

Animals provided by the supplier were weaned onto standard pig chow purchased from MFA Inc., Columbia, MO. 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, PA) over the time interval from day -7 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 2-4. Each day every animal was given an amount of feed equal to 5% of the mean body weight of all animals on study. Feed was administered in two equal portions of 2.5% of the mean body weight at each feeding. Feed was provided at 11:00 AM and 5:00 PM daily. Previous analysis of feed samples indicated the arsenic level was generally below the detection limit (0.1 ppm), which corresponds to a dose contribution from food of less than 5 ug/kg-day (less than 50 ug/day).

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

2.4 Dosing

Animals were exposed to sodium arsenate (abbreviated in this report as "NaAs") or a test material (site sediment) for 12 days, with the dose for each day being administered in two equal portions given at 9:00 AM and 3:00 PM (two hours before feeding). 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.

The dose levels administered were based on the arsenic content of the test material, with target doses of 300, 600, and 900 ug/day for NaAs and each test material. The mass of each test material needed to provide these doses of arsenic were calculated based on a preliminary estimation of the arsenic concentration in the test materials. Actual administered arsenic doses were re-calculated after the study was completed using the mean of two ICP-AES measurements and two ICP-MS measurements. These actual administered doses are presented in Appendix B.

2.5 Collection and Preparation of Samples

Urine

Samples of urine were collected from each animal for three consecutive 48-hour periods, on days 6/7, 8/9 and 10/11 of the study. Collection began at 9AM and ended 48 hours later. The urine was collected in a stainless steel pan placed beneath each cage, which drained into a plastic storage bottle. Each collection pan was fitted with a nylon screen to minimize contamination with feces, spilled food, or other debris. Plastic diverters were used to minimize urine dilution with drinking water spilled by the animals from the watering nozzle into the collection pan, although this was not always effective in preventing dilution of the urine with water. Due to the length of the collection period, collection containers were emptied at least twice daily into a separate holding container. This ensured that there was no loss of sample due to overflow.

At the end of each collection period, the urine volume was measured and 60-mL portions were removed for analysis. A separate 250-mL aliquot was retained as an archive sample. Each sample was acidified by the addition of concentrated nitric acid. The samples were stored refrigerated until arsenic analysis.

2.6 Arsenic Analysis

Urine samples were assigned random sample numbers and submitted to the 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.

Laboratory Quality Assurance

A number of quality assurance steps were taken during this project to evaluate the accuracy of the analytical procedures. Steps performed by the analytical laboratory included:

Spike Recovery

Randomly selected samples were spiked with known amounts of arsenic (usually 40 ug, as sodium arsenate) and the recovery of the added arsenic was measured. Recovery for individual samples ranged from 95% to 110%, with an average across all analyses of 103 ± 4.5% (N = 7).

Duplicate Analysis

Random samples were selected for duplicate analysis by the laboratory analyst. Duplicate results had a relative percent difference (RPD) of 0-17%, with an average of 2.6 ± 5.0% (N = 13).

Laboratory Control Standards

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

LCS Type	Certified Value	Average Recovery	SEM	N
E.R.A. P081 - Metals WasteWatR	366 ng/mL	97%	1.7%	42
N.R.C.C. Dolt-2 Dogfish Liver	16.6 +/- 1.1 ug/g dry wt	84%	0.0%	2
N.R.C.C. Tort-2 Lobster	21.6 +/- 1.8 ug/g dry wt	99%	3.3%	3
N.I.S.T. Oyster 1566b	7.65 +/- 0.65 ug/g dry wt	97%	0.8%	3

As seen, recovery of arsenic from these standards was good in all cases, and no samples were outside the acceptance criteria specified by the suppliers.

Blanks

Blank samples run along with each batch of samples never yielded a measurable level of arsenic, with all values being reported as less than 0.03 ug of arsenic.

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 (urines of known arsenic concentration) and a number of blind duplicates.

The results for the PE samples are shown in Figure 2-6. As seen, the PE samples included several different concentrations each of four different types of arsenic (As+3, As+5, MMA, and DMA). In all cases, there was good recovery of the arsenic.

The results for blind duplicates are shown in Figure 2-7. As seen, there was good agreement between results for the duplicate pairs.

Based on the results of all of the quality assurance samples and steps described above, it is concluded that the analytical results for samples of urine 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., test material and reference material) can be calculated from the ratio of the urinary excretion fraction of the two materials. This calculation is independent of the extent of tissue binding and of biliary excretion:

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

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 (ug/48hr) = Concentration (ug/L) · Volume (L/48hr)

- For each test material, the amount of arsenic excreted by each animal was plotted as a function of the amount administered (ug/48 hours), and the best fit straight line (calculated by linear regression) through the data (ug excreted per ug administered) was used as the best estimate of the urinary excretion fraction (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 describing the confidence around each slope (UEF) term 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 each RBA calculation. The 5th and 95th percentile values from the simulated distribution of RBA values were then taken to be the 90% confidence interval 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.

4.2 Urinary Excretion Fractions

Detailed results from the study are presented in Appendix B. The results for urinary excretion of arsenic are summarized in Figures 4-1 to 4-3. Although there is variability in the data, most 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). The following table summarizes the best fit slopes (urinary excretion fractions) for sodium arsenate and each of the test materials.

Summary of UEF Values

Test Material	Slope (UEF) ± SEM
NaAs	0.892 ± 0.033
TM1	0.326 ± 0.021
TM2	0.456 ± 0.021

4.3 Calculation of Relative Bioavailability

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

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

The results are summarized below:

Test Material	Relative Bioavailability	
	Best Estimate	90% Confidence Interval
TM1	37%	32% - 41%
TM2	51%	46% - 56%

5.0 DISCUSSION AND RECOMMENDATIONS

The *in vivo* RBA results for two composite sediments collected from the Aberjona River study area range from 37% to 51%. These results clearly indicate that arsenic in Aberjona River site sediments 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 sediments. Because each sediment sample tested during this study is a composite of three sub-samples collected from differing locations along the Aberjona River, each test material represents a fairly large spatial area, and the results for these two samples may be assumed to be generally applicable to the entire site.

Although RBA values can be applied in the site risk assessment process without any understanding of what factors are responsible for the observed RBA values, it is a matter of some interest to investigate the degree to which the RBA value is correlated with other factors. The following table compares the measured values for RBA with the arsenic concentration in the sample, the IVBA, and the primary mineral phase present in each test material:

Test Material	Concentration (ppm)	RBA	IVBA		Primary Form
			1 hr	4 hrs	
TM1	676	37%	14%	19%	Iron oxide
TM2	313	51%	35%	51%	Zinc sulfate
TM3	86.8	--	49%	66%	Iron sulfate

As seen, both RBA and IVBA show an inverse correlation with concentration in the sediment. This is plotted graphically in Figure 5-1. The basis of this apparent relationship is not known. Absolute values of IVBA at one hour tend to be lower than the measured RBA values, but the difference between RBA and IVBA tends to decrease after longer extraction times. Although the values for TM2 at 4 hours happen to be equal, the values for TM1 are not equivalent. These data suggest that IVBA is a good screen to evaluate the relative *in vivo* bioavailability of arsenic at different locations, but that it should not be used as a quantitative surrogate for *in vivo* RBA at this site. The data are not sufficient to establish an empiric relationship between mineral form and RBA, but the results suggest that arsenic in association with iron oxide is likely to be less bioavailable that other forms.

6.0 REFERENCES

Sanders J.G., Riedel G.F., and Osmann R.W. 1994. Arsenic Cycling and its Impact in Estuarine and Coastal Marine Ecosystems. In: Nriagu JO, ed. Arsenic in the environment, Part I: Cycling and Characterization. New York, NY: John Wiley & Sons, Inc., 289-308.

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.

Weis, C.P., and LaVelle, J.M. 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 STUDY DESIGN

Group	Number of Animals	Material Administered	Target Dose (ug As/day)
1	3	Control	0
2	4	Sodium Arsenate	300
3	4	Sodium Arsenate	600
4	4	Sodium Arsenate	900
5	4	Test Material 1	300
6	4	Test Material 1	600
7	4	Test Material 1	900
8	4	Test Material 2	300
9	4	Test Material 2	600
10	4	Test Material 2	900

TABLE 2-2 PRELIMINARY (SEMI-QUANTITATIVE) SPECIATION RESULTS

Sample	Arsenic Concentration (ppm)	Bioaccesibility (%)	PARTICLE FREQUENCY* Phase						PARTICLE SIZE (um) Phase					
			Iron sulfide	Iron oxide	Iron sulfate	Zinc-Iron Sulfate	Tin oxide	Sodium sulfate	Iron sulfide	Iron oxide	Iron sulfate	Zinc-Iron Sulfate	Tin oxide	Sodium sulfate
1	459	40	3	2		2			2-8	20		5-80		
2	527	31	Tr	2	3	2		Tr		4-100	8-110	12-25		
3	144	70	3	1	2	Tr				1-8		8-30		
4	145	40	1	3	2	1			1-40	8-150	15-125	7-35		
5	775	12	Tr	3		Tr	Tr			8-250				
6	176	55	3		Tr	2			3-7			12-40		
7	301	37	3	1	2	2		2	2-10		3-22	4-80		8-35
8	832	13	Tr	3		2				35-220		15		
9	407	15		3		2				30-225		7-30		
10	43.4	39	2	1	Tr				3-7					
11	64.0	49	1	1	Tr				2-10	15-35				
12	67.1	59	1	1		Tr			1-15	14				

* Code: 3 = Most Common
2 = Common
1 = Relatively Infrequent
Tr = Trace
= Majority of arsenic in probably in this phase

TABLE 2-3 COMPOSITION OF TEST MATERIALS

Analyte	Concentration (mg/kg)[a]		
	TM1	TM2	TM3
Aluminum	15000	11000	11000
Antimony	4.3	3.7	<1
Arsenic	676.3	312.8	86.8
Barium	75	98	60
Beryllium	0.96	0.62	0.54
Cadmium	15	16	1.9
Calcium	9100	10000	4100
Chromium	680	620	140
Cobalt	32	46	14
Copper	840	540	150
Iron	73000	38000	22000
Lead	410	350	130
Magnesium	2000	2600	4300
Manganese	510	610	430
Mercury	2.9	1.1	0.61
Nickel	28	35	22
Potassium	690	770	1300
Selenium	5.8	3.8	1.6
Silver	0.88	1.1	<1
Sodium	ND	<500	ND
Sulfides, Total	5.9	63	7.2
Thallium	1.7	4.4	1.4
Total Organic Carbon	210 g/kg	220 g/kg	120 g/kg
Vanadium	49	43	35
Zinc	3300	4500	830

ND = Not detected

[a] All values are in units of mg/kg except where noted otherwise. All metals except mercury were measured by USEPA method 6010B. Mercury was measured by USEPA method 7471A, total sulfides were measured by USEPA method 9030B/9034, and total organic carbon was measured by USEPA method 9060. All data are based on single measurements except arsenic, which is based on the average of duplicate analysis by ICP-MS and duplicate analysis by ICP-AES.

Table 2-4 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 ppm
Met+Cys	0.5876%	Zinc	118.0608 ppm
Tryptophan	0.2770%	Iron	135.3710 ppm
Histidine	0.5580%	Copper	8.1062 ppm
Leucine	1.8160%	Cobalt	0.0110 ppm
Isoleucine	1.1310%	Iodine	0.2075 ppm
Phenylalanine	1.1050%	Selenium	0.3196 ppm
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 ppm
Unsaturated Fat	3.7410%	Thiamine	9.1681 ppm
Linoleic 18:2:6	1.9350%	Riboflavin	10.2290 ppm
Linoleic 18:3:3	0.0430%	Niacin	30.1147 ppm
Crude Fiber	3.8035%	Pantothenic Acid	19.1250 ppm
Ash	4.3347%	Choline	1019.8600 ppm
Calcium	0.8675%	Pyridoxine	8.2302 ppm
Phos Total	0.7736%	Folacin	2.0476 ppm
Available Phosphorous	0.7005%	Biotin	0.2038 ppm
Sodium	0.2448%	Vitamin B12	23.4416 ppm
Potassium	0.3733%		

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

FIGURE 2-1 SAMPLE CHARACTERIZATION AND PREPARATION FLOW CHART

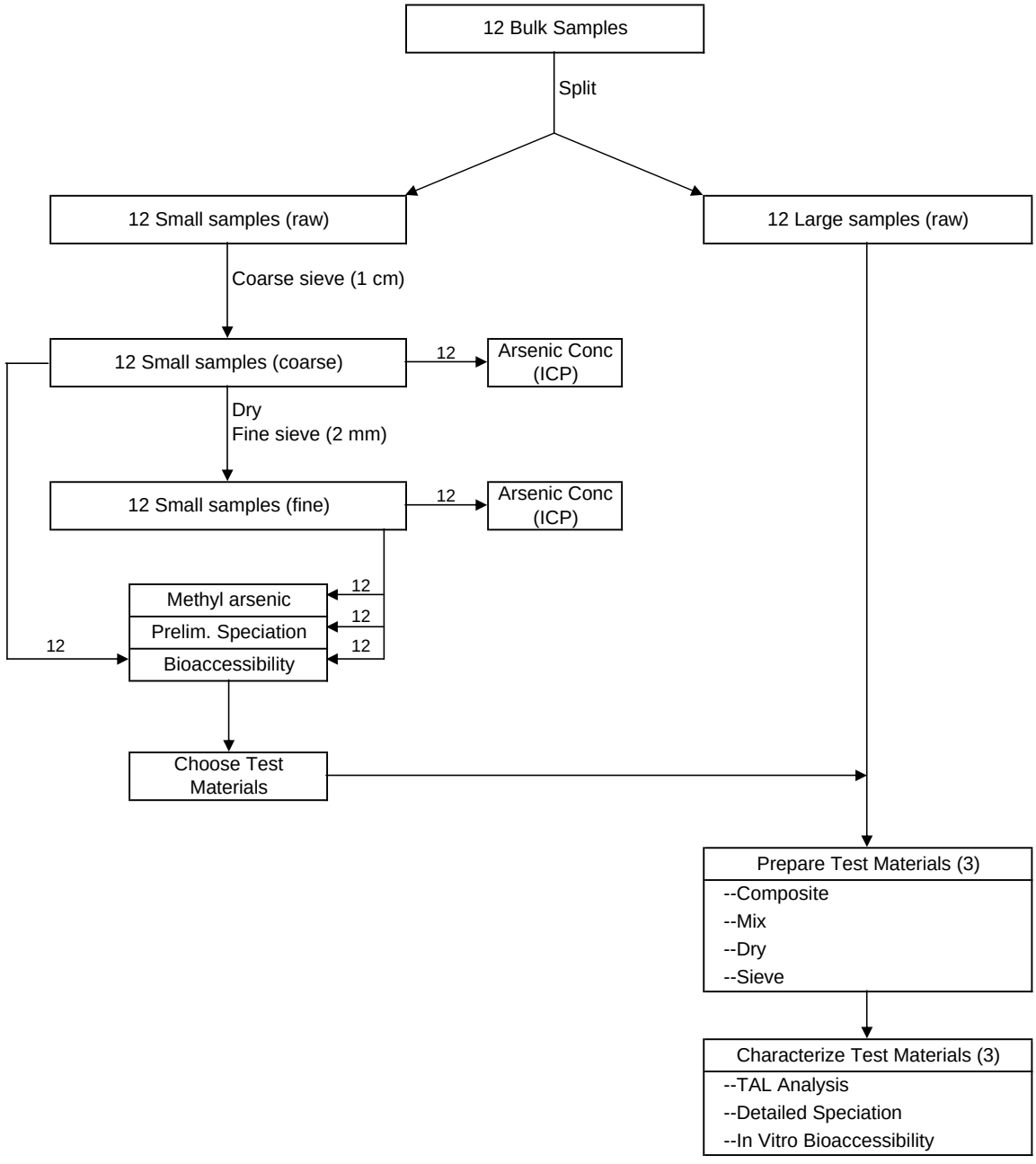


FIGURE 2-2 COMPARISON OF ARSENIC CONCENTRATIONS IN COARSE- AND FINE-SIEVED SAMPLES

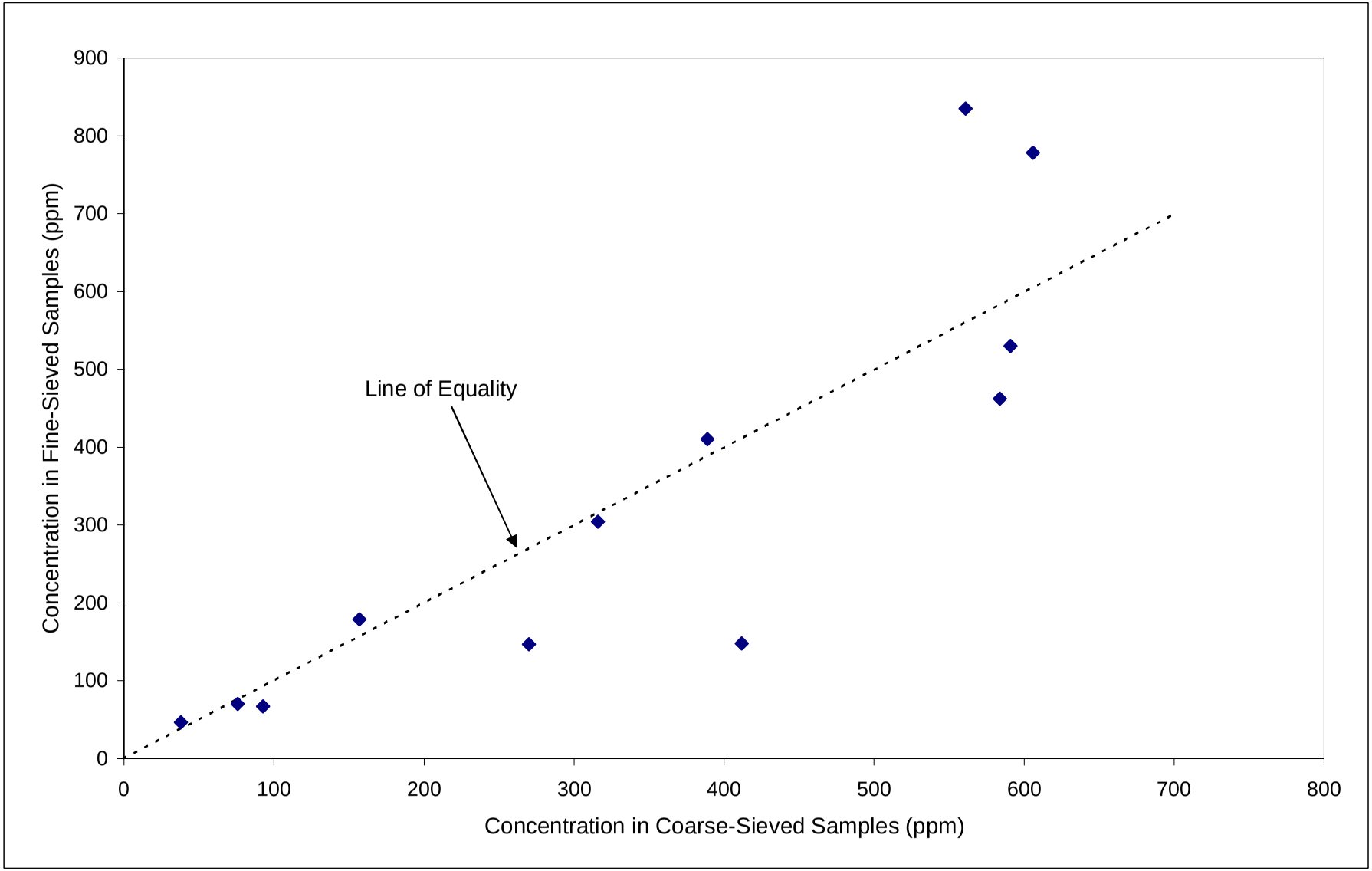
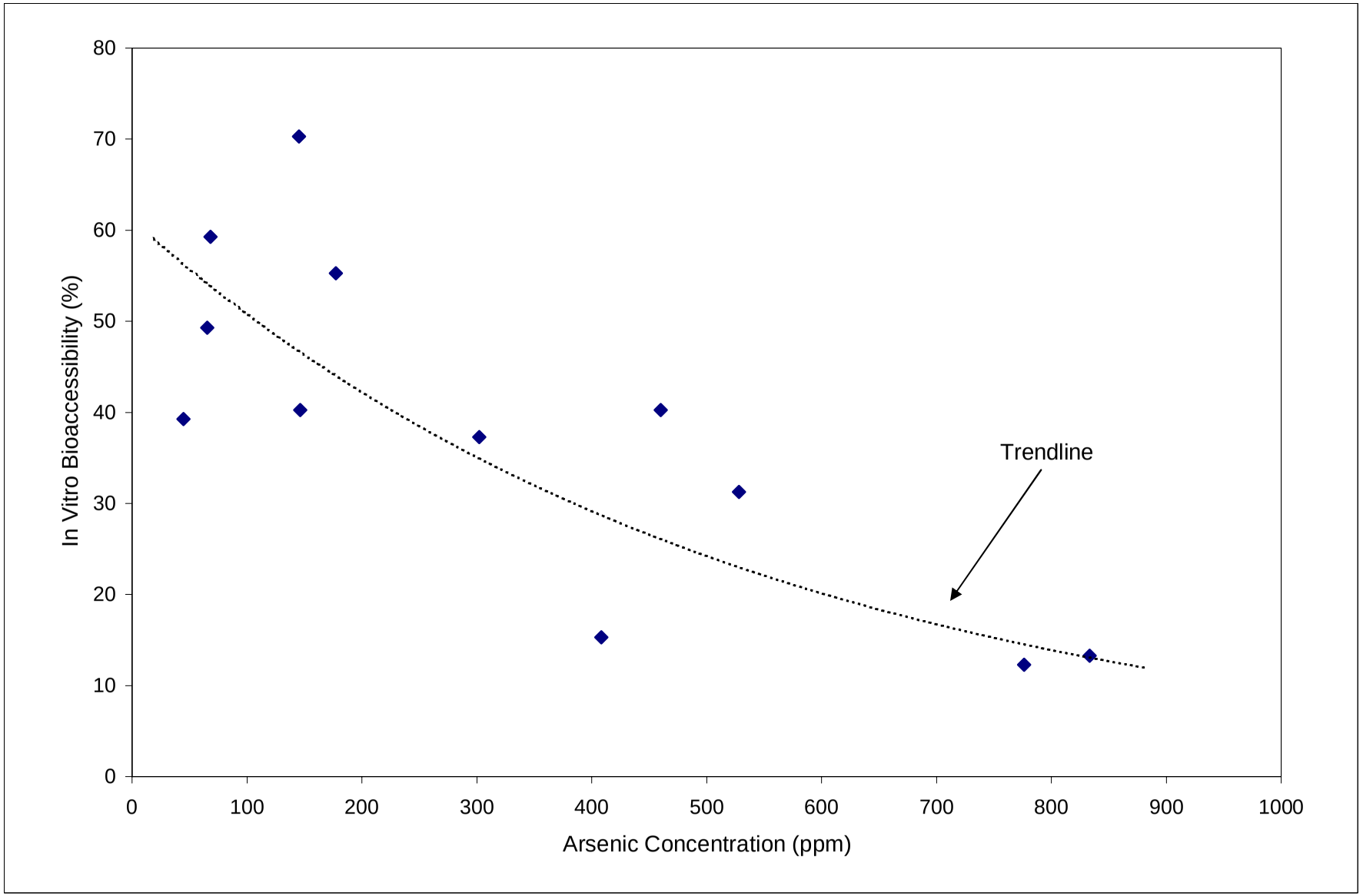


FIGURE 2-3 IN VITRO BIOACCESSIBILITY OF DRIED FINE-SIEVED SAMPLES



Prelim Char_tbls & figs.xls (Fig2-3_In Vitro)

FIGURE 2-4 COMPARISON OF IN VITRO BIOACCESSIBILITY OF DRIED AND UN-DRIED FINE-SIEVED SAMPLES

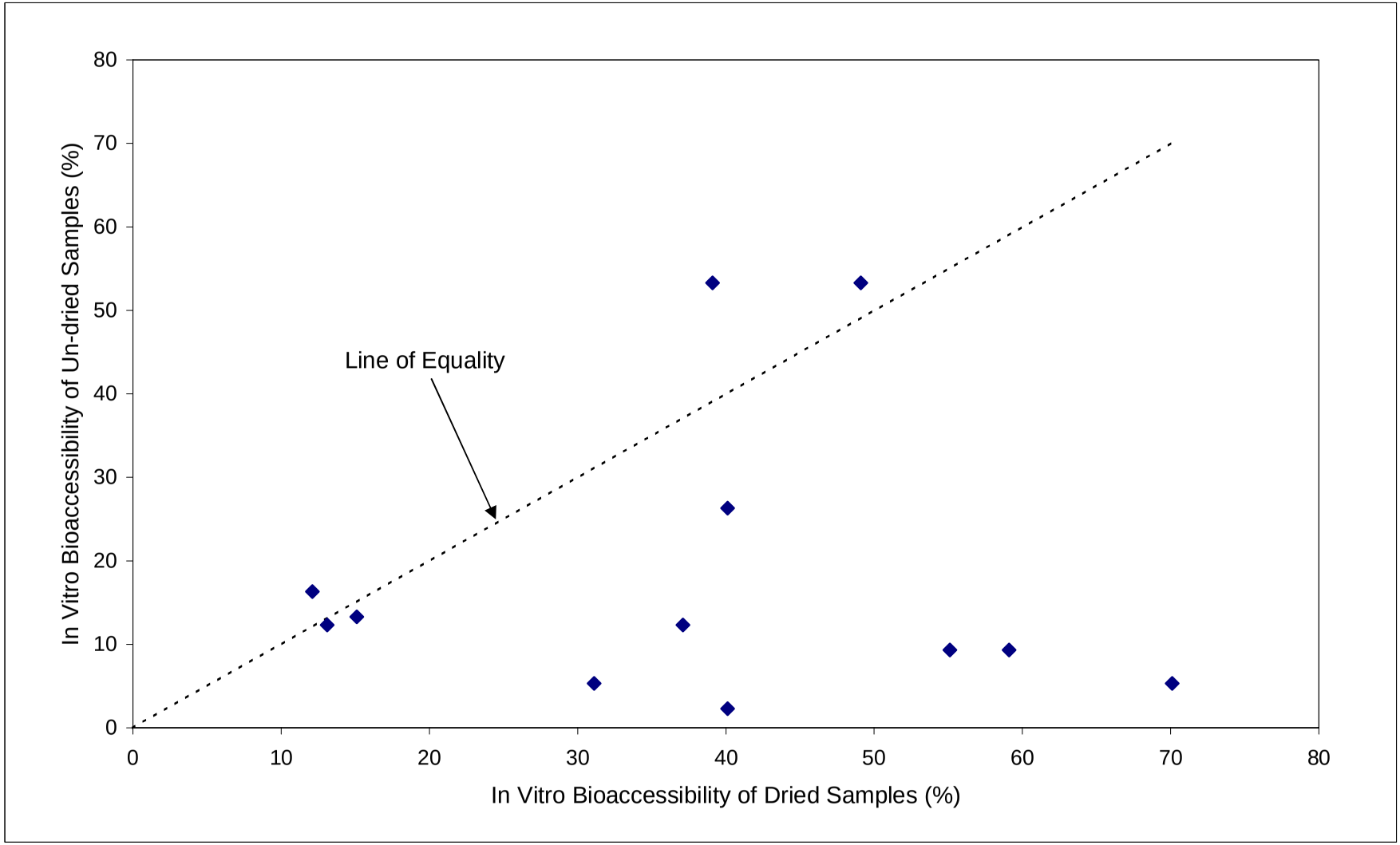
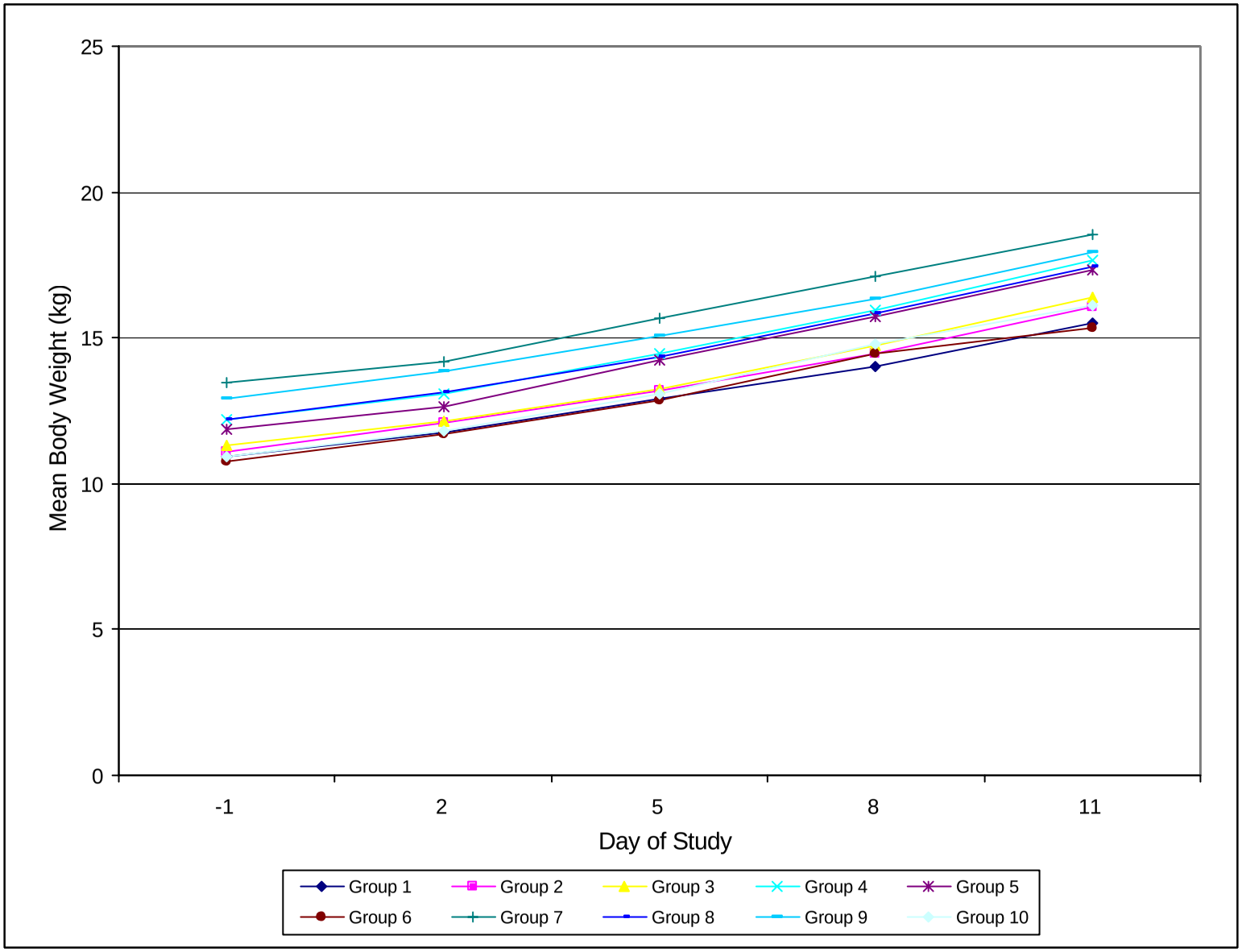
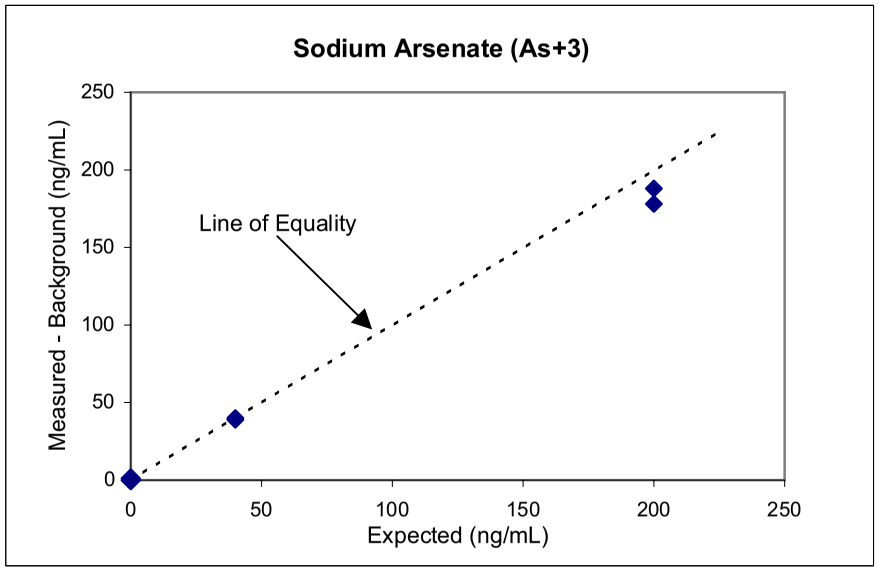


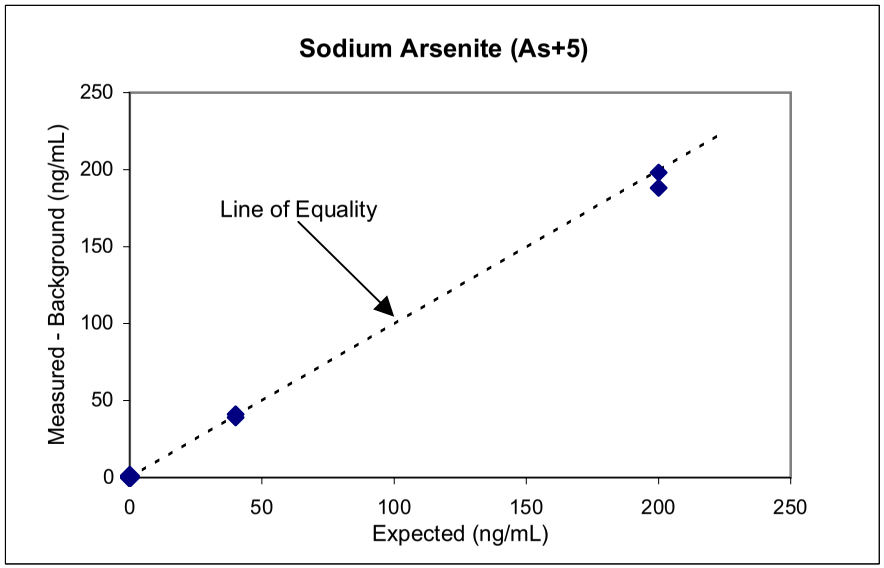
FIGURE 2-5 BODY WEIGHT GAIN

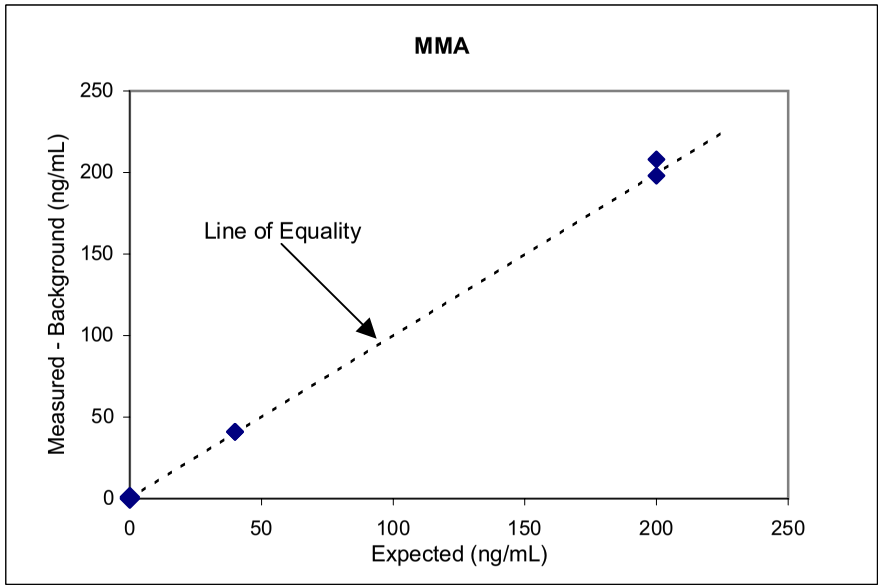


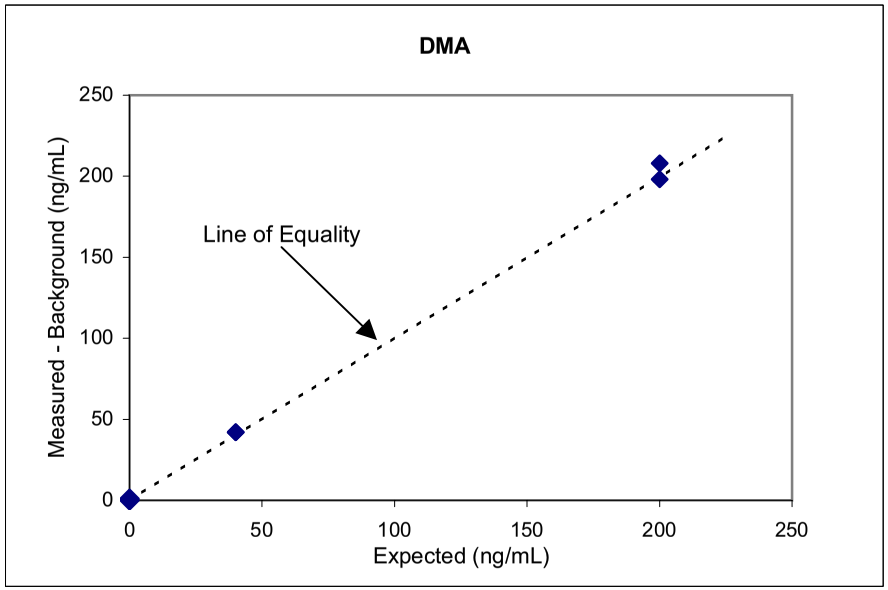
weights.xls (Fig2-5_group weights)

FIGURE 2-6 PERFORMANCE EVALUATION SAMPLES



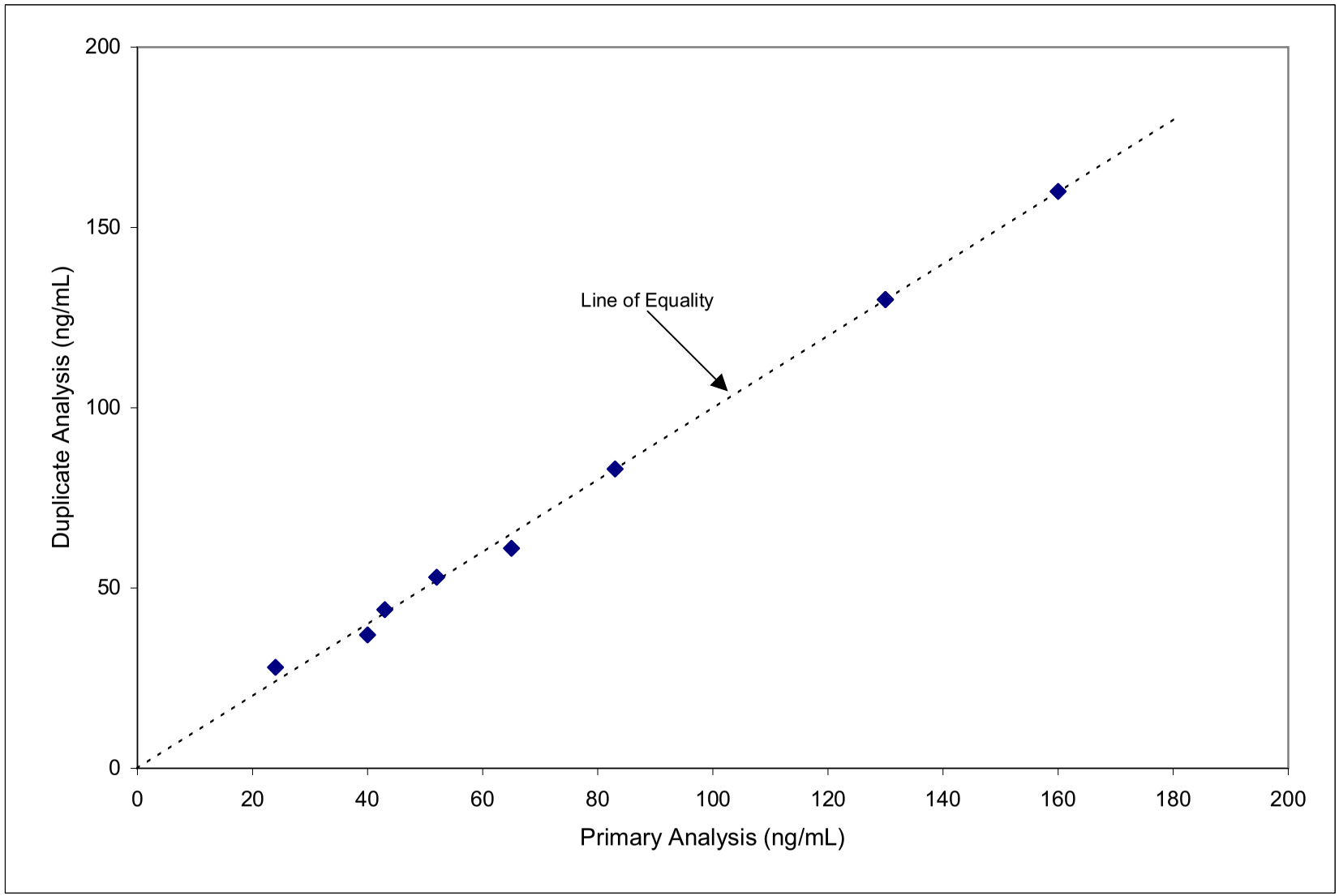






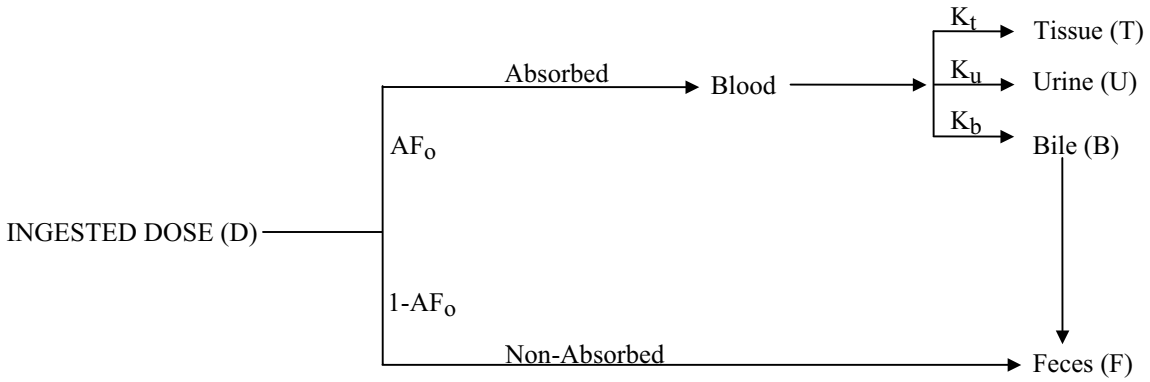
Blind QC analysis.xls (Fig2-6_PE)

FIGURE 2-7 BLIND DUPLICATE SAMPLES



Blind QC analysis.xls (Fig2-7_Dup)

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

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

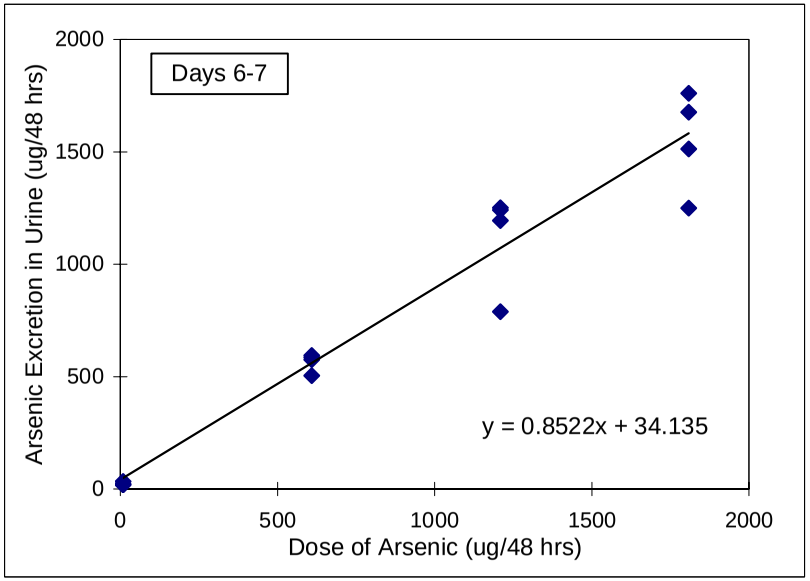
$= AF_o \cdot K_u$

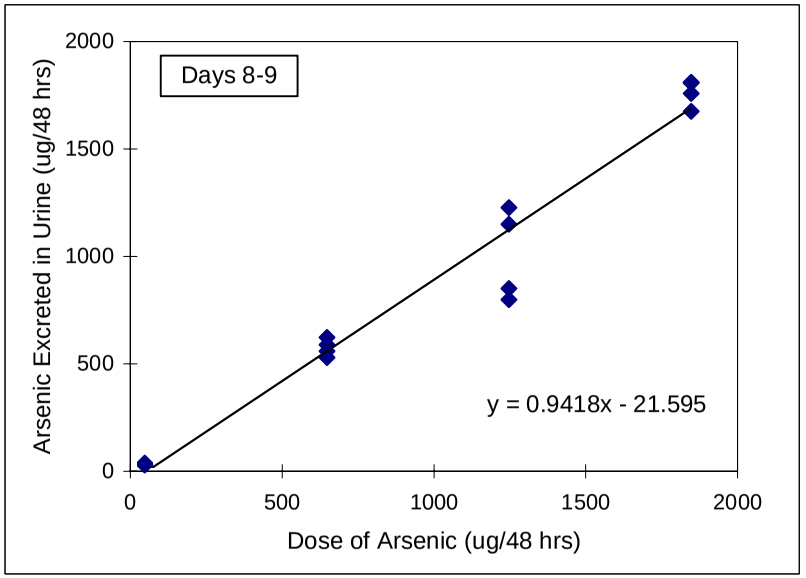
Relative Bioavailability (x vs. y) $= UEF(x) / UEF(y)$

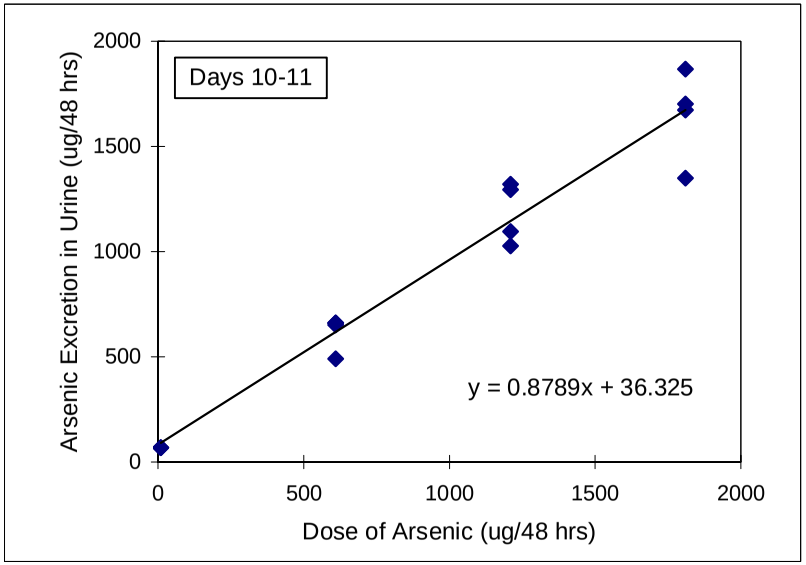
$= (AF_o(x) \cdot K_u) / (AF_o(y) \cdot K_u)$

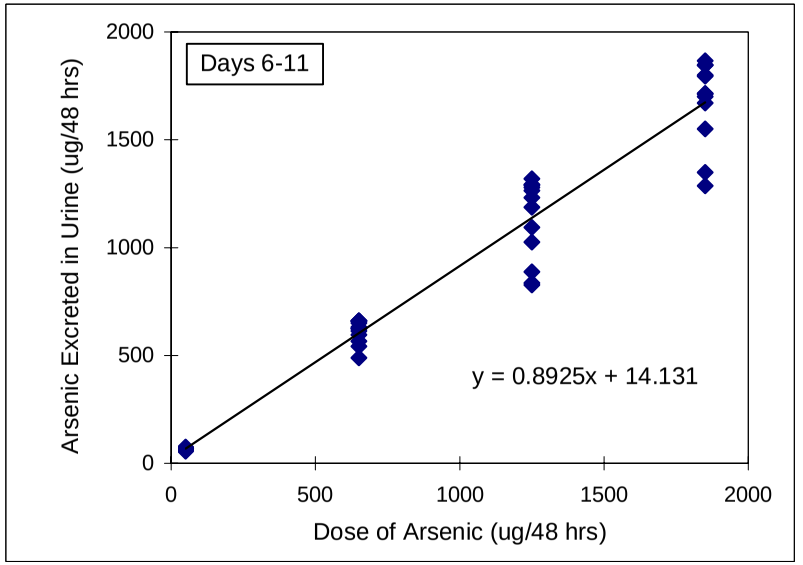
$= AF_o(x) / AF_o(y)$

FIGURE 4-1 URINARY EXCRETION OF ARSENIC FROM SODIUM ARSENATE



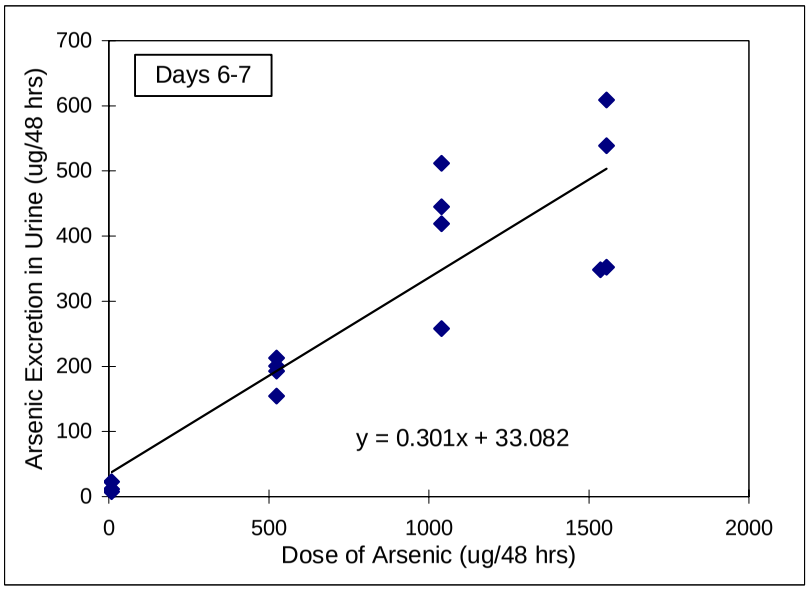


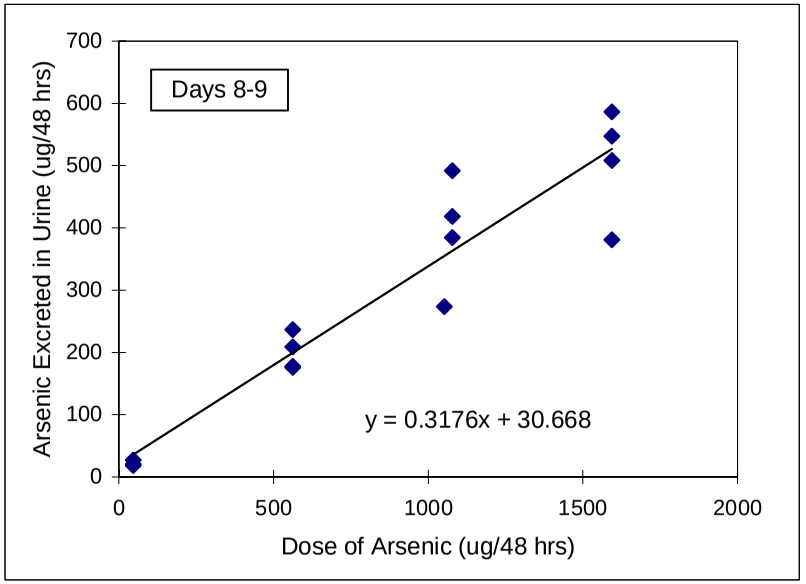


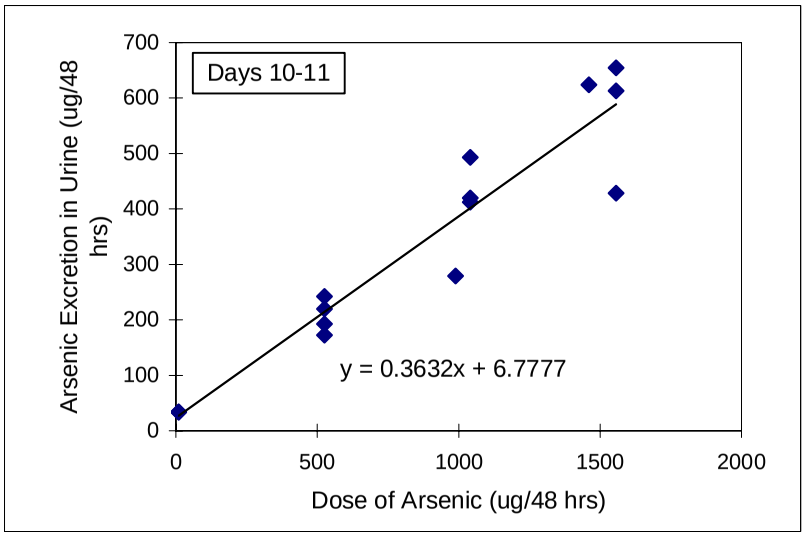


R1 UEF analysis.xls (NaAs)
12/16/2002

FIGURE 4-2 URINARY EXCRETION OF ARSENIC FROM TEST MATERIAL 1







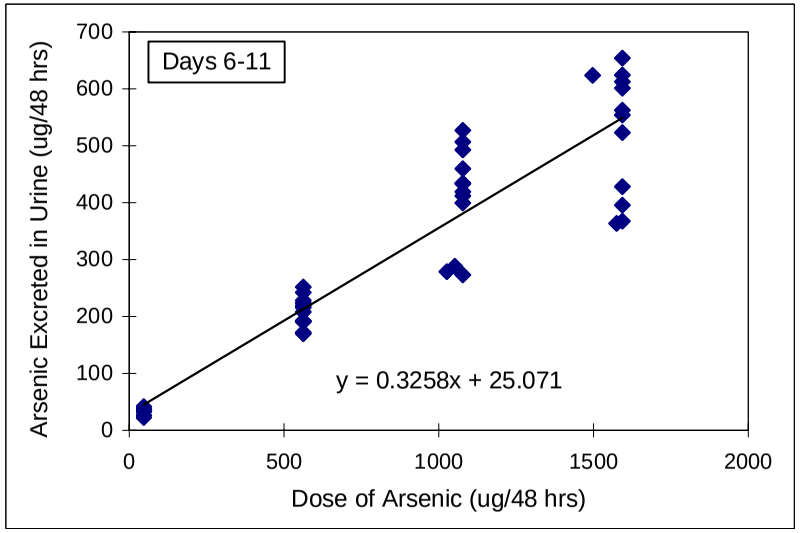
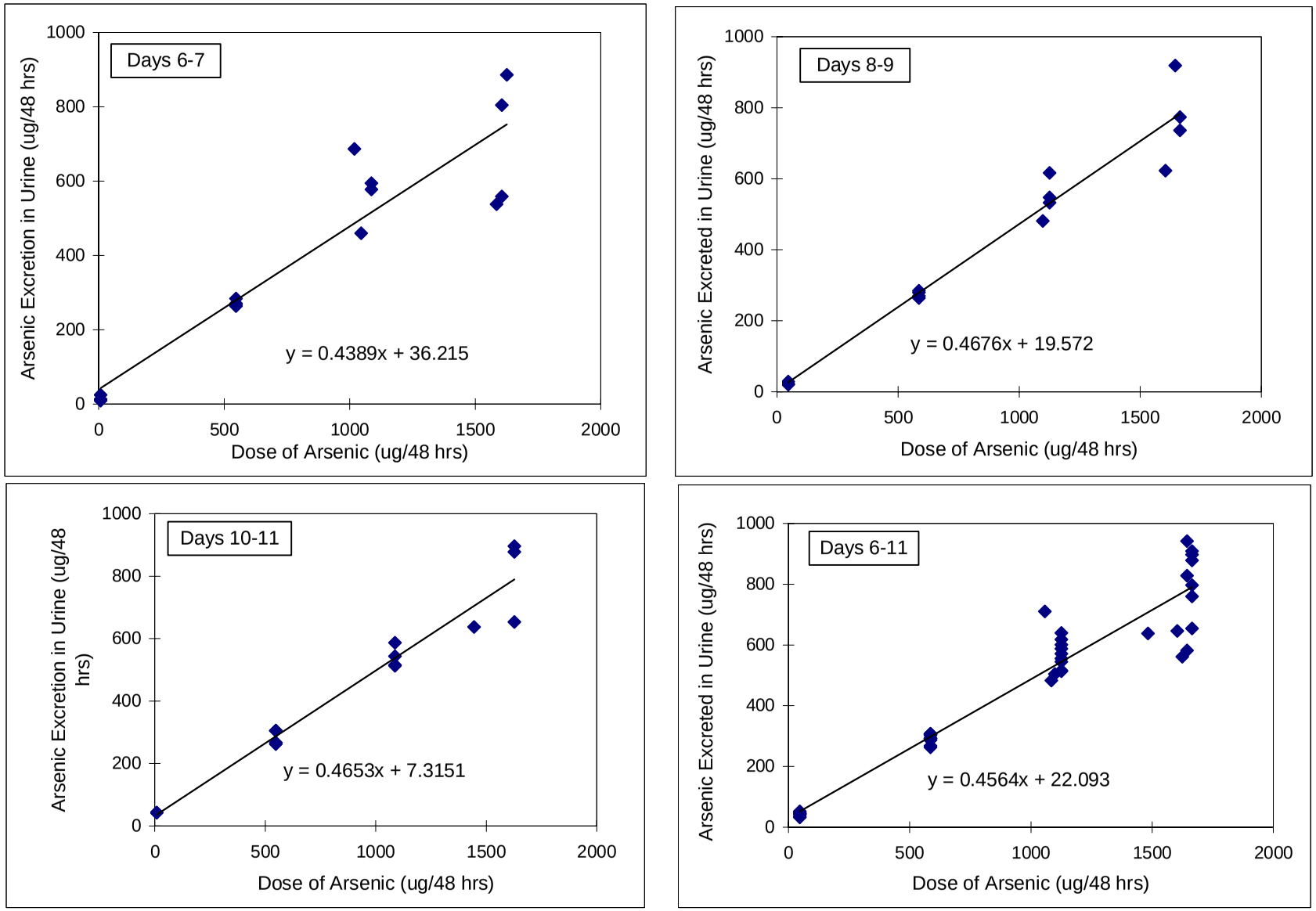
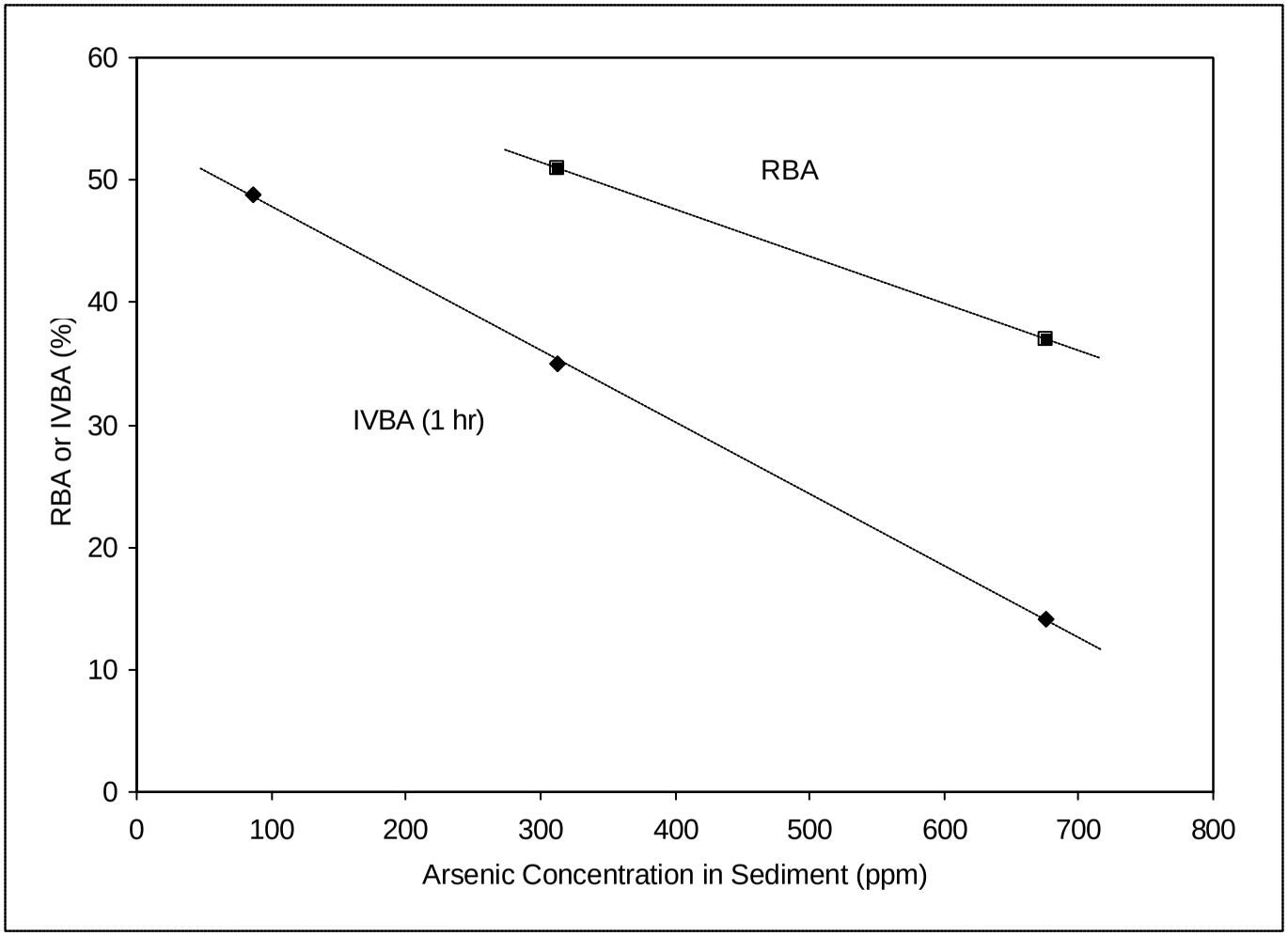


FIGURE 4-3 URINARY EXCRETION OF ARSENIC FROM TEST MATERIAL 2



R1 UEF analysis.xls (TM2)
12/16/2002

Figure 5-1. RBA and IVBA as a Function of Sediment Concentration

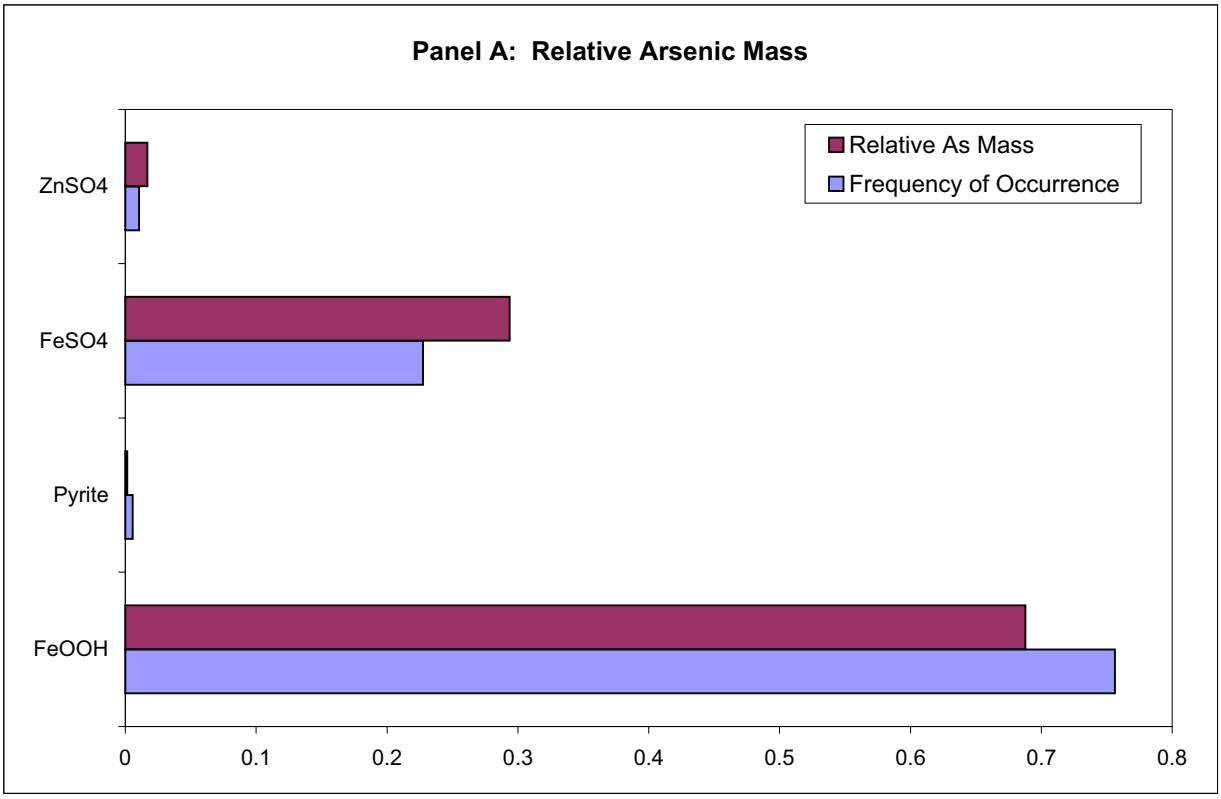


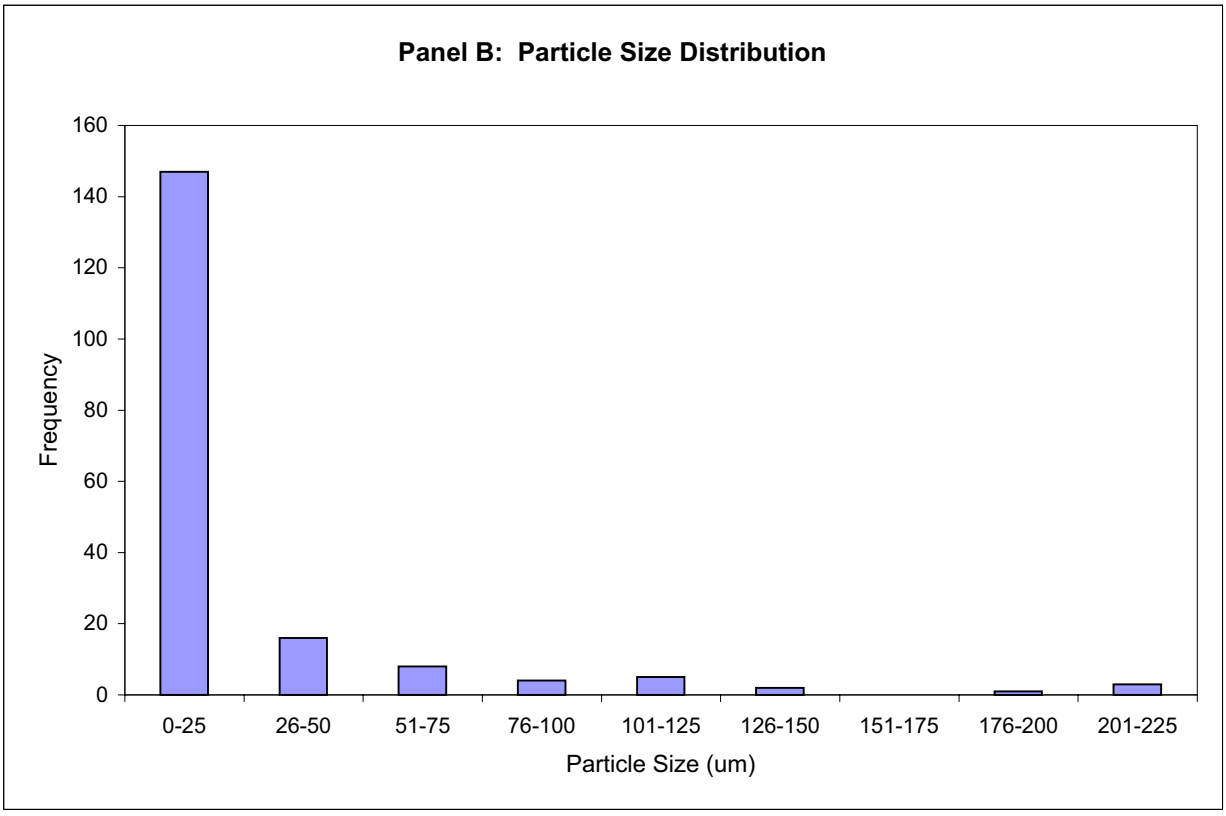
IVBA Results.xls (Summary and Graph)

APPENDIX A

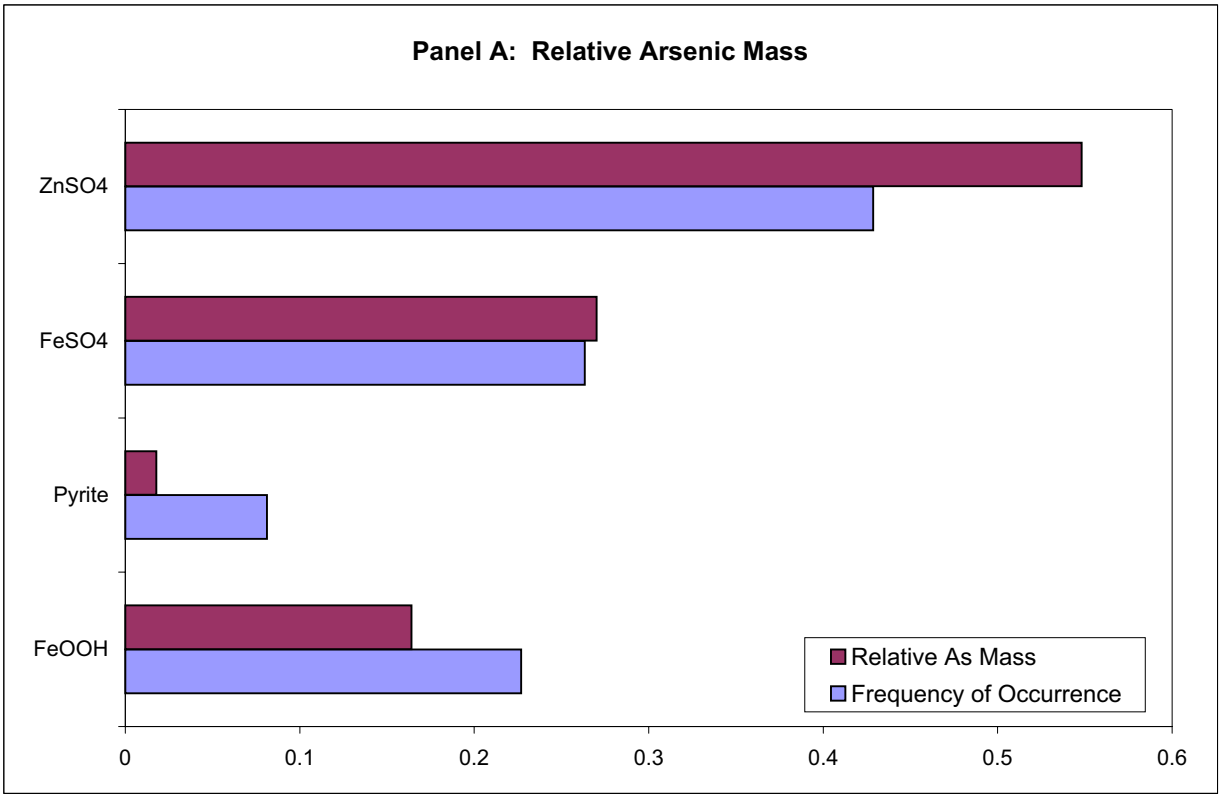
DETAILED ARSENIC SPECIATION RESULTS

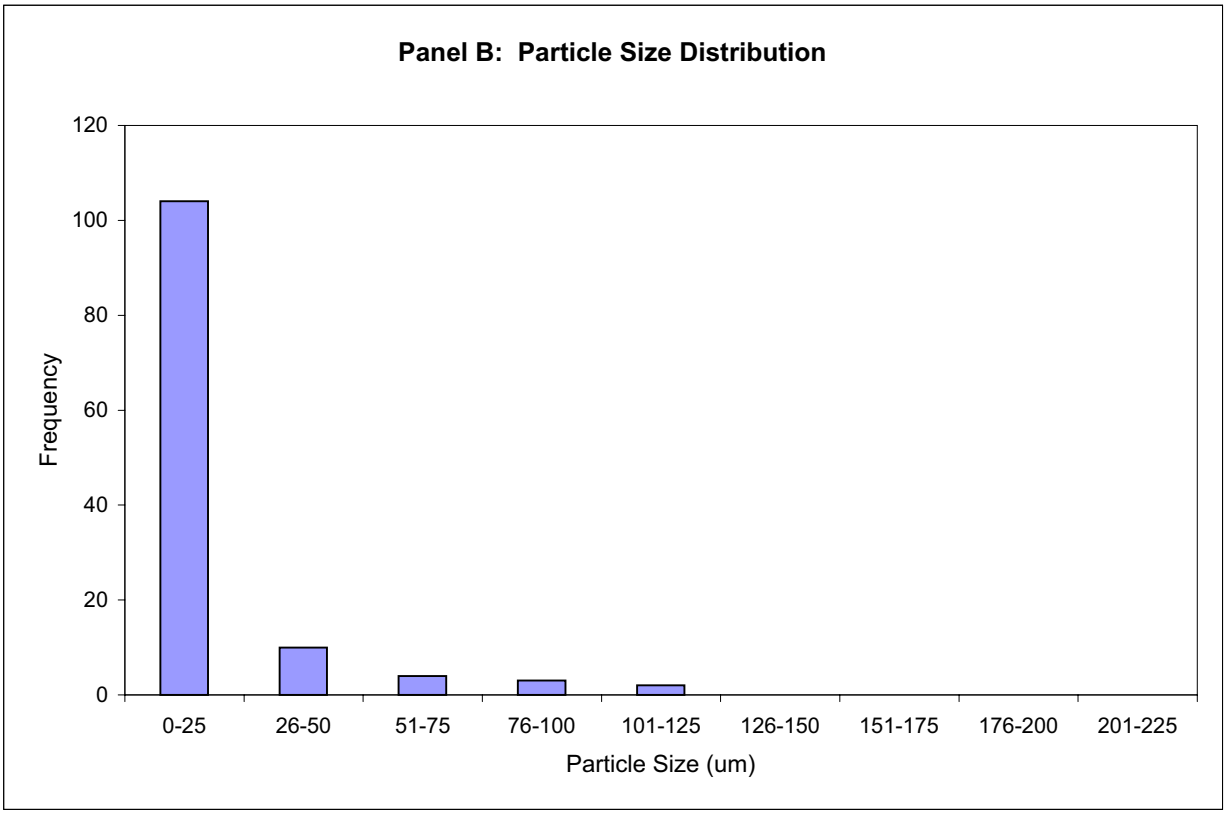
TEST MATERIAL 1 - SPECIATION AND PARTICLE SIZE DATA



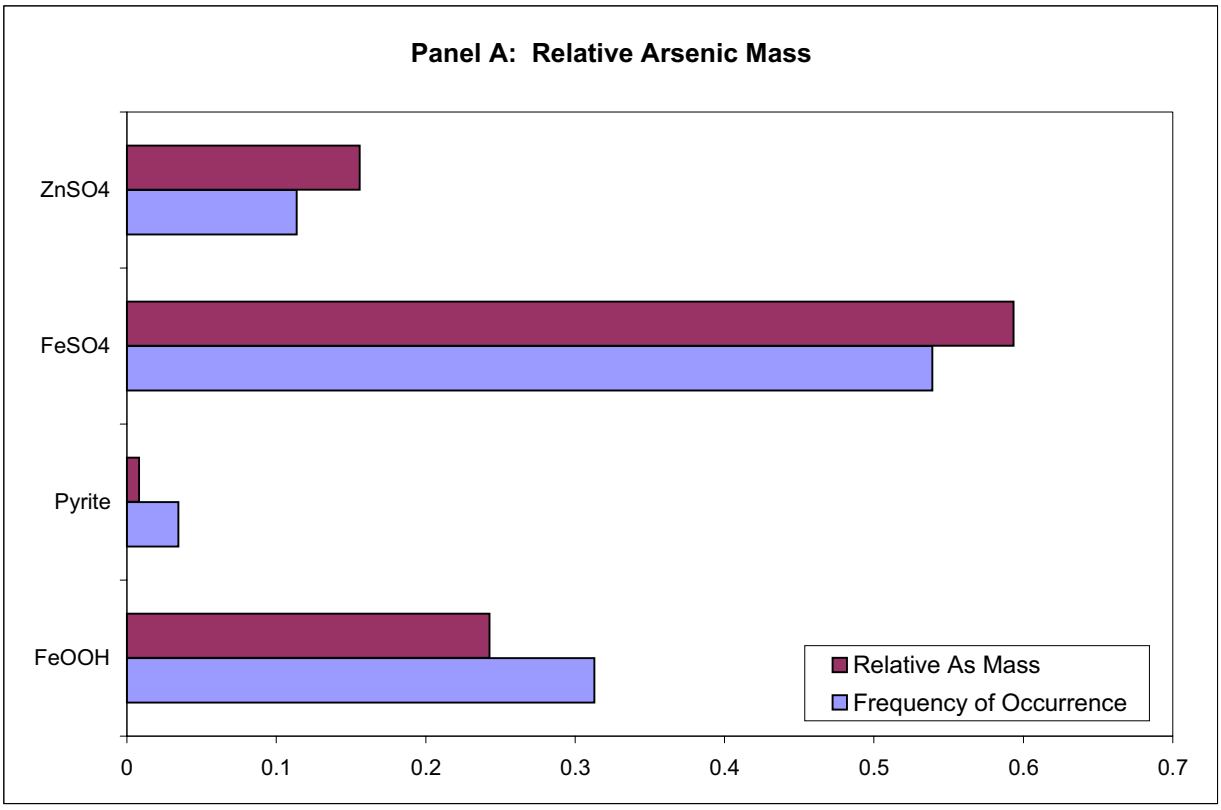


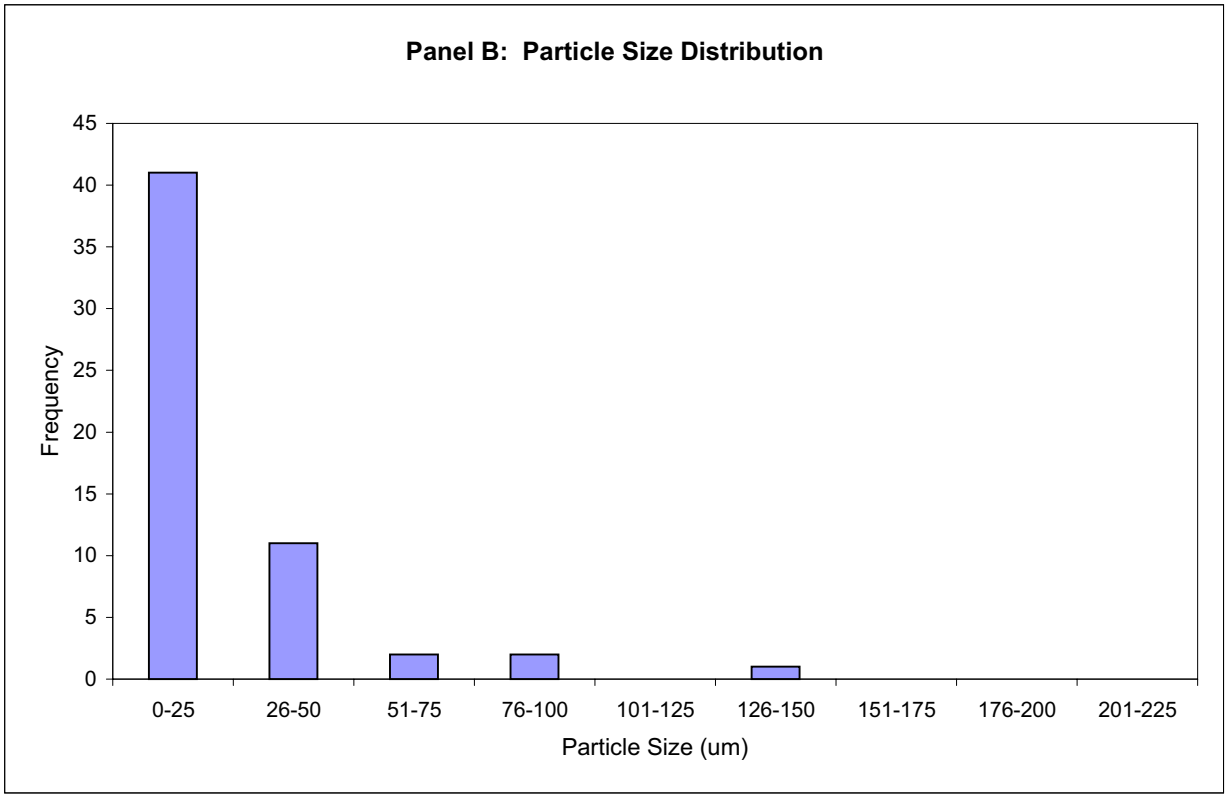
TEST MATERIAL 2 - SPECIATION AND PARTICLE SIZE DATA





TEST MATERIAL 3 - SPECIATION AND PARTICLE SIZE DATA





APPENDIX B

DETAILED RESULTS

TABLE B-1 SCHEDULE

Study Day	Day	Date	Dose Administration	Feed Special Diet	Weigh	Dose Prep	Cull Pigs/ Assign Dose Group	48 hr Urine Collection	Sacrifice
-8	Tuesday	8/27/02							
-7	Wednesday	8/28/02			X		X		
-6	Thursday	8/29/02							
-5	Friday	8/30/02							
-4	Saturday	8/31/02			X				
-3	Sunday	9/1/02							
-2	Monday	9/2/02		X					
-1	Tuesday	9/3/02	X	X	X	X			
0	Wednesday	9/4/02	X	X					
1	Thursday	9/5/02	X	X					
2	Friday	9/6/02	X	X	X	X			
3	Saturday	9/7/02	X	X					
4	Sunday	9/8/02	X	X					
5	Monday	9/9/02	X	X	X	X			
6	Tuesday	9/10/02	X	X				↕	
7	Wednesday	9/11/02	X	X				↕	
8	Thursday	9/12/02	X	X	X	X		↕	
9	Friday	9/13/02	X	X				↕	
10	Saturday	9/14/02	X	X				↕	
11	Sunday	9/15/02	X	X	X			↕	
12	Monday	9/16/02							X

TABLE B-2 GROUP ASSIGNMENTS

Pig Number	Dose Group	Material Administered	Target Dose of Arsenic (ug/day)
324 338 349	1	Control	0
326 330 339 350	2	NaAs	300
310 316 322 340	3	NaAs	600
303 315 329 341	4	NaAs	900
301 318 344 347	5	TM1	300
309 327 343 346	6	TM1	600
306 308 317 331	7	TM1	900
304 311 314 321	8	TM2	300
307 313 325 332	9	TM2	600
328 337 342 348	10	TM2	900

TABLE B-3 BODY WEIGHTS AND ADMINISTERED DOSES, BY DAY

Body weights were measured on days -7, -4, -1, 2, 5, 8, and 11. Weights for other days are estimated, based on linear interpolation between measured values.

Group	Pig #	Day -7		Day -4		Day -1		Day 0		Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7		Day 8		Day 9		Day 10		Day 11	
		BW kg	ugAs per day	BW kg	ugAs per day	BW kg	ugAs per day	BW kg	ugAs per day	BW kg	ugAs per day	BW kg	ugAs per day	BW kg	ugAs per day	BW kg	ugAs per day	BW kg	ugAs per day	BW kg	ugAs per day	BW kg	ugAs per day	BW kg	ugAs per day	BW kg	ugAs per day	BW kg	ugAs per day	BW kg	ugAs per day
1	324	10.15	0	10.25	0	11.15	0	11.48	0	11.82	0	12.15	0	12.57	0	12.98	0	13.40	0	13.75	0	14.10	0	14.45	0	14.87	0	15.28	0	15.70	0
1	338	8.9	0	9.45	0	10.9	0	11.0	0	11.2	0	11.3	0	11.7	0	12.1	0	12.45	0	12.8	0	13.1	0	13.45	0	14.1	0	14.8	0	15.4	0
1	349	10	0	9.45	0	10.75	0	11.1	0	11.4	0	11.75	0	12.1	0	12.5	0	12.85	0	13.3	0	13.8	0	14.2	0	14.6	0	15.0	0	15.4	0
2	326	11.05	300	11.2	300	11.9	300	12.3	300	12.6	300	13	300	13.3	300	13.7	300	14	300	14.4	300	14.8	300	15.25	300	15.8	300	16.4	300	16.9	300
2	330	9.65	300	10.3	300	11.35	300	11.5	300	11.7	300	11.85	300	12.3	300	12.7	300	13.15	300	13.5	300	13.9	300	14.25	300	14.8	300	15.4	300	16	300
2	339	8.2	300	9	300	9.85	300	10.3	300	10.8	300	11.2	300	11.5	300	11.8	300	12.15	300	12.6	300	13.0	300	13.45	300	14.0	300	14.5	300	15.05	300
2	350	10.55	300	10.45	300	11.25	300	11.6	300	12.0	300	12.3	300	12.7	300	13.1	300	13.45	300	13.9	300	14.4	300	14.9	300	15.4	300	15.9	300	16.35	300
3	310	11.55	600	11.65	600	12.65	600	12.8	600	13.0	600	13.2	600	13.5	600	13.9	600	14.2	600	14.8	600	15.5	600	16.1	600	16.5	600	16.9	600	17.3	600
3	316	9.65	600	10.2	600	11.7	600	12.0	600	12.3	600	12.55	600	12.9	600	13.3	600	13.65	600	14.0	600	14.4	600	14.75	600	15.4	600	16.1	600	16.7	600
3	322	10.45	600	10.95	600	11.8	600	12.2	600	12.5	600	12.9	600	13.3	600	13.8	600	14.2	600	14.9	600	15.6	600	16.25	600	16.9	600	17.6	600	18.3	600
3	340	7.8	600	8.2	600	9.05	600	9.3	600	9.6	600	9.85	600	10.2	600	10.5	600	10.85	600	11.2	600	11.5	600	11.75	600	12.2	600	12.7	600	13.2	600
4	303	11.35	900	11.25	900	12.5	900	12.70	900	12.9	900	13.1	900	13.6	900	14.1	900	14.65	900	15.2	900	15.8	900	16.4	900	16.9	900	17.4	900	17.85	900
4	315	10.45	900	10.75	900	11.95	900	12.2	900	12.5	900	12.75	900	13.2	900	13.7	900	14.2	900	14.6	900	14.9	900	15.25	900	15.8	900	16.4	900	16.95	900
4	329	11.05	900	11.8	900	12.9	900	13.4	900	13.8	900	14.25	900	14.7	900	15.2	900	15.6	900	16.0	900	16.5	900	16.9	900	17.4	900	18.0	900	18.5	900
4	341	8.85	900	9.95	900	11.45	900	11.7	900	12.0	900	12.3	900	12.7	900	13.0	900	13.4	900	14.0	900	14.6	900	15.15	900	15.9	900	16.7	900	17.45	900
5	301	13.1	257.802	13.45	257.802	14.65	257.802	15.0	258	15.3	258	15.6	257.802	16.2	258	16.9	258	17.5	257.802	18.2	258	18.8	258	19.45	257.802	20.0	258	20.6	258	21.1	257.802
5	318	11.2	257.802	11.3	257.802	12.35	257.802	12.5	258	12.7	258	12.9	257.802	13.4	258	13.9	258	14.45	257.802	15.0	258	15.6	258	16.1	257.802	16.6	258	17.2	258	17.7	257.802
5	344	10.6	257.802	10.25	257.802	11.1	257.802	11.3	258	11.5	258	11.75	257.802	12.3	258	12.8	258	13.3	257.802	13.8	258	14.2	258	14.7	257.802	15.2	258	15.6	258	16.1	257.802
5	347	8.35	257.802	8.4	257.802	9.45	257.802	9.8	258	10.1	258	10.4	257.802	10.8	258	11.3	258	11.7	257.802	12.0	258	12.4	258	12.7	257.802	13.3	258	13.8	258	14.35	257.802
6	309	8.7	515.604	9.9	515.604	10.8	515.604	11.0	516	11.3	516	11.5	515.604	11.8	516	12.2	516	12.5	515.604	13.1	516	13.7	516	14.3	515.604	14.7	516	15.1	516	15.5	515.604
6	327	9.85	515.604	10.15	515.604	11.25	515.604	11.6	516	11.9	516	12.15	515.604	12.6	516	13.1	516	13.6	515.604	14.2	516	14.8	516	15.4	515.604	15.7	516	15.9	516	16.2	515.604
6	343	9.4	515.604	9.1	515.604	10.1	515.604	10.4	516	10.7	516	10.95	515.604	11.4	516	11.8	516	12.15	515.604	12.6	516	13.0	516	13.4	489.823	13.6	516	13.8	516	14.05	464.043
6	346	9.4	515.604	9.9	515.604	11	515.604	11.4	516	11.8	516	12.25	515.604	12.6	516	12.9	516	13.25	515.604	13.7	516	14.2	516	14.7	515.604	15.0	516	15.4	516	15.7	515.604
7	306	9.7	773.405	13.6	773.405	14.8	773.405	15.0	773	15.2	773	15.45	773.405	16.1	773	16.7	773	17.25	773.405	17.7	773	18.1	773	18.45	773.405	19.0	773	19.6	773	20.15	773.405
7	308	11.15	773.405	11.95	773.405	12.7	773.405	12.9	773	13.1	773	13.3	773.405	13.7	773	14.1	773	14.45	773.405	15.0	773	15.5	773	15.95	773.405	16.4	773	16.8	773	17.2	773.405
7	317	12.75	773.405	12.25	773.405	12.6	773.405	12.9	773	13.1	773	13.4	773.405	13.9	773	14.4	773	14.95	773.405	15.4	773	15.8	773	16.25	773.405	16.8	773	17.3	773	17.75	773.405
7	331	12.85	773.405	12.85	773.405	13.8	773.405	14.1	773	14.3	773	14.6	773.405	15.1	735	15.6	773	16.15	773.405	16.7	754	17.2	773	17.7	773.405	18.1	773	18.6	754	19	696.065
8	304	10.1	269.655	10.8	269.655	12.1	269.655	12.4	270	12.7	270	13.05	269.655	13.4	270	13.7	270	14	269.655	14.3	270	14.7	270	15	269.655	15.7	270	16.3	270	17	269.655
8	311	11.4	269.655	11.95	269.655	12.75	269.655	13.0	270	13.3	270	13.5	269.655	13.9	270	14.4	270	14.8	269.655	15.3	270	15.8	270	16.3	269.655	16.9	270	17.6	270	18.2	269.655
8	314	10.45	269.655	10.8	269.655	11.5	269.655	11.9	270	12.3	270	12.65	269.655	13.0	270	13.4	270	13.8	269.655	14.5	270	15.1	270	15.8	269.655	16.2	270	16.5	270	16.9	269.655
8	321	11.95	269.655	12.1	269.655	12.45	269.655	12.8	270	13.1	270	13.4	269.655	13.9	270	14.3	270	14.75	269.655	15.2	270	15.7	270	16.15	269.655	16.7	270	17.2	270	17.65	269.655
9	307	13.7	539.31	13	539.31	13.6	539.31	14.0	539	14.3	539	14.65	539.31	14.9	539	15.2	539	15.5	525.828	15.8	539	16.1	472	16.45	539.31	16.9	539	17.4	539	17.85	539.31
9	313	12.55	539.31	12.9	539.31	13.4	539.31	13.8	526	14.2	539	14.6	512.345	15.0	539	15.4	539	15.85	539.31	16.1	539	16.4	539	16.6	539.31	17.4	539	18.2	539	19	539.31
9	325	11.45	539.31	11.7	539.31	12.25	539.31	12.5	539	12.7	539	12.95	539.31	13.4	539	13.9	539	14.35	539.31	15.0	512	15.6	526	16.2	525.828	16.8	526	17.4	539	17.95	539.31
9	332	11.95	539.31	11.5	539.31	12.4	539.31	12.7	539	12.9	539	13.2	539.31	13.7	539	14.2	539	14.65	539.31	15.2	539	15.7	539	16.15	539.31	16.4	539	16.7	539	16.9	539.31
10	328	11.05	808.966	11.25	808.966	12.1	808.966	12.5	809	12.8	809	13.15	808.966	13.6	809	14.1	809	14.5	808.966	15.1	809	15.7	809	16.35	788.741	16.8	809	17.3	809	17.75	808.966
10	337	8.5	808.966	9.1	808.966	9.75	808.966	10.1	789	10.4	809	10.75	788.741	11.2	809	11.7	769	12.1	808.966	12.5	769	13.0	809	13.4	768.517	13.9	789	14.3	667	14.75	768.517
10	342	10.9	808.966	11.15	808.966	11.8	808.966	12.1	809	12.4	809	12.75	808.966	13.1	809	13.4	809	13.75	808.966	14.5	809	15.2	789	15.9	808.966	16.3	809	16.6	809	16.95	808.966
10	348	9.05	808.966	9.2	808.966	10.1	808.966	10.3	809	10.5	809	10.65	808.966	11.1	789	11.6	809	12.05	808.966	12.6	789	13.1	809	13.55	808.966	14.0	809	14.5	809	15	808.966

Missed Doses:

Day 0 - Pig 313 did not eat entire afternoon dose (ate approximately 95%). Daily dose adjusted to 97.5%.

Day 0 - Pig 337 did not eat entire afternoon dose (ate approximately 95%). Daily dose adjusted to 97.5%.

Day 2 - Pig 313 did not eat entire afternoon dose (ate approximately 90%). Daily dose adjusted to 95%.

Day 2 - Pig 337 did not eat entire morning dose (ate approximately 95%). Daily dose adjusted to 97.5%.

Day 3 - Pig 331 did not eat entire afternoon dose (ate approximately 90%). Daily dose adjusted to 95%.

Day 3 - Pig 348 did not eat entire afternoon dose (ate approximately 95%). Daily dose adjusted to 97.5%.

Day 4 - Pig 337 did not eat entire afternoon dose (ate approximately 90%). Daily dose adjusted to 95%.

Day 5 - Pig 307 did not eat entire morning dose (ate approximately 95%). Daily dose adjusted to 97.5%.

Day 6 - Pig 331 did not eat entire morning dose (ate approximately 95%). Daily dose adjusted to 97.5%.

Day 6 - Pig 325 did not eat entire afternoon dose (ate approximately 90%). Daily dose adjusted to 95%.

Day 6 - Pig 337 did not eat entire morning dose (ate approximately 90%). Daily dose adjusted to 95%.

Day 6 - Pig 348 did not eat entire afternoon dose (ate approximately 95%). Daily dose adjusted to 97.5%.

Day 7 - Pig 307 did not eat entire afternoon dose (ate approximately 75%). Daily dose adjusted to 87.5%.

Day 7 - Pig 325 did not eat entire morning dose (ate approximately 95%). Daily dose adjusted to 97.5%.

Day 7 - Pig 342 did not eat entire morning dose (ate approximately 95%). Daily dose adjusted to 97.5%.

Day 8 - Pig 343 did not eat entire morning dose (ate approximately 90%). Daily dose adjusted to 95%.

Day 8 - Pig 325 did not eat entire morning dose (ate approximately 95%). Daily dose adjusted to 97.5%.

Day 8 - Pig 328 did not eat entire morning dose (ate approximately 95%). Daily dose adjusted to 97.5%.

Day 8 - Pig 337 did not eat entire afternoon dose (ate approximately 90%). Daily dose adjusted to 95%.

Day 9 - Pig 325 did not eat entire afternoon dose (ate approximately 95%). Daily dose adjusted to 97.5%.

Day 9 - Pig 337 did not eat entire afternoon dose (ate approximately 95%). Daily dose adjusted to 97.5%.

Day 10 - Pig 331 did not eat entire afternoon dose (ate approximately 95%). Daily dose adjusted to 97.5%.

Day 10 - Pig 337 did not eat entire morning or afternoon dose (ate approximately 70% and 95%, respectively). Daily dose adjusted to 82.5%.

Day 11 - Pig 343 did not eat entire afternoon dose (ate approximately 80%). Daily dose adjusted to 90%.

Day 11 - Pig 331 did not eat entire afternoon dose (ate approximately 80%). Daily dose adjusted to 90%.

Day 11 - Pig 337 did not eat entire afternoon dose (ate approximately 90%). Daily dose adjusted to 95%.

TABLE B-4 URINE VOLUMES - 48 HOUR COLLECTIONS

Units of Volume: mls

Group	Pig ID	Day		
		6-7	**8-9**	**10-11**
		9/10-9/11	9/12-9/13	9/14-9/15
1	324	5400	6780	11620
	338	6960	7280	13800
	349	6100	4340	4460
2	326	6870	7640	14940
	330	3060	1900	3350
	339	19330	8320	18380
	350	12850	7640	10100
3	310	11150	3260	14060
	316	24060	50480	40840
	322	16940	8720	12400
	340	4840	3480	8100
4	303	10270	12800	13490
	315	12220	23700	16150
	329	21400	21620	26660
	341	5540	7260	8990
5	301	3360	2240	2020
	318	4960	4830	3440
	344	3440	4380	4010
	347	10700	10740	11690
6	309	18340	16790	19700
	327	6280	6360	9800
	343	7040	4480	9240
	346	22050	15820	16650
7	306	8220	8220	11620
	308	15500	11400	12200
	317	2520	2350	2150
	331	8180	8680	11180
8	304	5660	6600	4440
	311	23820	23920	29080
	314	6000	5250	4660
	321	10300	14600	7440
9	307	17000	21760	18000
	313	24830	16420	14660
	325	4360	4840	4050
	332	8910	6760	4290
10	328	15700	14470	21760
	337	3320	1400	3800
	342	14000	14200	33350
	348	3680	3840	4800

Volume measured by:	TE, CL, HH	HH, BL	HH,TN
Date:	9/12/02-9/13/02	9/14/02	9/16/02

TABLE B-5 URINE ANALYTICAL RESULTS

Tag Number	Pig Number	Group	Day	Material Administered	Target Dose (ug/d)	Q	Arsenic Conc in Urine	DL	Units
R1-01-0194	324	1	6/7	Control	0	<	1	1	ng/mL
R1-01-0265	338	1	6/7	Control	0		1	1	ng/mL
R1-01-0173	349	1	6/7	Control	0		3	1	ng/mL
R1-01-0163	326	2	6/7	NaAs	300		83	1	ng/mL
R1-01-0200	330	2	6/7	NaAs	300		160	2	ng/mL
R1-01-0191	339	2	6/7	NaAs	300		29	1	ng/mL
R1-01-0228	350	2	6/7	NaAs	300		45	1	ng/mL
R1-01-0232	310	3	6/7	NaAs	600		110	2	ng/mL
R1-01-0199	316	3	6/7	NaAs	600		49	1	ng/mL
R1-01-0112	322	3	6/7	NaAs	600		73	1	ng/mL
R1-01-0250	340	3	6/7	NaAs	600		160	2	ng/mL
R1-01-0167	303	4	6/7	NaAs	900		170	2	ng/mL
R1-01-0220	315	4	6/7	NaAs	900		101	1	ng/mL
R1-01-0263	329	4	6/7	NaAs	900		70	1	ng/mL
R1-01-0233	341	4	6/7	NaAs	900		300	4	ng/mL
R1-01-0136	301	5	6/7	TM1	300		56	1	ng/mL
R1-01-0261	318	5	6/7	TM1	300		42	1	ng/mL
R1-01-0260	344	5	6/7	TM1	300		57	1	ng/mL
R1-01-0159	347	5	6/7	TM1	300		14	1	ng/mL
R1-01-0148	309	6	6/7	TM1	600		24	1	ng/mL
R1-01-0187	327	6	6/7	TM1	600		66	1	ng/mL
R1-01-0156	343	6	6/7	TM1	600		36	1	ng/mL
R1-01-0208	346	6	6/7	TM1	600		23	1	ng/mL
R1-01-0121	306	7	6/7	TM1	900		65	1	ng/mL
R1-01-0165	308	7	6/7	TM1	900		39	1	ng/mL
R1-01-0193	317	7	6/7	TM1	900		138	1	ng/mL
R1-01-0171	331	7	6/7	TM1	900		42	1	ng/mL
R1-01-0225	304	8	6/7	TM2	300		49	1	ng/mL
R1-01-0183	311	8	6/7	TM2	300		11	1	ng/mL
R1-01-0117	314	8	6/7	TM2	300		44	1	ng/mL
R1-01-0118	321	8	6/7	TM2	300		25	1	ng/mL
R1-01-0177	307	9	6/7	TM2	600		40	1	ng/mL
R1-01-0152	313	9	6/7	TM2	600		23	1	ng/mL
R1-01-0234	325	9	6/7	TM2	600		104	1	ng/mL
R1-01-0172	332	9	6/7	TM2	600		66	1	ng/mL
R1-01-0114	328	10	6/7	TM2	900		56	1	ng/mL
R1-01-0164	337	10	6/7	TM2	900		160	2	ng/mL
R1-01-0147	342	10	6/7	TM2	900		57	1	ng/mL
R1-01-0186	348	10	6/7	TM2	900		150	2	ng/mL
R1-01-0120	324	1	8/9	Control	0		2	1	ng/mL
R1-01-0237	338	1	8/9	Control	0		3	1	ng/mL
R1-01-0123	349	1	8/9	Control	0		3.6	1	ng/mL
R1-01-0139	326	2	8/9	NaAs	300		75	1	ng/mL
R1-01-0221	330	2	8/9	NaAs	300		270	5	ng/mL
R1-01-0107	339	2	8/9	NaAs	300		73	1	ng/mL
R1-01-0243	350	2	8/9	NaAs	300		71	1	ng/mL
R1-01-0189	310	3	8/9	NaAs	600		240	5	ng/mL
R1-01-0213	316	3	8/9	NaAs	600		24	1	ng/mL
R1-01-0111	322	3	8/9	NaAs	600		130	2	ng/mL

Tag Number	Pig Number	Group	Day	Material Administered	Target Dose (ug/d)	Q	Arsenic Conc in Urine	DL	Units
R1-01-0145	340	3	8/9	NaAs	600		240	5	ng/mL
R1-01-0132	303	4	8/9	NaAs	900		140	2	ng/mL
R1-01-0257	315	4	8/9	NaAs	900		70	1	ng/mL
R1-01-0240	329	4	8/9	NaAs	900		83	1	ng/mL
R1-01-0188	341	4	8/9	NaAs	900		240	5	ng/mL
R1-01-0215	301	5	8/9	TM1	300		77	1	ng/mL
R1-01-0133	318	5	8/9	TM1	300		48	1	ng/mL
R1-01-0218	344	5	8/9	TM1	300		39	1	ng/mL
R1-01-0255	347	5	8/9	TM1	300		19	1	ng/mL
R1-01-0138	309	6	8/9	TM1	600		29	1	ng/mL
R1-01-0170	327	6	8/9	TM1	600		65	1	ng/mL
R1-01-0251	343	6	8/9	TM1	600		60	1	ng/mL
R1-01-0141	346	6	8/9	TM1	600		24	1	ng/mL
R1-01-0127	306	7	8/9	TM1	900		66	1	ng/mL
R1-01-0258	308	7	8/9	TM1	900		51	1	ng/mL
R1-01-0205	317	7	8/9	TM1	900		160	5	ng/mL
R1-01-0161	331	7	8/9	TM1	900		58	1	ng/mL
R1-01-0242	304	8	8/9	TM2	300		39	1	ng/mL
R1-01-0253	311	8	8/9	TM2	300		11	1	ng/mL
R1-01-0166	314	8	8/9	TM2	300		52	1	ng/mL
R1-01-0262	321	8	8/9	TM2	300		19	1	ng/mL
R1-01-0105	307	9	8/9	TM2	600		28	1	ng/mL
R1-01-0134	313	9	8/9	TM2	600		32	1	ng/mL
R1-01-0185	325	9	8/9	TM2	600		98	1	ng/mL
R1-01-0113	332	9	8/9	TM2	600		80	1	ng/mL
R1-01-0144	328	10	8/9	TM2	900		63	1	ng/mL
R1-01-0101	337	10	8/9	TM2	900		440	10	ng/mL
R1-01-0210	342	10	8/9	TM2	900		54	1	ng/mL
R1-01-0196	348	10	8/9	TM2	900		190	5	ng/mL
R1-01-0202	324	1	10/11	Control	0	<	1	1	ng/mL
R1-01-0239	338	1	10/11	Control	0		1	1	ng/mL
R1-01-0142	349	1	10/11	Control	0		3	1	ng/mL
R1-01-0192	326	2	10/11	NaAs	300		40	1	ng/mL
R1-01-0224	330	2	10/11	NaAs	300		130	2	ng/mL
R1-01-0229	339	2	10/11	NaAs	300		33	1	ng/mL
R1-01-0108	350	2	10/11	NaAs	300		60	1	ng/mL
R1-01-0209	310	3	10/11	NaAs	600		74	1	ng/mL
R1-01-0207	316	3	10/11	NaAs	600		31	1	ng/mL
R1-01-0131	322	3	10/11	NaAs	600		100	1	ng/mL
R1-01-0219	340	3	10/11	NaAs	600		120	2	ng/mL
R1-01-0254	303	4	10/11	NaAs	900		96	1	ng/mL
R1-01-0125	315	4	10/11	NaAs	900		102	1	ng/mL
R1-01-0236	329	4	10/11	NaAs	900		68	1	ng/mL
R1-01-0264	341	4	10/11	NaAs	900		180	5	ng/mL
R1-01-0109	301	5	10/11	TM1	300		110	2	ng/mL
R1-01-0231	318	5	10/11	TM1	300		58	1	ng/mL
R1-01-0176	344	5	10/11	TM1	300		43	1	ng/mL
R1-01-0128	347	5	10/11	TM1	300		13	1	ng/mL
R1-01-0227	309	6	10/11	TM1	600		24	1	ng/mL
R1-01-0129	327	6	10/11	TM1	600		40	1	ng/mL
R1-01-0115	343	6	10/11	TM1	600		28	1	ng/mL
R1-01-0204	346	6	10/11	TM1	600		24	1	ng/mL

Tag Number	Pig Number	Group	Day	Material Administered	Target Dose (ug/d)	Q	Arsenic Conc in Urine	DL	Units
R1-01-0160	306	7	10/11	TM1	900		51	1	ng/mL
R1-01-0150	308	7	10/11	TM1	900		52	1	ng/mL
R1-01-0143	317	7	10/11	TM1	900		190	5	ng/mL
R1-01-0248	331	7	10/11	TM1	900		54	1	ng/mL
R1-01-0238	304	8	10/11	TM2	300		62	1	ng/mL
R1-01-0178	311	8	10/11	TM2	300		9.5	1	ng/mL
R1-01-0217	314	8	10/11	TM2	300		50	1	ng/mL
R1-01-0214	321	8	10/11	TM2	300		32	1	ng/mL
R1-01-0252	307	9	10/11	TM2	600		31	1	ng/mL
R1-01-0245	313	9	10/11	TM2	600		33	1	ng/mL
R1-01-0256	325	9	10/11	TM2	600		120	2	ng/mL
R1-01-0216	332	9	10/11	TM2	600		120	2	ng/mL
R1-01-0149	328	10	10/11	TM2	900		39	1	ng/mL
R1-01-0246	337	10	10/11	TM2	900		160	5	ng/mL
R1-01-0174	342	10	10/11	TM2	900		26	1	ng/mL
R1-01-0103	348	10	10/11	TM2	900		130	2	ng/mL
R1-01-0222	2340	3	6/7	NaAs	600		160	2	ng/mL
R1-01-0180	2306	7	6/7	TM1	900		61	1	ng/mL
R1-01-0244	2307	9	6/7	TM2	600		37	1	ng/mL
R1-01-0104	2329	4	8/9	NaAs	900		83	1	ng/mL
R1-01-0247	2346	6	8/9	TM1	600		28	1	ng/mL
R1-01-0110	2314	8	8/9	TM2	300		53	1	ng/mL
R1-01-0212	2330	2	10/11	NaAs	300		130	2	ng/mL
R1-01-0182	2344	5	10/11	TM1	300		44	1	ng/mL
R1-01-0151	2348	10	10/11	TM2	900		130	2	ng/mL
R1-01-0157	AsCtrl	PE		Control	0		3	1	ng/mL
R1-01-0206	AsCtrl	PE		Control	0		2	1	ng/mL
R1-01-0119	AsIA200	PE		Sodium arsenate	200		180	4	ng/mL
R1-01-0124	AsIA200	PE		Sodium arsenate	200		190	5	ng/mL
R1-01-0198	AsIA40	PE		Sodium arsenate	40		42	1	ng/mL
R1-01-0158	AsIA40	PE		Sodium arsenate	40		41	1	ng/mL
R1-01-0122	AsIB200	PE		Sodium arsenite	200		190	4	ng/mL
R1-01-0175	AsIB200	PE		Sodium arsenite	200		200	5	ng/mL
R1-01-0106	AsIB40	PE		Sodium arsenite	40		43	1	ng/mL
R1-01-0230	AsIB40	PE		Sodium arsenite	40		41	1	ng/mL
R1-01-0241	AsOA200	PE		MMA	200		200	4	ng/mL
R1-01-0130	AsOA200	PE		MMA	200		210	5	ng/mL
R1-01-0135	AsOA40	PE		MMA	40		43	1	ng/mL
R1-01-0169	AsOA40	PE		MMA	40		43	1	ng/mL
R1-01-0116	AsOB200	PE		DMA	200		200	4	ng/mL
R1-01-0203	AsOB200	PE		DMA	200		210	5	ng/mL
R1-01-0249	AsOB40	PE		DMA	40		44	1	ng/mL
R1-01-0154	AsOB40	PE		DMA	40		44	1	ng/mL