RELATIVE BIOAVAILABILITY OF ARSENIC IN SOILS FROM EL PASO COUNTY, TEXAS

Prepared for:

U.S. Environmental Protection Agency
Region VI
Dallas, Texas

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May 2003

ACKNOWLEDGEMENTS

The work described in this report is the product of a team effort involving a number of people. In particular, the authors would like to acknowledge the efforts and support of the following:

- Margaret E. Dunsmore, BS, for helping with all aspects of animal handling and dosing, as well as urine collection and sample preparation.

- Dr. Edward Hinderberger of L.E.T., Inc., Columbia, Missouri, for providing prompt and reliable chemical analysis of all of the biological samples for total arsenic concentrations.

- Weston Solutions, Inc., for collecting the soil samples.

- Severn Trent Laboratories, Inc., for sieving and analyzing the soil samples.

EXECUTIVE SUMMARY

A study using juvenile swine as test animals was performed to measure the gastrointestinal absorption of arsenic from two soil samples from El Paso County, Texas (Test Material 1 and Test Material 2). The relative bioavailability of arsenic was assessed by comparing the absorption of arsenic from the test materials to that of a reference material (sodium arsenate). The arsenic concentrations of Test Material 1 and Test Material 2 were 74 ppm and 73 ppm, respectively. Groups of five swine were given oral doses of sodium arsenate or a test material twice a day for 15 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 8, 11, and 14). The urinary excretion fraction (UEF) (the ratio of the amount excreted per 24 hours divided by the dose given per 24 hours) was calculated for sodium arsenate and the test materials using linear regression analysis. The relative bioavailability (RBA) of arsenic in the test material 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)	0.825 ± 0.045 (37)	[1.00]
Test Material 1	0.362 ± 0.031 (51)	0.44 (0.37-0.52)
Test Material 2	0.302 ± 0.021 (52)	0.37 (0.32-0.42)

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 Test Material 1 is 44% (90^{th} % CI = 37% - 52%) and 37% (32% - 42%) for Test Material 2. These values are 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 these soil samples 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 these soils.

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RELATIVE BIOAVAILABILITY OF ARSENIC IN SOILS FROM EL PASO COUNTY, TEXAS

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 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 was to use juvenile swine as a test system in order to determine the oral bioavailability of arsenic in two soil samples from El Paso, Texas, relative to the bioavailability of a soluble form of arsenic. The relative bioavailability estimates may be used to improve accuracy and decrease uncertainty in estimating exposures to arsenic in soil in human health risk assessments for these test materials.

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 soil samples (Test Material 1 and Test Material 2). The reference material was administered to groups of five animals at two different dose levels and the test materials were administered to groups of five animals at three different dose levels, each for 15 days (a detailed schedule is presented in Appendix A, Table A-1). Additionally, the study included a non-treated group of three animals to serve as a control for determining background arsenic levels. All doses were administered orally.

2.1 Test Material

2.1.1 Sample Description

The two soil samples were collected from locations approximately 1.5 miles east of the American Canal in El Paso County, Texas.

2.1.2 Sample Preparation

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

2.1.3 Arsenic Concentration

The concentration of arsenic in the sieved test materials was measured by Severn Trent Laboratories, Inc., in duplicate by inductively coupled plasma atomic emission spectroscopy (ICP-AES). The resulting arsenic values were 74 ppm for Test Material 1 (TM1) and 73 ppm for Test Material 2 (TM2).

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 animals were intact males of the Pig Improvement Corporation (PIC) genetically defined Line 26, and were purchased from Chinn Farms, Clarence, MO.

The number of animals purchased for the study was several more than required by the protocol. These animals were purchased at an age of about 4-5 weeks (weaning occurs at age 3 weeks) and housed in individual stainless steel cages with a 12/12 light/dark cycle. Ambient temperatures ranged from 82-86°F and exhaust fans were activated several times a day. The

animals were held under quarantine for one week to observe their health before beginning exposure to test materials. Each animal was examined by a certified veterinary clinician (swine specialist) and any animals that appeared to be in poor health during this quarantine period were excluded from the study. To minimize weight variations between animals and groups, extra animals most different in body weight (either heavier or lighter) four days prior to exposure (day -6) were also excluded from the study. The remaining animals were assigned to dose groups at random (group assignments are presented in Appendix A, Table A-2). When exposure began (day zero), the animals were about 5-6 weeks old and weighed an average of about 12.7 kg. The animals were weighed every three days during the course of the study. On average, animals gained about 0.45 kg/day and the rate of weight gain was comparable in all groups, ranging from 0.40 to 0.54 kg/day. These body weight data are summarized in Figure 2-1 and are also presented in Appendix A, Table A-3. 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, MO) by the supplier. In order to minimize arsenic exposure from the diet, the animals were transitioned from the MFA feed to a special feed (Zeigler Brothers, Inc., Gardners, PA), and this feed was maintained for the duration of the study. The feed was nutritionally complete and met all requirements of the National Institutes of Health–National Research Council. The typical nutritional components and chemical analysis of the feed 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 at 11:00 AM and 5:00 PM daily. Analysis of a single feed sample indicated that the arsenic level was below the detection limit (50 ng/g), which corresponds to a dose contribution from food of less than 2 µg/kg-day. In addition, previous analysis of feed samples indicated that the arsenic level was generally below the detection limit.

Drinking water was provided *ad libitum* via self-activated watering nozzles within each cage. Analysis of samples from randomly selected drinking water nozzles indicated the arsenic concentration was less than or equal to 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 a test material for 15 days, with the dose for each day being administered in two equal portions beginning at 9:00 AM and 3:00 PM (two hours before feeding), with two minute intervals allowed for individual pig dosing. Dose material was placed in the center of one or more small portions (about 5 grams) of moistened feed (this is referred to as a “doughball”), and this was administered to the animals by hand[1]. Because the arsenic concentrations of the test materials

[1] Doses for Days 0-2 were prepared using an alternative method that involved mixing the test material soil with dry feed, then moistening the mixture and forming it into doughballs.

were relatively low in this study, it was necessary to use as many as 5-10 doughballs to administer the doses. In these instances, the amount of feed administered was adjusted accordingly. If uneaten portions of doughballs were discovered, these were retrieved and offered again for consumption. Occasionally, some animals did not consume some or all of their dose. In these instances, the missed doses were estimated and recorded and the time-weighted average dose calculation for each animal was adjusted downward accordingly (see Appendix A, Table A-3).

The dose levels administered were based on the arsenic content of the test material, with target doses of 25 and 50 µg/kg-day for the reference material and 40, 80, and 160 µg/kg-day for each test material. The actual administered arsenic doses are presented in Appendix A, Table A-3.

2.5 Collection and Preservation of Urine Samples

Samples of urine were collected from each animal for 24-hour periods on days 0, 5, 8, 11, and 14 of the study. Collection began at 9:00 AM and ended 24 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 periodically (typically twice daily) into a separate holding container to ensure that there was no loss of sample due to overflow.

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

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.

2.6.1 Laboratory Quality Assurance

A number of quality assurance (QA) 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 urine samples were spiked with known amounts of arsenic (usually 400 µg, as sodium arsenate) and the recovery of the added arsenic was measured. Recovery for individual samples ranged from 100% to 113%, with an average across all analyses of 104 ± 3% (N = 25).

Duplicate Analysis

The laboratory analyst selected random urine samples for duplicate analysis. Duplicate results had a relative percent difference (RPD) of 0% to 12.0%, with an average of 2.4% ± 3.7% (N = 24).

Laboratory Control Standards

Five 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:

Check Sample	Certified Value	Average Recovery	SEM	n
E.R.A. P081 - Metals WasteWatR	366 ng/ml	99%	1.7%	92
N.R.C.C. Dolt-2 Dogfish Liver	16.6 +/- 1.1 Mcg/g dry wt	93%	3.5%	4
N.R.C.C. Tort-2 Lobster	21.6 +/- 1.8 Mcg/g dry wt	102%	3.8%	4
N.I.S.T. Oyster 1566b	7.65 +/- 0.65 Mcg/g dry wt	105%	6.4%	4
N.I.S.T. 1640	0.0267 +/- 0.0004	97%	--	1

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

Blank samples run along with each batch of samples never yielded a measurable level of arsenic, with all values being reported as less than 1 ng of arsenic (N = 13).

2.6.2 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 a number of blind duplicates.

The results for the PE samples are shown in Figure 2-2. As seen, 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 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., a test material and reference material) can be calculated from the ratio of the urinary excretion fraction of the two materials. This calculation is independent of the extent of tissue binding and of biliary excretion:

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

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/24 hrs) = Concentration (µg/L) · Volume (L/24 hrs)

- Because previous swine arsenic bioavailability studies have shown that urinary arsenic excretion patterns are stable after five days of dosing (USEPA, 1997), UEF and RBA calculations were based on data from days 8, 11, and 14 only (not days 0 and 5).

- For each test material, the amount of arsenic excreted by each animal was plotted as a function of the amount administered (µg/24 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 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 the 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 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, data from one animal on two different days (pig #1550 from group 1 on days 11 and 14) were deemed unreliable for this reason and excluded.

In addition, the datum for pig #80 (group 4, low dose of TM1) on day 14 was excluded because the amount of arsenic excreted was substantially higher than for the other four animals in that group, as well as all other animals in the study. This datum is indicated as an outlier in Figure 4-2.

4.3 Urinary Excretion Fractions and Relative Bioavailability

Detailed results from the study are presented in Appendix A. The urinary excretion results on days 8, 11, and 14 for NaAs, TM1, and TM2 are summarized in Figures 4-1, 4-2, and 4-3, respectively. (Urinary excretion results for all days, including days 0 and 5, are presented in Appendix A, Figures A-1, A-2, and A-3.) 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:

$$\text{RBA(test } \textit{vs.} \text{ NaAs)} = \text{UEF(test)} / \text{UEF(NaAs)}$$

The following table summarizes the best fit slopes (urinary excretion fractions) for sodium arsenate and the utility pole soil, as well as the estimated RBA:

Material Administered	UEF ± SEM (n)	RBA (90% CI)
Sodium Arsenate (reference material)	0.825 ± 0.045 (37)	[1.00]
Test Material 1	0.362 ± 0.031 (51)	0.44 (0.37-0.52)
Test Material 2	0.302 ± 0.021 (52)	0.37 (0.32-0.42)

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 is 44% for TM1 and 37% for TM2.

The RBA estimates for the two test materials are markedly lower than the default value range of 80%-100% that is usually employed for arsenic in soil when reliable site-specific data are lacking. This indicates that the arsenic in these soils 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 these soils.

5.0 REFERENCES

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APPENDIX A

DETAILED RESULTS