MOUSE ASSAY FOR DETERMINATION OF ARSENIC BIOAVAILABILITY IN CONTAMINATED SOILS

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Abstract: A mouse assay for measuring the relative bioavailability (RBA) of As in soil was developed. In this report, results are presented of RBA assays of 16 soils, including multiple assays of the same soils, which provide a quantitative assessment of reproducibility of mouse assay results, as well as a comparison of results from the mouse assay with results from a swine and monkey assay applied to the same test soils. The mouse assay is highly reproducible; three repeated assay on the same soils yielded RBA estimates that ranged from 1 to 3% of the group mean. The mouse, monkey, and swine models yielded similar results for some, but not all test materials. RBA estimates for identical soils (9 test soils and 3 standard reference materials [SRM]) assayed in mice and swine were significantly correlated (r=0.70). Swine RBA estimates for 6 of the 12 test materials were higher than estimates from the mouse assay. RBA estimates for 3 standard reference materials (SRM) were not statistically different (mouse/swine ratio ranged from 0.86–1.00). When 4 test soils from the same orchard were assessed in the mouse, monkey, and swine assays, the mean soil As RBA were not statistically different. Mouse and swine models predicted similar steady state urinary excretion fractions (UEF) for As of 62% and 74%, respectively, during repeated ingestion doses of sodium arsenate, the water soluble As form used as the reference in the calculation of RBA. In the mouse assay, the UEF for water soluble As^{V} (sodium arsenate) and As^{III} (sodium [meta] arsenite) were 62% and 66%, respectively, suggesting similar absolute bioavailabilities for the two As species. The mouse assay can serve as a highly cost-effective alternative or supplement to monkey and swine assays for improving As risk assessments by providing site-specific assessments of RBA of As in soils.

Key Words: arsenic, bioavailability, human health risk assessment, *in vivo*, metal contaminated soils, mouse

Running Head: Bioavailability of Arsenic in Mice

Abbreviations: AsIII: arsenite; AsV: arsenate; CI: Confidence Interval; CV: coefficient of variation; g: gram; INAA: Instrumental Neutron Activation Analysis; NIST: National Institute of Standards and Technology; RBA: Relative Bioavailability; SD: Standard Deviation; SRM: Standard Reference Material; UEF: Urinary Excretion Fraction; U.S. EPA: United States Environmental Protection Agency

The U.S. Environmental Protection Agency (EPA) estimates of cancer risk associated with ingestion of As-contaminated soils based on a cancer slope factor derived from findings of epidemiological studies in populations chronically exposed to As in drinking water (U.S. EPA, 2013). However, if oral bioavailability of As in soil and water are not equivalent, then cancer risk from ingestion of As-contaminated soil may not be assessed accurately (Bradham and Wentsel, 2010; U.S. EPA, 2007a; 2007b). Several factors can affect oral bioavailability of As in soil, including As speciation, modifying effects of other elements in soil, and physical and chemical properties of soil (Kelly et al., 2002; NRC, 2003; U.S. EPA, 2007a). These variables affecting soil As RBA were critical for the U.S. EPA decision to revise the default assumption of 100% As bioavailability in soils to an upper bound default value of 60%, based on a compilation of in vivo As RBA studies that included the in vivo mouse studies reported here (U.S. EPA, 2012). Therefore, accurate estimation of soil As bioavailability underlies reliable estimation of human health risks from ingestion of As-contaminated soil (U.S. EPA, 1989, 2007a; 2007b).

Difficulties inherent in measuring soil As bioavailability in humans (Stanek et al., 2010) inspired development of bioassays for estimating the relative bioavailability (RBA) of soil As (relative to a completely water soluble form of As such as sodium arsenate) in monkeys and swine, based on similarities in gastrointestinal physiology of these species to humans (Basta et al., 2007; Denys et al., 2012; Brattin and Casteel, 2013; Freeman et al., 1995; Juhasz et al., 2007; Roberts et al., 2002, 2007). Bioaccessibility assays that reflect As RBAs in the juvenile swine model have been evaluated (Basta et al., 2007; Juhasz et al., 2007; Denys et al., 2012). A recent study describes a mouse assay that economically, quickly, and reproducibly measures RBA of soil As correlated with bioaccessibility data (Bradham et al., 2011). Relatively low purchase and husbandry costs and extensive physiological characterization of inbred mouse strains offer an attractive cost-

effective alternative to RBA assays in monkeys and swine; however, no published studies have compared RBA estimates across species.

In this report, results are presented of RBA assays of an extended set of soils, including multiple assays of the same soils, which provide a quantitative assessment of reproducibility of mouse assay results, not previously reported for either the cynomolgus monkey or juvenile swine assays (Brattin and Casteel, 2012; Roberts et al., 2007). A comparison of estimates of oral As RBA for a group of identical soils assayed in mice and immature swine, which indicates good correspondence between the two assays, is also described. Two other problems are addressed in this report. Evidence is provided that the bioavailability of water soluble arsenate (As^{V}) and arsenite (As^{III}) are similar in mice, consistent with observations made in swine (Juhasz et al., 2007), and; implemented analytical and simulation solutions are offered for estimating confidence limits on the RBA, when it is based on the ratio of independently measured bioavailability estimates (e.g., soil As/sodium arsenate).

MATERIALS AND METHODS

Test Soils and Standard Reference Materials

Test soils evaluated in mouse and swine models were collected from sites where mining/smelting activities or pesticide application resulted in As contamination. All test soils were dried (<40°C), sieved to <250 μm, homogenized, and riffled (Blume et al., 1991). Three standard references materials (SRM), SRM 2710 and SRM 2710a (National Institute of Standards and Technology), and USGS Flat Creek reference material (U.S. Geological Survey, currently being certified) were also evaluated in bioavailability assays. Arsenic concentrations in test soils and SRM were determined by Instrumental Neutron Activation Analysis (INAA) at the

Department of Nuclear Engineering, North Carolina State University, Raleigh. The mean As mass detection limit was 0.035 μg (approximately 0.2 μg/g soil).

Arsenic speciation in soils was examined using the Materials Research Collaborative Access Team's (MRCAT) beamline 10-ID, Sector 10 at the Advanced Photon Source (APS), Argonne National Laboratory (ANL), Argonne, IL. The electron storage ring operated at 7 GeV in top-up mode. A liquid nitrogen-cooled double-crystal Si(111) monochromator was used to select incident photon energies and a platinum-coated glass mirror was used for harmonic rejection. The beam monochromator was calibrated by assigning the first derivative inflection point of sodium arsenate to 11874 eV and this calibration standard was collected simultaneously with each sample scan. Three As K-edge X-ray absorption spectroscopy (XAS) spectra were collected in fluorescence mode (16-element solid state Ge detector, Canberra) at room temperature for every soil and reference sample. Data analysis was conducted using IFEFFIT software (Ravel and Newville, 2005). Replicate scans for each sample were merged, then normalized, and converted into *k* space. A principal component analysis coupled with linear-combination fitting (LCF) was used to identify the major As species in the samples. LCF were performed using XAS k^2 space spectra from reference standards to As phases in the soil samples. Reference materials for LCF, based on principal component analysis, included arsenate sorbed to ferrihydrite (sorbed As^{V}), scorodite [$Fe(As^{V}O_4)$], realgar ($As^{III}S$), lollingite ($Fe^{III}As_2$) and arsenopyrite ($FeAs^{III}S$). Data for LCF fits reveal As speciation in each soil as ratios of these mineral forms.

Mouse Assay Procedure

The mouse assay used to assess As bioavailability in test soils and SRM was described by Bradham et al.(2011). All assays were performed in 4- to 6-week-old 16-20 g female

C57BL/6 mice (Charles River Laboratories, Raleigh, NC). The basal diet for mouse assays was powdered AIN-93G purified rodent diet (Reeves et al., 1993) obtained from Dyets (Bethlehem, PA). The As concentration in the basal diet was below the INAA detection limit. Based on this detection limit and measured diet consumption, As dosage from ingestion of basal diet was less than 30 μg/kg/day. Amended diets were prepared by blending of test soils or SRM with basal diet. For test soils or SRM, the soil:diet ratio was typically 1% (w/w). Arsenate (As^{V})- and arsenite (As^{III})-amended diets were prepared by addition of sodium arsenate heptahydrate or sodium (meta) arsenite (Sigma, St. Louis, MO), respectively, to powdered AIN-93G purified rodent diet.

Mice were housed in an American Association for the Accreditation of Laboratory Animal Care-accredited facility and animal procedures were approved by the Institutional Animal Care and Use Committee of the National Health and Environmental Effects Research Laboratory. Before the assay, animals were group housed in plastic box cages with pine shaving bedding with a 12 hr light:dark photoperiod at 20–22^{o}C. Mice had free access to AIN-93G purified rodent diet and tap water that contained <11 μg As/L (Kenyon et al., 2008). During assays, three mice were housed together for 10 days in a single metabolic cage that separated urine and feces with unlimited access to test diet and drinking water. Urine and feces were collected daily and food consumption was measured daily. For sample collection and data analysis, the unit of observation was the cage (i.e., combined excreta of three mice). Typically, an assay included 4 cages of animals (12 mice) that received the same amended diet. Urine and feces from each individual cage were pooled over the course of the assay and processed for As analysis by INAA.

Estimation of Arsenic RBA in Mice

Data from each mouse assay were used to calculate the urinary excretion fraction (UEF) of As from ingestion of an amended diet as the ratio of cumulative excretion of As in urine (μg) to cumulative dietary intake of As (μg) as shown in Equation 1:

$$UEF\% = 100 \cdot \frac{Cum\ Urinary\ As}{Cum\ As\ Dose} \qquad \text{Eq. (1)}$$

RBA was calculated as the ratio of the UEF for As in a specific soil-amended diet to the UEF for As in a diet containing sodium arsenate heptahydrate (see Equation 2):

$$RBA\% = \frac{UEF\%\ Soil}{UEF\%\ Arsenate} \qquad \text{Eq. (2)}$$

Each UEF in Equation 2 is derived from multiple estimates of UEF for groups of three mice housed together in a single metabolic cage (the unit of measure in the assay is data from a single cage). Therefore, estimating confidence limits on the RBA requires estimating the confidence limit on a ratio of mean values for UEF where each mean has an associated uncertainty that needs to be estimated from the sample distributions.

Confidence limits on each RBA were estimated based on Fieller's Theorem for estimating confidence limits on the ratio of means, where the numerator and denominator are mean UEF derived from multiple estimates of UEF for the soil and the reference arsenical (sodium arsenate), respectively (Fieller, 1954). Normal distributions for estimates of both UEF were assumed (see below) with adjustments for unequal variance (SAS PROC 'TTEST'). Bootstrap methods were also explored and yielded estimates of confidence limits that were similar to those obtained from Fieller's Theorem (see Appendix Table A-1).

Statistical comparisons were made using SAS/STAT® software, Version 9.3 of the SAS System for Windows SAS software. Unless noted, a p-value ≤ 0.05 was considered to be significant in statistical comparisons. Parameters for the regression model for RBA estimates obtained from the mouse and swine assay were calculated iteratively in a Monte Carlo simulation in which each RBA was represented as a normal distribution defined by the mean and SE (Denys et al., 2012). Lower and upper 95% confidence limits on the r^2 were estimated from the 2.5 and 97.5 percentiles, respectively, of 10,000 iterations of the Monte Carlo simulation.

RESULTS

Arsenic in Test Soils and SRM

Arsenic concentrations ranged from 280 to 4495 µg/g in test soils and from 601 to 879 µg/g in SRM (see Table 1). As^V species were the dominant As forms in most test soils and in SRM. However, test soils #4, #6, #7 and #15 contained appreciable levels of As^{III} species. Arsenic in test soil #7, obtained from a slag pile, consisted of 78% As^{III} (realgar and arsenopyrite). Test soil #15 had a mixture of As^V (84%) and As^{III} (16%) sorption complexes on iron oxides. Concentrations of Fe, Mn, and Al for each soil are included in Table 1, as well as the pH of each soil.

Reproducibility of RBA Estimates from the Mouse Assay

Six assays of sodium arsenate-amended diet yielded coefficients of variation (CV, SD/mean) that ranged from 4 to 11%. The mean was 61.9%±4.6 (95% CI: 59.9–63.8, CV: 7.4%).

Reproducibility of RBA estimates was assessed by repeated assays of test soils and SRM. These results show that As RBA estimates from mouse assays were highly reproducible. When

assays of SRM were repeated thrice, the separate estimates were within 1–3% of the mean (see Table 2). Triplicate assays with SRM #14 yielded a mean RBA of 42.9%±1 (SD, CV: 2.3%); individual RBA estimates were within 2% of the mean. Assays of SRM #15 at different soil:diet ratios resulted in dietary As concentrations of 3.9 to 16.1 μg/g and an As dosages of 0.58 to 2.6 mg/kg/day. Over this range of As dosage, RBA of SRM #15 was not affected by dosage (see Figure 1). The overall mean for the three RBA estimates of SRM #15 was 42.1%±0.2% (CV: 0.6%) with individual estimates of RBA within 1% of the overall mean. Duplicate assays of SRM #16 yielded RBA estimates of 12.7 and 16.4% (mean: 14.6%). Duplicate assays with test soil #7 yielded RBA estimates of 11.6 and 10.9% (mean: 11.2%).

Comparison of Bioavailability of As^{V} and As^{III} in Mouse Assay

Soil As RBA relative to the UEF was calculated for sodium arsenate (As^{V}, Equation 2), which was used as an index for bioavailability of a completely water soluble As species. However, both As^{V} and As^{III} forms were present in most test soils and As^{III} was the dominant form of As in test soil #7. If bioavailabilities of water soluble inorganic As^{V} and As^{III} were not equivalent, As RBA based on a sodium arsenate UEF might not accurately reflect the actual RBA of As^{III} in the soil that becomes soluble (bioaccessible) in the gastrointestinal tract (GIT). Therefore, As UEF was compared in mice fed diets amended with either sodium arsenate (As^{V}) or meta sodium arsenite (As^{III}), and similar estimates for UEF obtained. The mean UEF for As in mice receiving sodium arsenate was 61.9%±4.6 (SD) and mean for the sodium arsenite-amended diet was 66.0%±2.5 (SD). The means were not significantly different (). On this basis, sodium arsenate appears to be a reasonable reference for estimating soil As RBA.

Comparison of Mouse, Monkey, and Swine RBA Estimates

Nine test soils and three SRM assayed in mice were also tested in a juvenile swine assay (Brattin and Casteel, 2013; U.S. EPA, 2012). Details of the swine and monkey assays are described in U.S. EPA (2012). The results are compared in Figure 2. All soils used for comparison had verifiable and continuous chains of custody that ensured identical soil samples were tested in both assays. RBA estimates were significantly correlated (r=0.70); the r^2 indicated that the model account for 49% of the variability in the data (r^2=0.49, 95% CI: 0.29, 0.63) RBA for the three SRM were not statistically different by a paired t-test (mouse/swine ratio ranged from 0.86 to 1, mean: 0.94). Swine assays tended to yield higher RBA estimates for test soils (mouse/swine ranged from 0.43 to 0.76, mean: 0.68). Mouse and swine RBA estimates for all test materials (test soil and SRM) were significantly different by a paired t-test (), sign test () or signed rank test (). Mean UEF for sodium arsenate was 61.9%±4.6 (SD) in mice and 74.2%±6 (SD) in swine. (); Although, these values are significantly different (t-test), this magnitude of difference (mouse/swine ratio = 0.83) does not account for the larger differences in RBA estimates measured in the mouse and swine assays.

Of the 12 RBA pairs shown in Figure 2, 4 test soils collected from a single orchard (test soils #8–11) were also tested in a cynomolgus monkey assay (Roberts et al., 2007; U.S. EPA, 2012). RBA estimates for these test soils are shown in Table 3. RBA estimates from the three assays were not significantly different (F-test, Bonferoni multiple range test). For the 4 individual test soils, mouse/monkey RBA ratios ranged from 0.55 to 1.4 (mean: 1). Mouse/swine RBA ratios for the individual test soils ranged from 0.43 to 0.84 (mean 0.7). When viewed from a site-wide regulatory perspective, these differences are relatively small. If these four samples had been used to assess the As bioavailability of the site, the mouse, monkey

and swine values would have been statistically indistinguishable. Additionally, it is not unusual to base site-wide RBA estimates on only a few samples, when bioassay costs are prohibitive and when a single dominant As source is expected to result in uniform As bioavailability across the site. In this case, a single dominant As source was application of arsenate pesticides and, as expected, variability in RBA estimates was relatively low (CV=23% for mouse assay, 13% for monkey assay, and 23% for swine assay). In this example, if these 4 soil samples were used to represent the RBA for this orchard site, the site-wide RBA estimates from the mouse (mean: 29%, 95% CI: 18, 40), monkey (31%, 95% CI: 22, 41), and swine (mean: 44%, 95% CI: 28, 59) assays were statistically equivalent (t-test,).

DISCUSSION

Animal models for predicting oral RBA of soil As serve two important objectives in human health risk assessment. They provide estimates of As RBA *in lieu* of being able to make direct estimates of soil As bioavailability in humans. Animal models also provide a basis for developing *in vitro* approaches for predicting RBA which, if successful, may decrease, if not eliminate, use of animals for this same purpose. The mouse assay described here has several attractive features and could serve as an alternative or supplement to monkey and swine assays. Data presenting in this report show that the mouse assay is highly reproducible. Three repeated assay on the same soils yielded RBA estimates that ranged from 1 to 3% of the group mean. The mouse assay also is highly cost effective. It is estimated that an RBA for a test soil can be obtained with the mouse assay for approximately one-tenth of the cost of a swine assay and for approximately one-thirtieth of the cost of a monkey assay. This makes it more feasible to evaluate a larger number of soils at a given and develop a more thorough evaluation of site-wide soil As RBA.

To our knowledge, precision of As RBA estimates, as determined from repeated assays of the same soils, has not been reported for any animal model. Low between-assay variation in UEF and RBA estimates in the mouse assay likely reflect uniform conduct of assays and reduction of between-animal variation by use of inbred mice. Uniform genetic constitution and standardized husbandry for inbred mice in assays likely minimizes variation in preabsorptive (Pinyayev et al., 2011) and postabsorptive metabolism and disposition of As (Tseng, 2007). Use of three mice per cage as the experimental unit reduced the influence of intraindividual variability in UEF estimation.

Differences in RBA values for different test materials may be largely determined by As mineralogy and physical and chemical properties of soils (Table 1) which affect solubility of As in the GIT (Ruby et al., 1999). Supporting evidence for this are studies that showed that extractability of As into low pH aqueous solvents that resemble gastric fluid accounts for much of the variability in RBA estimates obtained from the animal bioassays, including the mouse, swine, and monkey assays (Basta et al., 2007; Bradham et al., 2011; Freeman et al., 1995; Juhasz et al., 2007; Roberts et al., 2007; Denys et al., 2012).

No published studies have been conducted (or are ever likely to be conducted) to rigorously validate whether or not animal bioassays accurately predict soil As RBA in humans. Therefore animal models, such as the cynomolgus monkey and swine, were selected based largely on evidence of physiological similarity of the GIT systems to humans (Brattin and Casteel, 2013; Casteel et al., 1997, 2006; Juhasz et al., 2007; Roberts et al., 2007). Rodent models offer the potential for more cost effective RBA assessments provided that results can be harmonized across assays. Data presented in this study suggest that the mouse, monkey, and swine models yielded similar results for some, but not all test materials. When 4 test soils from

the same orchard were assessed in the mouse, monkey, and swine assays, the mean soil As RBA were not significantly different . RBA estimates for 3 SRM assayed in mouse and swine also were not significantly different. However, for the 9 test soils, the swine assay tended to yield higher RBA estimates than the mouse assay. Mouse and swine RBA estimates for the entire data set of identical soils (9 test soils and 3 SRM) assayed in mice and swine were significantly correlated (r=0.70); the linear regression model applied to the data accounted for 49% of the variability (r^2=0.49) (2) Mouse and swine models predict similar steady state UEF for As during repeated ingestions doses of sodium arsenate, the water soluble As form used as the reference in the calculation of RBA mouse: 62%, swine:74%). The As UEF following oral sodium arsenate in the cynomolgus monkey assay (4-day urine collection following a single oral gavage dose) was approximately 40% (Roberts et al., 2007). (3) In the mouse assay, the UEF for water soluble As^V (sodium arsenate) and As^{III} (sodium [meta] arsenite) were similar, suggesting similar absolute bioavailabilities for the two As species. Juhasz et al. (2007) measured plasma As AUC in swine following oral or intravenous dosing with As^V or As^{III} and concluded that the bioavailabilities were 92.5%±22.3 (SD) and 103.9%±25.8 (SD), respectively.

For 6 of 12 test materials assayed in mice and swine, 95% confidence limits on RBA estimates from mouse and swine assays did not overlap and, in general, swine RBA estimates trended to be higher than mouse RBA estimates. There is currently no explanation for this trend and it may reflect differences in the assay design (e.g., soil dosing, diets) and/or differences in GIT processing of soil As in mice and swine. Several factors may have contributed to differences in bioavailability estimates obtained in these assays. In mouse assays, UEF was estimated for a single dosage level; whereas, swine assays used three As dosage levels to estimate UEF from the slope of the linear regression relationship between As dose and urinary As excretion (Brattin and

Casteel, 2013). Average As dosages were also higher in mouse assays than in swine assays (320–6100 μg/kg/day and 40–350 μg/kg/day, respectively). However, these differences should not appreciably effect the RBA estimates, because, in both assays, UEF was independent of the As dosage over the range of doses tested (580 to 2360 μg As/kg/day in mice, 40 to 350 μg As/kg/day in swine). Average soil dosages were also higher in mouse assays than in swine assays (1150–1650 mg/kg/day and 30–660 mg/kg/day, respectively).

Mouse and swine assays differed in mode and pattern of delivery of test material. In mouse assays, test material was added to basal diet so that test material was ingested concurrently with feed; swine received test material twice daily in a dough ball that was given two hrs before each of two daily meals (Brattin and Casteel, 2013). Monkeys receive oral gavage doses of test material after a 12-hr fast and 4 hrs prior to feeding (Roberts et al., 2007).

Incorporation of test material into diet might modify bioavailability. For example, the presence of inorganic phosphate in diet may alter arsenate absorption due to competition between arsenate and phosphate oxyanions for sodium-coupled phosphate transporters in the GIT barrier (Eto et al., 2006; Villa-Bellost and Sorribas, 2009). Preliminary studies in cynomolgus monkeys show that concurrent oral dosing with inorganic phosphate (approximately 50 mg/kg) together with sodium arsenate decreased absorption of sodium arsenate (0.3 mg As/kg) and increased soil As RBA (Roberts et al., 2010). Phosphate levels in the diets were approximately 4.8 mg/g diet in the mouse assay (based on Reeves et al., 1993) and 18 mg/g diet in the swine assay (based on Casteel et al., 2009). Phosphate:As ratios in the mouse assay ranged from approximately 100 to 700 and were typically 700 (at a dietary arsenic concentration of 7 μg/g diet). In swine assays, arsenic was administered together with approximately 10 g of the test diet (two 5 g dough balls each day). Based on the As dose range of 40 - 350 μg As/kg

body weight/day and typical body weight of 10 kg, the phosphate:arsenic ratios ranged from approximately 50 to 450. On-going studies are evaluating the effects of modification to dietary phosphate levels on As RBA in mice.

CONCLUSION

In conclusion, the mouse assay yields highly reproducible and cost-effective estimates of RBA for As present in soils contaminated by mining and agricultural activities. Comparison for 12 materials evaluated in mouse and swine assays found RBA estimates from the two assays to be significantly correlated with a trend of higher RBA estimates from the swine assay. When applied to multiple samples from the same site, the mouse, swine, and monkey assays yielded similar estimates of site-wide RBA. Additional studies may improve assay performance, and lead to harmonization of results across bioassays conducted in various animal models.

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APPENDIX

Fieller's theorem allows the calculation of a confidence interval for the ratio of two means where the underlying distributions of the numerator and denominator are normal (Fieller, 1954). Although, sample sizes for typical assays (N=4 cages) were too small to allow a rigorous evaluation of normality of most sample UEF distributions, normality could be evaluated for selected samples with larger sizes. The mean UEF of sodium arsenate-amended diets was estimated using 24 independent estimates obtained in repeated assays. The mean UEF for these assays was 61.9%±4.6 (SD); the distribution showed low skew (1.07) and the assumption of normality was not rejected by standard goodness-of-fit tests (K-S statistic $p>0.95$; Shapiro Wilk W $p=0.50$). Similarly, 12 independent estimates of UEF for diets amended with SRM #14 also showed low skew (1.09). Here, the mean was 26.5±2.1% (SD) and the assumption of normality was not rejected (SD, K-S statistic $p=0.99$, Shapiro Wilk W $p=0.54$). Similarly, 12 independent estimates of UEF for diets amended with SRM #15 (26.0%±1.9, skew = 0.17) and goodness-of-fit tests indicated that a standard distribution provided a reasonable model for the data (K-S statistic $p=0.99$; Shapiro Wilk W $p=0.91$). It is not surprising the UEF distribution would be symmetrical, because each UEF value is actually an average of dose and excretion data from three mice, housed together in a single cage.

The estimates of confidence limits based on Fieller's Theorem compared well with estimates based on bootstrap methods (see Table A-1). In the parametric bootstrap, normal distributions for the UEF values for the diets amended with soil or sodium arsenate were represented by their respective sample means and SD, and each was randomly sampled (with replacement) N times, where N was the UEF sample size. In the non-parametric bootstrap, the discreet distribution of UEF for each material were randomly sampled with replacement, N

times. Confidence limits based on the bootstrap were very similar to those estimated from Fieller's Theorem.

TABLE 1. Sources, Elemental Composition and Arsenic Speciation of Soils.

			Soil Properties					Arsenic Speciation[a]						
								Arsenate (AsV)		Arsenite (AsIII)				
Sample ID	Arsenic Source	Sample Site	As[b] (mg/kg)	Fe[c] (g/kg)	Mn[c] (g/kg)	Al[c] (g/kg)	pH	Sorbed As^{V} (%)	Scorodite (%)	Sorbed As^{III} (%)	Lollingite (%)	Realgar (%)	Asenopyrite (%)	X^2red[d]
Test Soils														
1	Mine/ smelter waste	Mine/ smelter	280	72.3	0.0	3.9	2.1	79.5	20.5	–	–	–	–	0.007
2	Mine/ smelter waste	Mine/ smelter	4495	120.1	0.4	12.3	2.6	67.6	32.4	–	–	–	–	0.011
3	Smelter waste	Smelter	182	24.1	0.4	20.5	6.8	76.0	–	–	24	–	–	0.0220
4	Smelter waste	Residential	990	20.9	0.5	11.8	6.1	52.0	21.2	–	–	26.8	–	0.004
5	Smelter waste	Residential	829	20.5	0.7	9.4	6.3	96.7	3.3	–	–	–	–	0.004
6	Smelter waste	Residential	379	18.9	0.2	9.0	5.0	53.1	15.2	–	–	31.7	–	0.003
7	Smelter waste	Slag pile	837	294.4	2.7	13.2	7.2	18.7	1.6	–	–	47.7	32.1	0.001
8	Pesticides	Garden	769	59.2	0.9	6.3	5.6	64.0	36	–	–	–	–	0.0172
9	Pesticides	Orchard	336	25.5	0.2	27.4	6.6[e]	100	–	–	–	–	–	0.0442
10	Pesticides	Orchard	446	49.2	0.9	71.7	6.6[e]	100	–	–	–	–	–	0.0302
11	Pesticides	Orchard	437	38.6	1.2	44.2	6.6[e]	100	–	–	–	–	–	0.0526
12	Pesticides	Orchard	422	37.0	1.5	43.3	6.6[e]	100	–	–	–	–	–	0.0398
13	Pesticides	Orchard	340	32.0	2.1	44.8	5.6	100	–	–	–	–	–	0.0071
Standard Reference Materials														
14 (NIST 2710)	Mine/ smelter waste	Mine/ smelter	601	29.2	8.5	17.2	5.0	95.0	5.0	–	–	–	–	0.0070
15 (NIST 2710A)	Mine/ smelter waste	Mine/ smelter	1513	34.0	1.7	10.0	4.0	66.8	23.2	–	–	9.9	–	0.0100
16 (USGS-FC)	Mine/ smelter waste	Mine/ smelter	879	36.98	2.6	5.3	5.9	83.6	–	16.4	–	–	–	0.0066

NIST, National Institute of Standards and Technology

[a]Determined by linear combination of As XAS

[b]Determined by Instrumental Neuron Activation Analysis

[c]Extracted using EPA Method 3051A and analyzed by ICP-OES 6010C

[d]Reduced chi-squared values = $[(data-fit)^2] / [data^2]$

[e]Determined from pH characterization study of same site

TABLE 2. Repeated RBA Estimates for Soils Based on Mouse Bioassay.

Sample ID	Assay Number	Mean RBA	LCL	UCL	% of Group Mean
Test Soil					
7	1	11.5	10.7	12.3	103.1
	2	10.8	10.0	11.7	96.9
Mean		11.2	10.6	11.8	
Standard Reference Materials					
14 (NIST 2710)	1	42.8	39.7	45.0	99.8
	2	43.9	36.0	52.0	102.4
	3	41.9	37.7	46.3	97.8
Mean		42.9	40.5	45.4	
SD		1.0			
15 (NIST 2710A)	1	41.9	38.5	45.6	99.7
	2	42.3	36.2	48.7	100.6
	3	41.9	36.6	47.3	99.6
Mean		42.1	39.8	44.4	
SD		0.24	–	–	
16 (USGS-FC)	1	12.7	12.1	13.4	87.3
	2	16.4	15.5	17.4	112.7
Mean		14.6	12.9	16.3	

RBA is expressed as % relative to sodium arsenate.
LCL, lower 95% confidence limit; UCL, upper 95% confidence limit

TABLE 3. Repeated RBA Estimates for Soils Based on Mouse Bioassay.

	RBA % (95% Confidence Limits)				
Assay	Test Soil #8	Test Soil #9	Test Soil #10	Test Soil #11	Site Average
Mouse	26 (23, 29)	35 (31, 40)	21 (16, 26)	35 (31, 39)	29 (18,40)
Monkey	33 (23, 43)	28 (22, 34)	38 (24, 52)	25 (15, 35)	31 (22, 41)
Swine	31 (24, 40)[a]	41 (37, 44)	49 (40, 59)	53 (48, 57)	44 (28, 59)

[a]Estimated as standard error x 1.96 (Z=1.96 for standard normal), where SE values were reported in U.S. EPA (2012)

TABLE A-1. Comparison of Confidence Limits Estimated from Fieller's Theorem and Bootstrap Methods.

			Fieller's Theorem		BS - Normal[b]		BS – Non-Parametric[c]	
Sample ID	N[a]	Mean RBA	LCL	UCL	LCL	UCL	LCL	UCL
1	4/24	39.9	36.2	43.8	36.8	43.1	37.5	42.4
2	3/24	14.5	11.2	17.8	12.7	16.3	13.0	15.8
3	4/24	26.7	22.8	30.7	23.8	29.7	24.2	28.7
4	4/24	48.7	43.4	54.2	44.4	53.1	45.8	52.4
5	4/24	49.7	45.0	54.5	45.8	53.7	47.0	53.0
6	4/24	51.6	47.0	56.3	47.7	55.5	48.3	54.4
7	8/24	11.2	10.6	11.8	10.5	11.9	10.6	11.7
8	4/24	24.0	20.9	27.2	21.6	26.4	22.3	26.0
9	4/24	26.3	23.4	29.4	23.9	28.7	24.5	28.2
10	4/24	35.2	30.9	39.6	31.9	38.6	33.0	38.2
11	4/24	20.9	15.9	26.0	17.5	24.4	17.8	23.2
12	4/24	35.0	31.2	38.9	32.0	38.1	32.4	37.1
13	4/24	33.2	27.7	38.7	29.3	37.2	30.5	36.6
14	12/24	42.9	40.5	45.4	40.0	45.8	40.5	45.0
15	12/24	42.1	39.8	44.4	39.4	44.9	39.8	44.1

BS, bootstrap, LCL, 95% lower confidence limit; RBA, relative bioavailability; UCL, 95% upper confidence limit

[a]Number of UEF estimates for the soil/number of absolute bioavailability (ABA) estimates for sodium arsenate.

[b]Bootstrap of N random draws from normal (mean, SD) distribution of ABAs.

[c]Booststrap of N draws from discrete (mean) distribution of ABAs.

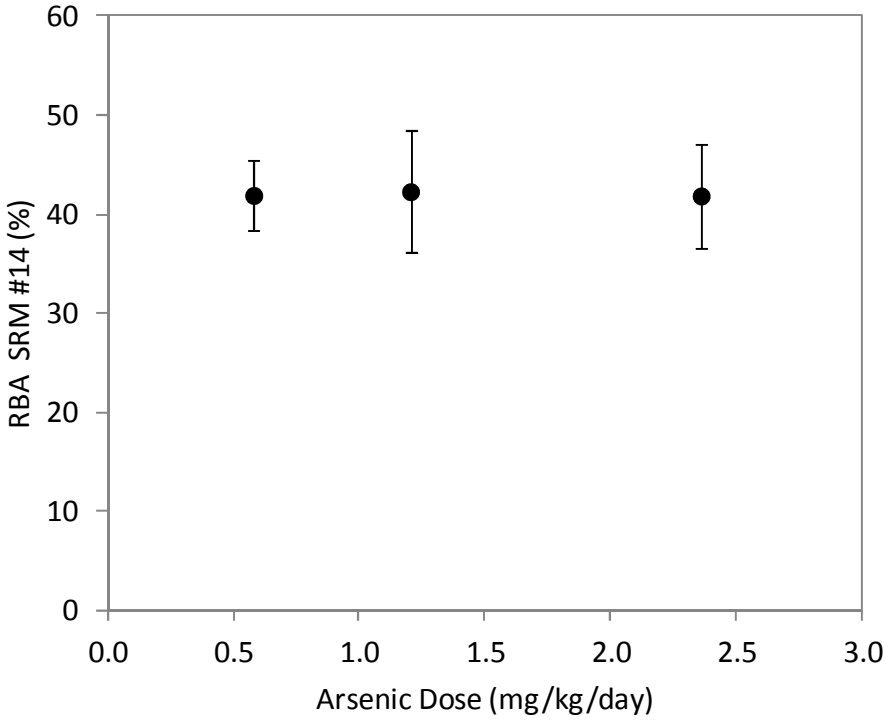

FIGURE 1. Effect of arsenic dose on RBA of SRM #14 (NIST 2710A). Error bars for mice are 95% CIs. RBA is not significantly associated with dose (r^2=0.03, p=0.89).

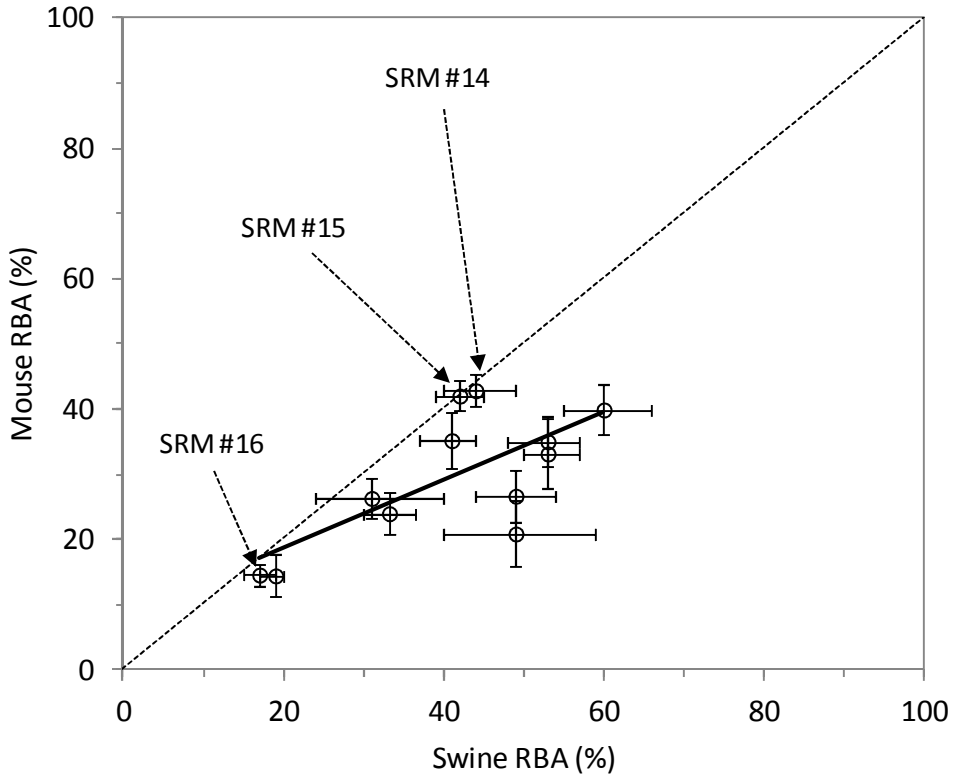

FIGURE 2. Comparison of RBA estimates based on mouse and swine bioassays applied to the same test materials (n=12). Error bars for mice are 95% CIs. Dashed line is line of identity. Solid line is the linear regression model (r^2=0.49, 95% CI: 0.29, 0.63, p=0.02).