Relative Bioavailability and Bioaccessibility and Speciation of Arsenic in Contaminated Soils

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Abbreviations: ABA: Absolute Bioavailability; Al: aluminum; As^{V}: arsenate; As: arsenic;; As^{III}: arsenite; Fe: iron; HCl: hydrochloric acid; ICP-OES: Inductively Coupled Plasma-Optical Emission Spectroscopy; IVBA: In vitro bioaccessibility; mg kg^{-1}: milligrams per kilogram; Mn: manganese; NIST: National Institute of Standards and Technology; RBA: Relative Bioavailability; SD: Standard Deviation; SRM: Standard Reference Material; US EPA: United States Environmental Protection Agency; XAS: X-Ray Absorption Spectroscopy.

ABSTRACT

Background: Assessment of soil arsenic bioavailability may profoundly affect the extent of remediation required at contaminated sites by improving human exposure estimates. Because small adjustments in soil arsenic bioavailability estimates can significantly alter risk assessments and remediation goals, convenient, rapid, reliable, and inexpensive tools are needed to determine soil arsenic bioavailability.

Objectives: This study evaluated inexpensive methods for assessing arsenic bioavailability in soil as a means to improve human exposure estimates and potentially reduce remediation costs.

Methods: Nine soils from residential sites affected by mining or smelting activity and two NIST standard reference materials were evaluated for arsenic bioavailability, bioaccessibility, and speciation. Arsenic bioavailability was determined using an in vivo mouse model while arsenic bioaccessibility was determined using the Solubility/Bioavailability Research Consortium in vitro assay. Arsenic speciation in soil and selected soil physicochemical properties were also evaluated in order to determine whether these parameters could be used as predictors of arsenic bioavailability and bioaccessibility.

Conclusion: In the mouse assay, bioavailabilities of arsenic in soils were compared to that for sodium arsenate. Relative bioavailabilities of soil arsenic ranged from 11-53% (mean = 33%). In vitro soil arsenic bioaccessibility values were strongly correlated with soil arsenic relative bioavailability values (R^2 = 0.92). Among physicochemical properties, combined concentrations of iron and aluminum accounted for 80 and 62% of the variability in estimates of relative bioavailability and bioaccessibility, respectively. The multifaceted approach described here yielded congruent estimates of arsenic bioavailability and evidence of interrelations among physicochemical properties and bioavailability estimates.

INTRODUCTION

The metalloid arsenic (As), a group 1 human carcinogen (IARC 2004), is the second most common inorganic contaminant at Superfund sites (U.S. EPA 2001). Hence, cancer risk associated with ingestion of As-contaminated soils (Calabrese et al. 1996; Davis et al. 1991; Dudka and Miller 1999) often drives risk assessments for human exposure to metal contaminants at Superfund sites (U.S. EPA 2007c). With increasing urbanization, exposure to As-contaminated soils grows more likely as residential areas extend into the vicinity or, in some cases, intrude onto Superfund sites (Scheckel et al. 2009). Reliable analysis of human health risks from ingestion of As-contaminated soil depends on estimating the bioavailability of As in the soil (U.S. EPA 1989). Current exposure estimates from ingestion of As-contaminated soils often do not consider differences between the bioavailability of As in water and soil (Ehlers and Luthy 2003). The use of default values that assume equivalent bioavailabilities for As in the two matrices can overestimate risk associated with ingestion of As-contaminated soil (Bradham and Wentsel 2010; U.S. EPA 2007b, 2007c). Speciation of As in soil, concentrations of other metals or metalloids, and other soil properties (e.g., pH and mineralogy) can affect the bioavailability of soil As and the amount available for systemic disposition (Kelly et al. 2002; NRC 2003; U.S. EPA 2007b). Because even small adjustments in soil As bioavailability estimates can significantly affect estimated risk and cleanup goals (U.S. EPA 2007c), methods are needed that quickly and inexpensively provide accurate and reliable data that can be applied to cleanups of As-contaminated sites worldwide.

Studies of soil As bioavailability have used species as diverse as rodents, swine, and monkeys (Casteel et al. 1997; Freeman et al. 1995; Lorenzana et al. 1996; Nagar et al. 2009; Ng et al. 1998; Pascoe et al. 1994; Rees et al. 2009; Roberts et al. 2002). Time and cost considerations may limit use of some species in bioavailability assays (U.S. EPA 2007b). In the present study, the mouse was the test species of choice because of low purchase and husbandry costs, ease of handling, improved predictive value of data due to the feasibility of an increased sample size in assays, and the potential for widespread use of a

mouse-based assay in many laboratories. Mice are well characterized physiologically and can be manipulated experimentally (e.g., altered dietary components, altered genotype) to determine the effects of biological variation on the gastrointestinal absorption of metals and metalloids. Extant data on gastrointestinal absorption of ingested arsenicals facilitate use of the mouse as a test species in assays of soil As bioavailability (Hughes et al. 2003, 2005, 2008). Although there are differences between mice and humans in metabolism and disposition of arsenicals (Vahter 1999), similarities are sufficient to permit use of mouse data to create physiologically based pharmacokinetic models which can be scaled for humans (El-Masri and Kenyon 2008; Evans et al. 2008; Gentry et al. 2004a, 2004b; Hughes et al. 1999).

Use of complementary experimental approaches to assess bioavailability has been advocated as a strategy to develop models that reduce uncertainty in risk assessment (NRC 2003). Here, animal-based and in vitro assays have been linked with physicochemical characterization of soils in a unified approach to develop accurate and reliable methods for risk assessment of As-contaminated soils. Results for test soils and standard reference materials (SRMs) suggest that concerted use of in vivo and in vitro methods combined with physicochemical characterization of soils provides a stronger scientific basis for the refinement of risk assessments for As-contaminated soils. In addition, correlations between physicochemical properties of soils and estimates of As bioavailability and bioaccessibility indicated that use of physicochemical properties could profitably inform the refinement of both animal-based and in vitro assays.

METHODS

Soil origin, processing, and physicochemical characterization - Please see Supplemental Material for full description of soil origin, processing, and physicochemical characterization. Soils used in this study were collected from sites affected by mining and smelter activities. Physicochemical properties were determined in duplicate samples of each 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. A principal component analysis coupled with linear combination fitting (LCF) was used to identify the major As species in the samples. Linear combination fits (LCF) were performed using XAS k^2 space spectra from reference standards to As phases in the soil samples.

Arsenic concentrations in all soil and biological samples were determined by Instrumental Neutron Activation Analysis (INAA) at the Department of Nuclear Engineering, North Carolina State University, Raleigh (mean As mass detection limit of 0.035 µg). All bioavailability and bioaccessibility calculations were based on INAA values.

Mouse Bioavailability Assay – The Institutional Animal Care and Use Committee of the U.S. EPA National Health and Environmental Effects Research Laboratory approved a protocol for mouse use that assured humane treatment and alleviation of suffering. Four to six week-old female C57BL/6 mice (Charles River Laboratory, Raleigh, NC) were acclimated in groups of three in a 12 hour light–12 hour dark photocycle at 20-22^{o}C. Mice had free access to rodent diet (TestDiet, Richmond, IN) and tap water that contained less than 11 µg of As per liter (Kenyon et al. 2008). Composition of AIN-93G purified rodent diet (Reeves et al., 1993) obtained from Dyets (Bethlehem, PA) is given in Supplemental Material, Table 1. Soil-amended diets were prepared by thorough mixing of test soil with powdered AIN-93G purified rodent diet to a 1% (w/w) soil:diet ratio. Arsenate-amended diet prepared by addition of sodium

arsenate heptahydrate (Sigma, St. Louis, MO) to powdered AIN-93G purified rodent diet was used to determine the bioavailability of a freely soluble As salt. Diets were stored at 4^{o} C until used.

At an assay's beginning, three mice housed together during acclimation were transferred *en group* to a metabolic cage that separated urine and feces (Nalgene, Rochester, NY). Twelve mice in four metabolic cages constituted an experimental run. Metabolic cages were maintained for 10 days under environmental conditions given above with unlimited access to test diet and drinking water. For sample collection and data analysis, the unit of observation was the cage and the standard assay for a soil had a sample size of four (except soil 9 where sample size was three). To examine assay variability and reproducibility, bioavailability of As in soils 4 and 10 were assayed two and three times, respectively, over a two-year period.

Daily food consumption for each cage was calculated as the difference between the weight of the food hopper immediately after each morning's filling and before replenishment the following morning. Cumulative food consumption for each cage was the sum of daily food consumption. Urine and feces were collected each morning from each metabolic cage. Combined body weights of the three mice in each metabolic cage were determined immediately before initial transfer into the metabolic cage and at termination. Mice were euthanized by CO_2 anesthesia on day 10.

Daily urine or feces collections for each cage were stored at -20^{o}C until processed to produce a single cumulative urine sample and single cumulative feces sample. After thorough mixing, multiple aliquots of the cumulative urine sample for each cage were taken for determination of As concentration by INAA. Cumulative urinary excretion of As was calculated as the product of As concentration in the cumulative urine sample and the volume of the cumulative urine sample. Cumulative feces samples were homogenized with a Spex CertiPrep 6850 Freezer/Mill (Metuchen, NJ). Multiple aliquots of cumulative feces sample were taken for determination of As concentration by INAA. Cumulative fecal excretion of

As was calculated as the product of As concentration in the cumulative feces sample and the mass of the cumulative feces sample.

Absolute bioavailability (ABA) of As from ingestion of a soil or arsenate-amended diet was calculated as the ratio of cumulative excretion of As in urine and cumulative dietary intake of As (NRC 2003, U.S. EPA 2007c). ABA is commonly calculated and expressed on a percentage basis:

% ABA = [Cumulative µg As excreted in urine/Cumulative µg As consumed]*100 [1]

Relative bioavailability (RBA) was calculated as the ratio of the ABA for As in a specific soil-amended diet to the ABA for As in a diet containing sodium arsenate (NRC 2003, U.S. EPA 2007c). RBA is commonly expressed on a percentage basis:

% RBA = [ABA for As in a specific diet/ABA of As in sodium arsenate]*100 [2]

Bioaccessibility assays - Please see Supplemental Material for a full description of bioaccessibility assays. Bioaccessible As was determined using an in vitro method developed by the Solubility/Bioavailability Research Consortium (SBRC assay) (Kelly et al. 2002). In vitro assays were performed in triplicate for each soil and included addition of one gram test soil to 100 mL of 0.4 M glycine, pH 1.5 gastric fluid in 125 mL HDPE bottle, rotating end-over-end in a water bath at 37^{o}C for one hour. All soils tested in the bioaccessibility protocol were identical to those administered to mice in the in vivo and mineralogy studies described above. All in vitro extraction solutions were refrigerated at 4 ^{o}C for preservation and subsequent analysis by ICP-OES (U.S. EPA 2007d).

In vitro bioaccessibility (IVBA) was calculated and expressed on a percentage basis using the following equation:

IVBA (%) = [in vitro extractable (mg kg^{-1})/total contaminant (mg kg^{-1})]*100 [3]

Statistical analysis - Simple linear regression was used to evaluate the relationship between in vivo As RBA data and in vitro bioaccessibility data and to examine the role of selected soil physicochemical

properties on As RBA and bioaccessibility. All analyses were performed using R version 2.9.1 (R Development Core Team, Vienna, Austria), and figures were created using GraphPad Prism version 5.0 (GraphPad, San Diego, CA).

RESULTS

Soil Characterization -Table 1 summarizes selected characteristics of test soils. Total As concentration in test soils ranged from 173 to 6899 mg kg^{-1}. As speciation by oxidation state varied among soils (See Supplemental Material, Figure 1). Soils 1, 3, 4, 7 and 11 had varying ratios of arsenite (As^{III}) to arsenate (As^{V}) species; soils 2, 5, 6, 8, 9, and 10 contained only arsenate. Realgar was identified in soils 1, 3, 4, and 11 and arsenopyrite was identified in soils 4 and 7. Sorbed arsenate and scorodite are common As species in soil environments and often result from the oxidation of As ore materials such as realgar or arsenopyrite. Concentrations of Fe, Mn, and Al in soils ranged from 18.9 to 294.4 g kg^{-1}, 0 to 8.5 g kg^{-1}, and 3.9 to 21.7 g kg^{-1} respectively. Soil pH ranged from 2.1 to 7.3.

Mouse Bioavailability Assay - The gross clinical condition of mice was unaffected by ingestion of any of the amended diets; amendment of diet with soil or sodium arsenate did not significantly affect cumulative diet consumption (data not shown). Thus, amendment of AIN-93G rodent diet with 1% (w/w) soil or arsenate did not affect diet palatability for mice. Mean cumulative consumption of As strongly correlated with the concentration of As in the diet (See Supplemental Material, Figure 2). Mouse assay performance was evaluated by determining the percentage of cumulative As intake recovered in cumulative urine and feces collections. Arsenic recoveries in excreta averaged 83.7% (range 67 to 96%) for sodium arsenate- or soil-amended diets. For all dietary additives, percentage recovery and dietary As concentration were not correlated ($R^2 = 0.227$, $P = 0.398$ by Pearson Product Moment Correlation).

Increasing cumulative ingestion of As from amended diets was associated with increasing cumulative urinary excretion of As (Figure 1). Figure 2a shows As ABA estimates from diets amended with arsenate, test soils, or SRMs. Duplicate assays with arsenate-amended diet yielded an As ABA of ~60%. As ABA estimates for test soils ranged widely from ~7 to ~33%. Duplicate assays with diets amended with soil 4 (4a, 4b) yielded As ABA estimates of 6.7 and 7.1%. Triplicate assays with diets amended with NIST-2710 Montana Soil SRM (10a, 10b, 10c) yielded As ABA estimates ranging from

25.9 to 27.2%. For comparison, NIST-2710a SRM-amended diets dosed at multiple levels yielded an As ABA of ~ 26% for each dosage level (Supplemental Material, Figure 2). Figure 2b shows As RBA estimates for test soils and SRMs. Relative to arsenate bioavailability, As RBA estimates for test soils ranged from 11 to 53%. As RBA estimates for NIST-2710 Montana soil-amended diet and NIST2710a soil-amended diet were ~44%. Supplemental Material, Table 2 summarizes data from mouse assays.

Correlations among Estimates of Bioaccessibility and Bioavailability and Physicochemical Properties- IVBA values ranged from 6.8 to 67% (SD 0 – 3%). NIST SRMs (Soils 10 and 11) were extracted multiple times over the course of the study in accordance with the SBRC assay (SDs were 4.1 and 1.7, respectively). Predictability of As RBA estimates from the mouse assay by the estimates of bioaccessibility from the SBRC assay was assessed by linear regression analysis. The derived regression model accounted for 92% of the variability in As bioavailability observed in the mouse assay (R^2 = 0.92, Pearson correlation = 0.96) (Figure 3).

Predictability of As bioavailability or bioaccessibility from the physicochemical properties and speciation of As in soils was examined by simple linear regression analysis (Table 2). Physicochemical properties of soil that were significant predictors ($P < 0.10$) of As RBA estimates were also significant predictors of IVBA estimates, with the exception of the percent arsenopyrite term. Among predictors, FeAl (Fe+Al concentration) accounted for the largest amount of variation in RBA and IVBA estimate (R^2 = 0.58 and 0.40, respectively). Log (FeAl) improved the predictive value of this term (R^2 = 0.80 and 0.62 for RBA and IVBA respectively). Although multivariable linear regression analysis has been used to estimate As bioavailability (Yang et al. 2002), application of this method in the present study did not materially improve predictions of As RBA or IVBA.

DISCUSSION

The concordance of RBA and bioaccessibility estimates obtained in mouse and in vitro assays with common physicochemical characteristics of soils suggested that these approaches could be used in a complementary manner to reduce uncertainty in assessment of risk associated with exposure to As-contaminated soils.

The mouse assay proved adaptable for use with soils with a wide range of As concentrations and physicochemical properties. Amended diets were palatable and, as anticipated from earlier studies (Xie et al., 2004), mice remained in apparent good health throughout the experimental period. In this study, calculation of the absolute bioavailability of arsenic used results from the mouse assay for a diet amended with 7 ppm of arsenic as sodium arsenate. This amendment produced arsenic dosage levels of 8.9 and 9.2 mg kg^{-1} in duplicate studies (Supplemental Material Tables 1 and 2). The dosage level for arsenate-amended diets exceeded those for contaminated soils 3, 5, 6, 8, 10b, approximately equaled (i.e., with overlapping standard deviations) those for soils 4a, 4b, 10a,10c, and was lower than those for soils 1, 2, 7, 9, 11. Hence, for most soils tested, the concentration of arsenate added to the diet equaled or exceeded that present in diet after soil amendment. Although additional studies with arsenate-amended diets are needed to confirm that estimates of bioavailability of arsenate or As in soil are unaffected by arsenic concentration in amended diets, studies in arsenate-treated laboratory mice suggest that dosage level does not affect the rate of urinary clearance of arsenic (Hughes et al., 1994; Hughes and Thompson, 1996; Kenyon et al., 2008). Similarities in the pattern and extent of urinary clearance of arsenic in mice which have received sodium arsenate over a wide range of dosage levels suggests that dosage level does not influence uptake of arsenate across the gastrointestinal barrier or its clearance into urine. In the absence of a change in the rate of urinary clearance of arsenic over a wide dosage range, it is likely that mice

ingesting diets amended with arsenate or arsenic-containing soils will reach whole body steady state body burden during the experimental period used in this study (Hughes et al., 2003).

Similar estimates of As bioavailability obtained for soils 4 and 10 in assays over a two-year period indicated that assay performance was stable (Figure 2a and b). In adult female mice receiving repeated daily oral doses of sodium arsenate, the body burden of As reaches steady state after eight or nine days of dosing (Hughes et al. 2003, 2010). Under steady state condition, concentrations of As in tissues and outputs of As in urine and feces will reach plateau values that will remain unchanged throughout the dosing interval. Although concentrations of As in urine and feces are both good indicators of current exposure, the predominance of urine as the route for As clearance after oral administration of inorganic As (Hughes et al. 2003) makes it ideal for estimating the extent of absorption of dietary As. Summing amounts of As excreted in urine and feces during the experimental period can be used to approximate recovery of As in the mouse assay. For the materials evaluated in the mouse assay, recoveries of ingested As in excreta ranged from 67 to 96%. However, these values should be regarded as minimal estimates as they do not include As that is retained in tissues of mice.

The mouse assay can be further refined by examining the role of dietary composition on the estimates of soil As bioavailability obtained with this model. Compared with AIN-93 purified diets, the human diet commonly consumed in developed countries derives more calories from fat, contains less fiber, and may not be optimal in terms of mineral and vitamin composition. These differences in dietary composition could affect the bioavailability of arsenic in two ways. First, the elemental composition of the diet can affect As uptake across the gastrointestinal barrier. For example, an increasing concentration of phosphate reduces in vitro uptake of arsenate by Caco-2 intestinal cells derived from human colonic adenocarcinoma cells (Calatayud et al. 2010) and gastrointestinal uptake of As in rats dosed orally with arsenate (Gonzalez et al. 1995). Second, in humanized gnotobiotic mice the microbiota of the

gastrointestinal tract is quickly altered by consumption of a diet with a high fat and high sugar content (Turnbaugh et al. 2009). Alteration of the microbiota of the gastrointestinal tract produced by changes in dietary composition could alter gastrointestinal uptake of ingested arsenate. Recent studies show that the anaerobic microbiota from the mouse cecum extensively metabolize arsenate to produce inorganic thioarsenicals and methylated oxy- and thioarsenicals (Pinyayev et al. 2011). The mouse model can readily be adapted to examine effects of dietary composition of diets on the bioavailability of As in soils.

Soil As RBA estimates obtained in juvenile swine and monkeys have ranged from 0 to 52% (Casteel et al. 1997; Freeman et al. 1995; Lorenzana et al. 1996; Rees et al. 2009; Roberts et al. 2002; Rodriguez et al. 1999). Comparisons of As RBA data obtained in mice and juvenile swine are problematic due to differences in experimental design and dosing levels. However, there are four soils that have been evaluated in both species. For three soils (soils 9, 10, 11 in this study), As RBA estimates from mouse and juvenile swine differed by 4, 0, and 1%, respectively (U.S. EPA 2009). For the fourth soil (soil 8 in this study) As RBA estimates differed for mouse (40.9%) and juvenile swine (60%). Differences in As RBAs for mouse and juvenile swine may reflect physiological differences between species. Additional soils should be evaluated in both species to identify possible sources of variability and permit a detailed comparison of the assays.

A recent NRC report has recommended development and validation of in vitro assays that can replace in vivo assays and can provide reliable and accurate data that reduces uncertainty in risk assessment (NRC 2007). This recommendation prompted development of bioaccessibility assays that reflect processes that control As bioavailability in the human gastrointestinal tract (Basta et al. 2007, Juhasz et al. 2007, Kelly et al. 2002, Rodriguez et al. 1999, Ruby et al. 1999). High correlation (R^2 = 0.92, Pearson correlation = 0.96) between the As bioaccessibility data from the SBRC assay and As RBA estimates from the mouse assay is consistent with the high correlation of estimates of As RBA from juvenile swine with As bioaccessibility estimates from the SBRC assay (R^2 = 0.75, Pearson correlation =

0.87) (Juhasz 2009). The correlation of findings from the SBRC assay and the mouse assay suggests that the bioaccessibility assay provides useful information about the characteristics of As-containing soils that influence As RBA as measured in the mouse assay. In addition, strong agreement of estimates from the SBRC in vitro assay and the mouse assay suggest that the mouse assay can be used to validate performance of bioaccessibility assays.

Metal speciation and the concentrations of Fe, Al, and Mn are known to affect solubilities and bioavailabilities of metals in soils (Bradham et al. 2006; Kelly et al. 2002; NRC 2003; Scheckel et al. 2009). Here, the effects of As speciation and metal concentrations on estimates of soil As RBA and bioaccessibility obtained in the mouse assay and SBRC assay were evaluated by linear regression analyses. Significant inverse correlations ($P < 0.10$) were found between concentrations of extractable Fe or Al in soils and between sums of concentrations of extractable soil Fe and Al (Fe+Al) and estimates of soil As RBA and bioaccessibility. For example, the log-transformed sum of extractable Fe and Al concentrations accounted for 80% and 62% of the variability in estimates of As RBA and bioaccessibility, respectively. The high predictive value of log (Fe+Al) suggested that sorption of As to Fe and Al oxides reduced As solubilization and thereby reduced As RBA and bioaccessibility. Similar results were found by Beak et al. (2006a, 2006b) for As bioaccessibility using a modified Rodriguez et al (1999) in vitro method which investigated As sorption on ferrihydrite ($Fe^{3+}_5O_3(OH)_9$) and corundum (Al_2O_3). Thus, determination of the concentrations and forms of Fe and Al in soils may be useful in assessing As bioavailability. There are several clay minerals that contain ferrous and ferric iron which, upon release via weathering, will form iron oxides and hydroxides in soil environments (Bowell 1994). Similar processes are also identified for aluminum and manganese oxides in soils (Jenne 1968; McKeague et al. 1971). Lower As RBA estimates for soils containing sulfide forms of As (realgar or arsenopyrite) may reflect slow dissolution kinetics of these mineral species. Although arsenopyrite was only present in two of the test soils, its presence significantly reduced As bioavailability estimates ($P < 0.10$). This finding is

consistent with reports showing that As in arsenopyrite is bound tightly; therefore, As bioavailability is likely to be low (Roberts et al. 2007). Additional studies would be useful to identify other metals and metalloids in soils that are potential modifiers of As bioavailability and bioaccessibility and to determine concentration dependencies of these interactions.

CONCLUSION A multifaceted approach combining in vivo assays, in vitro assays, and physicochemical characterization of soils yielded comparable estimates of As bioavailability and provided evidence of interrelations among physicochemical properties and estimates of As bioavailability. The range of As RBA estimates in this study (11 to 53%) implies that use of a default value of 100% for As bioavailability in human health risk assessments may overestimate risk associated with exposure to As-contaminated soils. Further studies with the mouse assay and the in vitro assay coordinated with physiochemical characterization of test soils can confirm and extend the results obtained in this study and identify refinements in experimental design and data analysis that can improve the accuracy and reliability of estimates of bioaccessibility and bioavailability.

SUPPLEMENT MATERIAL AVAILABLE

Figures and tables showing the XANES spectra for the soils, the composition of basal purified rodent diet, summary data on As consumption, dosage level, excretion, and bioavailability for materials evaluated in mouse assays, and the relationship between concentrations of As in mouse diet and mean cumulative consumption of As over the experimental period can be found in the Supplement Material.

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Table 1. Description, elemental composition and arsenic speciation in test soils[a]

		Soil Properties					Arsenic Speciation*				
							Arsenate (As^V)		Arsenite (As^{III})		
Soil ID	Description[b]	As[c] (mg/kg)	Fe[d,e] (g/kg)	Mn[d,e] (g/kg)	Al[d,e] (g/kg)	pH[e]	Sorbed As^V (%)	Scorodite (%)	Realgar (%)	Arsenopyrite (%)	χ^2 red[f]
1	urban residential	990	20.9	0.5	11.8	6.1	52.0	21.2	26.8	-	0.004
2	urban residential	829	20.5	0.7	9.4	6.3	96.7	3.3	-	-	0.004
3	urban residential	379	18.9	0.2	9.0	5.0	53.1	15.2	31.7	-	0.003
4	smelter slag	837	294.4	2.7	13.2	7.2	18.7	1.6	47.7	32.1	0.001
5	residential	244	46.0	0.8	21.7	7.3	96.2	3.8	-	-	0.002
6	residential	173	63.4	0.7	20.9	6.6	66.8	33.2	-	-	0.002
7	smelter slag	6899	144.5	0.9	15.0	5.2	18.3	47.1	-	34.6	0.001
8	residential	280	72.3	0.0	3.9	2.1	79.5	20.5	-	-	0.007
9	smelter slag	4495	120.1	0.4	12.3	2.6	67.6	32.4	-	-	0.011
10	NIST 2710	601	29.2	8.5	17.2	5.0	95.0	5.0	-	-	0.007
11	NIST 2710a	1513	34.0	1.7	10.0	4.0	66.8	23.2	9.9	-	0.01

[a] < 250 µm particle size fraction used for all analyses
[b] Source of arsenic contaminated soil
[c] Determined by Instrumental Neuron Activation Analysis
[d] Extracted using EPA Method 3051A and analyzed by ICP-OES 6010C
[e] Data represents the mean of duplicate analyses
[f] Reduced chi-squared values = $[(\text{data-fit})^2] / [\text{data}^2]$
* Determined by linear combination of As XAS

Table 2 - Results of linear regression analyses to explore the influence of select soil properties on arsenic relative bioavailability (RBA) and in vitro bioaccessibility (IVBA).

predictor	RBA			IVBA		
	equation	R^2	P value	equation	R^2	P value
sorbed As(V) (%)	RBA = 0.2x + 17.1	0.14	0.26	IVBA = 0.3x + 18.4	0.11	0.31
scorodite (%)	RBA = -0.4x + 38.9	0.10	0.35	IVBA = -0.7x + 50.9	0.16	0.22
realgar (%)	RBA = 0.1x + 31.1	0.01	0.80	IVBA = 0.2x + 36.1	0.01	0.73
arsenopyrite (%)	RBA = -0.7x + 36.2	0.28	0.09*	IVBA = -0.7x + 42.5	0.16	0.23
AsV (%)	RBA = 0.2x + 19.0	0.05	0.50	IVBA = 0.1x + 26.9	0.02	0.70
AsIII (%)	RBA = -0.2x + 34.7	0.05	0.50	IVBA = -0.1x + 40.2	0.02	0.70
As (mg/kg)	RBA = x + 37.3	0.17	0.21	IVBA = x + 45.2	0.15	0.23
Fe (g/kg)	RBA = -0.1x + 43.5	0.48	0.02**	IVBA = -0.2x + 51.4	0.32	0.07*
Al (g/kg)	RBA = -1.9x + 57.3	0.34	0.06*	IVBA = -2.7x + 73.3	0.32	0.07*
Mn (g/kg)	RBA = 0.7x + 31.0	0.01	0.77	IVBA = 1.1x + 36.3	0.01	0.76
pH	RBA = -2.2x + 43.3	0.05	0.52	IVBA = -1.2x + 44.0	0.01	0.82
FeAl (mol/kg)	RBA = -8.8x + 48.7	0.58	0.01***	IVBA = -10.5x + 57.9	0.40	0.04**
log(FeAl) (mol/kg)	RBA = -53.1x + 41.6	0.80	0.00***	IVBA = -67.5x + 50.1	0.62	0.00***

* $P \leq 0.10$, ** $P \leq 0.05$, *** $P \leq 0.01$

LEGENDS

Figure 1. Relationship between cumulative arsenic intake and cumulative urinary arsenic excretion. Soils identified in Table 1. Replicate assays shown for soil 4 (4a, 4b) and soil 10 (10a, 10b, 10c); NaAs is sodium arsenate-amended diet. (Mean and standard deviation shown)

Figure 2. Absolute (a) and relative (b) bioavailabilities of arsenic from amended diets. Results expressed as function of cumulative arsenic intake. Soils identified in Table 1. Replicate assays shown for soil 4 (4a, 4b) and soil 10 (10a, 10b, 10c); NaAs is sodium arsenate-amended diet. (Mean and standard deviation shown)

Figure 3. Correlation between estimates of arsenic bioaccessibility and bioavailability**.** Linear regression analysis of correlation between estimates of bioaccessibility (% IVBA) and estimates of relative bioavailability (% RBA). Soils identified in Table 1. (Mean and standard deviation shown)

Figure 1

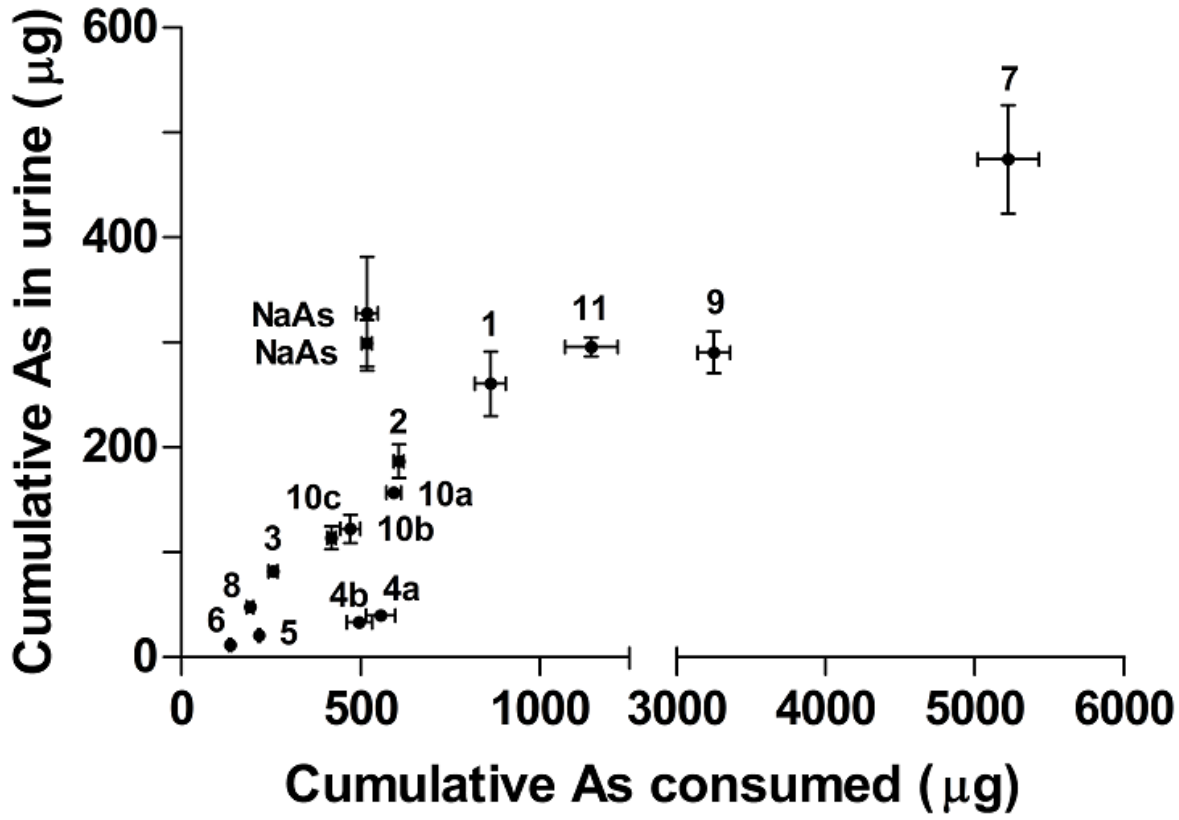

Cumulative As in urine (μg)
600
400
200
0
0
500
1000
3000
4000
5000
6000
Cumulative As consumed (μg)
NaAs
NaAs
1
11
9
7
2
10c
10a
10b
3
8
4b
4a
6
5

Figure 2

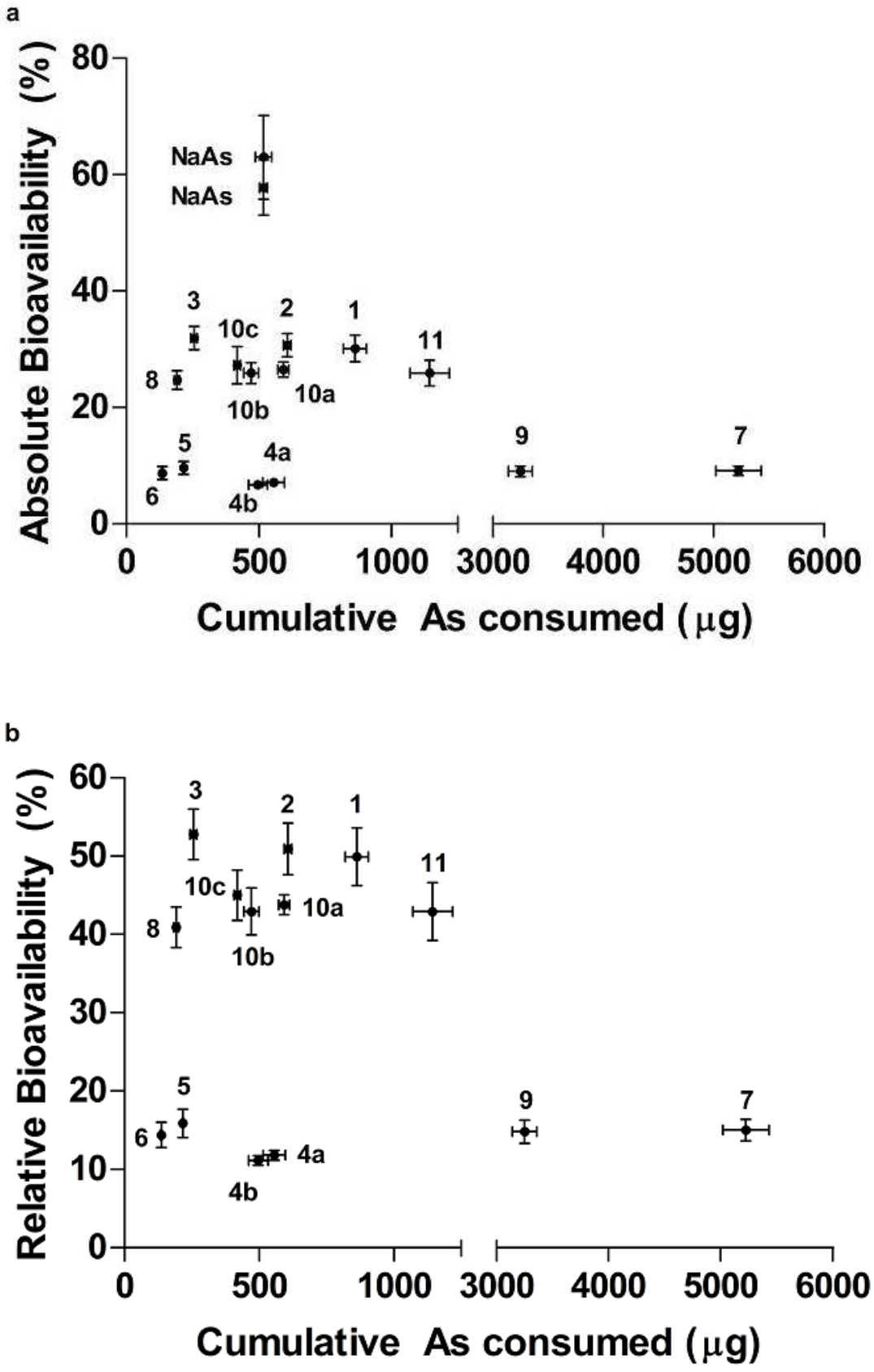
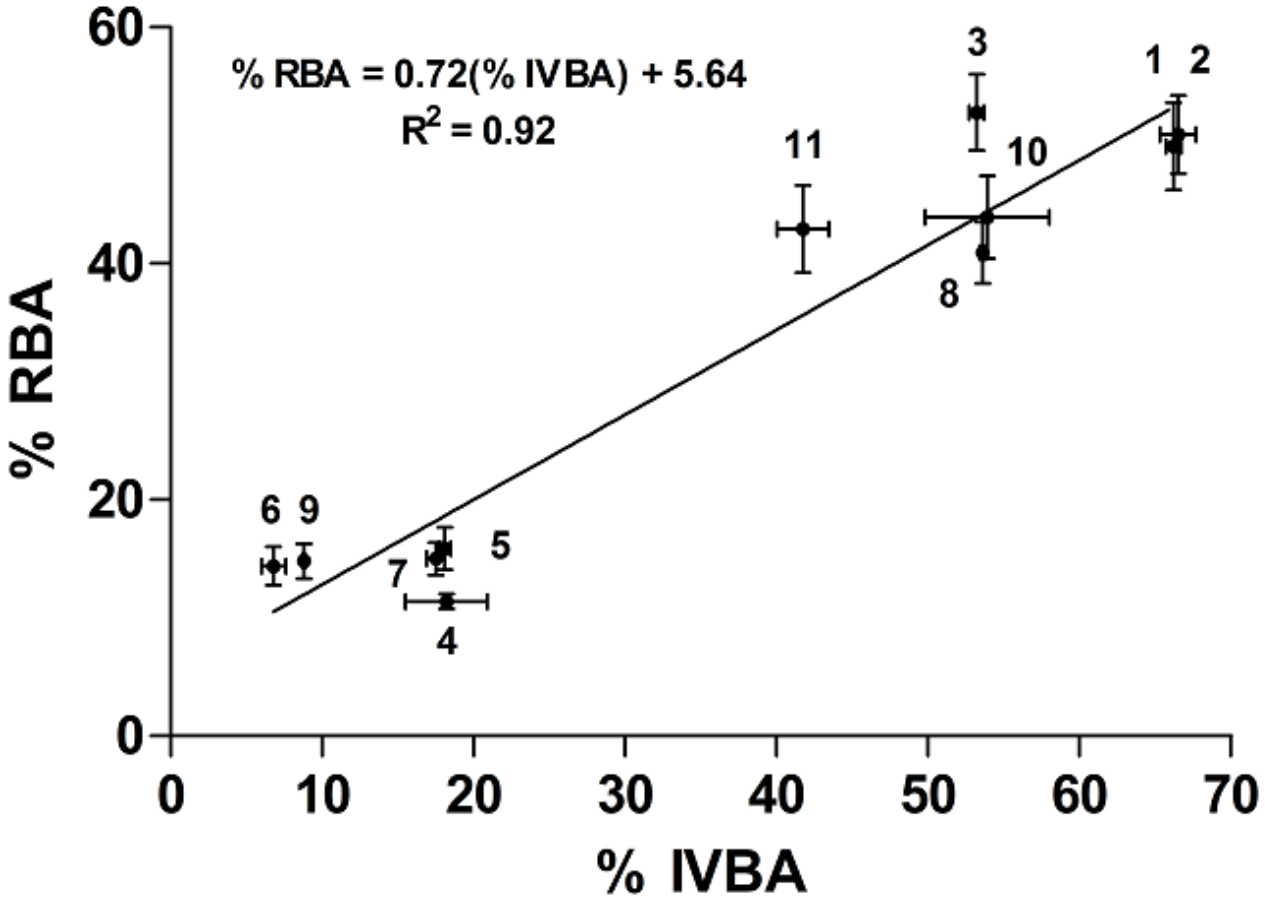

a
Absolute Bioavailability (%)
80
60
40
20
0
NaAs
NaAs
3
10c
2
1
11
8
10b
10a
9
7
5
4a
6
4b
0
500
1000
3000
4000
5000
6000
Cumulative As consumed (μg)
b
Relative Bioavailability (%)
60
50
40
30
20
10
0
3
2
1
11
10c
10a
8
10b
5
9
7
6
4a
4b
0
500
1000
3000
4000
5000
6000
Cumulative As consumed (μg)

Figure 3


% RBA = 0.72(% IVBA) + 5.64
R2 = 0.92
% RBA
% IVBA
0
20
40
60
0
10
20
30
40
50
60
70
1
2
3
4
5
6
7
8
9
10
11

Supplemental Material:

Relative Bioavailability and Bioaccessibility and Speciation of Arsenic in Contaminated Soils

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METHODS

Soil processing and physicochemical characterization - Soils used in this study were dried (< 40°C) and sieved to < 250 μm. The U.S. EPA considers particles < 250 μm most likely to adhere to hands and be subsequently ingested by hand-to-mouth contact, especially in young children (U.S. EPA 2007a, 2007b). Soil samples were homogenized, riffled, and aliquots were split for each of the participating labs by procedures described in Blume et al. (1991).

Selected extractable inorganics in each soil were determined by extracting the < 250 μm soil using U.S. EPA Method 3051A (2007c) with analysis by Varian Vista-MPX Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) according to U.S. EPA Method 6010C (2007d). Soil pH was determined using 1:1 soil:water suspension using a combination pH electrode (Thomas 1996).

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 N_2 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 energy was calibrated by assigning the first derivative inflection point of the L_{III}-absorption edge of gold metal (11919 eV) foil. Three As K_α (11867 eV) 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. Linear combination fits (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 ($As^{III}S$), and arsenopyrite ($FeAs^{III}S$). Data for LCF fits reveal As speciation in each soil as ratios of these mineral forms.

Mouse Bioavailability Assay - Female C57BL/6 mice (four to six weeks old) were purchased from Charles River Laboratory (Raleigh, NC). During acclimation, these mice were housed in groups of three in polycarbonate cages with cellulose bedding and environmental enhancements (e.g., cardboard tubes) in a 12 hour light–12 hour dark photocycle at 20-22 ^{o}C. Mice had free access to rodent diet (TestDiet, Richmond, IN) and sipper-type water bottles containing tap water filtered on-site with inline sand and charcoal filters and re-chlorinated to a final concentration of 3 to 5 ppm Cl.

The basal diet was AIN-93G purified powdered rodent diet (Dyets, Bethlehem, PA), which is formulated to support growth, pregnancy, and lactation in mice (Supplemental Material, Table 1) (Reeves et al. 1993). Soil-amended diets were prepared by thorough mixing of 10 g of test soil with 990 g of AIN-93G purified rodent diet with a goal of a 1% (w/w) soil:diet ratio. A sodium arsenate-amended diet prepared by addition of sodium arsenate heptahydrate (Sigma, St. Louis, MO) to AIN-93G purified rodent diet was performed in duplicate to determine the bioavailability of a freely soluble As salt for comparison with bioavailabilities of As in different soils. All diets were stored at 4 ^{o} C until used.

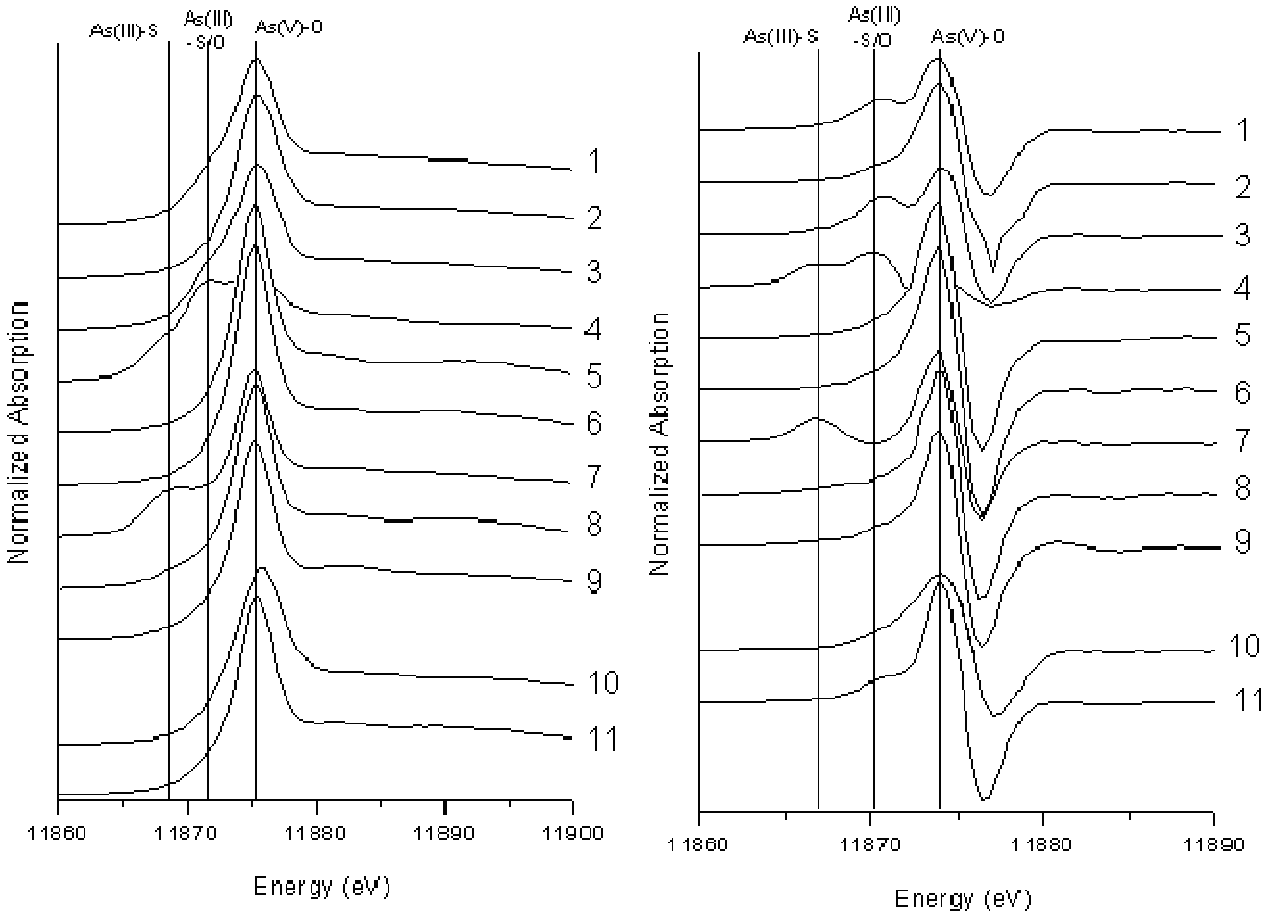
Mice were placed in metabolic cages on the morning of day 1 with free access to diet and drinking water for 9 days. On the mornings of day 2 through 10, urine and feces were collected

from each metabolic cage. Food hoppers were removed on the afternoon of day 9 and mice were fasted overnight with free access to drinking water until the morning of day 10.

For each cage, daily urine or feces collections were pooled to create cumulative urine and feces samples. These samples were stored in freezers at -20 ^{o}C until processed. Pooling of urine and feces collections over the experimental period yielded a single sample for each metabolic cage. The pooled urine sample for each cage was thawed at room temperature and its volume determined. This sample was well mixed and multiple aliquots were taken for determination of the concentration of As by INAA (mean As mass detection limit of 0.035 µg).

Bioaccessibility assays - Bioaccessible As was determined using an in vitro method developed by the Solubility/Bioavailability Research Consortium (SBRC method) for comparison with in vivo data (Kelly et al. 2002). All soils tested in the bioaccessibility protocol were identical to those administered to mice in the in vivo and mineralogy studies described above. In vitro procedures were conducted by adding one gram of test soil to 100 mL of buffered glycine solution (0.4 M glycine, pH 1.5) in a 125 mL HDPE bottle, rotating end-over-end in a water bath at 37 ^{o}C (body temperature) for one hour. Extractions were performed in triplicate for each test soil. Blanks, National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) soils (Montana soils 2710 and 2710a), and spikes were analyzed to meet quality assurance and quality control requirements. All labware were cleaned, washed, and rinsed with deionized water prior to use according to a standard protocol. Analysis of the extracts by ICP-OES included QA/QC procedures as described above. The method detection limit (MDL) in extraction fluid was calculated to be 0.1 mg L^{-1} for Method 6010C.

Normalized XANES spectra **Derivative of the XANES spectra**



Supplemental Material, Figure 1. Normalized and derivative of the XANES spectra. Soils 1 through 3 were collected from urban residential sites. Soils 4, 7, and 9 are slag soils from smelter sites. Soils 5, 6, and 8 came from non-urban residential sites. Soil 10 is NIST 2710 and soil 11 is NIST 2170a.

Supplemental Material, Table 1. Composition of basal AIN-93G purified rodent diet[a]

Ingredient	Grams per kilogram	Kilocalories per kilogram
Casein	200	716
Sucrose	100	400
Cornstarch	397.486	1430.9496
Dyetrose	132	501.6
L-cystine	3	12
Cellulose	50	0
Soybean oil	70	630
t-Butylhydroquinone	0.014	0
Mineral mix	35	30.8
Vitamin mix	10	38.7
Choline bitartrate	2.5	0
	1000	3760.0496

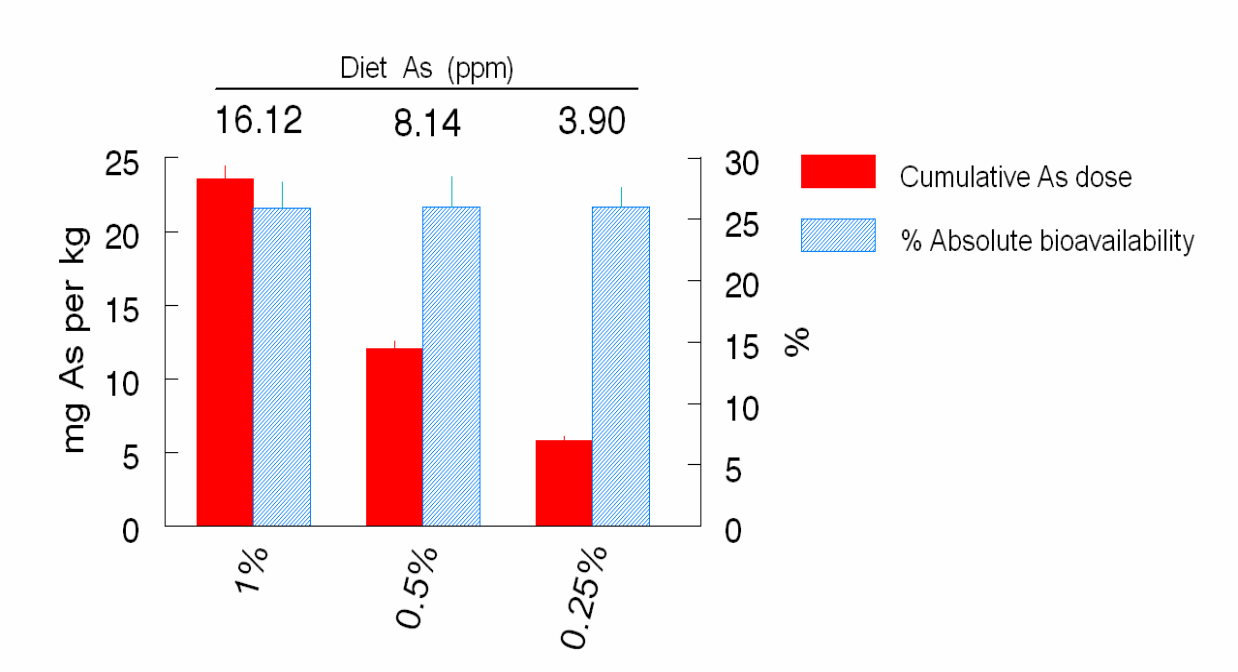
a. Composition of AIN-93 G purified rodent diet provided by Dyets (Bethlehem, PA) is in accordance with recommendations of the American Institute of Nutrition Ad Hoc Writing Committee of the reformulation of the AIN-76A rodent diet (Reeves et al., 1993).

Supplemental Material, Table 2. Summary data on arsenic consumption, dosage level, excretion, and bioavailability for materials evaluated in mouse assays[a].

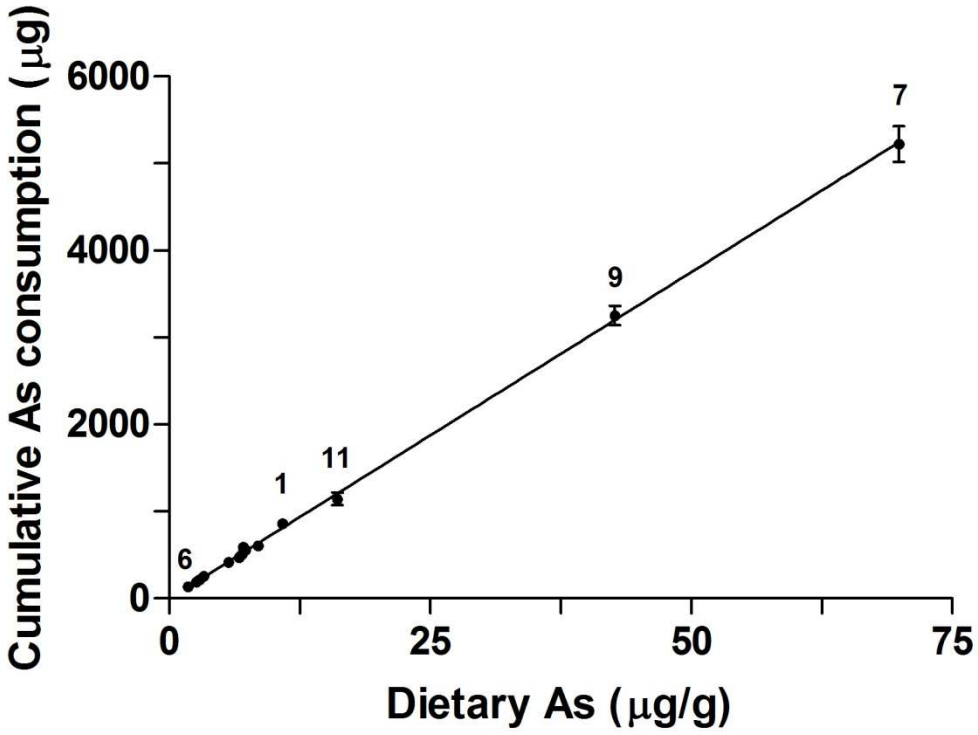
Dietary Amendment	Cumulative As consumption (µg)	As dosage (mg/kg)	Cumulative As Excretion (% of dose)			% Bioavailability	
			Urine	Feces	Sum	Absolute	Relative[b]
Sodium arsenate #1	518.0 30.4	9.2 0.5	63.0 7.2	11.6 2.2	74.6 6.6	63.0 7.2	100
Sodium arsenate # 2	517.7 14.2	8.9 0.3	57.8 4.8	8.3 1.7	66.1 3.3	57.8 4.8	
Soil							
1	862.8 43.5	15.8 0.9	30.1 2.3	47.5 6.4	77.6 4.1	30.1 2.3	49.9 3.7
2	606.9 14.3	11.9 0.1	30.8 2.0	36.6 1.8	67.4 2.8	30.7 2.0	50.9 3.3
3	256.2 12.3	5.3 0.2	31.6 1.9	56.1 0.7	87.7 2.4	31.9 2.0	52.8 3.2
4A	556.0 41.1	10.4 0.8	7.1 0.3	81.6 16.1	88.8 15.8	7.1 0.3	11.8 0.6
4B	496.8 36.1	10.4 0.5	6.7 0.3	83.3 3.1	89.9 3.2	6.7 0.3	11.1 0.6
5	217.3 3.3	4.1 0.2	9.6 1.1	68.9 1.5	78.5 1.7	9.6 1.1	15.9 1.8
6	136.0 4.0	2.6 0.1	8.7 1.0	87.6 3.7	96.3 2.8	8.7 1.1	14.4 1.6
7	5224.1 204.5	101.7 2.2	9.1 0.8	78.8 2.4	87.9 3.0	9.1 0.8	15.0 1.4
8	191.8 9.2	3.9 0.1	24.8 1.6	56.8 1.1	81.6 1.3	24.7 1.6	40.9 2.6
9	3252.0 109.4	60.3 3.3	8.9 0.9	79.5 4.7	88.4 3.8	9.0 0.9	14.8 1.5
10A	593.3 20.6	10.1 0.2	26.4 1.3	54.1 1.9	80.5 0.6	26.5 1.3	43.8 2.2
10B	419.2 11.5	6.5 0.3	27.1 3.2	54.9 2.8	82.0 4.0	27.2 3.2	45.0 5.3
10C	470.9 27.8	9.5 0.7	25.9 1.8	55.6 1.4	81.5 0.5	25.9 1.8	42.9 3.0
11	1144.7 73.8	23.7 1.0	25.8 2.2	57.8 2.1	83.6 3.5	25.9 2.2	42.9 3.7

a – Mean (upper) and standard deviation (lower). Sample size of 4 for all soils except soil 9 where sample size is 3.

b - Relative % bioavailability for soils calculated as: (absolute % bioavailability for soil)/mean absolute % bioavailability for sodium arsenate). Here, mean absolute % bioavailability for sodium arsenate was 60.4.



Supplemental Material, Figure 2. Diets amended by adding by weight 1, 0.5, or 0.25% NIST-2710a soil to the powdered purified rodent chow resulted in mice receiving arsenic at multiple dosage levels over approximately a 4-fold range. Estimates of absolute bioavailability of arsenic from this soil were the same for each dosage level and estimation of absolute bioavailability was independent of dosage level.



Supplemental Material, Figure 3. Relationship between concentrations of As in mouse diet and mean cumulative consumption of As over the experimental period. Equation for linear regression ($y = 75.38x - 4.7644$, $r^2 = 0.999$).

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