Independent data validation of an in vitro method for prediction of relative bioavailability of arsenic in contaminated soils

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Abstract

In vitro bioaccessibility assays (IVBA) estimate arsenic (As) relative bioavailability (RBA) in contaminated soils to improve accuracy in human exposure assessments. Previous studies correlating soil As IVBA with RBA have been limited by use of few soil types and sources of As, and the predictive value of As IVBA assays have not been validated using an independent set of As-contaminated soils. In this study, a robust linear model was developed to predict As RBA in mice using an IVBA assay and the predictive capability of the model was independently validated using a unique set of As-contaminated soils. Forty As-contaminated soils varying in soil type and contaminant source were included in this study, with 31 soils used for initial model development and nine soils used for independent model validation. The initial model reliably predicted As RBA values in the independent data set, with a mean As RBA prediction error of 5.4%. Following validation, 40 soils were used for final model development, resulting in a linear model with the equation: RBA = 0.65 * IVBA + 7.8 and R^2 of 0.81. The in vivo-in vitro correlation and independent data validation presented provide critical verification necessary for regulatory acceptance in human health risk assessment.

Introduction

Arsenic (As) is the most frequently occurring contaminant on the Priority List of Hazardous Substances, which lists substances of greatest public health concern to people living at or near U.S. National Priority Listing sites.[1] Human exposure to As via ingestion of As-contaminated soils can have serious health impacts including increased cancer risk.[2,3,4] Accurate assessment of human health risks from exposure to As-contaminated soils depends on estimating its bioavailability, defined as the fraction of ingested As absorbed across the gastrointestinal barrier and available for systemic distribution and metabolism. Arsenic bioavailability varies among soils and is influenced by site-specific soil physical and chemical characteristics and internal biological factors. U.S. Environmental Protection Agency (USEPA) guidance describes the need for development of soil As bioavailability methods and data to improve the accuracy of human exposure and risk calculations at As-contaminated sites.[5]

Difficulties inherent in measuring site-specific soil arsenic bioavailability in humans[6] have prompted development of in vivo animal bioassays to determine As relative bioavailability (RBA) in soil.[7-14] Although, mice and humans differ in metabolism and disposition of arsenicals, similarities are sufficient to permit use of mouse data to create physiologically based pharmacokinetic models that can be scaled for humans[7]. For these assays, the bioavailability of soil As is expressed relative to the bioavailability of a completely water soluble form of As (i.e., sodium arsenate). Currently, the USEPA requires the use of in vivo models for assessing the RBA of As- contaminated soils.[15] However, time and cost considerations often limit their use in risk assessment and result in the use of default values for As RBA.[7]

As an alternative to in vivo bioassays, in vitro bioaccessibility (IVBA) assays have been developed to measure the extent of As solubilization in simulated gastrointestinal fluids.[7, 12, 16-21] IVBA assays are attractive alternatives to in vivo assays because they are cost-effective and reduce reliance on animal studies. A prime assumption underlying these IVBA assays is that the fraction of As solubilized in vitro is similar to the fraction of As that can cross the gastrointestinal barrier.[22] If an IVBA method is an appropriate surrogate, then it must be shown to reliably predict in vivo RBA.[5] While some studies have examined the relationship between As RBA and IVBA, [7,12,16,19,20,23] validation of this relationship using an independent set of soils is the next critical step for regulatory acceptance.

Multiple in vivo animal models and in vitro methods have been proposed to assess As RBA and IVBA, respectively, in contaminated soils.[7,12,16,19,20,23] A recent study described a mouse assay as a cost effective and reproducible alternative to other animal assays.[7,8] Until recently, precision of As RBA estimates determined from repeated assays of the same soils had not been reported for any animal model. Low between-assay variation in urinary excretion fraction (UEF) and RBA estimates in the mouse assay results in a highly reproducible, inexpensive in vivo model.[8] A strong relationship was noted between As RBA estimated from the mouse assay and As IVBA determined using a simplified gastric phase method[7] hereafter referred to as the Solubility/Bioavailability Research Consortium (SBRC) method.[24] A study evaluating the correlation between the mouse model and 5 commonly employed in vitro methods, which varied in operational parameters from simplified gastric methods[24] to complex physiological methods aimed at replicating human digestive systems,[25] reported that the strongest correlation was found between the results obtained with the mouse model and the SBRC method.[26] A similar study comparing results from a juvenile swine model and the SBRC method also found a strong correlation.[20]

The objective of this study was to build upon a previously published linear regression model[7] to predict As RBA in mice using an IVBA assay and to develop a more robust model across multiple soil types, As contaminant sources, and As concentrations. A second objective was to validate the predictive capability of this model using an independent set of As-contaminated soils. Although earlier studies have evaluated correlations between As RBA and IVBA, these studies have lacked model validation using an independent set of soils and been limited with respect to variety of soil types and contaminant sources used to construct the model. Validation of model performance using data independent to those used to construct the model is imperative for IVBA data to be used routinely for incorporation into human health risk assessments.[27] This is particularly important because the predictive capability of the model may be overestimated when evaluated solely with samples used to construct the model.[28]

Materials and Methods

Test Soils and Standard Reference Materials

This study used 37 As-contaminated soils in which As was introduced by mining and smelting, pesticide or defoliant use in agricultural or orchard sites, railway corridors, cattle tick dip sites, or occurred as a natural soil constituent. Standard reference materials (SRMs), SRM 2710 and SRM 2710a (National Institute of Standards and Technology), and a USEPA reference material were also evaluated. No soils spiked or amended with As were included in this study. All test soils were collected from the top 1-2" of soil, dried (<40°C), sieved to <250 μm, homogenized, and riffled[29] for mixing and splitting samples.

Total As concentrations in test soils and SRMs 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). Additional soil element concentrations (Al, Fe, Mn, and P) were determined by microwave digestion in accordance with USEPA SW-Method 3051 with analysis by Inductively Coupled Plasma-Atomic Emissions Spectroscopy in accordance with USEPA SW-Method 6010C.

A subset of test soils (soils 1-5; 8-27) were also characterized for As speciation 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. Additional information on As speciation determination is provided in Supporting Information.

Assessment of As relative bioavailability

Arsenic RBA was determined using an in vivo mouse model.[7,8] All assays were performed in four- to six-week-old female C57BL/6 mice (Charles River Laboratories, Raleigh, NC). Additional details on mouse assay methodology is provided in Supporting Information. 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 arsenic (μg) as shown in Equation 1:

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

Arsenic 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).

Assessment of As bioaccessibility

Arsenic IVBA was determined using the SBRC in vitro method (USEPA Method 9200.86-2).[24] See Supporting Information for additional details on IVBA methodology.

Arsenic IVBA was calculated and expressed on a percentage basis according to equation 3.

$$As\ bioaccessibility\ (\%) = \left(\frac{in\ vitro\ As}{total\ As} \right)\ x\ 100 \qquad \text{Eq. (3)}$$

Where:

In vitro As = As extracted during the in vitro assay

Total As = Amount of As in the contaminated soil used for bioaccessibility determination

As relative bioavailability prediction: model formulation and validation

In this study, the correlation between As RBA and IVBA was determined for 40 soils. A training set of 31 soils (#1-31) were used for initial model development, and nine additional soils (#32-40), previously described by Juhasz et al,[12] were used to independently validate the in vivo-in vitro correlation. The soils used for the independent data validation are from Australia and contain As from different contamination sources (e.g., cattle dip), mineralogy, and As concentrations versus the soils from the U.S. Following validation, the regression model was then fitted using all 40 soils.

A Bayesian hierarchical approach to linear regression was used to evaluate the ability of the SBRC in vitro assay to predict As RBA in the form:

$$\text{RBA (\%)} = a + (b)\ \text{IVBA (\%)} + \epsilon$$

where,

a = y-intercept

b = slope

ϵ = normally distributed prediction error

This approach has the advantage over simple linear regression of accounting for variation among replicate measurements of individual soils, as well as variation among different soils

(see Supporting Information for a more detailed summary of the Bayesian model formulation).

The predictive capability of the model was assessed using the coefficient of determination (R^2) and absolute error (AE) in As RBA prediction. Here, R^2 is defined as the fraction of the variance in the observations that is resolved by the model predictions (i.e., the means of the predictive distributions) relative to a null (constant-only) model. [30] AE is defined as the absolute percent difference between the model-predicted As RBA value and the As RBA value observed in the mouse assay.

To evaluate model robustness, model parameters (slope and y-intercept) and As RBA prediction performance under each model development scenario (training data only, independent validation data only, and all soils) were compared. A "leave-one-out" cross validation (CV) was also performed for the overall (i.e., 40 soil) fitted model to further assess model robustness by evaluating model prediction "out-of-sample" over a wider range of observations.[31] In this case, As RBA for each of the 40 soil types was predicted, in turn, after removing the target soil type from the observation data set and re-calibrating the model based on the remaining 39 observations.

RESULTS

Test Soils and Standard Reference Materials

Test soils and SRMs displayed a range of As and other elemental concentrations, pH values, and speciation (Tables SI-1 and SI-2). Total As concentration in test soils ranged from 108 to 6,899 mg kg^{-1} (Table 1). The concentration of major elements, including aluminum (Al) and iron (Fe) ranged from 0.7 - 72.1 g/kg and 14.4 - 276.2 g/kg, respectively. The concentration

212 of manganese (Mn) and phosphorous (P) ranged from 0.5 - 9,321 and 4 - 6,745 mg kg^{-1},
213 respectively. Soil pH ranged from 2.2 to 8.8. Arsenic speciation was categorized into three
214 coordination environments, As(V)-oxygen bonding (arsenate sorbed to oxides and scorodite),
215 As(III)-oxygen bonding (arsenite sorbed to oxides and schneiderhöhnite), and As-sulfide
216 bonding (realgar, loellingite, and arsenopyrite). With respect to speciation, mining soils had
217 varying ratios of all three arsenic coordination environments (Table SI-2). Mining Soil 12
218 was mostly As(III)-oxygen bonding (26%) and As-sulfide bonding (60%), but the remaining
219 mining soils contained mostly As(V)-oxygen bonding species. Orchard soils were
220 predominately sorbed As(V) phases with the exception of Soil 21, which had about 10%
221 sorbed As(III). The reference material soils were predominantly sorbed As(V) and scorodite
222 with minor addition of sorbed As(III).
223
224 ***As relative bioavailability and bioaccessibility in test soils***
225 Arsenic RBA values observed in the mouse assay ranged from 1.9-52.8 % (Table 1). Arsenic
226 IVBA in test soils and SRMs ranged from 0.0-74.3 % (Table 1), while sodium arsenate IVBA
227 was 100%. In addition to a strong correlation with As RBA values, acceptable within-
228 laboratory repeatability and between-laboratory reproducibility must be established in order
229 for an in vitro method to be accepted.[5] Although, this study was not designed as an inter-
230 laboratory trial, information is provided in the discussion regarding the repeatability and
231 reproducibility of the SBRC method.
232
233 The current study provided SBRC values for 23 soils determined at two independent
234 laboratories. Observed standard deviations (SDs) ranged from 0.1 to 6.7%. Comparison of

between-lab variability resulted in a strong correlation (slope = 1.0; y-intercept = 3.7; R^2 = 0.92) (Figure 1) indicating that the assay was reproducible.

Regression Model Performance – Utility of in vitro bioaccessibility data for predicting As relative bioavailability

An initial linear model was developed using the training data set (n=31 soils) to evaluate the ability of IVBA values to predict As RBA in mice. The initial linear model had a slope of 0.67 (standard error (SE) of 0.06) and y-intercept of 7.1 (SE of 1.8) (Table 2). Goodness of fit, as measured by R^2, was 0.83. This finding is similar to studies by Juhasz et al.[20,21] and Brattin et al.,[19] which have reported strong correlations with the SBRC gastric method correlation and in vivo RBA swine data (R^2=0.75 and R^2 = 0.72, respectively). Bradham et al.[7] reported a strong correlation between the SBRC method and in vivo RBA mouse data (R^2=0.92) for mining soils.

For independent validation, this initial linear model was used to predict As RBA for nine additional soils (#32-40) with comparison to measured values. The model accurately predicted As RBA for all nine soils in the validation set with a mean and median absolute error (AE) of 5.4 and 6.0% respectively (range of 1.7 to 8.4%) (Table 3). The R^2 for the validation predictions was 0.73.

Following independent validation, all 40 soils were fitted to an updated linear regression model (Figure 2 and Table 2). Parameters for this model were similar to the initial model with a slope of 0.65 (SE of 0.05), y-intercept of 7.8 (SE of 1.6), and R^2 of 0.81 (R = 0.91). Mean and median AE in As RBA prediction across all 40 soils were 4.9% and 4.8%, respectively. In addition, 39 of the 40 predicted As RBA values were within 10% of the

RBA value observed in the mouse assay; only soil 17 (AE of 16.7%) exceeded the target range. A potential explanation for poor agreement between IVBA and RBA in soil #17 is that high Al levels observed in this soil (66.9 g kg^{-1}) differentially influenced As dissolution in vitro versus in vivo due to either pH specific sorption kinetics or the influence of organic matter in mouse diet on As sorption onto Al surfaces, resulting in the observation of low As IVBA values relative to RBA. Interestingly, the only soil in the data set with higher aluminum levels, soil #36 (72.1 g kg^{-1}) also had a much lower As IVBA than RBA (9.0% versus 21.5% respectively).

To evaluate model robustness, slope and y-intercept model parameters and As RBA prediction accuracy were compared under the multiple model development scenarios used in this study (i.e., 1. training data set, 2. independent data set, and 3. all 40 soils) (Table 2). Model performance was consistent across all scenarios, with slope and intercept values all within one SE of each other. Mean and median AE in As RBA predictions were within 0.0% and 0.8%, respectively.

Results of the leave-one-out cross validation (CV) were used to assess model robustness by estimating the model's ability to predict "out-of-sample" across all 40 soils used for model development. This approach also showed consistent estimates in slope and y-intercept across the CV model runs. Slope varied from 0.63 to 0.67 and y-intercept varied from 7.2 to 8.3. Overall model goodness of fit (R^2) for the CV predictions was 0.79 (compared to 0.81 for the "full" model fit to all 40 soils).

Discussion

The RBA values reported in this study fall within the As RBA range previously reported for juvenile swine and monkey bioassays.[11,13,16,19,20,23,32,33] A recent study by the USEPA Technical Review Workgroup Bioavailability Committee compiled all available estimates of soil As RBA across juvenile swine, primate, and mouse assays (103 As RBA values) and reported that only 5% of As RBA values were greater than 60% (USEPA 2012). Based on these studies, As RBA values reported in this study were consistent with these findings and represent a wide range in As RBA values. Differences in bioavailability values for different soils may be largely determined by As mineralogy and physical and chemical properties of soils (Table SI-1) that influence solubility of As in the gastrointestinal system.[22] Studies have shown that As bioaccessibility extractability accounts for much of the variability in RBA estimates obtained from the animal bioassays, including the mouse, swine, and monkey assays.[7,9,11,12,14,23] Some clay minerals contain ferrous and ferric iron that, upon release via weathering, will form iron oxides and hydroxides in soil environments,[34] which sorb As reducing As bioavailability. Similar processes have also been identified for aluminum and manganese oxides in soils.[35,36] Lower As RBA estimates were observed for soils containing sulfide forms of As (realgar or arsenopyrite), which may reflect slow dissolution kinetics of these mineral species. 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.

Comparison of between-lab variability resulted in a strong correlation (slope = 1.0; y-intercept = 3.7; $R^2 = 0.92$) (Figure 1) indicating that the assay was reproducible.

Results of the between-lab variability in As bioaccessibility values using the SBRC method support previously published observations that the SBRC method is reproducible between

309 labs. Juhasz et al.[26] previously demonstrated a strong relationship (slope = 1.12; y intercept =
310 0.61; R^2 = 0.98) between SBRC As bioaccessibility measurements made in their study with
311 data previously published by Bradham et al.[7] Koch et al. [37] conducted an extensive round
312 robin study evaluating 17 bioaccessibility methods, including the SBRC method, by 14
313 laboratories for NIST SRM 2710. For the SBRC method, the between-lab reproducibility SD
314 was 9.5-13% and the individual lab reproducibility SD was 5-8%. A recent study by Brattin
315 et al.[19] reported results of a 4 laboratory comparison of the SBRC method resulting in a
316 within-lab precision of less than 3% (SD) and average SD of 0.8% for the 4 labs. The
317 between-lab variation resulted in an overall average of 3% SD,[19] illustrating the performance
318 of the SBRC in vitro assay.
319
320 Taken together, comparisons of the multiple model development scenarios along with results
321 of the cross validation indicate that model performance is robust with regards to both model
322 parameterization (slope and y-intercept) and As RBA prediction accuracy, as measured by
323 mean and median AE. It is important to note that some range of uncertainty or variability in
324 actual As RBA relative to model predicted As RBA can be expected, due to authentic inter-
325 sample variability in As RBA and/or to measurement error in in vitro bioaccessibility or
326 RBA.[5] Therefore, the actual As RBA may be either lower or higher than the best estimate
327 predicted value using IVBA data and the regression model (see 95% predictive intervals,
328 Figure 2). Only one of the 40 observations fell outside of the 95% prediction intervals during
329 the cross validation, indicating that the model provides adequate, and perhaps slightly
330 conservative, uncertainty quantification.
331
332 A desirable property of the in vivo-in vitro relationship is a coefficient of correlation (R)
333 greater than or equal to 0.8 which reflects a strong correlation between As RBA and IVBA

data.[27] The model presented here, which incorporated As RBA and IVBA data from soils with a wide range of As concentrations derived from a variety of anthropogenic and geogenic sources yielded a strong in vivo-in vitro correlation (R = 0.91) that met this criterion. Eleven mining and smelter-impacted soils included in this data set had previously been correlated to As IVBA derived from the SBRC gastric in vitro assay using simple least squares linear regression with a reported R^2 of 0.92.[7] A strong in vivo-in vitro relationship (R^2 = 0.90) for the SRBC method has been reported.[26] This study found no significant difference in the slope and y-intercepts ($P > 0.05$) of these relationships illustrating the robustness and reproducibility of SBRC as a predictor of As RBA. These investigators also evaluated other in vitro assays reporting no significant difference in the slopes of in vivo-in vitro correlations when SBRC, IVG, PBET, DIN and UBM gastric and intestinal phases (Solubility and Bioavailability Research Consortium, Deutsches Institut fur Normung, In Vitro Gastrointestinal, Physiologically Based Extraction Technique, and the Unified BARGE Method, respectively) were used to derive the in vivo-in vitro relationships.[26] However, a significantly ($P < 0.05$) smaller y-intercept was determined for the in vivo-in vitro correlation using SBRC compared to the other in vitro methods. This is important to note as the use of in vivo-in vitro correlations with large y-intercepts may over-predict As adsorption, particularly in soils with low As RBA. Other studies[20,21] determined that SBRC, IVG, PBET, DIN and UBM assays (including gastric and intestinal phases) all predicted As RBA with varying degrees of confidence (R^2 = 0.52-0.75). However, comparison of the in vivo and in vitro results from these studies demonstrated that the SBRC gastric method provided the best prediction of in vivo RBA (R^2=0.75).[20,21] Similarly, a strong correlation has been reported between As RBA determined in juvenile swine and As IVBA determined using SBRC (slope = 0.62, y intercept = 19.68, R^2 = 0.72).[19] However, this study included soils spiked with exogenous As, which strongly affected the overall R^2 value.

The approach to measuring As RBA (single versus multiple doses; Area Under the Curve (AUC) versus Steady State Urinary Excretion (SSUE)) may influence in vivo outcomes in terms of whether single versus multiple As doses are administered and whether absorption is determined using AUC or SSUE.[26] The USEPA noted that an advantage of steady state models is that they more closely mimic the status of receptors who receive continuous daily exposure to contaminated soil and dust.[15] In addition, under steady state conditions, urinary As excretion is constant so that urinary excretion factors can be estimated by averaging As concentrations from multiple samples over time. Although As RBA comparisons have been made between mouse and swine models using the SSUE approach,[8] it is unknown to what extent these conditions influence As RBA measurement. Variability in bioaccessibility measurements from in vitro analyses may result from subtle differences in the conduct of assays.[18] To address the uncertainty associated with in vivo-in vitro correlation variability, comparative studies of As RBA with different animal models and endpoints would be advantageous. In addition, assessment of sources of inter-laboratory variability associated with both in vitro and in vivo measurements could be beneficial.

Oral ingestion of metal contaminated soil and dust is often a "risk driver" for human exposure at contaminated sites, resulting in remedial action. Even a small bioavailability adjustment to site-specific RBA may result in significant remediation cost savings[5]. Therefore, reliable, quick, and inexpensive methods for assessing As RBA in soil are needed to reduce exposure estimate uncertainties in human health risk assessment and reduce clean-up costs. The in vivo-in vitro correlation and independent data validation presented here for the SBRC method provides critical supporting information for use in human health risk assessment.

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Supporting Information Available

Soil properties and speciation, bayesian model formulation, equations used for model formulation, references for model formulation. This material is available free of charge via the Internet at http://pubs.acs.org.

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555

556 Table 1. Soil source, arsenic concentration, IVBA and RBA values for the 40 soils included
557 in this study.

Soil ID	Source	Soil [As] (mg/kg)[a]	RBA (%) ± SD	IVBA (%) ± SD		Soil ID	Source	Soil [As] (mg/kg)[a]	RBA (%) ± SD	IVBA (%) ± SD
1	mining	244	15.3 ± 1.7	18.1 ± 0.4		**21**	orchard	396	46.0 ± 1.9	48.1 ± 0.8
2	mining	173	13.9 ± 1.6	6.8 ± 0.8		**22**	mining	197	28.7 ± 4.2	22.0 ± 0.2
3	mining	6900	14.5 ± 1.3	17.5 ± 0.6		**23**	mining	884	22.9 ± 5.3	17.0 ± 0.4
4	mining	280	39.5 ± 2.5	53.6 ± 0.2		**24**	mining	293	17.8 ± 0.8	12.3 ± 0.3
5	mining	4490	14.3 ± 1.4	8.8 ± 0.1		**25**	mining	223	19.6 ± 2.6	17.3 ± 0.1
6	mining	491	17.0 ± 0.7	22.8 ± 0.6		**26**	mining	494	17.8 ± 2.5	15.5 ± 0.1
7	mining	207	18.6 ± 4.2	25.7 ± 0.4		**27**	mining	738	11.1 ± 1.2	13.4 ± 3.5
8	mining	182	26.4 ± 2.6	32.9 ± 0.2		**28**	mining	777	4.3 ± 0.9	0.0 ± 0.0
9	mining	990	48.2 ± 3.6	73.1 ± 0.6		**29**	mining	943	2.9 ± 0.3	0.1 ± 0.0
10	mining	829	49.2 ± 3.1	74.3 ± 1.3		**30**	mining	898	1.9 ± 0.3	0.1± 0.0
11	mining	379	51.1 ± 3.2	53.2 ± 0.5		**31**	mining	668	3.5 ± 0.4	0.0 ± 0.0
12	mining	837	11.4 ± 0.5	18.2 ± 2.7		**32**	railway corridor	981	35.9 ± 1.9	54.3 ± 2.5
13	SRM	601	42.3 ± 2.1	53.9 ± 4.1		**33**	railway corridor	246	44.6 ± 4.2	47.0 ± 2.1
14	SRM	1510	41.5 ± 2.4	41.8 ± 1.7		**34**	railway corridor	108	23.5 ± 2.6	27.0 ± 0.8
15	SRM	879	16.2 ± 0.6	14.5 ± 0.2		**35**	railway corridor	184	22.8 ± 2.5	11.9 ± 0.1
16	orchard	322	26.1 ± 2.0	18.8 ± 0.3		**36**	cattle dip	965	21.5 ± 2.1	9.0 ± 0.4
17	orchard	462	34.9 ± 3.0	16.1 ± 0.4		**37**	mining	573	6.4 ± 0.4	3.5 ± 0.4
18	orchard	401	20.7 ± 3.2	18.0 ± 0.2		**38**	mining	583	14.0 ± 0.3	21.3 ± 0.2
19	orchard	422	34.7 ± 2.6	27.9 ± 0.8		**39**	gossan	239	20.2 ± 2.6	12.4 ± 0.6
20	orchard	340	32.8 ± 3.5	35.4 ± 1.9		**40**	cattle dip	313	28.8 ± 2.4	36.5 ± 1.3

558 a Determined by Instrument Neutron Activation Analysis; SD = standard deviation; SRM = Standard Reference Material

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561 Table 2. Model parameters (slope, intercept and R^2) and As RBA prediction accuracy under

562 the various regression model development scenarios.

	Model Parameters			RBA Prediction Accuracy			
Model	**# soils**	**Slope (± SE)**	**Intercept (% ± SE)**	**R^2**	**Mean AE (%)**	**Median AE (%)**	**Range AE (%)**
Training data	31	0.67 ± 0.06	7.1 ± 1.8	0.83	4.9	4.6	0.3 – 17.0
Validation data	9	0.56 ± 0.15	10.3 ± 4.5	0.73	4.9	5.4	0.1 – 15.7
All data	40	0.65 ± 0.05	7.8 ± 1.6	0.81	4.9	4.8	0.0 – 16.7

563 **SE = standard error; AE = absolute error**

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567 Table 3. Results of independent model validation.

Soil ID	IVBA (%)	Predicted RBA - model (%)	Observed RBA - mice (%)
32	54.3	43.5	35.9
33	47.0	38.6	44.6
34	27.0	25.2	23.5
35	11.9	15.1	22.8
36	9.0	13.1	21.5
37	3.5	9.4	6.4
38	21.3	21.4	14.0
39	12.4	15.4	20.2
40	36.5	31.6	28.8
		Mean AE (%)	**5.5**

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Figure 1. Comparison of between-laboratory reproducibility for in vitro bioaccessibility method.

Figure 2. Results of fitting the linear regression model for the prediction of As relative bioavailability using SBRC in vitro assay. The 31 training data points are shown as circles and the 9 validation data points are shown as triangles. The overall fitted model was RBA (%) = 0.65 * IVBA (%) + 7.8, with dotted lines representing the 95% prediction intervals.

Figure 1.

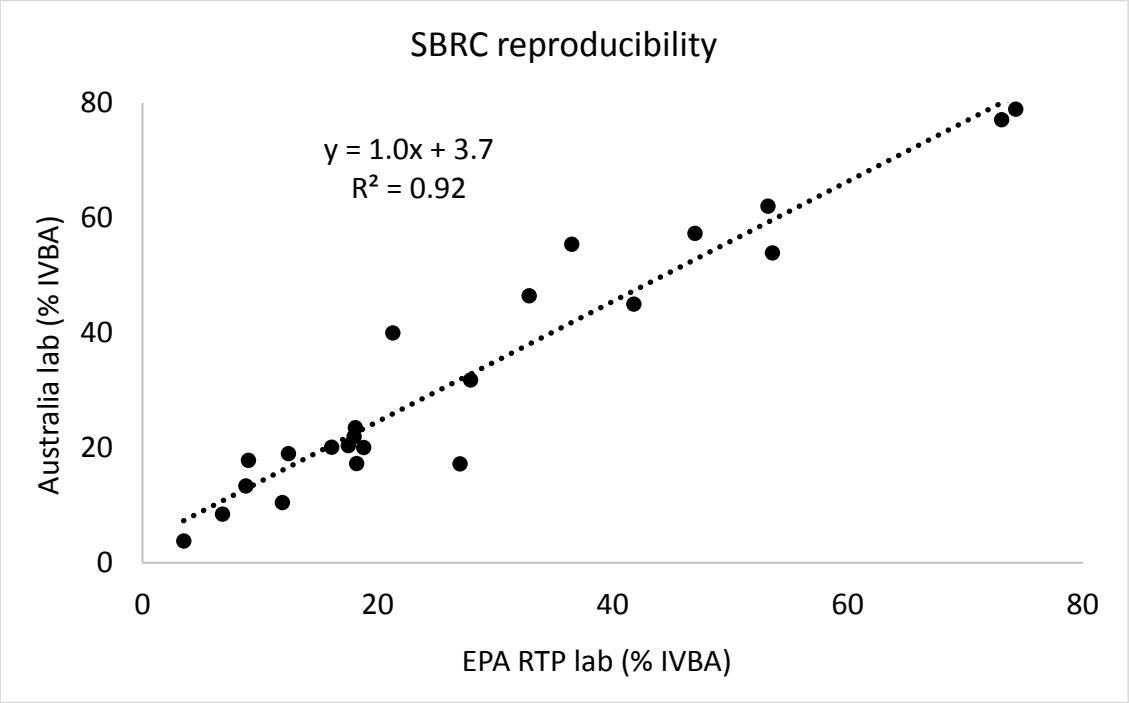
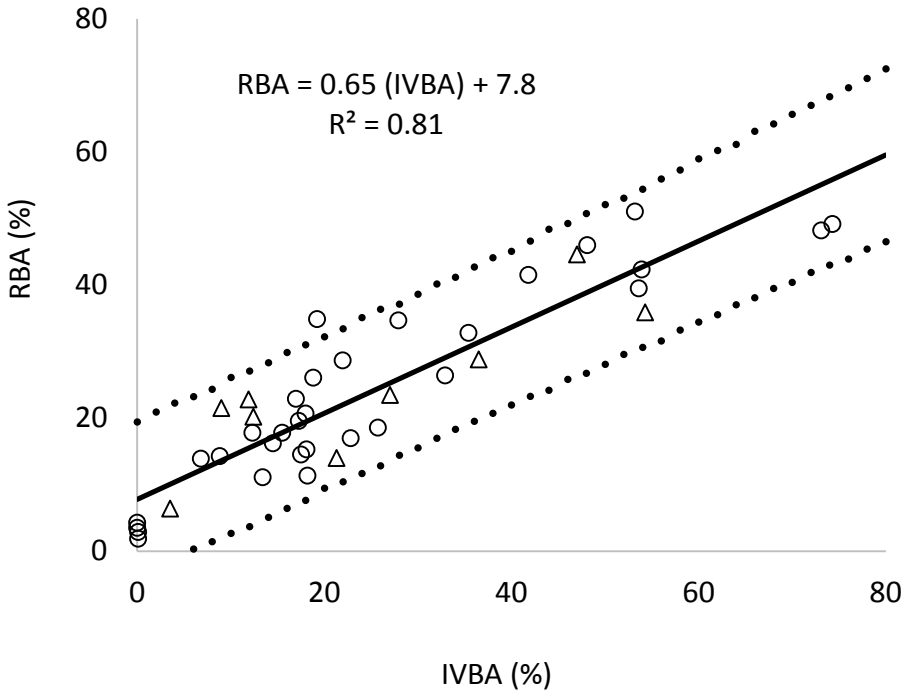

SBRC reproducibility
y = 1.0x + 3.7
R² = 0.92
Australia lab (% IVBA)
EPA RTP lab (% IVBA)
0
20
40
60
80

Figure 2.



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Supporting Information

Independent data validation of an in vitro method for prediction of relative bioavailability of arsenic in contaminated soils

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Supporting Information Contents (7 pages)

Soil arsenic speciation determination

A subset of test soils (soils 1-27) were characterized for As speciation 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 energy was calibrated by assigning the first derivative inflection point of the K-edge of sodium arsenate (11874 eV). Three As K-edge (11867 eV) X-ray absorption spectroscopy (XAS) spectra were collected in deadtime corrected fluorescence mode (16-element solid state Ge detector, Canberra) and transmission mode with an ionization chamber at room temperature for every soil and reference sample. Data analysis was conducted using IFEFFIT software.[1] 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 arsenic species in the samples. LCFs were performed using the first derivative of the XANES spectra from reference standards to arsenic phases in the soil samples over a fit range of -20 to 130 eV. Reference materials for LCF, based on principal component analysis, included arsenate (As(V)) and arsenite (As(III)) sorbed to ferrihydrite, scorodite [$Fe(As^{V}O_4)$], schneiderhöhnite [$Fe^{2+}Fe^{3+}{}_3(As^{III}{}_5O_{13})$], realgar (AsS), lollingite ($FeAs_2$) and arsenopyrite (FeAsS). Data for LCF fits reveal As speciation in each soil as ratios of these mineral forms.

Mouse assay methodology

Arsenic RBA was determined using an in vivo mouse model performed in four- to six-week-old female C57BL/6 mice. The basal diet for mouse assays was powdered AIN-93G purified rodent diet[2] 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 SRMs with basal diet. For test soils or SRMs, the soil:diet ratio was typically 1% (w/w). Arsenate (As^{V})-amended diets were prepared by addition of sodium arsenate

heptahydrate (Sigma, St. Louis, MO) 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.

Mice had free access to amended diet and tap water for 9 days, with urine and feces collection and measurement of food consumption performed daily for 10 days. For sample collection and data analysis, the unit of observation was the cage (i.e., combined excreta of three mice). Typically, an assay included four 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 arsenic analysis by INAA.

As IVBA determination

Arsenic IVBA was determined using the SBRC in vitro method. In vitro assays included addition of 1 g test soil to 100 mL gastric fluid consisting of 0.4 M glycine at pH 1.5 in a 125-mL high-density polyethylene bottle and rotated end-over-end in a water bath at 37°C for 1 hr. All samples were extracted in duplicate or triplicate. Quality control standards, including reagent blanks, blank spikes, sample matrix spikes, and NIST 2710A SRM, were included with each batch (12 bottles per batch). All QC sample observations were within allowable quality control limits as defined by USEPA Method 9200.2-86.[3,4] In vitro extraction solutions were refrigerated at 4°C for preservation and analyzed by Inductively Coupled Plasma-Mass Spectrometry (USEPA SW-846 Method 6020).[4] Quality control standards for the ICP-MS were included with all analyses as described in USEPA SW-846 Method 6020. All soils tested in the IVBA protocol were identical to those administered to mice in the in vivo studies and used in the mineralogy studies described above.

Bayesian model formulation

In the Bayesian model formulation, each RBA replicate $y_{i,j}$ of soil type *i* and replicate number *j* is assumed to come from a normal distribution:

$$y_{i,j} \sim N(\tilde{y}_i, \tilde{\sigma}_i) \quad (1)$$

where $\tilde{y}_i$ is the true (unknown) RBA value for soil type i, and $\tilde{\sigma}_i$ is the standard deviation of the replicates. Based on a preliminary analysis of the replicates, $\tilde{\sigma}_i$ appears to be proportional to $\tilde{y}_i$, such that it can be approximated as:

$$\tilde{\sigma}_i = \overline{y_i}\beta_\sigma \quad (2)$$

where $\overline{y_i}$ is the mean RBA of the replicates of soil type i, and β_σ is a scaling parameter determined by the model. The IVBA replicates, $x_{i,j}$, are modeled in the same fashion as the RBA replicates. RBA and IVBA can then be related to each other as follows:

$$\tilde{y}_i = f(\tilde{x}_i) + \epsilon \quad (3)$$

where $f(\tilde{x}_i)$ is the prediction of RBA using IVBA; and ϵ is the normally-distributed prediction error, i.e., $\epsilon \sim N(0, \sigma_\epsilon)$. In this study, we considered the model form of $f(\tilde{x}_i)$:

$$f(\tilde{x}_i) = a + b\tilde{x}_i \quad (4)$$

In eq 4, the relationship between RBA and IVBA is a linear function with intercept parameter *a* and slope parameter *b*.

Model parameters were estimated through Bayseian inference, using WinBUGS software[5] called from R[6] via R2WinBUGS.[7,8] The MCMC sampling was performed in three parallel 'chains' of up to 25,000 samples each, and the first half of each chain was removed as a 'burn-in period'.[9] The remaining chain portions were then thinned to 2500 samples each to reduce autocorrelation, and checked to ensure that they had converged on equivalent posterior parameter distributions. Convergence was evaluated using the $\hat{R}$ statistic,[10] and when $\hat{R}$ is less than 1.1 one for all model parameters, convergence is considered achieved.

Test soil elemental concentrations and properties

Table SI-1. Select physico-chemical properties of soils (< 250 μm particle size fraction) in this study.

Soil ID	pH	Soil totals (mg kg^{-1})				
		As[a]	Al[b]	Fe[b]	Mn[b]	P[b]
1	7.5	244	24040	42580	812	1690
2	6.4	173	19970	60650	688	1400
3	5.5	6900	15970	139410	966	1090
4	2.3	280	4482	77880	17	62
5	2.7	4490	14400	140130	435	948
6	6.9	491	12950	42830	548	1800
7	6.7	207	21520	39380	1150	2320
8	6.9	182	21930	22940	441	780
9	6.4	990	12520	17650	517	1060
10	6.5	829	10220	15670	619	631
11	5.1	379	10660	14380	264	178
12	7.1	837	14740	276280	2750	1310
13	5.0	601	19090	31250	9320	1020
14	4.0	1510	10860	38590	1820	989
15	5.9	879	4136	36050	2600	6740
16	6.2	322	37690	26560	232	1170
17	6.2	462	66850	46650	854	1410
18	5.6	401	53480	40960	1350	1440
19	5.9	422	47000	35210	1540	1770
20	5.7	340	20930	23170	1670	1640
21	5.6	396	12750	20350	466	1280
22	5.2	197	27360	23440	303	50
23	6.4	884	28440	29960	738	51
24	6.5	293	40120	35510	407	34
25	5.4	223	34150	28330	350	45
26	6.0	494	28010	37840	567	36
27	6.6	738	19740	31190	877	43
28	2.9	885	2740	174230	0.5	194
29	3.1	566	678	106440	6	46
30	3.3	802	1700	199070	2	159
31	3.5	552	3680	179000	13	184
32	8.3	981	11350	31950	766	861
33	8.8	246	22980	20480	373	197
34	7.8	108	26450	33660	279	157
35	6.4	184	20710	31240	487	16
36	5.7	965	72080	97660	1520	24
37	6.6	573	9060	30350	421	66
38	7.6	583	4700	25490	378	25
39	8.6	239	10700	23860	232	4
40	5.2	313	18860	19150	1000	16

Table SI-2. Speciation data for soils used in this study. Values are given in percent (%).

Soil ID	As(V)-Oxygen Coordination		As(III)-Oxygen Coordination		As-Sulfide Coordination			R-factor[1]
	As(V) Sorbed to Oxides	Scorodite	As(III) Sorbed to Oxides	Schneider höhnite	Realgar	Loellingite	Arseno-pyrite	
1	97			3				0.005
2	87		13					0.001
3	51	16	13				21	0.003
4	65	19		6		11		0.004
5	61	29		5		5		0.003
8	58		20	23				0.003
9	54	22	24					0.001
10	80	5	15					0.001
11	55	10	13		22			0.002
12	14		14	12	38	9	13	0.006
13	95	5						0.001
14	75	11	14					0.001
15	77		12				11	0.002
16	100							0.001
17	100							0.001
18	100							0.002
19	100							0.003
20	100							0.002
21	90		10					0.002
22	44		4	16	36			0.005
23	77				23			0.001
24	54			23	23			0.002
25	79			10	11			0.001
26	71			14	14			0.001
27	78				22			0.001

[1] **R-factor = $[\Sigma((\text{data-fit})^2)]/[\Sigma(\text{data}^2)]$**

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