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Independent Data Validation of an in Vitro Method for the Prediction of the Relative Bioavailability of Arsenic in Contaminated Soils

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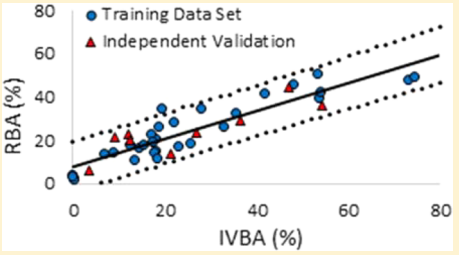
Supporting Information

ABSTRACT: In vitro bioaccessibility (IVBA) assays 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 the use of few soil types and sources of As, and the predictive value of As IVBA has 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 IVBA, 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 an 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.[2−4] 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 of 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] IVBAs are attractive alternatives to in vivo assays because they are cost-effective and reduce reliance on animal studies. A prime assumption underlying these IVBAs 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

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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 termed the solubility/bioavailability research consortium (SBRC) method.[24] A study evaluating the correlation between the mouse model and five 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 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 from 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, or 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 in. 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 of North Carolina State University. The mean As mass detection limit was 0.035 μg (approximately 0.2 μg/g of 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 emission spectroscopy in accordance with USEPA SW-Method 6010C.

A subset of test soils (soils 1−5 and 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 about As speciation determination is provided in the Supporting Information.

Assessment of As Relative Bioavailability. Arsenic RBA was determined using an in vivo mouse model.[7,8] All assays were performed in 4- to 6-week-old female C57BL/6 mice (Charles River Laboratories, Raleigh, NC). Additional details about the mouse assay methodology are provided in the 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 (micrograms) to cumulative dietary intake of arsenic (micrograms) as shown in eq 1:

$$\text{UEF\%} = 100\frac{\text{Cum Urinary As}}{\text{Cum As Dose}} \quad (1)$$

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 eq 2):

$$\text{RBA\%} = \frac{\text{UEF Soil}}{\text{UEF Arsenate}} \quad (2)$$

Each UEF in eq 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 the Supporting Information for additional details of IVBA methodology.

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

$$\text{As bioaccessibility (\%)} = \left(\frac{\text{in vitro As}}{\text{total As}}\right) \times 100 \quad (3)$$

where in vitro As is the amount of As extracted during the in vitro assay and total As is the 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 United States. Following validation, the regression model was then fit 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

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

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Table 1. Soil Sources, Arsenic Concentrations, and IVBA and RBA Values for the 40 Soils Included in This Study

soil	source	soil [As] (mg/kg)[a]	RBA (%) ± SD[b]	IVBA (%) ± SD[b]	soil	source	soil [As] (mg/kg)[a]	RBA (%) ± SD[b]	IVBA (%) ± SD[b]
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[c]	601	42.3 ± 2.1	53.9 ± 4.1	33	railway corridor	246	44.6 ± 4.2	47.0 ± 2.1
14	SRM[c]	1510	41.5 ± 2.4	41.8 ± 1.7	34	railway corridor	108	23.5 ± 2.6	27.0 ± 0.8
15	SRM[c]	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

[a]Determined by instrument neutron activation analysis. [b]SD is the standard deviation. [c]SRM is the standard reference material.

where a is the y-intercept, b the slope, and ε the 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 the 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 recalibrating the model based on the 39 remaining 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 of the Supporting Information). The total As concentration in test soils ranged from 108 to 6899 mg kg^{-1} (Table 1). The concentration of major elements, including aluminum (Al) and iron (Fe), ranged from 0.7 to 72.1 g/kg and from 14.4 to 276.2 g/kg, respectively. The concentration of manganese (Mn) and phosphorus (P) ranged from 0.5 to 9321 mg kg^{-1} and from 4 to 6745 mg kg^{-1}, respectively. The soil pH ranged from 2.2 to 8.8. Arsenic speciation was categorized into three coordination environments, As(V)−oxygen bonding (arsenate sorbed to oxides and scorodite), As(III)−oxygen bonding (arsenite sorbed to oxides and schneiderhöhnite), and As−sulfide bonding (realgar, loellingite, and arsenopyrite). With respect to speciation, mining soils had varying ratios of all three arsenic coordination environments (Table SI-2 of the Supporting Information). Mining soil 12 included mostly As(III)−oxygen bonding (26%) and As−sulfide bonding (60%), but the remaining mining soils included mostly As(V)−oxygen bonding. Orchard soils were predominately sorbed As(V) phases with the exception of soil 21, which had ∼10% sorbed As(III). The reference material soils were predominantly sorbed As(V) and scorodite with a minor addition of sorbed As(III).

As Relative Bioavailability and Bioaccessibility in Test Soils. Arsenic RBA values observed in the mouse assay ranged from 1.9 to 52.8% (Table 1). Arsenic IVBA in test soils and SRMs ranged from 0.0 to 74.3% (Table 1), while sodium arsenate IVBA was 100%. In addition to a strong correlation with As RBA values, acceptable within-laboratory repeatability and between-laboratory reproducibility must be established for an in vitro method to be accepted.[5] Although this study was not designed as an interlaboratory trial, information about the repeatability and reproducibility of the SBRC method is provided in the Discussion.

This study provided SBRC values for 23 soils determined at two independent 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

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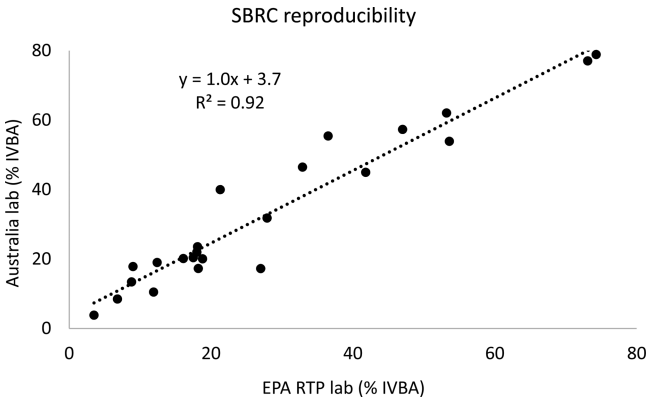

Figure 1. Comparison of between-laboratory reproducibility for the in vitro bioaccessibility method.

values to predict As RBA in mice. The initial linear model had a slope of 0.67 [standard error (SE) of 0.06] and a *y*-intercept of 7.1 (SE of 1.8) (Table 2). The goodness of fit, as measured by R^2, was 0.83. This finding is similar to those of studies by Juhasz et al.[20,21] and Brattin et al.,[19] which have reported strong correlations with the SBRC gastric method 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 1.7−8.4%) (Table 3). The R^2 for the validation predictions was 0.69.

Following independent validation, all 40 soils were fit to an updated linear regression model (Figure 2 and Table 2). Parameters for this model were similar to those of the initial model with a slope of 0.65 (SE of 0.05), a *y*-intercept of 7.8 (SE of 1.6), and an R^2 of 0.81 (R = 0.91). The 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 sorption of As 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 an As IVBA much lower than its RBA (9.0 and 21.5%, respectively).

Table 3. Results of Independent Model Validation

soil	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 absolute error (%)	5.5

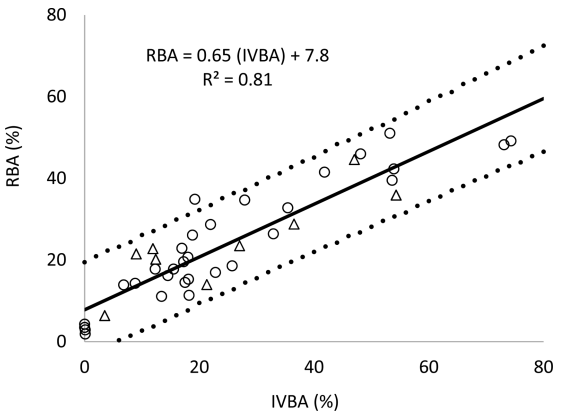

Figure 2. Results of fitting the linear regression model for the prediction of As relative bioavailability using the SBRC in vitro assay. The 31 training data points are shown as circles, and the nine validation data points are shown as triangles. The overall fitted model was RBA (%) = 0.65 × IVBA (%)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

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. The 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 of the slope and *y*-intercept across the CV model runs. The slope varied from 0.63 to 0.67 and the *y*-intercept from 7.2 to 8.3. The 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).

Table 2. Model Parameters (slope, intercept, and R^2) and As RBA Prediction Accuracy under the Various Regression Model Development Scenarios

	model parameters			RBA prediction accuracy			
model	no. of soils	slope ± SE	intercept ± SE (%)	R^2	mean AE[b] (%)	median AE[b] (%)	AE[b] range (%)
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	0.69	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

[a]SE is the standard error. [b]AE is the absolute error.

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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). On the basis of these studies, As RBA values reported in this study were consistent with these findings and represent a wide range of 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 of the Supporting Information) that influence the 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 the slow dissolution kinetics of these mineral species. Additional studies would be useful for identifying other metals and metalloids in soils that are potential modifiers of As bioavailability and bioaccessibility and determining 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 can be reproduced between laboratories. Juhasz et al.[26] previously demonstrated a strong relationship (slope = 1.12; y-intercept = 0.61; R^2 = 0.98) between SBRC As bioaccessibility measurements made in their study and data previously published by Bradham et al.[7] Koch et al.[37] conducted an extensive round robin study evaluating 17 bioaccessibility methods, including the SBRC method, by 14 laboratories for NIST SRM 2710. For the SBRC method, the between-lab reproducibility SD was 9.5−13% and the individual lab reproducibility SD was 5−8%. A recent study by Brattin et al.[19] reported results of a four-laboratory comparison of the SBRC method resulting in a within-lab precision of <3% (SD) and average SD of 0.8% for the four laboratories. The between-lab variation resulted in an overall average of 3% SD,[19] illustrating the performance of the SBRC in vitro assay.

Taken together, comparisons of the multiple model development scenarios along with results of the cross validation indicate that model performance is robust with regard to both model parametrization (slope and y-intercept) and As RBA prediction accuracy, as measured by mean and median AE. It is important to note that some range of uncertainty or variability in actual As RBA relative to model-predicted As RBA can be expected, because of authentic intersample variability in As RBA and/or measurement error in in vitro bioaccessibility or RBA.[5] Therefore, the actual As RBA may be either lower or higher than the best estimate-predicted value using IVBA data and the regression model (see 95% predictive intervals in Figure 2). Only one of the 40 observations fell outside of the 95% prediction intervals during the cross validation, indicating that the model provides adequate, and perhaps slightly conservative, uncertainty quantification.

A desirable property of the in vivo−in vitro relationship is a coefficient of correlation (R) 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] Juhasz et al. reported a strong in vivo−in vitro relationship (R^2 = 0.90) for the SRBC method.[26] This study found no significant difference in the slopes 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, in vitro gastrointestinal, physiologically based extraction technique, Deutsches Institut fur Normung, 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 overpredict 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 dose vs multiple doses; area under the curve (AUC) vs 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] the extent to which these conditions influence As RBA measurement is unknown. 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

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with different animal models and end points would be advantageous. In addition, assessment of sources of interlaboratory 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 cleanup costs. The in vivo−in vitro correlation and independent data validation presented here for the SBRC method provide critical supporting information for use in human health risk assessment.

■ ASSOCIATED CONTENT

Supporting Information

Soil properties and speciation, Bayesian model formulation, equations used for model formulation, and references for model formulation. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b00905.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) *Toxicological profile for arsenic*; Division of Toxicology and Environmental Medicine/Applied Toxicology Branch, Agency for Toxic Substances and Disease Registry: Atlanta, 2007.

(2) Calabrese, E. J.; Stanek, E.; Barnes, R. Methodology to estimate the amount and particle size of soil ingested by children: Implications for exposure assessment at waste sites. *Regul. Toxicol. Pharmacol.* **1996**, *24*, 264−268.

(3) Davis, A.; Ruby, M. V.; Bergstrom, P. D. Bioavailability of arsenic and lead in soils from the Butte, Montana mining district. *Environ. Sci. Technol.* **1992**, *26*, 461−468.

(4) Dudka, S.; Miller, W. P. Permissible concentrations of arsenic and lead in soils based on risk assessment. *Water, Air, Soil Pollut.* **1999**, *113*, 127−132.

(5) Guidance for Evaluating the Oral Bioavailability of Metals in Soils for Use in Human Health Risk Assessment. OSWER 9285.7-80; U.S. Environmental Protection Agency: Washington, DC, 2007.

(6) Stanek, E. J.; Calabrese, E. J.; Barnes, R. M.; Danku, J. M.; Zhou, Y.; Kostecki, P. T.; Zillioux, E. Bioavailability of arsenic in soil: Pilot study results and design considerations. *Hum. Exp. Toxicol.* **2010**, *29*, 945−960.

(7) Bradham, K. D.; Scheckel, K. G.; Nelson, C. M.; Seales, P. E.; Lee, G. E.; Hughes, M. F.; Miller, B. W.; Yeow, A.; Gilmore, T.; Harper, S.; Thomas, D. J. Relative bioavailability and bioaccessibility and speciation of arsenic in contaminated soils. *Environ. Health Perspect.* **2011**, *119*, 1629−1634.

(8) Bradham, K. D.; Diamond, G. L.; Scheckel, K. G.; Hughes, M. F.; Casteel, S. W.; Miller, B. W.; Klotzbach, J. M.; Thayer, W. C.; Thomas, D. J. Mouse assay for determination of arsenic bioavailability in contaminated soil. *J. Toxicol. Environ. Health, Part A* **2013**, *76*, 815−826.

(9) Basta, N. T.; Foster, J. N.; Dayton, E. A.; Rodriguez, R. R.; Casteel, S. W. The effect of dosing vehicle on arsenic bioaccessibility in smelter-contaminated soil. *J. Environ. Sci. Health, Part A: Toxic/Hazard. Subst. Environ. Eng.* **2007**, *42*, 1275−1281.

(10) Brattin, W. J.; Casteel, S. W. Measurement of arsenic relative bioavailability in swine. *J. Toxicol. Environ. Health, Part A* **2013**, *76*, 449−457.

(11) Freeman, G. B.; Schoof, R. A.; Ruby, M. V.; Davis, A. O.; Dill, J. A.; Liao, S. C.; Lapin, C. A.; Bergstrom, P. D. Bioavailability of arsenic in soil and house dust impacted by smelter activities following oral administration in cynomolgus monkeys. *Fundam. Appl. Toxicol.* **1995**, *28*, 215−222.

(12) Juhasz, A. L.; Smith, E.; Weber, J.; Rees, M.; Rofe, A.; Kuchel, T.; Sansom, L.; Naidu, R. Comparison of in vivo and in vitro methodologies for the assessment of arsenic bioavailability in contaminated soils. *Chemosphere* **2007**, *69*, 961−966.

(13) Roberts, S. M.; Weimar, W. R.; Vinson, J. R.; Munson, J. W.; Bergeron, R. J. Measurement of arsenic bioavailability in soil using a primate model. *Toxicol. Sci.* **2002**, *67* (2), 303−310.

(14) Roberts, S. M.; Munson, J. W.; Lowney, Y. W.; Ruby, M. V. Relative oral bioavailability of arsenic from contaminated soils measured in the cynomolgus monkey. *Toxicol. Sci.* **2007**, *95* (1), 281−288.

(15) Compilation and review of data on relative bioavailability of arsenic in soil. OSWER 9200.1-113; U.S. Environmental Protection Agency: Washington, DC, 2012.

(16) Rodriguez, R. R.; Basta, N. T.; Casteel, S. W.; Pace, L. W. An in vitro gastrointestinal method to estimate bioavailable arsenic in contaminated soils and solid media. *Environ. Sci. Technol.* **1999**, *33*, 642−649.

(17) Ruby, M. V.; Davis, A.; Schoof, R.; Eberle, S.; Sellstone, C. M. Estimation of lead and arsenic bioavailability using a physiologically based extraction test. *Environ. Sci. Technol.* **1996**, *30*, 422−430.

(18) Wragg, J.; Cave, M.; Basta, N.; Brandon, E.; Casteel, S.; Denys, S.; et al. An inter-laboratory trial of the unified BARGE bioaccessibility method for arsenic, cadmium and lead in soil. *Sci. Total Environ.* **2011**, *409*, 4016−4030.

(19) Brattin, W.; Drexler, J.; Lowney, Y.; Griffin, S.; Diamond, G.; Woodbury, L. An in vitro method for estimation of arsenic relative bioavailability in soil. *J. Toxicol. Environ. Health, Part A* **2013**, *76*, 458−478.

(20) Juhasz, A. L.; Smith, E.; Weber, J.; Naidu, R.; Rees, M.; Rofe, A.; Kuchel, T.; Sansom, L. Assessment of four commonly employed in vitro arsenic bioaccessibility assays for predicting in vivo arsenic relative bioavailability in contaminated soils. *Environ. Sci. Technol.* **2009**, *43*, 9887−9894.

(21) Juhasz, A. L.; Weber, J.; Smith, E. Influence of saliva, gastric and intestinal phases on the prediction of As relative bioavailability using the unified Bioaccessibility Research Group of Europe method. *J. Hazard. Mater.* **2011**, *197*, 161−168.

(22) Ruby, M. V.; Schoof, R.; Brattin, W.; Goldade, M.; Post, G.; Harnois, M.; et al. Advances in evaluating the oral bioavailability of inorganics in soil for use in human health risk assessment. *Environ. Sci. Technol.* **1999**, *33*, 3697−3705.

DOI: 10.1021/acs.est.5b00905
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

(23) Denys, S.; Caboche, J.; Tack, K.; Rychen, G.; Wragg, J.; Cave, M.; Jondreville, C.; Feidt, C. In vivo validation of the unified BARGE method to assess the bioaccessibility of arsenic, antimony, cadmium, and lead in soils. *Environ. Sci. Technol.* **2012**, *46*, 6252−6260.
(24) Kelley, M. E.; Brauning, S. E.; Schoof, R. A.; Ruby, M. V. *Assessing Oral Bioavailability of Metals in Soil*; Battelle Press: Columbus, OH, 2002.
(25) DIN, Deutsches Institut fur Normung e. V Soil Quality: Absorption availability of organic and inorganic pollutants from contaminated soil material. DIN E 19738, 2000.
(26) Juhasz, A. L.; Smith, E.; Nelson, C.; Thomas, D.; Bradham, K. Variability Associated with As in Vivo-in Vitro Correlations When using Different Bioaccessibility Methodologies. *Environ. Sci. Technol.* **2014**, *48*, 11646−11653.
(27) Juhasz, A. L.; Basta, N. T.; Smith, E. What is required for the validation of in vitro assays for predicting contaminant relative bioavailability? Considerations and criteria. *Environ. Pollut.* **2013**, *180*, 372−375.
(28) Kutner, M. H.; Nachtsheim, C. J.; Neter, J.; Li, W. *Applied Linear Statistical Models*; McGraw-Hill Irwin: Burr Ridge, IL, 2005.
(29) Blume, L. J.; Schumacher, B. A.; Schaffer, P. W.; Cappo, L. A.; Papp, M. L.; Van Remortel, R. D.; et al. *Handbook of Methods for Acid Deposition Studies, Laboratory Analyses for Soil Chemistry*. EPA/600/S4-90/023; U.S. Environmental Protection Agency: Las Vegas, NV, 1991.
(30) Gelman, A.; Hill, J. *Data Analysis Using Regression and Multilevel/Hierarchical Models*; Cambridge University Press: New York, 2007.
(31) Chatfield, C. Model Uncertainty. *Encyclopedia of Environmetrics*; John Wiley & Sons: Hoboken, NJ, 2006.
(32) Casteel, S. W.; Cowart, R. P.; Weis, C. P.; Henningsen, G. M.; Hoffman, E.; Brattin, W. J.; et al. Bioavailability of lead to juvenile swine dosed with soil from the Smuggler Mountain, NPL Site of Aspen, Colorado. *Fundam. Appl. Toxicol.* **1997**, *36*, 177−187.
(33) Rees, M.; Sansom, L.; Rofe, A.; Juhasz, A.; Smith, E.; Weber, J.; Naidu, R.; Kuchel, T. Principles and application of an in vivo swine assay for the determination of arsenic bioavailability in contaminated matrices. *Environ. Geochem. Health* **2009**, *31*, 167−177.
(34) Bowell, R. J. Sorption of arsenic by iron oxides and oxyhydroxides in soils. *Appl. Geochem.* **1994**, *9*, 279−286.
(35) Jenne, E. A. Controls on Mn, Fe, Co, Ni, Cu, and Zn concentrations in soils and water: The significant role of hydrous Mn and Fe oxides. *Trace Inorganics in Water*; Advances in Chemistry Series; American Chemical Society: Washington, DC, 1968; Vol. 73, pp 337−387.
(36) McKeague, J. A.; Brydon, J. E.; Miles, M. N. Differentiation of forms of extractable iron and aluminum in soils 1. *Soil Sci. Soc. Am. J.* **1971**, *35*, 33−38.
(37) Koch, I.; Reimer, K. J.; Bakker, M. I.; Basta, N. T.; Cave, M. R.; Denys, S.; et al. Variability of bioaccessibility results using seventeen different methods on a standard reference material, NIST 2710. *J. Environ. Sci. Health, Part A: Toxic/Hazard. Subst. Environ. Eng.* **2013**, *48*, 641−655.

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Environ. Sci. Technol. XXXX, XXX, XXX−XXX