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Predicting oral relative bioavailability of arsenic in soil from in vitro bioaccessibility

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ABSTRACT

Several investigations have been conducted to develop in vitro bioaccessibility (IVBA) assays that reliably predict in vivo oral relative bioavailability (RBA) of arsenic (As). This study describes a meta-regression model relating soil As RBA and IVBA that is based upon data combined from previous investigations that examined the relationship between As IVBA and RBA when IVBA was determined using an extraction of soil in 0.4 *M* glycine at pH 1.5. Data used to develop the model included paired IVBA and RBA estimates for 83 soils from various types of sites such as mining, smelting, and pesticide or herbicide application. The following linear regression model accounted for 87% of the observed variance in RBA (R^2 = .87): RBA(%) = 0.79 × IVBA(%) + 3.0. This regression model is more robust than previously reported models because it includes a larger number of soil samples, and also accounts for variability in RBA and IVBA measurements made on samples collected from sites contaminated with different As sources and conducted in different labs that have utilized different experimental models for estimating RBA.

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A recent compilation of data from a variety of arsenic (As)-contaminated sites demonstrated that oral bioavailability of As in soil tends to be lower than that of water soluble sodium arsenate (U.S. Environmental Protection Agency [EPA], 2012a, 2012b). Expressed as relative oral bioavailability (RBA, the percent ratio of bioavailability of As in soil to that of dissolved sodium arsenate), the mean and 95th percentile RBA values for more than 100 soil samples were 30% and 60%, respectively. Many factors affect the bioavailability of As in soil, including chemical form of As, as well as physical and chemical characteristics of As-bearing soil particles, and these factors are known to vary both within and between sites (Bradham et al., 2011; Brattin et al., 2013; Roberts et al., 2007; Ruby et al., 1999; Scheckel et al., 2009). The EPA recommended that site-specific assessments of soil As RBA be performed where such assessments are deemed feasible and valuable for improving the characterization of risk at the site (U.S. EPA, 1989, 2007a, 2007b).

Currently accepted methods for assessing oral RBA of soil As require animal bioassays (U.S. EPA, 2012b). The time and expense of animal bioassays have prompted interest in developing more rapid and less expensive in vitro bioaccessibility (IVBA) assays that may be employed to reliably predict oral RBA of soil As (Bradham et al., 2011, 2015; Brattin et al., 2013; Denys et al., 2012; Juhasz et al., 2007a, 2007b, 2009, 2011, 2014a, 2014b; Makris et al., 2008; Medlin, 1997; Roberts et al., 2007; Rodriquez et al., 1999; Ruby et al., 1996; Wragg et al., 2011). IVBA assays measure the solubility of As when soil is incubated in an aqueous solution that often is intended to mimic temperature, pH, and other compositional characteristics of

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Supplemental data for this article can be accessed at 10.1080/15287394.2015.1134038 and include brief descriptions of in vivo RBA assay methods, IVBA and RBA estimates, and soil arsenic concentrations for all soil samples considered in this analysis (Table S-1), and plots of data from each laboratory (Figures S-1–S-3).

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gastrointestinal tract (GIT) fluids. The underlying assumption for the use of an IVBA assay to predict in vivo RBA is that solubility of a chemical in GIT fluids is an important determinant of GIT absorption; therefore, it is expected that IVBA will be highly correlated with RBA measured in vivo. Testing this assumption requires that regression models relating IVBA and RBA be evaluated with paired measurements of IVBA and RBA conducted on a variety of soils that reflect a range of As concentrations, soil types, and chemical forms of As that occur in soil at affected sites.

This study focused on an IVBA assay that was developed for and is currently used widely in the assessment of lead RBA (Drexler and Brattin, 2007). The assay involves a gastric-phase extraction of soil in a relatively simple extraction medium (0.4 *M* glycine buffer at pH 1.5 ± 0.5). Several investigations found that the 0.4 *M* glycine/pH 1.5 assay provided estimates of As IVBA that were highly correlated with in vivo As RBA measured in mice and swine (Bradham et al., 2011, 2013; Brattin et al., 2013; Juhasz et al., 2009, 2014a). However, each of these investigations derived a unique regression model relating IVBA and RBA. Several factors may have contributed to differences in the regression models derived in each study, including use of different animal bioassays for measuring RBA, interlab differences in IVBA measurement error, and variability in soil characteristics or As speciation of the samples assayed by each lab. Although one could opt to select a specific regression model from a given study for human health risk assessment applications, this is unlikely to be optimal because there are no apparent distinguishing quality or conceptual factors that may serve as a basis for deciding which regression model more accurately predicts soil As RBA in humans.

An alternative to selecting a single study as the basis for a regression model is to derive a model based on collective data from multiple investigations. This single meta-model would incorporate the observed variability in the relationship between IVBA and RBA found in multiple studies, and would provide more realistic prediction limits for assessing uncertainty in RBA estimates utilized in risk assessment. Accordingly, in this analysis, data from multiple investigations were combined into an aggregate (pooled) data set and a regression model was derived for predicting RBA from IVBA measured in the 0.4 *M* glycine/pH 1.5 assay. The resulting pooled regression model is more robust than previously reported models because it includes both a larger number of soil samples and a wider variety of sources of As contamination such as mining, smelting, and herbicides or pesticides. In addition, the pooled model also accounts for variability in RBA and IVBA measurements performed in different labs that utilized different experimental models for estimating RBA.

Methods

Data included in the meta-analysis

Studies that contributed data to the meta-analysis found good correlations ($R^2 > .6$) between As IVBA and RBA in soils, when IVBA was assessed with the 0.4 *M* glycine/pH 1.5 assay (Bradham et al., 2011, 2015; Brattin et al., 2013; Juhasz et al., 2009, 2014a). From these investigations, only data from authentic site samples were selected for meta-analysis. Samples prepared by adding As to soil in the lab, with or without aging, were excluded from consideration. Investigators for these studies were contacted and each agreed to provide individual sample data for the meta-analysis, including confidence estimates for each RBA and IVBA value. Data from the individual labs provided the following numbers of IVBA–RBA pairs: lab A, $n = 40$ (Bradham et al. 2011, 2013, 2015); lab B, $n = 19$ (Brattin et al. 2013); and lab C, $n = 24$ (Juhasz et al. 2009, 2014a).

IVBA assay methods

All IVBA assays followed the extraction protocol described in U.S. EPA (2012c). Each soil sample was dried and sieved (<250 μm), and 1 g sieved soil was incubated with constant agitation (end-over-end rotation) at 37°C for 1 h in 100 ml solvent made up of 0.4 *M* glycine, pH 1.5. Arsenic in the soil was quantified by Instrumental Neutron Activation Analysis, or by microwave-assisted digestion (EPA SW-846 Method 3051A), with analysis by inductively coupled plasma–atomic emission spectrometry (ICP-AES; EPA SW-846 Method 6010c) (U.S. EPA, 2007c) or ICP–mass spectrometry (ICP-

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MS; EPA SW-846 Method 6020A) (U.S. EPA, 2007d). Arsenic in the extraction fluid was measured by ICP-MS (EPA SW-846 Method 6020) or inductively coupled plasma–optical emission spectroscopy (ICP-OES, EPA SW-846 Method 6010c).

In vivo RBA assay methods

In vivo RBA assays used in the various studies have been published and are described briefly in the Supplemental Data. Assays conducted in C57BL/6 mice were performed as reported in Bradham et al. (2011) (referred to in this report as the mouse urinary excretion fraction or UEF assay). In vivo RBA assays in juvenile swine reported in Brattin et al. (2013) were performed as described in Brattin and Casteel (2013) (referred to in this report as the swine UEF assay). In vivo RBA assays conducted in juvenile swine, reported in Juhasz et al. (2009, 2014b), were performed as described in Rees et al. (2009) (referred to in this report as the swine area under the curve or AUC assay).

Statistical analyses

All statistical analyses were performed using SAS/STAT software, Version 9.3 of the SAS System for Windows SAS software or Aegis Technologies Advanced Continuous Simulation Language (acslX). A p value ≤.05 was considered significant in all comparisons.

Two types of regression analyses were performed on these data. Preliminary regression modeling relied on ordinary least squares (OLS) linear regression. These preliminary analyses were used to identify major explanatory variables for RBA, outliers, and regression diagnostics (e.g., heteroscedasticity, normality of residuals). Final regression modeling of the pooled data used weighted least squares regression. This approach allowed incorporation of uncertainty in individual IVBA and RBA estimates into the regression model.

Linear regression was performed using the general linear model (SAS PROC GLM), with RBA as the dependent variable. The general linear model used in these analyses is given in Eq. (1):

$$Y_i = \beta_0 + \beta_1 X_{i1} + \beta_2 X_{i2} + \beta_3 X_{i3} + \epsilon_i \quad (1)$$

where β_0 is the intercept and β_1 is the slope coefficient for IVBA (X_1). Because each lab estimated RBA using different animal bioassays, an explanatory categorical variable (*laboratory*) was included in the general linear model (β_2, X_2 in Eq. (1)). The predominant source of As (e.g., ore or ore processing, pesticide application, herbicide application) at the site from which the soil sample was collected was also explored as a categorical variable (*As source*, β_3, X_3 in Eq. (1)). Requirements for homoscedasticity and normality of errors were tested using White's test (SAS PROC REG/SPEC) and the Shapiro–Wilks statistic (SAS PROC REG/UNIVARIATE), respectively. Pooled data were tested for heterogeneity of slopes for different labs (SAS PROC GLM, X*GROUP effect). Studentized residuals (>3 or <–3) were used to identify statistical outliers. Cook's D statistic (>4/n) was used to identify influential outliers.

Based on the results of the multivariate regression analysis, the model was reduced to a monovariate, with IVBA as the independent variable (see Results section for explanation). Weighted linear regression was performed with uncorrelated weights assigned to each soil (W_i) calculated from Eq. (2):

$$W_i = \frac{w(X_i)\, w(Y_i)}{w(X_i) + (Y_i)\,(\beta^2)} \quad (2)$$

where $w(X_i)$ are IVBA weights ($1/SD_i^2$), $w(Y_i)$ are RBA weights ($1/SE_i^2$), and β is the slope of the linear regression line fit by minimizing the weighted sum of squared residuals (Thirumalai et al., 2011; York et al., 2004).

Results

Attributes of the data set

The data set is summarized in Table 1 and individual sample data are presented in the Supplemental Data (Table S-1). As shown, there was wide variability in As concentrations and values for IVBA and RBA values across the 83 samples. This wide variability is important to help constrain and define the model. The correlations between IVBA and RBA data from each lab are shown in the Supplemental Data (Figures S-1–S-3). Means of IVBA or RBA

Table 1. Summary of IVBA and RBA Estimates Used in the Meta-Analysis.

	Data source			
	Lab A	Lab B	Lab C	Pooled
RBA assay	Mouse UEF	Swine UEF	Swine AUC	Mouse, swine
Sample *N*	40	19	24	83
Soil As (ppm)				
Mean	790	670	664	726
SD	1214	852	577	978
Median	455	383	468	401
Range	108, 6899	181, 3857	42, 2270	42, 6899
IVBA (%)				
Mean	25.0	29.5	29.0	27.2
SD	19.5	18.4	23.0	20.2
Median	18.2	22.0	18.4	19.0
Range	0.0, 74.3	6.0, 78.0	3.8, 80.0	0.0, 80.0
RBA (%)				
Mean	24.3	37.3	29.3	28.7
SD	13.9	12.9	21.1	16.7
Median	21.4	40.3	21.2	23.8
Range	1.9, 51.6	17.8, 60.2	7.0, 80.5	1.9, 80.5

Note. As, arsenic; IVBA, in vitro bioaccessibility; RBA, relative bioavailability; SD, standard deviation; UEF, urinary excretion fraction; AUC, area under the curve.

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from sites contaminated predominantly from ore or ore processing (gossan, mining, smelting), pesticides (orchards and livestock dip sites), or herbicides (railway corridors) were not significantly different (Table 2).

Preliminary evaluations

A preliminarily regression analysis of pooled data using OLS regression was used to identify important independent variables in the regression model. The effect of interlab variability was explored with multiple regression analysis in which laboratory was included in the regression model as a categorical variable representing the lab source of each IVBA-RBA observation. The effect of predominant source of As contamination was included in the regression model as a categorical variable (As source) representing ore or ore processing, pesticide application, or herbicide application. Laboratory, As source, and IVBA were found to be significant variables in the pooled model and together accounted for approximately 85% of the variance in RBA (R^2 = .85). A test of heterogeneity of slopes for data from each lab or As source was not significant, indicating that combined data may be described by a common slope. When IVBA, laboratory, and As source were included in the model, the regression coefficient (slope) for *IVBA* was 0.71 (±0.04 SE) and the intercept was 12.4 (±2.2 SE). When laboratory was excluded from the model, leaving As source and IVBA as the sole explanatory variables, R^2 decreased from .85 to .79. When both As source and laboratory were excluded from the model, leaving IVBA as the sole explanatory variable, R^2 was .76. This suggests that approximately 76% of the variance in RBA was attributable to IVBA and approximately 9% was attributed to As source (3%) and laboratory (6%). These results indicate that, for this data set, IVBA is the dominant variable determining RBA. On

Table 2. Summary of IVBA and RBA Estimates by Primary Contamination Source.

	Primary source of contamination		
	Ore or ore processing[a]	Insecticide application[b]	Herbicide application[c]
Sample *N*	52	21	10
Soil As (ppm)			
Mean	899	450	404
SD	1183	280	393
Median	586	364	252
Range	88, 6899	200, 1221	42, 1114
IVBA (%)			
Mean	27.8	20.8	37.0
SD	21.3	13.7	23.0
Median	20.1	18.0	34.9
Range	0.0, 78.4	5.7, 55.4	10.5, 80.0
RBA (%)			
Mean	27.3	28.1	37.5
SD	16.4	13.3	23.1
Median	23.3	29.1	30.0
Range	1.9, 70.5	10.1, 52.8	10.1, 80.5

Note. As, arsenic; IVBA, in vitro bioaccessibility; RBA, relative bioavailability; SD, standard deviation.
[a]Gossan, mining, smelting.
[b]Orchards, livestock dip sites.
[c]Railway corridors.

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this basis, the monovariate model with only IVBA as the explanatory variable was adopted for estimating parameter values for the regression relationship between IVBA and RBA.

Final model fitting

Development of the final model used weighted regression in which the squared error between each observed and predicted RBA value is weighted by the uncertainty in the measured RBA and IVBA values (estimates having higher uncertainty were assigned smaller weights). Weighting for RBA uncertainty is needed because of the relatively wide range of uncertainties in reported RBA values (0.3–19.9%). Ratios of SE/mean RBA also appeared to vary across labs, although mean SE/mean ratios were not significantly different: A, 0.07 (±0.034 SD), B, 0.082 (±0.039 SD), and C, 0.20 (±0.13 SD). Uncertainty in IVBA values was generally smaller and more uniform than RBA. Mean coefficients of variation (CV, ratio of mean/standard deviation) for IVBA in each lab were <5%, typical for the As IVBA assay (Brattin et al., 2013). Mean IVBA CVs were similar across labs and lower than mean/SE ratios for RBA: A, 0.04, B, 0.05, and C, 0.021. The weighted regression model is shown in. Figure 1 The estimated slope is 0.79 ± 0.01 (SE) and intercept is 3.0 ± 0.1 (SE). The equation of the model is

$$\mathrm{RBA}(\%) = 0.79 \times \mathrm{IVBA}(\%) + 3.0 \qquad (3)$$

This model explains approximately 87% of the variance in RBA (weight-adjusted R^2 = .87). The 95% prediction limit for a single RBA measurement was ±19% RBA.

The model shown in Figure 1 excludes one observation that was an influential outlier based on its Studentized residual = −3.6 and Cooks D statistic (= 0.12) > $4/n$ (= 0.05) (sample number 34, Supplemental Data, Table S-1). The IVBA for this sample was 41.7%, and its RBA was 6.8%. The high IVBA/RBA ratio for this soil (6.1) exceeded ratios for all other soils (range: 0.25–1.6). Excluding this sample exerted little effect on the pooled regression model. With the observation included, the regression model (R^2 = .85) had slope 0.76 (±0.01) and intercept 3.0 (±0.1 SE).

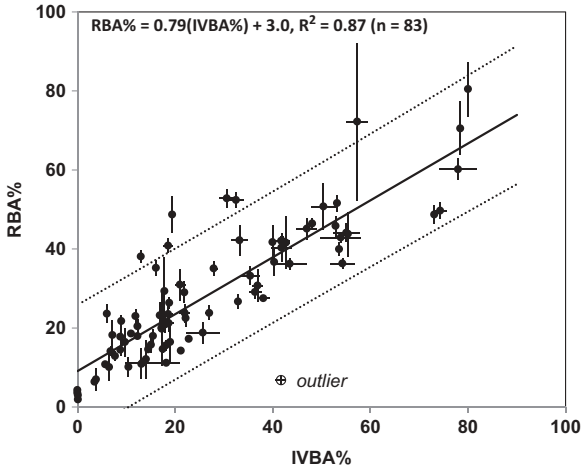


Figure 1. Weighted linear regression of RBA and IVBA based on data pooled from three laboratories. Points are observations ±SE (RBA) and ±SD (IVBA). The solid line is the weighted regression model. Dotted lines are 95% prediction limits for the weighted model. The data point labeled *outlier* was not used in fitting regression models.

Discussion

Several investigations have been conducted by scientists worldwide to develop IVBA assays that reliably predict in vivo RBA of As (Bradham et al., 2011; 2015; Brattin et al., 2013; Roberts et al., 2007; Denys et al., 2012; Juhasz et al., 2007a, 2007b, 2009, 2011, 2014a, 2014b; Makris et al., 2008; Medlin, 1997; Rodriguez et al., 1999; Ruby et al., 1996; Wragg et al., 2011). This meta-analysis builds on published studies that examined the relationship between IVBA and RBA of As in soil when IVBA was measured from an extraction of soil in 0.4 *M* glycine at pH 1.5 (Bradham et al., 2011; 2015; Brattin et al., 2013; Juhasz et al., 2009; 2014a). Using different in vivo RBA bioassays, these studies found that the 0.4 *M* glycine/pH 1.5 IVBA assay, originally developed for predicting in vivo RBA of soil lead (U.S. EPA, 2012c), performed well in predicting in vivo As RBA in mice and swine. The Juhasz et al. (2009, 2014a) studies compared the performance of existing IVBA assays, whereas Brattin et al. (2013) optimized IVBA assay conditions de novo and found that optimal performance was achieved with 0.4 *M*

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glycine/pH 1.5. Performance of the IVBA assay (R^2) was not improved at higher pH or with addition of phosphate, hydroxylamine, or hypochlorite, or with increased buffer strength. In addition, when 4 independent labs used the 0.4 *M* glycine/pH 1.5 assay to estimate IVBA of 12 identical soil samples, interlab variation in mean IVBA estimates was less than 5% (mean 1.7%; Brattin et al., 2013). Collectively, these studies provided evidence for reliability and reproducibility of the 0.4 *M* glycine/pH 1.5 IVBA assay for predicting soil As RBA.

Each of these investigations derived a unique regression model relating IVBA and RBA (Bradham et al., 2011, 2015; Brattin et al., 2013; Juhasz et al., 2009, 2014a). Differences in the regression models developed by different labs are likely due to use of different animal bioassays for measuring RBA, interlab variation in IVBA measurement error, and variability in soil characteristics or As speciation of samples assayed by each lab. The use of different animal models to measure RBA is particularly important, because the intended application of the IVBA assay is for predicting RBA in humans. However, no animal models or IVBA assays have been evaluated directly for predicting RBA in humans, nor are such evaluations in humans feasible or likely to be undertaken in the near future. Rather than attempting to justify selection of a specific animal model, this analysis combined data from studies that used different animal models and different methods to estimate RBA. In the combined data, laboratory (representing the source of each data pair) was a significant variable in the regression model; however, additional variance in RBA explained by including laboratory was relatively small (6%), compared to 76% for the model with IVBA alone. The mouse and swine assays yielded similar results when applied to aliquots of the same soil samples (Juhasz et al., 2014b; Bradham et al., 2013), which provides further support for their inclusion in the meta-analysis. The predominant source of the As at the sites from which the samples were collected (ore or ore processing, pesticide application, or herbicide application) was also a significant explanatory variable in the regression model; however, it explained only approximately 3% of the variance in RBA. This variation may reflect differences in As species related to the source of contamination (Bradham et al. 2013; Brattin et al. 2013).

The final weighted regression model is considered to be more robust than the OLS model because it incorporates uncertainty in the individual estimates of RBA and IVBA. The weighted model is based on a large (n = 83) sample set that includes soils affected by various As waste processes, including mining, smelting, and pesticide or herbicide application (Table S-1), and data from three different RBA bioassays. By combining data from multiple studies, the model incorporates interlab variability in the IVBA–RBA relationship, thereby providing more realistic prediction limits for RBA, which are important considerations in risk assessment. Given the size and breadth of the sample sources, we postulate that new data are unlikely to alter the regression parameter estimates appreciably; however, the model may be updated and reevaluated as new IVBA–RBA data become available. Of particular interest would be paired RBA and IVBA measurements for additional soils that might serve as a validation data set, allowing comparison of predicted and measured RBA in a set of soils that were not included in data used to derive the regression model.

This analysis relied completely on in situ contaminated soils and excluded spiked samples that were created by addition of sodium arsenate to a site soil. This approach was utilized because the regression model is intended for use in predicting As RBA of soils found at sites where contamination may have been in place for many years or decades, whereas IVBA and RBA measures of spiked soils may change with aging depending on the physical–chemical properties of the soil (Brattin et al., 2013; Juhasz et al., 2007a, 2007b, 2009). Moreover, soils spiked with sodium arsenate usually display IVBA and RBA values close to 100%, which is higher than typically found at sites of contamination. A compilation and analysis of soil As RBA estimates that included more than 100 in situ contaminated soils collected from a wide variety of metal contaminated sites found that the 95th percentile RBA was 60% (U.S. EPA, 2012a). The 95th percentile RBA for the pooled data used in this analysis was

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54.3% and the highest RBA was 80.5%. Excluding sodium arsenate spiked samples from the analysis yielded a regression model that is more representative of As RBA likely to be encountered at most contaminated sites.

The observation of one influential outlier in data collected for this analysis suggests that 0.4 *M*/pH 1.5 IVBA assay may not reliably predict RBA in every soil. In this case, the soil sample (number 34 in Supplemental Data, Table S-1) exhibited an IVBA (41.7%) that was sixfold higher than its RBA (6.8%). The soil was collected from a site affected by historical arsenate pesticide applications. However, this was not the sole contributing factor to the large IVBA/RBA ratio, because 17 other pesticide sites did not display large IVBA/RBA ratios (range 0.4–1.3). Presumably, the large IVBA/RBA ratio arose from yet-to-be identified factors unique to this soil that influence As absorption and are not accounted for by the IVBA assay. Although only 1 of 84 soil samples included in this analysis was a statistical outlier, an important future objective would be to gather more information on such soils to identify measurable soil or As characteristics that might help identify samples that may not be well-represented by the current model.

The regression model based on the pooled data provides strong support for the 0.4 *M* glycine/pH 1.5 IVBA assay (U.S. EPA, 2012c) to predict oral RBA of As in soil. The IVBA method was shown in multiple labs to provide a strong correlation with RBA (Bradham et al., 2011, 2015; Brattin et al., 2013; Juhasz et al., 2009; 2014a) and demonstrated excellent interlab reproducibility (Brattin et al., 2013). The 0.4 *M* glycine/pH 1.5 IVBA assay has several important attributes that make it highly suitable for risk assessment. In addition to reliably predicting in vivo RBA, it is a relatively simple assay that does not require special lab equipment other than a means for monitoring pH and agitating samples at 37°C. Laboratories that are qualified to run EPA Standard Operating Procedure 9200.2-86 (U.S. EPA, 2012c) have all the necessary apparatus for performing soil extractions. The extraction protocol is identical to that employed in the IVBA assay which EPA has validated for predicting the RBA of lead in soil for human health risk assessment (U.S. EPA, 2007b). Adoption of this extraction protocol as a standard protocol for As enables RBA estimates for lead and As to be obtained in a single extraction of the same soil sample, potentially realizing a cost savings at sites where lead and As are co-contaminants in soil.

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Supporting Information

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Brief descriptions of *in vivo* RBA assay methods, IVBA and RBA estimates and soil arsenic concentrations for all soil samples considered in this analysis (Table S-1), regression models for data from each laboratory (Figures S-1–S-3). This material is available free of charge via Internet at http://pubs.acs.org.

In vivo RBA Assay Methods. *In vivo* RBA assays in mice were performed as reported in Bradham et al. (2011, referred to in this report as the *mouse UEF assay*). In brief, female C57BL/6 mice, 4 to 6 weeks of age, were housed in metabolic cages (three mice per cage) allowing collection of urine and feces. The Institutional Animal Care and Use Committee of the U.S. EPA National Health and Environmental Effects Research Laboratory approved the protocol for mouse use, which assured humane treatment and alleviation of suffering. Mice were maintained on a low-arsenic basal diet (AIN-93G) that had an arsenic concentration of <1 ppm, which contributed negligible levels of arsenic in urine. Groups of 12 mice were fed a diet amended with either sodium arsenate or the test soil, for a period of ten days, during which urine and feces were collected daily and food consumption was measured daily. Each group of mice was fed a single arsenic dose level. RBA of arsenic in soil was calculated as the ratio of urinary excretion fractions (UEF) in animals that ingested arsenic in soil or sodium arsenate, where UEF was the ratio of the cumulative urinary arsenic excretion (µg per 10 days) and the cumulative dietary arsenic intake (µg per 10 days, Equations S1 and S2).

$$UEF = \frac{Cum\ Urinary\ As}{Cum\ As\ Dose} \quad \text{Eq. (S1)}$$

$$RBA = 100 \cdot \frac{UEF_{Soil}}{UEF_{NaAsO_4}} \quad \text{Eq. (S2)}$$

In vivo RBA assays reported in Brattin et al. (2013) were performed as described in Brattin and Casteel (2013, referred to in this report as the *swine UEF assay*). In brief, male juvenile swine were housed in individual stainless steel cages that enabled collection of urine. Swine had free access to water and were fed a diet having an arsenic concentration of <1 ppm. Groups of swine (5–7 weeks of age, 7–12 kg) received repeated daily doses of arsenic in soil or sodium arsenate for a period of 14 days. The doses were delivered in a dough ball (approximately 5 g of moist feed), twice daily, 2 hours before each feeding. Three dose levels of arsenic as sodium arsenate and 2 or 3 dose levels of soil arsenic were administered (4–5 animals per dose group), with doses selected to achieve similar ranges for soil arsenic and sodium arsenate. Periodic 48-hour urine collections were made during the dosing period. RBA of arsenic in soil was calculated as the ratio of UEF in animals that ingested arsenic in soil or sodium arsenate (Equation S2). UEF was estimated by regression analysis using simultaneous weighted regression of arsenic mass excreted vs arsenic mass administered. This automatically incorporates all the data and adjusts for a non-zero value in control animals.

In vivo RBA assays reported in Juhasz et al. (2009, 2014a) were performed as described in Rees et al. (2009, referred to in this report as the *swine AUC assay*). In brief, juvenile female swine

were surgically prepared with indwelling jugular vein catheters. Each of three pigs were administered a single oral gavage dose of sodium arsenate solution or an aqueous suspension of soil at different times (i.e., each pig served as its own reference). Blood samples were collected over a 26-hour period following dosing. RBA of arsenic in soil was calculated as the ratio of dose (µg)-adjusted area under the curve (AUC) of the arsenic concentration in plasma (µg hr/L) in animals that ingested arsenic in soil or sodium arsenate (Equation S3):

$$RBA\ (\%) = 100 \cdot \frac{AUC_{Soil}}{AUC_{NaAsO_4}} \cdot \frac{Dose_{NaAsO_4}}{Dose_{Soil}} \qquad \text{Eq. (S3)}$$

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Table S-1. Data Used for Meta-analysis of IVBA Assay for Predicting oral RBA of Arsenic

ID	As Source	Soil As (ppm)	IVBA (%)	IVBA SD (%)	RBA (%)	RBA SE (%)	RBA Assay
1	Mining/smelting	676	13.0	0.7	38.1	1.6	Swine UEF
2	Mining/smelting	313	32.5	1.6	52.4	2.0	Swine UEF
3	Pesticide (orchard)	290	21.0	1.1	31.0	4.0	Swine UEF
4	Pesticide (orchard)	388	18.6	0.9	40.8	1.8	Swine UEF
5	Pesticide (orchard)	382	19.4	0.4	48.7	4.7	Swine UEF
6	Pesticide (orchard)	364	30.6	1.5	52.8	2.3	Swine UEF

7	Mining/smelting	234	8.8	0.3	17.8	3.2	Swine UEF
8	Mining/smelting	367	6.0	0.3	23.6	2.4	Swine UEF
9	Mining/smelting	181	50.4	2.5	50.7	5.9	Swine UEF
10	Mining	200	78.0	3.9	60.2	2.7	Swine UEF
11	Mining	3957	11.0	0.6	18.6	0.9	Swine UEF
12	Mining/smelting	590	55.1	2.8	44.1	2.3	Swine UEF
13	Mining/smelting	1400	42.2	0.6	41.8	1.4	Swine UEF
14	Mining/smelting	312	41.8	2.1	40.3	3.6	Swine UEF
15	Mining/smelting	983	33.2	1.7	42.2	3.8	Swine UEF
16	Mining/smelting	390	40.3	0.7	36.7	3.3	Swine UEF
17	Mining/smelting	813	22.0	1.1	23.8	2.4	Swine UEF
18	Mining/smelting	368	18.7	0.9	21.2	2.1	Swine UEF
19	Mining/smelting	516	18.6	0.9	23.5	2.6	Swine UEF
20	Herbicide (railway corridor)	267	57.3	2.2	72.2	19.9	Swine AUC
21	Herbicide (railway corridor)	42	42.7	0.8	41.6	6.6	Swine AUC
22	Herbicide (railway corridor)	1114	17.2	0.4	20.0	9.5	Swine AUC
23	Herbicide (railway corridor)	257	10.5	0.1	10.1	2.5	Swine AUC
24	Herbicide (railway corridor)	751	22.2	0.0	22.5	2.2	Swine AUC
25	Herbicide (railway corridor)	91	80.0	0.3	80.5	6.9	Swine AUC
26	Pesticide (dip site)	713	17.8	0.1	29.3	8.7	Swine AUC
27	Pesticide (dip site)	228	55.4	0.6	43.8	5.6	Swine AUC
28	Mining	807	40.0	0.1	41.7	4.4	Swine AUC

29	Mining	577	3.8	0.0	7.0	2.9	Swine AUC
30	Gossan	190	19.0	0.2	16.4	5.2	Swine AUC
31	Gossan	88	14.0	0.2	12.1	4.9	Swine AUC
32	Pesticide	275	5.7	0.2	10.8	0.7	Swine AUC
33	Pesticide	210	7.7	0.4	12.9	1.2	Swine AUC
34	Pesticide	81	41.7	1.1	6.8	1.2	Swine AUC
35	Pesticide	358	6.5	0.1	10.1	3.5	Swine AUC
36	Pesticide	200	13.1	0.3	10.9	3.9	Swine AUC
37	Pesticide	215	7.2	0.2	18.2	3.8	Swine AUC
38	Pesticide	981	9.7	0.2	16.4	3.6	Swine AUC
39	Pesticide	1221	15.1	0.6	15.7	1.9	Swine AUC
40	Mining	949	52.9	0.1	45.8	2.6	Swine AUC
41	Mining	1126	36.9	1.1	30.7	4.1	Swine AUC
42	Mining	1695	38.1	1.3	27.5	0.7	Swine AUC
43	Mining	1306	78.4	0.4	70.5	6.8	Swine AUC
44	Mining	2270	43.5	3.4	36.2	1.5	Swine AUC
45	Mining	244	18.1	0.40	15.5	1.3	Mouse UEF
46	Mining	173	6.8	0.80	14.1	1.2	Mouse UEF
47	Mining	6899	17.5	0.60	14.7	1.0	Mouse UEF
48	Mining	280	53.6	0.20	39.9	1.7	Mouse UEF
49	Mining	4495	8.8	0.10	14.5	1.6	Mouse UEF
50	Mining	448	22.8	0.6	17.2	0.5	Mouse UEF
51	Mining	195	25.7	3.4	18.8	2.7	Mouse UEF
52	Mining/smelting	837	18.2	2.70	11.2	0.3	Mouse UEF
53	Mining/smelting	182	32.9	0.20	26.7	1.8	Mouse UEF
54	Mining/smelting	990	73.1	0.60	48.7	2.4	Mouse UEF
55	Mining/smelting	829	74.3	1.30	49.7	2.1	Mouse UEF
56	Mining/smelting	379	53.2	0.50	51.6	2.1	Mouse UEF

57	Pesticide (orchard)	322	18.8	0.30	26.3	1.4	Mouse UEF
58	Pesticide (orchard)	462	16.1	0.40	35.2	2.0	Mouse UEF
59	Pesticide (orchard)	401	18.0	0.20	20.9	2.2	Mouse UEF
60	Pesticide (orchard)	422	27.9	0.80	35.0	1.8	Mouse UEF
61	Pesticide (orchard)	340	35.4	1.90	33.2	2.4	Mouse UEF
62	Pesticide (orchard)	396	48.1	0.80	46.4	1.4	Mouse UEF
63	Pesticide (dip site)	965	9.0	0.40	21.7	1.5	Mouse UEF
64	Pesticide (dip site)	313	36.4	1.30	29.1	1.7	Mouse UEF
65	Herbicide (railway corridor)	246	47.0	2.10	45.1	2.7	Mouse UEF
66	Herbicide (railway corridor)	108	27.0	0.80	23.8	1.9	Mouse UEF
67	Herbicide (railway corridor)	184	11.9	0.20	23.0	1.8	Mouse UEF
68	Herbicide (railway corridor)	981	54.3	2.50	36.3	1.3	Mouse UEF
69	Mining	573	3.5	0.30	6.4	0.3	Mouse UEF
70	Mining	583	21.2	0.20	14.2	0.3	Mouse UEF
71	Gossan	239	12.3	0.70	20.4	1.9	Mouse UEF
72	Mining	197	21.9	0.20	29.0	2.7	Mouse UEF
73	Mining	884	16.9	0.40	23.2	3.3	Mouse UEF
74	Mining	293	12.3	0.30	17.9	0.7	Mouse UEF
75	Mining	223	17.3	0.10	19.8	1.9	Mouse UEF
76	Mining	494	15.5	0.10	18.0	1.8	Mouse UEF
77	Mining	738	13.4	3.50	11.2	0.9	Mouse UEF
78	Mining	777	0.0	0.00	4.3	0.7	Mouse UEF
79	Mining	943	0.1	0.00	3.0	0.2	Mouse UEF
80	Mining	898	0.1	0.00	1.9	0.2	Mouse UEF

81	Mining	668	0.0	0.00	3.6	0.3	Mouse UEF
82	Mining/smelting (SRM)	601	54.0	4.10	42.9	1.2	Mouse UEF
83	Mining/smelting (SRM)	1513	41.8	1.70	42.1	1.1	Mouse UEF
84	Mining/smelting (SRM)	879	14.5	0.20	14.6	0.8	Mouse UEF

As, arsenic; AUC, area under the curve; ID, sample identification number; IVBA, *in vitro* bioaccessibility; RBA, relative bioavailability; SD, standard deviation; SE, standard error; SRM, standard reference material; UEF, urinary excretion fraction

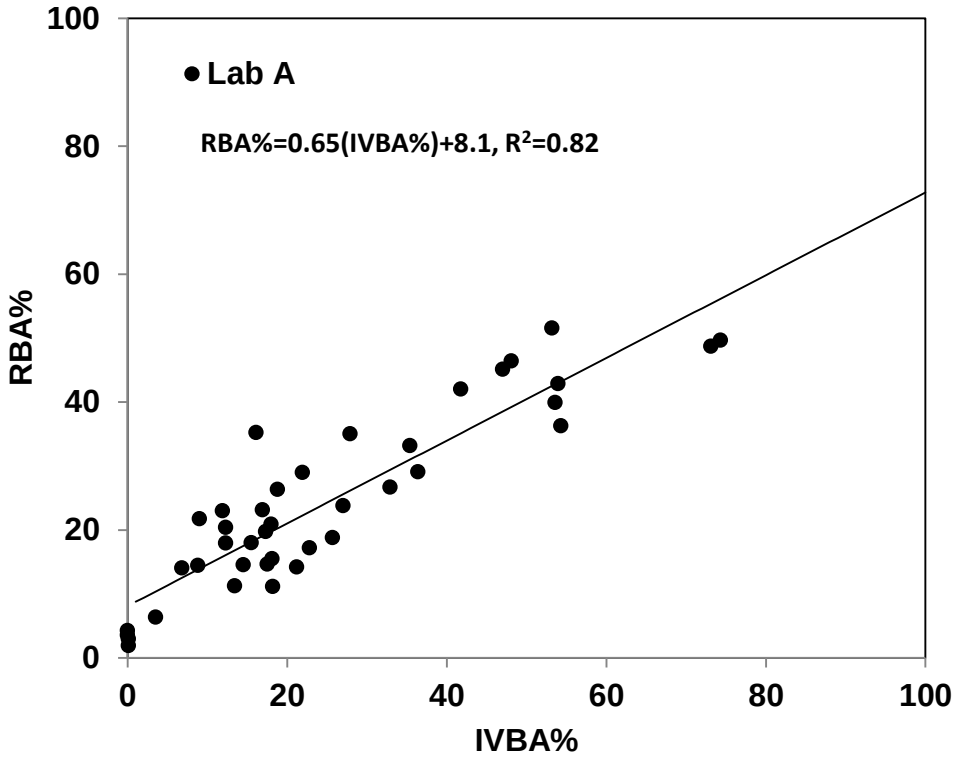


Figure S-1. Ordinary least squares linear regression model for data from Laboratory A (n=40; RBA estimated with mouse UEF assay; Bradham et al 2011).

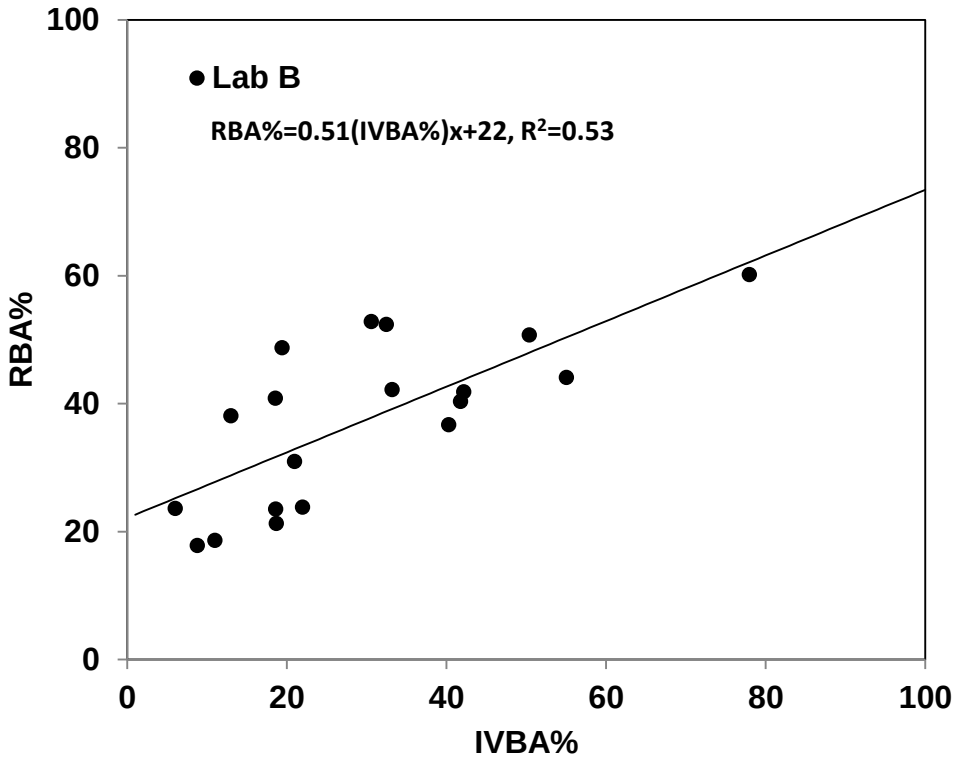


Figure S-2. Ordinary least squares linear regression model for data from Laboratory B (n-19; RBA estimated with swine UEF assay; Brattin and Casteel, 2013).

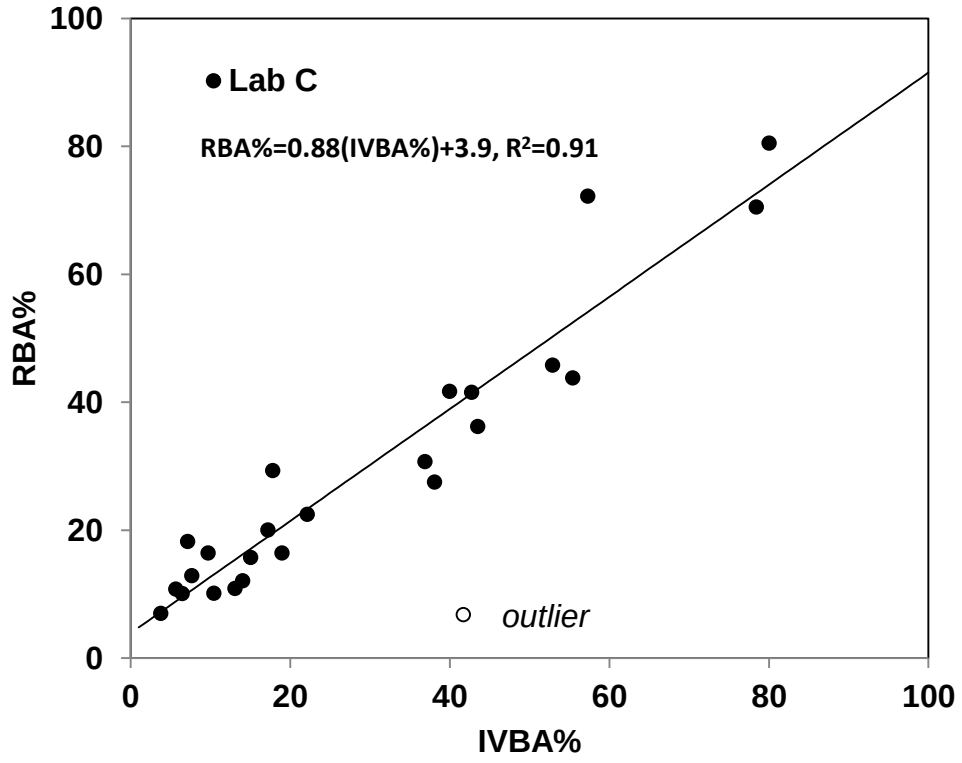


Figure S-3. Ordinary least squares linear regression model for data from Laboratory C (n=24; RBA estimated with swine AUC assay; Rees et al. 2009). The data point labeled *outlier* was not used in fitting the regression model.