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WASHINGTON, D.C. 20460

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SOLID WASTE AND
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MEMORANDUM

SUBJECT: Release of Standard Operating Procedure for an In Vitro Bioaccessibility Assay for Lead and Arsenic in Soil and Validation Assessment of the In Vitro Arsenic Bioaccessibility Assay for Predicting Relative Bioavailability of Arsenic in Soils and Soil-like Materials at Superfund Sites

FROM: Schatzi Fitz-James, Acting Director Schatzi Fitz-James
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TO: Superfund National Program Managers, Regions 1-10

The purpose of this memorandum is to transmit the Technical Review Workgroup (TRW) for Metals and Asbestos technical documents entitled "Standard Operating Procedure for an In Vitro Bioaccessibility Assay for Lead and Arsenic in Soil" and "Validation Assessment of In Vitro Arsenic Bioaccessibility Assay for Predicting Relative Bioavailability of Arsenic in Soils and Soil-like Materials at Superfund Sites." The Standard Operating Procedure provides an update to EPA Method 1340 (Standard Operating Procedure for an In Vitro Bioaccessibility Assay for Lead in Soil, April 2012, EPA 9200.2-86) by including an assessment of arsenic bioaccessibility. The Validation Assessment Report presents the basis for the Agency's determination that the In Vitro Bioaccessibility Assay (IVBA) method has satisfied the validation and regulatory acceptance criteria for application of the method for arsenic.

EPA Method 1340 was first published as an SW-846 Method by EPA Office of Resource Conservation and Recovery in 2013 for the assessment of lead bioaccessibility as a method to calculate Relative Bioavailability (RBA) and is now regularly used at Superfund sites. Since then, the TRW has worked to incorporate the assessment of arsenic bioaccessibility into this same method. Arsenic and lead are commonly found together at Superfund sites and accurately measuring their RBA has a significant impact on the risk assessment and on the selection of soil cleanup levels. The addition of arsenic to this method allows the arsenic RBA to be measured rapidly and inexpensively. The method does not require the use or sacrifice of animals, and the reduced cost per sample allows risk assessors to obtain a more representative number of soil samples per exposure unit. Additionally, the incorporation of arsenic into the already existing method for lead means that laboratories already have experience performing the assay.

These two documents can be accessed on the US EPA Superfund Website: https://www.epa.gov/superfund/soil-bioavailability-superfund-sites-guidance#arsenic. Please contact Matt Lambert at lambert.matthew@epa.gov or 703-603-7174 if you have any questions or concerns.

Attachments:

1. "Standard Operating Procedure for an In Vitro Bioaccessibility Assay for Lead and Arsenic in Soil"
2. "Validation Assessment of In Vitro Arsenic Bioaccessibility Assay for Predicting Relative Bioavailability of Arsenic in Soils and Soil-like Materials at Superfund Sites."

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Validation Assessment of *In Vitro* Arsenic Bioaccessibility Assay for Predicting Relative Bioavailability of Arsenic in Soils and Soil-like Materials at Superfund Sites

1. Introduction

This report summarizes the basis for the Agency's determination that the IVBA method for arsenic has satisfied the validation and regulatory acceptance criteria for application of the method in an appropriate regulatory context. Validation and regulatory acceptance criteria developed by the U.S. Environmental Protection Agency (U.S. EPA, 2007a), as adapted from the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM, 1997), have been applied to an *in vitro* arsenic bioaccessibility (IVBA) assay described in detail by Brattin et al. (2013). The arsenic IVBA method estimates site-specific relative bioavailability (RBA) of arsenic in soils quickly and inexpensively relative to *in vivo* methods. The arsenic IVBA assay is well suited for regulatory use in arsenic risk assessment for several reasons: (1) the assay does not sacrifice animals; (2) the reduced cost and analysis time from use of the IVBA assay in place of *in vivo* RBA assays will facilitate greater numbers of soil samples analyzed at each site to improve representativeness; (3) regulatory acceptance of the arsenic IVBA assay would lower bioavailability assessment costs by enabling simultaneous assessments of RBA for both arsenic and lead using the existing Standard Operating Procedure (SOP) for the IVBA extraction protocol, which has been previously validated for assessment of RBA of lead in soil (U.S. EPA 2009, 2012a); and (4) some of the U.S. EPA Regional laboratories and commercial laboratories have analytical and quality control experience with the SOP gained from use of the identical assay for lead.

2. Validation Assessment of the *In Vitro* Arsenic Bioaccessibility Assay

This section discusses the validation criteria established in the Agency soil bioavailability guidance (U.S. EPA, 2007a). Criteria for method validation and regulatory acceptance were consolidated because many of the criteria overlap.

2.1. Scientific and regulatory rationale for the test method, including a clear statement of its proposed use, should be available.

The scientific and regulatory rationale for the arsenic IVBA method is presented in the following:

> U.S. EPA. (2007a) Guidance for Evaluating the Bioavailability of Metals in Soils for Use in Human Health Risk Assessment. OSWER 9285.7-80. May 2007. Available online at https://semspub.epa.gov/work/11/175333.pdf
>
> U.S. EPA. (2012b) Recommendations for Default Value for Relative Bioavailability of Arsenic in Soil. OSWER 9200.1-113. December 2012. Available online at https://semspub.epa.gov/work/11/175338.pdf

Regulatory and scientific rationale: The *Guidance for Evaluating the Bioavailability of Metals in Soils for Use in Human Health Risk Assessment* (U.S. EPA, 2007a) articulates the regulatory rationale for determining the bioavailability of metals from soils when assessing human health risks at hazardous waste sites:

> *Accounting for potential differences in oral bioavailability of metals in different exposure media can be important to site risk assessment (U.S. EPA, 1989). This is true for all chemicals, but is of special importance for ingested metals. This is because metals can exist in a variety of chemical and physical forms, and not all forms of a given metal are absorbed to the same extent. For example, a metal in contaminated soil may be absorbed to a lesser extent than when ingested in drinking water or food. Thus, if the oral RfD or CSF for a metal is based on studies using the metal administered in water or food, risks from ingestion of the metal in soil might be overestimated. Even a relatively small adjustment in oral bioavailability can have significant impacts on estimated risks and cleanup goals.* (U.S. EPA, 2007a)

The *Recommendations for Default Value for Relative Bioavailability of Arsenic in Soil* (U.S. EPA, 2012b) document articulates the regulatory rationale for site-specific assessment of arsenic bioavailability in soils:

> *The current default assumption for assessing risk from arsenic in soil is that the bioavailability of arsenic in soil is the same as the bioavailability of arsenic in water (relative bioavailability [RBA] soil/water = 100%). However, recent bioavailability studies conducted in animal models show that bioavailability of arsenic in soil is typically less than that of highly water soluble forms of arsenic (e.g., sodium arsenate dissolved in water). This suggests that bioavailability of arsenic in soil will typically be less than that of arsenic dissolved in drinking water (i.e., RBA<100%). At sites where this applies, the default assumption of RBA=100% will result in an overestimation of risk.* (U.S. EPA, 2012b)
>
> *In general, the Agency (U.S. EPA, 2007a) recommends that efforts be made to collect data that support site-specific estimates, rather than relying on the default value recommended in this memorandum which may not accurately represent arsenic RBA at any specific site. Use of the national default in place of site specific estimates may underestimate or overestimate risk. Where development of site-specific RBA estimates is not feasible (e.g., screening-level assessments), the default value of 60% can be used, recognizing that the default value is an estimate that is not likely to be exceeded at most sites and is preferable to the assumption of an RBA equal to 100%.* (U.S. EPA, 2012b)

2.2. Relationship of the test method endpoint(s) to the endpoint of interest must be described.

The endpoint of interest for risk assessment is a prediction of the oral RBA of arsenic in soil (ratio of oral bioavailability of arsenic in soil to that of water-soluble arsenic) based on a measurement of IVBA of arsenic in soil (solubility of arsenic in soil at gastric pH). The test soil sample is assayed for IVBA, and the corresponding RBA is predicted from a regression model relating IVBA and RBA. This same approach has been validated by EPA for predicting RBA of lead in soil from IVBA (U.S. EPA, 2009).

The IVBA assay for predicting RBA of arsenic in soil is the same extraction procedure validated for predicting the RBA of lead in soil (U.S. EPA, 2009, 2012a). In brief, the IVBA assay consists of incubating a 1 g soil sample with end-over-end mixing in 100 mL of 0.4 M glycine buffer (pH 1.5) for 1 hour at 37°C (body temperature).

The regression model for predicting RBA of arsenic in soil from IVBA is based on a meta-analysis of concordant data from studies in mice and swine (Bradham et al., 2011, 2013; Brattin et al., 2013; Juhasz et al., 2009, 2014a). Data were combined into a validation dataset consisting of paired IVBA and RBA measurements made on 83 soils collected from different sites and mineral types, including mining, smelting, and pesticide or herbicide application (see Section 2.3 for mineral types). Paired measurements of IVBA and RBA for each of the 83 soil samples were included in a weighted linear regression model (Equation 1) in which IVBA and RBA were based on their respective variances (1/variance). The estimated slope is 0.79 ± 0.01 (SE) and intercept is 3.0 ± 0.1 (SE). The equation of the model is:

$$\mathrm{RBA}(\%) = 0.79 \cdot \mathrm{IVBA}(\%) + 3.0 \qquad \text{Eq. (1)}$$

This model explains approximately 87% of the variance in RBA (weight-adjusted $R^2 = 0.87$). The 95% prediction limit for a single RBA measurement was ±19% RBA. A detailed description of the derivation of the regression model is provided in Diamond et al. (2016). This regression model could be updated periodically by incorporating more data sets as they become available.

2.3. A detailed protocol for the test method must be available and should include a description of the materials needed, a description of what is measured and how it is measured, acceptable test performance criteria (e.g., positive and negative control responses), a description of how data will be analyzed, a list of the materials for which the test results are applicable, and a description of the known limitations of the test, including a description of the classes of materials that the test can and cannot accurately assess.

Standard Operating Procedure: The arsenic IVBA assay extraction protocol is the same as SOP 92000.2-86 for the IVBA assay for lead in soil (U.S. EPA, 2012a, 2017). EPA has developed an SOP specifically for arsenic that includes the SOP 09000.2-86 extraction protocol along with the corresponding analytical procedures for measuring arsenic in the soil and soil-like materials and extracts. The IVBA method is included under the validated methods tab on the SW-846 website as Method 1340 for lead, which will be updated to include arsenic.

Aside from the standard laboratory glassware, reagents, supplies, and equipment, the materials needed for the IVBA assay include 0.4 M glycine (free base, reagent-grade glycine in deionized water, adjusted to a pH of 1.50 ± 0.05 at 37°C using trace metal-grade concentrated hydrochloric acid), and either a water bath or an incubated air chamber with sample rotator is necessary for the extraction of the samples at 37°C. In addition, reference standards NIST 2710a SRM or Flat Creek SRM need to be purchased for use as the control soils in the QA/QC samples. These materials and equipment do not require a large investment from laboratories interested in performing the IVBA assay.

The IVBA assay is meant to measure the fraction of the amount of ingested arsenic that would be solubilized at the low pH of the stomach. The samples are sieved at 150 μm to mimic the fraction of soil that is likely to stick to human hands and thereby be ingested (U.S. EPA, 2016). The samples are then extracted in a 0.4 M glycine solution, pH 1.5 at 37°C for 1 hour with rotation to mimic gastric conditions. Following the extraction by IVBA assay, the concentration of arsenic in the extraction solution is measured by ICP-MS or ICP-AES. The total concentration of arsenic in the sample is measured by SW-846 Method 3051A.

As part of the quality control/quality assurance for the IVBA assay, the method requires that a set of quality control samples be run in a batch of samples. Quality control samples are reagent blank (extraction fluid that is not run through the extraction procedure), method blank (extraction fluid that has been run through the extraction procedure), laboratory control sample (LCS; extraction fluid spiked with arsenic that is run through the extraction procedure), matrix spike (spiked matrix, e.g., soil, that is run through the extraction procedure), duplicate sample, and control soil. Control limits and frequency for each quality control sample for arsenic are shown in Table 1.

Table 1. Recommended Control Limits for Quality Control Samples for Arsenic

Quality Control Samples	Frequency	Control Limits for Arsenic
Reagent blank	once per batch (minimum 1 in 20 samples)	<25 µg/L arsenic
Method blank	once per batch (minimum 1 in 20 samples)	<50 µg/L arsenic
LCS (10 mg/L)	once per batch (minimum 1 in 20 samples)	85–115% recovery
Matrix spike (10 mg/L)	once per batch (minimum 1 in 10 samples)	75–125% recovery
Duplicate sample	once per batch (minimum 1 in 10 samples)	±20% RPD
NIST 2710a[a]	once per batch (minimum 1 in 20 samples)	32.9–49.1%

RPD = Relative percent difference
[a] Appendix A

The % IVBA for a sample is determined from the analytical results by Equation 2.

$$\text{IVBA}(\%) = [(\text{As}_{\text{ext}} \times \text{V}_{\text{ext}})/(\text{As}_{\text{soil}} \times \text{Soil}_{\text{mass}}) \times 100 \qquad \text{Eq. (2)}$$

where:
As_{ext} = mass concentration of arsenic in the IVBA extract (mg/L)
V_{ext} = IVBA extract solution volume (L)
As_{soil} = total arsenic concentration (as determined by SW-846 Method 3051A or equivalent) (mg/kg)
$\text{Soil}_{\text{mass}}$ = mass of soil extracted by IVBA (kg)

Equation 1 is applied to the % IVBA results to determine the % RBA (see section 2.2).

Applicable test materials: Application of the IVBA method SOP is expected to yield predictions of RBA for individual soil samples that fall within the prediction interval of the assay (±19 RBA%). The prediction interval was based on results from various sources, including mining, smelting, or pesticide applications. Although arsenic mineralogy has not been evaluated for all soils in the data set, the following arsenic mineral phases were identified: sorbed As^{V} and As^{III}, arsenic trioxide, arsenopyrite, lollingite, realgar, scorodite, and a variety of arsenic-metal oxides (Bradham et al., 2011, 2013, 2015; Brattin et al., 2013; Juhasz et al., 2007). It is possible that some soils may fall outside of the established prediction interval as a result of an unusual arsenic mineralogy or soil composition not represented in the validation dataset. Therefore, whenever a sample is suspected of containing an unusual and/or untested source material or arsenic mineralogy, this should be identified as a potential data gap and source of uncertainty in the resulting prediction of RBA. As additional samples with a variety of new and different arsenic forms are tested by both *in vivo* and *in vitro* methods, the range of applicability of the method should be refined and expanded.

Assay limitations: The following uncertainties may apply to applications of the IVBA assay.

i. **Sample arsenic concentration limits**: The arsenic concentrations of soils tested in the development of the regression model relating IVBA and RBA and its associated prediction interval for the IVBA assay ranged from 40 to 13,000 ppm. This validation range should be sufficient for most applications of the methodology. Although there is no basis for predicting what errors would necessarily be introduced into the predictions of RBA if sample concentrations outside this range were used in the IVBA assay, use of such samples without validating comparisons with results of an *in vivo* assay will introduce additional uncertainty into estimates of RBA. However, applications of the IVBA assay to such high arsenic concentrations (e.g., >7,000 ppm) are unlikely to change risk management decisions; thus, this limitation is not a serious constraint for the utility of the method to support cleanup decisions. If additional data suggest~~s~~ modification of the limits, then the Agency will issue additional guidance. In addition, the minimum soil concentration in the sample is determined by that which is measurable in the assay using the SOP.

ii. **Particle size**: Soil samples in the validation dataset were sieved for particles less than 250 µm. Particle size can be expected to affect dissolution of arsenic embedded in soil particles (Karna et al., 2017). Therefore, additional uncertainty will be associated with RBA estimates from IVBA assays of soil samples having particle sizes excluded from the validation dataset (i.e., >250 µm) U.S. EPA recommends a sieving size of <150 µm to represent the particle fraction having the highest likelihood of incidental ingestion (Ruby and Lowney, 2012; U.S. EPA, 2016). Arsenic IVBA in soils sieved to <250 µm were not different from IVBA measured in soils sieved to <150 µm (Karna et al., 2017).

iii. **Uncertainty in predicted RBA value**: The IVBA assay for arsenic measures IVBA for a test soil and converts this to an estimate of RBA using a regression equation estimated from a meta-analysis of 83 samples. The predicted RBA is the most likely (highest probability) estimate corresponding to the IVBA, but the actual RBA (if measured *in vivo*) might be either higher or lower than the predicted value. The 95% prediction limit for the arsenic IVBA-RBA regression model is relatively narrow in the context of its application to risk assessment, ±19 RBA%. This means that there will be a 95% probability that individual RBA measurements will be ±19 of the RBA% predicted from IVBA. In general, the most likely estimate of RBA is the most appropriate value for use in risk assessments because there is an equal probability of the true RBA being above or below the predicted value; however, other values from within the RBA prediction interval could also be evaluated as part of an uncertainty analysis.

iv. **Predicting RBA in humans**: The IVBA assay was developed to predict arsenic RBA in humans, although there are no data in humans to provide a direct validation of RBA predictions in humans. Therefore, the arsenic IVBA assay was evaluated with estimates of RBA made from studies conducted in two different juvenile swine bioassays and a mouse bioassay. The use of animals for establishing arsenic RBA values to be used in regulatory contexts has several precedents: (1) a national default soil arsenic RBA, to be used when site-specific estimates are not available (it is always better to collect and analyze site-specific data than to rely on a default value), was derived based on a large sample of soil RBA measurements made in mice, monkeys, and swine (U.S. EPA, 2012a,c); (2) an IVBA assay was validated for predicting lead RBA based on soil RBA measurements made in a swine assay (U.S. EPA, 2009); and (3) animal bioassays (e.g., mice, monkeys, swine) remain valid for establishing site-specific soil arsenic and lead RBA, but are not recommended because it is better to run IVBA analyses on many samples (e.g., a statistical sample) than to rely on a smaller number of samples analyzed in animal bioassays (U.S. EPA, 2007b, 2010). Significantly greater costs and time to complete will limit the number of animal bioassays.

Although there is no quantitative support for discerning which animal bioassay provides a more accurate prediction of arsenic RBA in humans, RBA estimates obtained from the mouse and swine assays are in close agreement (Bradham et al., 2013; Juhasz et al., 2014b).

2.4. The extent of within-test variability and the reproducibility of the test within and among laboratories must have been demonstrated. The degree to which sample variability affects this test reproducibility should be addressed.

Within-test variability: Precision of the IVBA protocol was assessed with analyses of soils included in the validation dataset, which included contributions from three laboratories. Each laboratory achieved consistent and relatively low coefficients of variation (CV=standard deviation/mean): 2.1, 4.0, and <5% (Brattin et al., 2013; Diamond et al., 2016).

Inter-laboratory reproducibility: An inter-laboratory comparison of the IVBA was conducted with four participating laboratories: ACZ Laboratories Inc.; EPA Region 7 laboratory; EPA Region 8 laboratory; and University of Colorado at Boulder (Brattin et al., 2013). Each laboratory applied the IVBA method to analyses (in triplicate) of 12 test soils. Average within-laboratory variability (coefficient of variation, CV) ranged from 1.3 to 11.0%. The inter-laboratory coefficient ranged from 2.2 to 15% (mean: 5.4%).

Effects of sample variability: The prediction interval for the IVBA assay was derived based on analysis of 83 soil samples from a variety of site types: mining, smelting, or pesticide application. The IVBA range for the soil samples was 0–80% (mean: 27.2 ± 20 SD). The within-laboratory coefficient of variation for IVBA was <0.05 (Diamond et al., 2016).

2.5. The test method performance must have been demonstrated using reference materials or test materials representative of the types of substances to which the test method will be applied, and should include both known positive and known negative agents.

Performance with reference materials: Precision of the IVBA protocol was assessed with replicate arsenic analyses of standard reference materials (SRMs; National Institute of Standards and Technology [NIST] SRM 2710A) conducted by the EPA Office of Research and

Development National Exposure Research Laboratory [ORD NERL]) over several years (Appendix B). The mean relative percent difference ranged from -10.2 to 9.6% (mean: -0.14 ± 5.3% SD).

Performance with representative materials: The prediction interval for the IVBA assay was derived based on analysis of samples having a variety of arsenic mineral phases from a variety of different types of sites: mining, smelting, and pesticide application.

2.6. Sufficient data should be provided to permit a comparison of the performance of a proposed substitute test with that of the test it is designed to replace.

The IVBA assay is a cost-effective and time-saving alternative to *in vivo* RBA assays that can improve data quality by increasing the number of samples analyzed while reducing costs and turn-around time. For the dataset used to derive the regression model, the model accounted for approximately 87% of the observed variance in RBA. The 95% prediction interval for the model is ±19 RBA%, based on 83 soil samples from a variety of site types that are expected to be typical applications of the assay for site risk assessment (mining, smelting, and or pesticide application). The standard errors for the RBA estimates for this sample of 83 soils ranged from 0.2 to 20% (median 2%), and the ratios of the SE to the mean RBA (SE/mean) ranged from 0.02 to 0.48 (median 0.09).

2.7. Data supporting the validity of a test method should be obtained and reported in accordance with Good Laboratory Practices (GLPs).

Data supporting validity of the IVBA assay are reported in detail in a published report (Diamond et al., 2016). Data used in the analysis is provided in Appendix C.

2.8. Data supporting the assessment of the validity of the test method must be available for review.

Data supporting the assessment of the validity of the IVBA assay are available online at http://www.tandfonline.com/doi/full/10.1080/15287394.2015.1134038.

2.9. The methodology and results should have been subjected to independent scientific review.

The arsenic IVBA methodology was reviewed by EPA scientists and evaluated in several peer-reviewed publications (Bradham et al., 2011, 2013, 2015; Brattin et al., 2013; Juhasz et al., 2009, 2014a,b). The report describing derivation of the prediction regression model was reviewed by the EPA Office of Superfund Remediation and Technology Innovation (OSRTI) Technical Review Workgroup Bioavailability Committee, EPA ORD peer-review for release of publication, and editorial peer-review for publication (Diamond et al., 2016).

2.10. The method should be time and cost effective.

Costs of assessment of a soil sample using the IVBA assay are expected to range from approximately 10-fold to 100-fold less than the costs of a bioassay. Time requirements for the IVBA assay are expected to range from approximately 10-fold to 50-fold less than that required to conduct an *in vivo* bioassay (i.e., days compared to several weeks). Additional cost and time efficiencies are expected for applications at sites where arsenic and lead are chemicals of interest because the same IVBA extraction protocol can be used to predict arsenic and lead RBA. These efficiencies can be used to analyze a greater number of samples.

2.11. The method should be one that can be harmonized with similar testing requirements of other agencies and international groups.

Other international efforts (e.g., Australia, Canada, European Union, United Kingdom) are pursuing the development of methods for *in vitro* assessment of RBA of arsenic and of other metals and inorganic contaminants in soil. The IVBA assay is directly applicable to these national and international programs. It satisfies the Bioaccessibility Research Canada (BARC) acceptance criteria for use in risk assessment (BARC, 2016; Koch and Reimer, 2012) and the IVBA assay has been used widely to characterize soil arsenic bioaccessibility; recent examples of international use include reports from Africa, Australia, Canada, China, and Great Britain (Dodd et al., 2013; Ettler et al., 2012; Juhasz et al., 2015; Koch and Reimer 2012; Kribek et al., 2014; Li et al., 2015a,b; Meunier et al., 2010; Morales et al., 2015; Silvetti et al., 2014; Wang et al., 2012; Yang et al., 2015). The meta-analysis that forms the basis for the predictive regression model for RBA included contributors from the United States and Australia (Diamond et al., 2016). Various EPA and non-government laboratories provided data to support the validation.

2.12. The method should be suitable for international acceptance.

The IVBA assay is suitable for international acceptance (see section 2.11 for further discussion).

2.13. The method must provide adequate consideration for the reduction, refinement, and replacement of animal use.

The IVBA assay replaces bioassays and will decrease the use of animals for assessing RBA of arsenic in soil.

3. Summary

The IVBA assay for arsenic has been evaluated against validation criteria established by EPA (U.S. EPA, 2007a) for validation of test methods to be used in a regulatory context. All validation criteria have been satisfied. SOPs have been established and tested for intra-laboratory precision and inter-laboratory reproducibility. The quantitative relationship between the IVBA assay output and output from *in vivo* animal bioassays, which the IVBA assay is meant to replace, has been reliably established. The description in the method SOP is expected to yield predictions of RBA that fall within acceptable prediction limits for applications in arsenic site risk assessment. The prediction interval is based on assays of samples collected from a variety of arsenic mineral phases from a variety of different sites and, as a result, the method is expected to be widely applicable to soil typically encountered at arsenic waste sites. Based on this assessment, EPA concludes that the IVBA method is valid for predicting RBA of arsenic in soils in support of site-specific risk assessments. The following regression model is recommended for applications to risk assessment (Equation 1):

$$\text{RBA}(\%)=\text{IVBA}(\%)\cdot 0.79+3.0(\%) \qquad \text{Eq. (1)}$$

The Agency strongly encourages use of this methodology when implemented in context with the decision framework described in its soil bioavailability guidance (U.S. EPA, 2007a).

4. References

BARC (Bioaccessibility Research Canada). (2016) Checklist for minimum criteria for *in vitro* bioaccessibility tests (June, 2014). Available online at http://bioavailabilityresearch.ca/downloads.html. Accessed January 26, 2016.

Bradham, KD; Scheckel, KG; Nelson, CM; Seales, PE; Lee, GE; Hughes, MF; Miller, BW; Yeow, A; Gilmore, T; Serda, SM; Harper, S; Thomas, DJ. (2011) Relative bioavailability and bioaccessibility and speciation of arsenic in contaminated soils. Environ Health Perspect 119:1629–1634.

Bradham, KD; Diamond, GL; Scheckel, KG; Hughes, MF; Casteel, SW; Miller, BW; Klotzbach, JM; Thayer, WC; Thomas, DJ. (2013) Mouse assay for determination of arsenic bioavailability in contaminated soils. J Toxicol Environ Health A 76:815–826.

Bradham, KD; Nelson, C; Juhasz, AL; Smith, E; Scheckel, K; Obenour, DR; Miller, BW; Thomas, DJ. (2015) Independent data validation of an *in vitro* method for the prediction of the relative bioavailability of arsenic in contaminated soils. Environ Sci Technol 49:6313–6318.

Brattin, W; Drexler, J; Lowney, Y; Griffin, S; Diamond, G; Woodbury, L. (2013) An *in vitro* method for estimation of arsenic relative bioavailability in soil. J Toxicol Environ Health, Part A: Current Issues 76(7):458–478.

Diamond, GD; Bradham, KD; Brattin, WJ; Burgess, M; JW; Griffin, S; Hawkins, CA; Juhasz, AL; Klotzbach, JM; Nelson C; Lowney, YW; Scheckel, KG; Thomas, DJ. (2016) Predicting oral bioavailability of arsenic in soil from *in vitro* bioaccessibility. J Toxicol Environ Health, Part A: Current Issues. 79:165–173.

Dodd, M; Rasmussen, PE; Chenier, M. (2013) Comparison of Two *In Vitro* Extraction Protocols for Assessing Metals' Bioaccessibility Using Dust and Soil Reference Materials. Hum Ecol Risk Assess 19(4):1014–1027.

Ettler, V; Kribek, B; Majer, V; Knesl, I; Mihaljevic, M. (2012) Differences in the bioaccessibility of metals/metalloids in soils from mining and smelting areas (Copperbelt, Zambia). J Geochem Explor 113:68–75.

ICCVAM (Interagency Coordinating Committee on the Validation of Alternative Methods). (1997) Validation and Regulatory Acceptance of Toxicological Test Methods: A Report of the Ad Hoc Coordinating Committee on the Validation of Alternative Methods. NIH Publication 97-3981. National Institute of Environmental Health Sciences, Research Triangle Park, N.C. Available online at http://ntp.niehs.nih.gov/iccvam/docs/about_docs/validate.pdf. Accessed January 26, 2016.

Juhasz, AL; Smith, E; Weber, J; Rees, M; Rofe, A; Kuchel, T; Sansom, L; Naidu, R. (2007) *In vitro* assessment of arsenic bioaccessibility in contaminated (anthropogenic and geogenic) soils. Chemosphere 69:69–78.

Juhasz, AL; Weber, J; Smith, E; Naidu, R; Rees, M; Rofe, A; Kuchel, T; Sansom, L. (2009) Assessment of four commonly employed in vitro arsenic bioaccessibility assays for predicting *in vivo* relative arsenic bioavailability in contaminated soils. Environ Sci Technol 43:9487–9494.

Juhasz, AL; Herde, P; Herde, C; Boland, J; Smith, E. (2014a) Validation of the predictive capabilities of the Sbrc-G *in vitro* assay for estimating arsenic relative bioavailability in contaminated soils. Environ Sci Technol 48:12962–12969.

Juhasz, AL; Smith, E; Nelson, C; Thomas, DJ; Bradham, K. (2014b) Variability associated with As *in vivo-in vitro* correlations when using different bioaccessibility methodologies. Environ Sci Technol 48:11646–11653.

Juhasz, A.L., Herde, P., Herde, C., Boland, J., Smith, E. (2015) Predicting Arsenic Relative Bioavailability Using Multiple *In Vitro* Assays: Validation of in Vivo-in Vitro Correlations. Environ Sci Technol 49(18):11167–11175.

Karna, R.R., Noerpel, M., Betts, A.R., Scheckel, K.G. (2017) Lead and arsenic bioaccessibility and speciation as a function of soil particle size. J. Environ. Qual. DOI:10.2134/jeq2016.10.0387

Koch, I; Reimer, KJ. (2012) Bioaccessibility extractions for contaminant risk assessment. In: Comprehensive Sampling and Sample Preparation, Volume 3; Pawliszyn, J; Le, XC; Li, X; et al.; Eds. Elsevier, Academic Press: Oxford, UK, pp 487–507.

Kribek, B; Majer, V; Pasava, J; Kamona, F; Mapani, B; Keder, J; Ettler, V. (2014) Contamination of soils with dust fallout from the tailings dam at the Rosh Pinah area, Namibia: Regional assessment, dust dispersion modeling and environmental consequences. J Geochem Explor Part C 144:391–408.

Li, HB; Li, J; Zhu, YG; Juhasz, AL; Ma, LQ. (2015a) Comparison of arsenic bioaccessibility in house dust and contaminated soils based on four *in vitro* assays. Sci Total Environ 532:803–811.

Li, J; Li, K; Cui, XY; Basta, NT; Li, LP; Li, HB; Ma, LQ. (2015b) *In vitro* bioaccessibility and *in vivo* relative bioavailability in 12 contaminated soils: Method comparison and method development. Sci Total Environ 532:812–820.

Meunier, L; Wragg, J; Koch, I; Reimer, KJ. (2010) Method variables affecting the bioaccessibility of arsenic in soil. J Environ Sci Health A Tox Hazard Subst Environ Eng 45(5):517–526.

Morales, NA; Martinez, D; Garcia-Meza, JV; Labastida, I; Armienta, MA; Razo, I; Lara, RH. (2015) Total and bioaccessible arsenic and lead in soils impacted by mining exploitation of Fe-oxide-rich ore deposit at Cerro de Mercado, Durango, Mexico. Environ Earth Sci 73(7):3249–3261.

Ruby, MV; Lowney YW. (2012) Selective soil particle adherence to hands: Implications for understanding oral exposure to soil contaminants. Environ Sci Technol 46:12759–12771.

Silvetti, M; Castaldi, P; Holm, PE; Deiana, S; Lombi, E. (2014) Leachability, bioaccessibility and plant availability of trace elements in contaminated soils treated with industrial by-products and subjected to oxidative/reductive conditions. Geoderma 214–215:204–212.

U.S. EPA (U.S. Environmental Protection Agency). (1989) Risk Assessment Guidance for Superfund. Volume I. Human Health Evaluation Manual (Part A). EPA/540/1-89/002 (as cited in U.S. EPA 2007a).

U.S. EPA (U.S. Environmental Protection Agency). (2007a) Guidance for Evaluating the Bioavailability of Metals in Soils for Use in Human Health Risk Assessment. OSWER 9285.7-80. May 2007. Available online at http://semspub.epa.gov/src/document/HQ/175333. Accessed January 26, 2016.

U.S. EPA (U.S. Environmental Protection Agency). (2007b) Estimation of Relative Bioavailability of Lead in Soil and Soil-like Materials Using *In Vivo* and *In Vitro* Methods. OSWER 9285.7-77. May 2007. Available online at http://semspub.epa.gov/src/document/HQ/175416. Accessed January 26, 2016.

U.S. EPA (U.S. Environmental Protection Agency). (2009) Validation Assessment of *In Vitro* Lead Bioaccessibility Assay for Predicting Relative Bioavailability of Arsenic in Soils and Soil-like Materials at Superfund Sites. U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response: Washington, DC. OSWER 9200.3-51. Available online at http://semspub.epa.gov/src/document/HQ/175417. Accessed January 26, 2016.

U.S. EPA (U.S. Environmental Protection Agency). (2010) Relative Bioavailability of Arsenic in Soils at 11 Hazardous Waste Sites using *In Vivo* Juvenile Swine. OSWER 9200.0-76. June 2010. Available online at http://semspub.epa.gov/src/document/HQ/175341 and http://semspub.epa.gov/src/document/HQ/175340 (Appendix A). Accessed January 26, 2016.

U.S. EPA (U.S. Environmental Protection Agency). (2012a) Standard Operating Procedure for an *In Vitro* Bioaccessibility Assay for Lead in Soil. EPA 9200.2-86. April 2012. Available online at http://semspub.epa.gov/src/document/HQ/174533. Accessed January 26, 2016.

U.S. EPA (U.S. Environmental Protection Agency). (2012b) Recommendations for Default Value for Relative Bioavailability of Arsenic in Soil. U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response: Washington, DC. OSWER 9200.1-113. Available online at http://semspub.epa.gov/work/11/175338.pdf. Accessed January 26, 2016.

U.S. EPA (U.S. Environmental Protection Agency). (2012c) Compilation and Review of Data on Relative Bioavailability of Arsenic in Soil. U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response: Washington, DC. OSWER 9200.1-113. Available online at http://nepis.epa.gov/Exe/ZyPDF.cgi/P100FKWO.PDF?Dockey=P100FKWO.pdf. Accessed January 26, 2016.

U.S. EPA (U.S. Environmental Protection Agency). (2016) Recommendations for Sieving Soil and Dust Samples at Lead Sites for Assessment of Incidental Ingestion. U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response: Washington, DC. OLEM Directive 9200.1-128. Available online at https://semspub.epa.gov/work/HQ/100000133.pdf. Accessed October 5, 2016.

U.S. EPA (U.S. Environmental Protection Agency). (2017) Standard Operating Procedure for an *In Vitro* Bioaccessibility Assay for Lead and Arsenic in Soil. OLEM 9200.2-164. February 2017. Available online at: not currently posted

Wang, C; Zhao, Y; Pei, Y. (2012) Investigation on reusing water treatment residuals to remedy soil contaminated with multiple metals in Baiyin, China. J Hazard Mat 237–238:240–246.

Yang, K; Im, J; Jeong, S; Nam, K. (2015) Determination of human health risk incorporating experimentally derived site-specific bioaccessibility of arsenic at an old abandoned smelter site. Environ Res 137:78–84.

APPENDIX A

Provisional Reference Values for Arsenic IVBA of NIST 2710A Standard Reference Material

Consensus values for In Vitro Bioaccessibility (IVBA) of arsenic in soil reference materials (RM) are needed to support the Standard Operating Procedures (SOP) for determination of arsenic IVBA in soil. EPA intends to conduct multi-laboratory evaluations of arsenic IVBA for NIST 2710A and USGS Flat Creek RMs. and has conducted similar evaluations of lead IVBA for these RMs. Until the arsenic IVBA evaluations are completed, EPA recommends using the provisional reference values for NIST 2710A in Table A-1. Although, the provisional reference values are based on data from only two laboratories, the estimated prediction interval (±20%) is in the range observed for lead IVBA reference values (Table A-2). The data on which the arsenic IVBA reference values are based are provided in Tables A-3 (summary) and A-4 (individual replicates).

Table A-1. Recommended Provisional Reference Value for Arsenic IVBA% of NIST 2710A

Laboratory	Reference Material	Laboratory Analysis	Total Soil Arsenic Method	Units	Number of Replicates	Lower 99% Prediction Limit	Mean	Upper 99% Prediction Limit	PI as Percent of Mean
All Labs[a]	NIST2710A	Arsenic IVBA	NIST Certificate[b]	%	131	32.9	41.0	49.1	± 19.8

[a] Data provided by Karen Bradham (EPA PRD NERL) and John Drexler (University of Colorado)

[b] NIST certificate median soil arsenic concentration: 1400 mg/kg

Table A-2. Reference Values for Lead IVBA% of Standard Reference Materials

Laboratory	Reference Material	Laboratory Analysis	Total Soil Lead Method	Units	Number of Replicates	Lower 99% Prediction Limit	Mean	Upper 99% Prediction Limit	PI as Percent of Mean
QATS Round Robin	NIST2710A	Lead IVBA	NIST Certificate	%	35	60.7	67.5	74.2	±10
QATS Round Robin	NIST2711A	Lead IVBA	NIST Certificate	%	35	75.2	85.7	96.2	±12.3
QATS Round Robin	Flat Creek	Lead IVBA	EPA 3051A	%	30, 35[a]	56.0	71.0	86.0	±21.1

[a] Based on n=35 estimates of total Pb (mg/kg) and 30 estimates of IVBA Pb (mg/kg)

Table A-3. Values for Arsenic IVBA% of NIST 2710A Based Data from Individual Laboratories and Combined Data

Laboratory[a]	Reference Material	Laboratory Analysis	Total Soil Arsenic Method	Units	Number of Replicates	Lower 99% Prediction Limit	Mean	Upper 99% Prediction Limit	PI as Percent of Mean
EPA NERL	NIST2710A	Arsenic IVBA	NIST Certificate	%	117	33.1	40.8	48.4	± 18.8
U Colorado	NIST2710A	Arsenic IVBA	NIST Certificate	%	14	30.7	43.0	55.2	± 28.5
All Labs	NIST2710A	Arsenic IVBA	NIST Certificate	%	131	32.9	41.0	49.1	± 19.8

[a] Data provided by Karen Bradham (EPA PRD NERL) and John Drexler (University of Colorado)

Table A-4. NIST 2710A Arsenic IVBA Replicate Data Used in Calculation of Provisional Reference Values

Replicate	Laboratory[a]	Soil Mass (g)	Extracted As (mg/L)	Total Soil As[b] (mg/kg)	As IVBA (%)
1	EPA NERL	1.00	5.59	1400	39.9
2	EPA NERL	1.00	5.56	1400	39.6
3	EPA NERL	1.00	5.33	1400	38.0
4	EPA NERL	1.00	5.14	1400	36.7
5	EPA NERL	1.00	6.40	1400	45.6
6	EPA NERL	1.00	6.40	1400	45.6
7	EPA NERL	1.00	5.98	1400	42.7
8	EPA NERL	1.00	6.15	1400	43.9
9	EPA NERL	1.00	5.46	1400	38.9
10	EPA NERL	1.00	5.82	1400	41.4
11	EPA NERL	1.00	6.39	1400	45.5
12	EPA NERL	1.00	5.25	1400	37.5
13	EPA NERL	1.00	5.26	1400	37.6
14	EPA NERL	1.00	5.19	1400	37.1
15	EPA NERL	1.00	5.54	1400	39.5
16	EPA NERL	1.00	5.43	1400	38.8
17	EPA NERL	1.00	5.52	1400	39.3
18	EPA NERL	1.00	5.20	1400	37.0
19	EPA NERL	1.00	5.08	1400	36.3
20	EPA NERL	1.00	5.19	1400	37.0
21	EPA NERL	1.00	5.24	1400	37.4
22	EPA NERL	1.00	6.01	1400	42.9
23	EPA NERL	1.00	5.57	1400	39.7
24	EPA NERL	1.00	5.58	1400	39.6
25	EPA NERL	1.00	5.66	1400	40.4
26	EPA NERL	1.00	5.25	1400	37.4
27	EPA NERL	1.00	5.25	1400	37.5
28	EPA NERL	1.00	5.51	1400	39.4
29	EPA NERL	1.00	4.89	1400	35.0
30	EPA NERL	1.00	5.61	1400	40.0
31	EPA NERL	1.00	5.36	1400	38.2
32	EPA NERL	1.01	5.94	1400	42.1
33	EPA NERL	1.00	5.86	1400	41.8
34	EPA NERL	1.00	5.84	1400	41.6
35	EPA NERL	1.00	4.83	1400	34.4
36	EPA NERL	1.00	5.12	1400	36.5
37	EPA NERL	1.00	5.29	1400	37.7
38	EPA NERL	1.00	5.88	1400	41.9

Table A-4. NIST 2710A Arsenic IVBA Replicate Data Used in Calculation of Provisional Reference Values

Replicate	Laboratory[a]	Soil Mass (g)	Extracted As (mg/L)	Total Soil As[b] (mg/kg)	As IVBA (%)
39	EPA NERL	1.00	5.69	1400	40.6
40	EPA NERL	1.00	5.88	1400	41.8
41	EPA NERL	1.00	5.70	1400	40.6
42	EPA NERL	1.00	5.44	1400	38.8
43	EPA NERL	1.00	5.35	1400	38.2
44	EPA NERL	1.00	5.38	1400	38.3
45	EPA NERL	1.00	5.37	1400	38.3
46	EPA NERL	1.00	5.42	1400	38.7
47	EPA NERL	1.00	5.30	1400	37.9
48	EPA NERL	1.00	5.10	1400	36.3
49	EPA NERL	1.00	6.00	1400	42.7
50	EPA NERL	1.00	5.21	1400	37.1
51	EPA NERL	1.00	5.19	1400	37.0
52	EPA NERL	1.00	6.29	1400	44.8
53	EPA NERL	1.00	5.92	1400	42.1
54	EPA NERL	1.00	5.64	1400	40.1
55	EPA NERL	1.00	5.60	1400	39.9
56	EPA NERL	1.00	5.73	1400	40.8
57	EPA NERL	1.00	5.90	1400	42.0
58	EPA NERL	1.00	5.59	1400	39.9
59	EPA NERL	1.00	5.55	1400	39.5
60	EPA NERL	1.00	5.73	1400	40.7
61	EPA NERL	1.00	5.95	1400	42.4
62	EPA NERL	1.00	5.83	1400	41.6
63	EPA NERL	1.00	5.63	1400	40.2
64	EPA NERL	1.00	5.64	1400	40.2
65	EPA NERL	1.00	6.18	1400	44.1
66	EPA NERL	1.00	5.70	1400	40.6
67	EPA NERL	1.00	5.39	1400	38.3
68	EPA NERL	1.00	5.85	1400	41.6
69	EPA NERL	1.00	6.14	1400	43.7
70	EPA NERL	1.00	6.05	1400	43.1
71	EPA NERL	1.00	6.53	1400	46.6
72	EPA NERL	1.00	6.13	1400	43.7
73	EPA NERL	1.00	6.35	1400	45.3
74	EPA NERL	1.00	6.21	1400	44.2
75	EPA NERL	1.00	5.24	1400	37.3
76	EPA NERL	1.00	5.60	1400	40.0

Table A-4. NIST 2710A Arsenic IVBA Replicate Data Used in Calculation of Provisional Reference Values

Replicate	Laboratory[a]	Soil Mass (g)	Extracted As (mg/L)	Total Soil As[b] (mg/kg)	As IVBA (%)
77	EPA NERL	1.00	6.05	1400	43.1
78	EPA NERL	1.00	5.99	1400	42.6
79	EPA NERL	1.00	5.45	1400	38.9
80	EPA NERL	1.00	5.73	1400	40.8
81	EPA NERL	1.00	5.79	1400	41.2
82	EPA NERL	1.00	5.55	1400	39.5
83	EPA NERL	1.01	6.09	1400	43.1
84	EPA NERL	1.00	5.68	1400	40.4
85	EPA NERL	1.00	5.28	1400	37.6
86	EPA NERL	1.00	5.26	1400	37.5
87	EPA NERL	1.00	5.50	1400	39.2
88	EPA NERL	1.01	5.67	1400	40.2
89	EPA NERL	1.00	5.36	1400	38.2
90	EPA NERL	1.01	5.70	1400	40.5
91	EPA NERL	1.00	5.68	1400	40.4
92	EPA NERL	1.01	5.48	1400	38.8
93	EPA NERL	1.01	5.35	1400	37.9
94	EPA NERL	1.00	5.62	1400	40.0
95	EPA NERL	1.00	5.63	1400	40.1
96	EPA NERL	1.01	5.94	1400	42.0
97	EPA NERL	1.00	6.57	1400	46.9
98	EPA NERL	1.00	5.77	1400	41.2
99	EPA NERL	1.00	6.14	1400	43.8
100	EPA NERL	1.00	6.50	1400	46.5
101	EPA NERL	1.01	6.36	1400	44.9
102	EPA NERL	1.01	6.14	1400	43.5
103	EPA NERL	1.01	6.62	1400	46.7
104	EPA NERL	1.01	6.21	1400	44.0
105	EPA NERL	1.01	6.70	1400	47.5
106	EPA NERL	1.00	6.45	1400	46.1
107	EPA NERL	1.00	5.73	1400	40.8
108	EPA NERL	1.01	5.87	1400	41.7
109	EPA NERL	1.01	5.98	1400	42.5
110	EPA NERL	1.00	6.04	1400	43.0
111	EPA NERL	1.00	5.42	1400	38.6
112	EPA NERL	1.00	5.49	1400	39.1
113	EPA NERL	1.01	6.15	1400	43.6
114	EPA NERL	1.01	6.63	1400	46.9

Table A-4. NIST 2710A Arsenic IVBA Replicate Data Used in Calculation of Provisional Reference Values

Replicate	Laboratory[a]	Soil Mass (g)	Extracted As (mg/L)	Total Soil As[b] (mg/kg)	As IVBA (%)
115	EPA NERL	1.01	5.93	1400	42.0
116	EPA NERL	1.01	6.14	1400	43.5
117	EPA NERL	1.00	6.44	1400	45.9
118	U. Colorado	1.00	5.10	1400	36.3
119	U. Colorado	1.02	5.22	1400	36.7
120	U. Colorado	1.01	5.69	1400	40.3
121	U. Colorado	1.01	6.55	1400	46.5
122	U. Colorado	1.00	6.69	1400	47.7
123	U. Colorado	1.00	6.34	1400	45.1
124	U. Colorado	1.00	6.75	1400	48.2
125	U. Colorado	1.00	6.45	1400	46.1
126	U. Colorado	1.00	6.34	1400	45.2
127	U. Colorado	1.01	6.46	1400	45.8
128	U. Colorado	1.02	5.79	1400	40.4
129	U. Colorado	1.01	5.69	1400	40.3
130	U. Colorado	1.00	5.68	1400	40.4
131	U. Colorado	1.01	6.02	1400	42.4

[a] Data provided by Karen Bradham *(EPA ORD NERL) and John Drexler, University of Colorado

[b] NIST certificate median soil arsenic concentration

APPENDIX B

Replicate IVBA results for NIST2710A (March 2010 – January 2015)
EPA Office of Research and Development National Exposure Research Laboratory

Replicate	IVBA (%)	RPD
1	42.4	3.9
2	40.0	-1.9
3	38.5	-5.7
4	37.2	-9.2
5	40.9	0.3
6	37.6	-8.1
7	39.5	-3.2
8	43.7	6.9
9	42.5	4.1
10	42.8	4.8
11	40.9	0.3
12	39.6	-2.9
13	38.8	-5.0
14	40.9	0.3
15	41.6	2.0
16	39.0	-4.4
17	42.5	4.1
18	36.8	-10.2
19	43.4	6.2
20	43.3	6.0
21	42.5	4.1
22	42.8	4.8
23	40.9	0.3
24	39.9	-2.2
25	39.6	-2.9
26	44.9	9.6
27	38.4	-6.0
Mean	40.8.	-0.14
SD	2.2	5.32
Min	36.8	-10.25
Maximum	44.9	9.63

APPENDIX C

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

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
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

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
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