# INDEPENDENT MEASURES FOR MORE CONFIDENT SELECTION AND APPLICATION OF ARSENIC BIOACCESSIBILITY METHODS TO PREDICT BIOAVAILABILITY

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## Introduction

Exposure risk associated with soils contaminated with arsenic (As) is assessed by human health risk assessment (HHRA). A critical component of HHRA is exposure assessment by various exposure pathways. In soils, often the most important pathway for As, the risk driver, associated with human exposure is incidental soil ingestion. A more accurate and site-specific HHRA accounts for bioavailability of As in a soil matrix as part of the exposure assessment. The most commonly used animal model for determining bioavailable As is the juvenile swine model. In order to overcome some of the difficulties and expenses associated with animal dosing trials used to assess bioavailability of contaminants in soil, extensive research efforts have been directed toward development of in vitro gastrointestinal methods, that simulate the gastrointestinal environment, to predict bioavailable As. While there are multiple efforts to advance in vitro methodology, few in vitro conditions are commonly employed in the United States; a 0.4 M glycine buffered gastric solution at pH 1.5 (SBRC) and an unbuffered gastric solution at pH 1.8 followed by an unbuffered intestinal solution at pH 6.5 (OSU). However, it is not known how well these methods will predict RBA in soils contaminated with As from sources outside those used in developing the regression equation. Therefore, external measures to support the selection and use of the appropriate in vitro method are needed.

## Methods

In vitro bioaccessible (IVBA) As was determined by the SBRC and OSU methods for Empire Mine, a former gold mining site in California. Arsenic RBA was determined using the juvenile swine model. Soils were fractionated into five fractions: (F1) non-specifically sorbed; (F2) specifically sorbed; (F3) amorphous and poorly-crystalline oxides of Fe and Al; (F4) well-crystallized oxides of Fe and Al and (F5) residual As. As speciation was determined by X-Ray Absorption Spectroscopy (XAS) and grouped into phases; (P1) Ca and calcium-iron arsenates, (P2) sulfates, (P3) As sorbed to Al oxides, (P4) As sorbed to Fe oxides, (P5) Fe arsenates, and (P6) sulfides. Bioaccessible As fractions and phases were compared to bioavailable As fractions and phases. These comparisons were linked to RBA prediction error using established regression equations for each method.

## Results

A summary of total, bioaccessible, and bioavailable As from the soils collected from Empire Mine are presented in Table 1.


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**Table 1.** Summary statistics for total As, in vitro bioaccessible As determined by SBRC, and OSU methods, as well as swine RBA for Empire Mine soils.

| | Total As | SBRC | OSU GE | OSU IE | RBA |
|---|---|---|---|---|---|
| Soil | mg/kg | | As (%) | | |
| min | 203 | 0.361 | 1.51 | 1.27 | 4.00 |
| mean | 4,006 | 3.67 | 4.00 | 4.36 | 15.9 |
| max | 12,095 | 14.4 | 9.27 | 10.7 | 23.7 |

Both the SBRC and OSU-IVG extracted approximately F1-F2 As fractions determined by SEP. However, RBA As fractions included not F1-F2 only but also approximately 1/3 (m=0.364) of F1-F3 (Figure 1A). Similarly RBA As includes a larger portion of P1-P4 As than IVBA (Figure 1B). The considerably greater slope of RBA vs. P1-P4 indicates that RBA As is greater than IVBA As because As contained in the P4 phase (arsenic sorbed to ferrihydrite and/or goethite) is more bioavailable than what is solubilized in vitro. When IVBA us used in developed regression equations, the SBRC method consistently under-predicts RBA for Empire Mine. However, while the OSU method extracted similar As fractions and phases as the SBRC method, the predictions range from under to over. This is due to a y-intercept >10 in the regression equations for OSU GE and OSU IE.

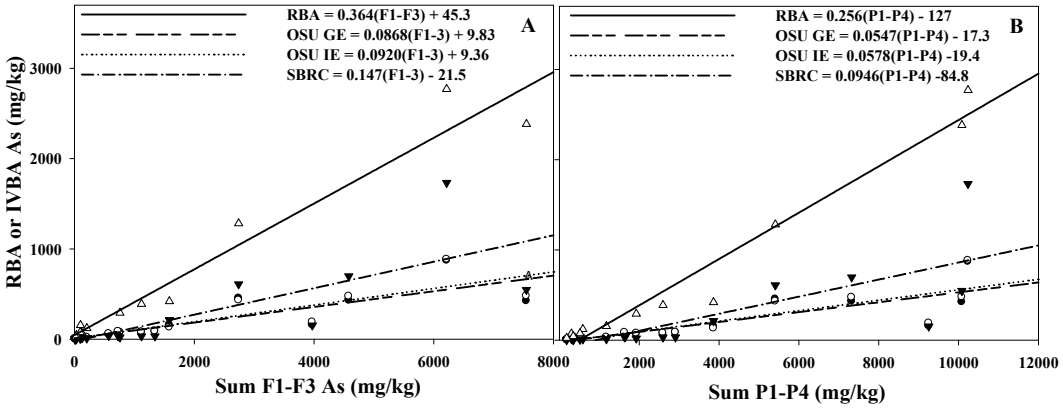


**Figure 1**. Comparison of bioavailable and bioaccessible As fractions (A) and phases (B).

## Conclusion

Using predictive equations developed from datasets from other studies demonstrated that prediction results vary drastically depending on the study soils used to develop the IVIVC. The SBRC method drastically under-predicts RBA for all but one Empire Mine soil. In vitro methods that meet IVIVC have a y-intercept that does not deviate significantly from zero are highly desirable (Juhasz et al.,2014). The probability that an in vitro result to under-predict RBA using a regression equation with a small y-intercept may be indicated by SEP and speciation. If the IVBA As is ≤ F1-2, there is a higher likelihood that the predicted RBA will be lower than the actual RBA than if IVBA is > F1-F2.

## References

Juhasz, A. L., E. Smith, et al. (2014). Variability associated with as in vivo-in vitro correlations when using different bioaccessibility methodologies. *Environ Sci Technol,* 48(19), 11646-11653.


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