

# COMPREHENSIVE EVALUATION OF IN VITRO BIOACCESSIBILITY METHODS TO PREDICT BIOAVAILABILITY OF ARSENIC IN CONTAMINATED SOILS

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## Introduction

Ingestion of contaminated soil often is the “risk – driver” for arsenic (As) and other trace element contaminated soils and the key exposure pathway in human and ecological risk assessment. The trace element interactions with the soil and sediment and general soil properties impact chemistry, solubility, and bioavailability. Often the calculated risk for a site can be reduced when the bioavailability of As in the soil is included in site specific risk calculation. Relative bioavailability (RBA) can be determined for specific sites using in vivo bioassays that mimic human gastrointestinal physiology (i.e., juvenile swine or adult mouse), however these methods are very expensive, time consuming, and include large numbers of animals. Scientists have developed in vitro bioaccessibility methods (IVBA) to predict RBA in an effort to replace in vivo bioassays. The ability of an in vitro method to predict RBA is determined from an in vitro-in vivo correlation (IVIVC). Several IVBA methods with validated IVIVC are gaining regulatory acceptance in the international community. The objective of this study was to determine the predictive capability of five international in vitro bioaccessibility methods for 27 As contaminated soils with a range of soil properties, contamination sources, and solid phase As speciation.

## Methods

The ability of five international published in vitro methods to predict animal RBA As was determined. The in vitro methods included; the Solubility Bioaccessibility Research consortium assay (SBRC), the Unified Barge Method (UBM), the Physiologically Based Extraction Test (PBET), the OSU In Vitro Gastrointestinal Method (OSU), and a slightly modified OSU method (MOSU). RBA was determined using both the juvenile swine and adult mouse models. Twenty two of the 27 soils were dosed to swine, 19 of the 27 soils were dosed to mice and 14 of the 27 soils were dosed to both animals. IVBA As vs RBA As was fitted using linear regression and evaluated for goodness of fit, slope, y-intercept, prediction error, and compared against proposed acceptance criteria. Soil properties relevant to As solubility and bioaccessibility were determined; including total As, reactive Fe and Al, pH, organic carbon content, and clay content. Solid phase speciation of As and Fe was determined using X-Ray Absorption Spectroscopy (XAS) and linear combination fitting (LCF). In addition Mössbauer spectroscopy was used to confirm Fe XAS speciation.

## Results

The arsenic concentrations within the soils ranged from 162 mg/kg to 12,500 mg/kg with an average of 2210 mg/kg. Twenty two different As mineral phases were identified by LCF analyses, the most dominant phases were As (V) absorbed to soil minerals such as ferrihydrite and goethite. Twenty five different Fe species were identified via LCF analyses. Similarly to As analyses Fe analyses identified soil minerals such as ferrihydrite and amorphous Fe oxides as the dominant Fe species within the study soils. Arsenic bioavailability determined using the adult mouse model ranged from 6 to 80% with average of 35%. RBA

determined using the juvenile swine model ranged from 4 to 60% with a mean of 32%. Due to differences between the animal physiology IVIVCs were made using RBAs from each animal separately.

Arsenic bioaccessibility was determined using five different international in vitro methods and ranged from <1 to >90%. A variety of linear regression methods have been used throughout literature to produce IVIVCs. Some of the methods are basic (simple linear regression) others are statistically complex and require specialty software. These advanced methods do not produce IVIVCs that are drastically different than those created using basic techniques such as simple linear regression and in turn simple linear regression was chosen for this study. Summary statistics for gastric phase IVIVCs for the adult mouse and juvenile swine model are shown below in Table 1 and 2 respectively. Wragg et al. (2011) suggested criteria for assessing the ability of an in vitro bioaccessibility method to predict relative bioavailability including a slope near unity (0.8 – 1.2) and that IVIVCs are strongly correlated ( $r^2 > 0.6$ ). All of the methods used in this study met the criteria for correlation suggested by Wragg et al. however, most of the methods have an  $r^2 > 0.7$ . Not all of the methods meet the slope criteria suggested by Wragg et al, the methods that most resemble human physiology are better at predicting RBA. In addition the methods worked for all soil types and contamination sources except for two. These two soils contained very high amounts of absorbed As(III) and amorphous Al oxides.

**Table 1.** Mouse RBA vs Gastric IVBA IVIVC Statistics

Method	SBRC	PBET	UBM	OSU	MOSU
Slope	0.74	0.74	0.81	0.85	0.72
Y-Intercept	13.6	14.2	8.70	10.9	-2.48
$r^2$	0.82	0.82	0.84	0.89	0.73

**Table 2.** Swine RBA vs Gastric IVBA IVIVC Statistics

Method	SBRC	PBET	UBM	OSU	MOSU
Slope	0.71	0.80	0.88	1.09	0.64
Y-Intercept	18.3	17.5	14.3	12.6	5.17
$r^2$	0.60	0.63	0.67	0.73	0.65

## Conclusion

In vitro bioaccessibility methods have the ability to accurately predict in vivo relative bioavailability. The methods that most resemble human physiology are better predictors for RBA. However, all RBA values are not equal for more conservative estimates it maybe be better to use IVIVCs generated using juvenile swine models because swine physiology most resembles humans. Results from this study will help support the adoption of methods to measure bioaccessible As that predict bioavailable As for many different soil and As source types. These methods also have the potential to be used for ecological risk assessments and to access the success of soil remediation techniques, ultimately helping to close contaminated areas and allow for the future use of degraded lands.

## References

- Wragg, J., Cave, M., Basta, N., Brandon, E., Casteel, S., Denys, S., ... Van de Wiele, T. (2011). An inter-laboratory trial of the unified BARGE bioaccessibility method for arsenic, cadmium and lead in soil. *Sci Total Environ*, 409(19), 4016–4030.