Fact Sheet: Arsenic RBA and IVBA

Relative Bioavailability and In Vitro Bioaccessibility of Arsenic in Soil

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Introduction and definitions

Bioavailability refers to the fraction or percentage of an ingested dose of arsenic that is absorbed into the systemic circulation (OSWER 9200.1-113). Where soil is contaminated with arsenic, the speciation of the arsenic and the characteristics of the soil can modify the amount of arsenic available for uptake into an organism. This fact sheet focuses on the bioavailability of arsenic to humans via the ingestion exposure pathway. *Absolute bioavailability* (ABA) refers to the fraction of an ingested dose of arsenic that crosses the gastrointestinal epithelium and becomes available for internal distribution. *Relative bioavailability* (RBA) is the ratio of ABA for soil arsenic to that of a water-soluble reference form of arsenic; typically, sodium arsenate. ABA and RBA are measured in animal models; however, RBA can also be predicted from *in vitro bioaccessibility* (IVBA) assays. For human health risk assessment purposes, relative bioavailability is important because we are most often interested in knowing the extent to which the absolute bioavailability of a chemical increases or decreases in different exposure matrices (e.g., food vs. water vs. soil) or with the physical or chemical form(s) of the chemical to which humans are exposed. *Bioaccessibility* refers to the fraction of an ingested dose of soil arsenic that is in a form that can interact with absorptive transport mechanisms (e.g., transcellular carriers or channels, paracellular diffusion). Soil arsenic must be bioaccessible in the gastrointestinal tract in order for it to be bioavailable. Processes contributing to bioaccessibility of soil arsenic may include release of arsenic from soil particles, dissolution of arsenic into gastrointestinal tract fluids, or chemical transformation. IVBA assays estimate bioaccessibility from measurements of *in vitro* solubility of soil arsenic. IVBA assays are designed to predict RBA from *in vitro* measurements of arsenic solubility.

Rationale for measuring soil arsenic RBA

Health risk for ingestion of arsenic-contaminated soils is estimated by applying a cancer slope factor or chronic oral Reference Dose (RfD), derived from human studies in populations chronically exposed to arsenic in drinking water, to estimated soil arsenic ingestion. However, if oral bioavailability of arsenic in soil is less than that from water, risk will be overestimated. Data from a variety of arsenic-contaminated sites demonstrated that oral bioavailability of arsenic in soil tends to be lower than that of water-soluble sodium arsenate (U.S. EPA 2012a). Expressed as RBA, the median and 95th percentile RBA values for more than 100 soil samples were 30% and 60%, respectively (U.S. EPA 2012a). EPA has recommended that site-specific RBA analysis of soil arsenic be performed when deemed feasible and valuable for improving the characterization of risk at the site (U.S. EPA, 1989, 2007a,b, 2012b). Soil arsenic RBA is measured in animal bioassays; however, it can be predicted from measurements of IVBA (U.S. EPA 2017a,b).

Bioassays for measuring soil arsenic RBA

Various animal models have been used to study oral bioavailability of inorganic arsenic in soil, including monkeys, mice, rabbits, and swine (OSWER 9200.1-113). Soil arsenic RBA bioassays have relied on two metrics of absorbed arsenic dose for estimating bioavailability: the area under the concentration-time curve (AUC) for plasma or blood, and the urinary excretion fractions (UEF).

Commonly used animal models are summarized in Table 1. Each model has its own strengths and weaknesses in terms of procedures needed to obtain biological samples (e.g., blood, plasma, urine), availability of animals, husbandry requirements and expense. Applications to risk assessment require accepting uncertainties in extrapolating RBA estimates made in animal models to humans. Contributors to uncertainty include interspecies variation in nutrition, gastrointestinal tract morphology, physiology and post-natal development.

Several studies have compared soil arsenic RBA measured on the same soils in different bioassays (Bradham et al., 2013, 2018; Li et al., 2016). Collectively, these studies indicate that mouse, swine, and monkey RBA assays yield similar RBA estimates when applied to the same soils; although, there appears to be a trend for the swine assay to predict higher RBAs than the mouse UEF assay.

The mouse assay EPA (Bradham et al., 2011, 2013, 2015) developed is a lower cost alternative to monkey and swine assays for measuring soil arsenic RBA. EPA considers the mouse assay to be a valid tool for conducting arsenic RBA research, including but not limited to development of and evaluation of IVBA assays, confirming results of RBAs generated from IVBA for problematic or unusual soil types or arsenic species, and identifying factors that contribute to variability in arsenic RBA at sites. Additional information and assistance in applications of the mouse arsenic oral RBA bioassay can be obtained from Dr. Karen Bradham (EPA ORD NERL) or by emailing the EPA OSRTI Technical Review Workgroup Bioavailability Committee hotline (bahelp@epa.gov).

IVBA methods

EPA has validated an IVBA assay for predicting soil arsenic RBA for human health risk assessment and recommends using the assay for characterizing site-specific soil arsenic RBA (U.S. EPA, 2017a, b). The assay involves a gastric-phase extraction of soil in a relatively simple extraction medium. The regression model used for predicting RBA from IVBA is as follows (Diamond et al., 2016):

$$RBA\% = 0.79 \times IVBA\% + 3\% \ (R^2=0.87) \qquad \text{Eq. (1)}$$

Application of RBA estimates to risk assessments

Methods and guidance for applying RBA estimates to risks assessment, screening and removal assessments can be found in U.S. EPA, 2020 (http://www.epa.gov/superfund/health/contaminants/bioavailability/guidance.htm). Additional

resources regarding measuring RBA and applying it to risk assessments can be found on the EPA Office of Superfund Remediation and Technology Innovation bioavailability webpages: https://www.epa.gov/superfund/soil-bioavailability-superfund-sites.

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Table 1. Arsenic Soil RBA Bioassays

Primary reference	*Brattin and Casteel 2013*	*Juhasz et al., 2007b*	*Roberts et al., 2007*	*Bradham et al., 2011*	*Li et al., 2016*
Animal	Swine	Swine	Monkey	Mouse	Mouse
Dosing schedule	2 times/day for 12 days administered in 2 equal portions	Single dose separated by 48-hour washout period	Single dose separated by at least 3-week washout period	Free access to amended feed for 10 days	Single dose
Number of dose levels	Control (basal diet), 3 dose levels for soil and reference	Control (basal diet), single dose level for soil and reference	Control (basal diet), single dose level for soil, 3 dose levels for reference	Control (basal diet), single dose level for soil and reference	Control (basal diet), 3 dose levels for reference or soil
Biological samples for estimating RBA	Urine: 48-hour samples collected from each animal on days 6-7, 8-9, and 10-11 during dosing	Blood: prior to dosing (baseline) and then repeatedly over a 26-hour post-dosing period	Urine: cumulative sample collected over a 4-day post-dosing period	Urine: cumulative sample collected for 8 days during dosing and 1 day post-dosing	Blood: repeatedly over a 48-hour post-dosing period
RBA metric	Urine EF	Blood AUC	Urine EF	Urine EF	Blood AUC

ABA, absolute bioavailability; As, arsenic; AUC, area under the curve for arsenic in blood; DR, dosing rate; RBA, relative bioavailability; Ref, reference material; EF, excretion fraction