OFFICE OF SUPERFUND REMEDIATION AND TECHNOLOGY INNOVATION
WASHINGTON, D.C. 20460

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MEMORANDUM

SUBJECT: Mouse Model for Measuring *In Vivo* Inorganic Arsenic Oral Relative Bioavailability (RBA)

FROM: Jennifer Hovis, Acting Director
Assessment and Remediation Division
Office of Superfund Remediation and Technology Innovation

JENNIFER HOVIS
Digitally signed by JENNIFER HOVIS
Date: 2025.03.19 07:39:40 -04'00'

TO: Superfund and Emergency Management Division Directors, Regions 1-10
Laboratory Services and Applied Sciences Division Directors, Region 1-10
Regional Toxics Integration Coordinators (RTICs), Regions 1-10

Purpose

The purpose of this memorandum is to inform the Regions about the development and availability of an *in vivo* mouse model to measure arsenic relative bioavailability (RBA) in soil and soil-like materials.

Definitions

In this memorandum, the term *bioavailability* refers to the fraction or percentage of an ingested dose of arsenic that is absorbed into the systemic circulation. Bioavailability of arsenic in soil can be expressed either in absolute terms (absolute bioavailability) or in relative terms (relative bioavailability):

Absolute bioavailability (ABA) is defined as the ratio of the amount of arsenic absorbed from the gastrointestinal tract and entering the blood and tissues to the amount ingested. This ratio is also referred to as the oral absorption fraction.

Relative bioavailability (RBA) is defined as the ratio of the ABA fraction of arsenic present in the exposure medium of interest to the ABA of arsenic in the exposure medium used in the critical study that formed the basis for the oral Reference dose

(RfD_O) or cancer slope factor (based on studies of populations exposed to arsenic in drinking water).

Bioaccessibility refers to the physiological solubility of arsenic in the gastrointestinal tract. Ingested arsenic must become bioaccessible in the gastrointestinal tract in order to be absorbed. This process may include physical transformation of arsenic-bearing particles (for example, breakdown of the particle to expose arsenic to gastrointestinal tract fluids), dissolution of arsenic, and chemical transformation of dissolved arsenic. Bioaccessibility can be measured with an *in vitro* bioaccessibility (IVBA) assay that measures solubility of soil arsenic in a gastric-like (i.e., low pH) extraction medium.

Background

EPA has recommended that site-specific assessments of soil arsenic RBA be performed where such assessments are deemed feasible and valuable for improving the characterization of human health risk at the site (U.S. EPA, 1989, 2007a,b, 2012, 2020). Soil arsenic RBA is measured in animal bioassays; however, it can be predicted from measurements of IVBA. An important goal of EPA's bioavailability research is to reduce the reliance on animal models for site-specific assessments of soil RBA by developing accurate and cost effective IVBA methods. EPA has validated an arsenic IVBA assay for predicting RBA (EPA Method 1340; U.S, EPA, 2017a,b).

While EPA generally encourages RBA site assessments be performed with *in vitro* methods rather than with animal bioassays where feasible and sufficient, animal models remain important research tools for human health risk assessment and may be needed for a variety of purposes. Important uses of animal models include:

1. Calibration, evaluation, and improvement of in vitro bioaccessibility assay (IVBA) predictions

2. Identifying site-specific factors that contribute to variability in arsenic RBA, including physiology of the gastrointestinal tract, soil geochemistry and arsenic speciation

3. Understanding and quantifying uncertainties in interspecies (e.g. animal-to human) extrapolation of RBA predictions

4. Developing new RBA prediction methods for other important soil contaminants (e.g., lead, PAH, PCDD/Fs, PFAs)

5. Evaluating the effectiveness of soil amendments in reducing the RBA of arsenic.

Several animal bioassays have been developed for measuring soil arsenic RBA, including bioassays in mice, monkeys and swine (Bradham et al., 2011; Brattin and Casteel, 2013; Juhasz et al., 2007; Roberts et al., 2007). These assays substantially differ with respect to methods,

feasibility (ethical and husbandry requirements) and cost (Bradham et al. 2018a, 2020). Cost is an important factor in the selection of animal models because all applications of bioassays enumerated above require evaluations of large numbers of soil samples (e.g., Diamond et al. 2016). Lower costs allow assessment of larger numbers of samples and improved characterization of RBA at sites. There is currently no empirical basis for determining which animal model provides the most reliable prediction of soil arsenic oral RBA in humans. This problem cannot be definitively addressed without human clinical studies. The difficulties of such studies (e.g., Stanek et al. 2010) make it unlikely that animal bioassays will ever be fully validated with human data. However, confidence in extrapolating RBA measured in animal models to humans is increased if bioassays conducted in different animal models can be shown to predict similar estimates for RBA. Furthermore, if several animal bioassays yield similar results, the assay having the lowest cost or that can be performed faster or more easily can be selected for research applications that otherwise would be prohibitive. Studies summarized below provide evidence that arsenic bioavailability (ABA and RBA) is similar when measured in different mammalian species, lending support to use of the mouse model to predict arsenic RBA in humans (see C*omparability to RBA Estimates from Swine and Monkey Assays*; *Comparison to ABA estimates from Swine and Monkey Assays and Humans*).

Mouse Arsenic RBA Assay

Development. The mouse arsenic RBA assay was developed as part of a collaborative effort of scientists at the Center for Environmental Measurement and Modeling (CEMM), Center for Computational Toxicology and Exposure (CCTE), and Center for Environmental Solutions and Emergency Response (CESER), with support from the Office of Superfund Remediation and Technology Innovation (Bradham et al. 2011). A principal objective of this collaboration was to develop a more cost-effective alternative to monkey and swine bioassays for arsenic bioavailability research.

Description of the Mouse RBA Assay. Mice (female C57BL/6 mice) are exposed to arsenic mixed into a semi-purified low arsenic diet (<20 ppb) for a period of 9 days; during this period, cumulative consumption of diet is measured, and cumulative urine is collected. One group of mice is exposed to diet amended with a reference arsenic compound (sodium arsenate) and one group is exposed to diet amended with the test soil. RBA is estimated by comparing the fraction of the cumulative arsenic dose excreted in urine (urinary excretion fraction, UEF) in the two groups of mice ($UEF_{test}/UEF_{reference}$).

Reproducibility of RBA Estimates. The mouse assay has been shown to provide highly reproducible estimates of arsenic RBA in soils contaminated with arsenic from a variety of sources, including industrial, mining and ore processing, and pesticide application. Repeated assays of a soil reference material (NIST 2710a) provided RBA estimates that were within 3% of the mean; coefficients of error (SE/mean) that ranged from 4% to 8% (mean: 6.5%); and a composite coefficient of variation for the RBA estimates (SD/mean) of 6.7% (Bradham et al. 2020). An interlaboratory comparison of RBA estimates for the same 10 soils or reference materials performed in two independent laboratories provided RBA estimates that differed by

less than 5% (Bradham et al. 2020). This was the first interlaboratory comparison of an assay designed to estimate the RBA for an environmental contaminant.

Comparability to RBA Estimates from Swine and Monkey Assays. Comparisons of RBA estimates for the same soils, obtained from the mouse assay and from two different swine assays indicated that the mouse and swine assays yield similar estimates of RBA (Bradham et al. 2013, 2018b). The mean relative percent difference was +13% (swine – mouse). Comparisons of the mouse assay to the monkey assay are limited to four soils from an orchard (Bradham et al. 2013). The RBA estimated for the site (based on the mean RBA for the four soils) was 31% (95% CI: 22, 41) based on the monkey assay and 29% (95% CI: 18, 40) based on the mouse assay.

Comparison to ABA estimates from Swine and Monkey Assays and Humans. In mice that consumed diet amended with sodium arsenate, the arsenic ABA was 85% (95% CI: 81, 89). This estimate of arsenic ABA for the mouse is comparable to estimates in humans who consumed arsenic in drinking water and diet, and to estimates of ABA in monkeys and swine exposed to sodium arsenate. The concordance of estimates for ABA in mice and humans provides further support for use of the mouse model in human health risk assessment (Diamond et al. 2022).

Applications to IVBA. Estimates of RBA from the mouse assay correlate with IVBA measured using EPA method 1340 (Bradham et al., 2015; Juhasz et al., 2014). The correlation is robust enough ($R^2 = 0.87$) to provide reliable predictions of RBA from IVBA. Approximately 40 RBAs estimated with the mouse assay were used, along with 43 swine RBAs, to support the development of a regression model for predicting soil arsenic RBA from IVBA (Diamond et al. 2016). The regression model and the IVBA method have been validated for regulatory use (EPA Method 1340; U.S, EPA, 2017a,b).

Mouse Assay Cost: The cost of running a mouse RBA bioassay is estimated to be 5 to 10% of the cost of a swine or monkey assay. The exact cost savings will depend on the number of soil samples evaluated and methods used to analyze the soil and biological samples (urinary arsenic).

Recommendations. The mouse assay is a cost-effective alternative to monkey and swine assays for measuring soil arsenic RBA (Diamond et al. 2022, Bradham et al 2018b, 2020). EPA considers the mouse assay to be a valid tool for conducting arsenic RBA research. This includes but is not limited to 1) development of and evaluation of IVBA assays, 2) confirming results of RBAs predicting from IVBA for problematic soils such as arsenic concentrations > 13,000 mg/kg, 3) arsenic sources not previously evaluated, (those evaluated include industrial, mining and ore processing, and pesticide application), 4) amended soils (soil with agents added to cause arsenic to be less soluble, less mobile, or less bioavailable), and 5) identifying factors that contribute to variability in arsenic RBA at sites.

Assistance and Information. Additional information and assistance in applications of the mouse arsenic RBA bioassay can be obtained from Dr. Karen Bradham (ORD CEMM, Bradham.karen@epa.gov).

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