Fact Sheet: Lead RBA and IVBA

Relative Bioavailability and In Vitro Bioaccessibility of Lead in Soil
January 4, 2021

Introduction and definitions

Bioavailability refers to the fraction or percentage of an ingested dose of lead that is absorbed into the systemic circulation (OSWER 9200.1-113). Where soil is contaminated with lead, the speciation of the lead and the characteristics of the soil can modify the amount of lead available for uptake into an organism. This fact sheet focuses on the bioavailability of lead to humans via the ingestion exposure pathway. *Absolute bioavailability* (ABA) refers to the fraction of an ingested dose of lead that crosses the gastrointestinal epithelium and becomes available for internal distribution. *Relative bioavailability* (RBA) is the ratio of ABA for soil lead to that of a water-soluble reference form of lead; typically lead acetate. 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 lead that is in a form that can interact with absorptive transport mechanisms (e.g., transcellular carriers or channels, paracellular diffusion). Soil lead must be bioaccessible in the gastrointestinal tract in order for it to be bioavailable. Processes contributing to bioaccessibility of soil lead may include release of lead from soil particles, dissolution of lead into gastrointestinal tract fluids, or chemical transformation. IVBA assays estimate bioaccessibility from measurements of *in vitro* solubility of soil lead. IVBA assays are designed to predict RBA from *in vitro* measurements of lead solubility.

Rationale for measuring soil lead RBA

EPA's assessment of human health risk for lead in soil is estimated by applying either the Integrated Exposure Uptake Biokinetic (IEUBK) Model for Lead in Children (U.S. EPA, 2007a) or the Adult Lead Methodology (ALM; U.S. EPA 2003) to estimate blood lead levels. The IEUBK model parameter *Absorption Fraction Percent* for soil (AFP_{soil}) is the value for the fraction of ingested soil lead that is absorbed into blood (ABA). The default value for AFP_{soil} in the IEUBK model is 30% (U.S. EPA, 1994). However, if oral bioavailability at a site is greater or less than 30%, lead risk will be over or underestimated at the site, respectively. EPA has recommended that site-specific RBA analysis of soil lead be performed when to increase confidence of risk estimates (U.S. EPA, 1989, 2007b,c). Previously, soil lead RBA was exclusively measured in animal bioassays; however, it is now routinely predicted from measurements of IVBA which accurately predict RBA and can be performed faster and with less expense than animal bioassays (U.S. EPA, 2017a,b). Measurements of soil lead RBA at the site can then be used to calculate a corresponding site-specific value for AFP_{soil} as follows (Equation 1):

$$AFP_{soil} = \text{RBA\%} / 100 * AFP_{water} \qquad \text{Eq. (1)}$$

where AFP_{water} is the IEUBK model default value for the *Absorption Fraction Percent* of lead in drinking water (50%), which represents soluble lead.

The corresponding absorption parameter in the ALM is the *Absorption Fraction for Soil and Dust* (AF_{S+D}) which is the value for the fraction of ingested soil lead that is absorbed into blood (equivalent to soil lead ABA). The default value for AF_{S+D} in the ALM is 0.12 (12%) which was based on the product of an RBA for soil lead of 60% and an absorption fraction for soluble lead in adults of 20% (i.e., 12/20=0.6; U.S. EPA, 2003). A site-specific value for AF_{S+D} can be calculated from measurements of soil RBA as follows (Equation 2):

$$AF_{S+D} = \text{RBA\%} / 100 * 0.20 \qquad \text{Eq. (2)}$$

Bioassays for measuring soil lead RBA

Various animal models have been used to study oral bioavailability of lead in soil, including rats, mice, and swine (OSWER 9200.1-113). Soil lead RBA bioassays have relied on measurements of blood lead or tissue lead (e.g., bone, kidney, liver) as metrics of absorbed lead dose for estimating bioavailability. Previously, the swine model U.S. EPA (Casteel et al., 2006; U.S. EPA, 2007d) developed was the most commonly used animal model. More recently, EPA has developed a mouse model for measuring soil lead RBA, which is less expensive than the swine model and provides RBA estimates that are similar to estimates from the swine model (Bradham et al., 2016).

The swine and mouse 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., serial vs terminal blood, bone sample vs whole skeleton), 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.

The mouse bioassay EPA developed is a more cost-effective alternative to swine bioassays for measuring soil lead oral RBA. The mouse and swine bioassays generated similar results when used to estimate RBA of the same soils (Bradham et al., 2016). Development and evaluation of the EPA mouse model is currently on-going (Bradham et al., 2019). Recent mouse studies have included pilot studies to evaluate the efficacy of soil amendments in reducing the bioavailability of soil lead (Bradham et al., 2018). Additional information and assistance in applications of the mouse lead 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 lead RBA for human health risk assessment and recommends using the assay for characterizing site-specific soil lead RBA (U.S. EPA, 2017). The assay involves a gastric-phase extraction of soil in a simple extraction medium. The

regression model for predicting RBA from IVBA is (Drexler and Brattin, 2007; U.S. EPA, 2017):

$$RBA\% = 0.878 \times IVBA\% - 2.8\% \quad (R^2=0.92) \qquad \text{Eq. (3)}$$

Application of RBA estimates to risk assessments

Methods and guidance for applying RBA estimates to baseline risk assessment, screening and removal assessments can be found in U.S. EPA, 2020. Additional resources regarding measuring RBA and applying it to risk assessments can be found at: https://www.epa.gov/superfund/soil-bioavailability-superfund-sites-guidance.

References

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Table 1. Lead Soil RBA Assays

Primary reference	***Casteel et al., 2006***	***Bradham et al., 2016***
Animal	Swine	Mouse
Dosing schedule	2 times/day for 15 days administered in 2 equal portions	Free access to amended feed for 9 days
Number of dose levels	Control (basal diet), 3 dose levels for soil and reference	Control (basal diet), 3 dose levels for soil and reference
Biological samples for estimating RBA	Blood samples collected from each animal on days 0, 1, 2, 3, 4, 6, 9, 12 and 15. Femur, kidney and liver samples collected on day 15.	Collected on day 9: Total skeleton, blood, kidney, liver
RBA metric	External dose-internal dose regression model, where internal dose estimated from: blood AUC, bone, kidney, liver, or aggregate of all tissues (*point estimate*)	External dose-internal dose regression model, where internal dose estimated from: blood AUC, bone, kidney, liver, or aggregate of all tissues (*point estimate*)