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

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MEMORANDUM

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

FROM: Jennifer Hovis, Acting Director
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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 lead 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 lead that is absorbed into the systemic circulation. Bioavailability of lead 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 lead absorbed from the gastrointestinal tract and enters 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 lead present in the exposure medium of interest to the ABA of soluble lead in drinking water or food.

Bioaccessibility refers to the physiological solubility of lead in the gastrointestinal tract. Ingested lead must become bioaccessible in the gastrointestinal tract in order to be

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

Background

EPA has recommended that site-specific assessments of soil lead 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, 2020). Soil lead 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 a lead IVBA assay for predicting RBA (SW-846 EPA Method 1340; U.S, EPA, 2009, 2013, 2017).

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 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 lead RBA, including physiology of the gastrointestinal tract, soil geochemistry and lead 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., arsenic, PAH, PCDD/Fs, PFAs)

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

Several animal bioassays have been developed for measuring soil lead RBA, including bioassays in mice and swine (Bradham et al. 2016; Casteel, 2006; Juhasz et al. 2009). These assays substantially differ with respect to methods, feasibility (husbandry requirements) and cost. 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., Casteel et al. 2006). 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 lead oral RBA in humans. This

problem cannot be definitively addressed without human clinical studies. The difficulties of such studies (e.g., Maddaloni et al. 1998) 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 lead RBA is similar when measured in swine and mouse assays, lending support to the use of the mouse model to predict lead RBA in humans (see Comparability to RBA Estimates from Swine Assays).

Mouse Lead RBA Assay

Development. The mouse lead RBA assay was developed as part of a collaborative effort of scientists at the EPA 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. 2016). A principal objective of this collaboration was to develop a more cost-effective alternative to swine bioassays for lead bioavailability research.

Description of the Mouse RBA Assay. Mice (female C57BL/6 mice) are exposed to lead mixed into a semi-purified low lead diet (<10 ppb) for a period of 9 days; during this period, cumulative consumption of diet is measured, and lead in total bone mass, blood and other tissues is measured at the end of the exposure period. One group of mice is exposed to diet amended with a reference lead compound (lead acetate) and one group is exposed to diet amended with the test soil. RBA is estimated by comparing the ratios of the tissue lead and cumulative dose (tissue/dose ratio, TDR) in the two groups of mice ($TDR_{test}/TDR_{reference}$). Because most of the lead body burden in located in bone, the RBA based on bone lead levels provides greater sensitivity and allows estimates of lead RBA in samples that have relatively low lead levels, such as house dust (Sowers et al. 2020).

Reproducibility of RBA Estimates. The mouse assay has been shown to provide highly reproducible estimates of lead RBA in soils contaminated with lead (Sowers et al. 2020). Repeated assays of a soil reference material (NIST 2710a) provided RBA estimates that were within 10% of the mean; coefficients of error (SE/mean) that ranged from 3% to 5% (mean: 3.4%); and a composite coefficient of variation for the RBA estimates (SD/mean) of 8.0%.

Comparability to RBA Estimates from Swine Assays. Comparisons of RBA estimates for the same soils, obtained from the mouse assay and from a swine assay, indicated that the mouse and swine assays yield similar estimates of RBA (Bradham et al. 2016, 2018; Casteel et al. 2006). The estimated RBA for a soil reference material (NIST 2710a) was 49% (90% CI: 31, 68) based on the mouse assay and 57% (95% CI: 39, 84) based on the swine assay (Bradham et al. 2016). The estimated RBA at a mining and smelting site was 118% (90% CI: 102, 138) compared to

repeated estimates based on a swine assay that ranged from 71% (95% CI: 56, 87) to 82% (95% CI: 61, 107; Bradham et al. 2018).

Applications to IVBA. Estimates of RBA from the swine assay correlate with IVBA measured using EPA method 1340 (Casteel et al. 2006). The correlation is robust enough (R^2 = 0.92) to provide reliable predictions of RBA from IVBA. The regression model and the IVBA method have been validated for regulatory use (EPA Method 1340; U.S, EPA, 2009, 2013, 2017).

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

Recommendations. The mouse assay is a cost-effective alternative to and swine assays for measuring soil lead RBA. EPA considers the mouse assay to be a valid tool for conducting lead 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 lead concentrations > 50,000 mg/kg, 3) sources not previously evaluated (those evaluated include industrial, mining and ore processing, and pesticide application), 4) amended soils (soil with agents added to cause lead to be less soluble, less mobile, or less bioavailable), 5) identifying factors that contribute to variability in RBA at sites (e.g., flooding conditions).

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

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