



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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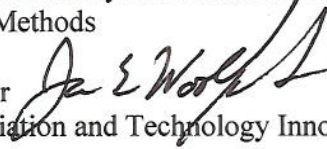
OFFICE OF
SOLID WASTE AND EMERGENCY
RESPONSE

OSWER 9285.7-77

JUL 3 2007

MEMORANDUM

SUBJECT: Estimation of Relative Bioavailability of Lead in Soil and Soil-like Materials Using *In Vivo* and *In Vitro* Methods

FROM: James E. Woolford, Director 
Office of Superfund Remediation and Technology Innovation

TO: Superfund National Policy Managers, Regions 1-10
Regional Toxics Integration Coordinators (RTICs), Regions 1-10

Purpose

This memorandum addresses an *in vivo* swine bioavailability bioassay and an *in vitro* bioaccessibility assay (further described in the attached document), which generally are scientifically sound and feasible methodologies for predicting the relative bioavailability (RBA) of lead in soil and soil-like materials. The Office of Superfund Remediation and Technology Innovation (OSRTI) believes that the Regions normally should consider these particular test methodologies to be validated methodologies for quantitative use in site-specific risk assessments. The use of the recommended *in vitro* methodology in site risk assessment is discussed in greater detail below.

This memorandum and the document released by this memorandum (U.S. EPA, 2007a) provide technical and policy guidance to the U.S. Environmental Protection Agency (EPA) staff on making risk management decisions for contaminated sites. It also provides information to the public and to the regulated community on how EPA intends to exercise its discretion in implementing its regulations at contaminated sites. It is important to understand, however, that this memorandum and attached document do not substitute for statutes that EPA administers or their implementing regulations, nor is it a regulation itself. Thus, these documents do not impose legally-binding requirements on EPA, states, or the regulated community, and may not apply to a

particular situation based upon the particular circumstances. Rather, these documents suggest approaches that may be used at particular sites as appropriate, given site-specific circumstances.

Background

Over the past several years, considerable effort has been directed at developing validated laboratory methods for determining bioavailability of soil-borne lead, arsenic, and other metals, including the development of rapid screening tools (e.g., *in vitro* bioaccessibility tests). The availability of new methods has reinforced the need for additional guidance on evaluating bioavailability data and incorporating this information into site-specific risk assessments. Beginning in mid-2002, the Office of Solid Waste and Emergency Response initiated an intra-agency workgroup to respond to the need for additional guidance. A bioavailability workshop was held in April 2003 that brought together a diverse group of experts from academia, industry, and government to discuss and provide input to EPA on bioavailability issues. The information shared at the workshop was used to develop recommended criteria for evaluating the validation and regulatory acceptance of alternative bioavailability test methods (see U.S. EPA, 2007b). EPA has used these recommended criteria to evaluate two separate test methods for predicting the relative bioavailability of lead. The results of this evaluation are reflected in this memorandum and the attached technical support document which are intended to facilitate national consistency in the use of lead bioavailability information in site-specific human health risk assessments.

The attached document reflects comments received from offices within the Office of Solid Waste and Emergency Response, the Regions, the Office of General Counsel, and from external peer reviewers. This document was also reviewed by the EPA Science Policy Council Steering Committee.

Implementation

ASSESSMENT OF LEAD BIOAVAILABILITY METHODS

The attached document describes methodologies for predicting lead RBA in soil and soil-like materials using either an *in vivo* swine bioavailability bioassay or an *in vitro* bioaccessibility assay (IVBA). These two methodologies generally satisfy the recommended method validation and regulatory acceptance criteria discussed in the *Guidance for Evaluating the Oral Bioavailability of Metals in Soils for Use in Human Health Risk Assessment* (U.S. EPA, 2007b). Thus, Regions should consider both the *in vivo* and the *in vitro* methodologies described in the attachment as potentially appropriate regulatory methodologies for determining the relative bioavailability of lead for quantitative use in site-specific risk assessments.

The *in vitro* methodology described in the attached document can provide a tool for characterizing site-specific RBA of lead in soil that is far less resource intensive than the *in vivo* model. A major advantage of utilizing this *in vitro* methodology may be that larger numbers of soil samples can be included in the characterization of soil lead bioaccessibility/bioavailability at a site. This typically would allow characterization of variability that might be associated with

location, proximity to sources of lead contamination, soil characteristics, or lead mineralogy at a site, which in turn could provide a more comprehensive assessment of site-specific RBA and greater confidence in lead risk estimates. The use of this *in vitro* method is also consistent with Agency objectives to reduce reliance on animal testing (U.S. EPA, 1999). Therefore, the Agency supports and encourages use of this methodology in appropriate circumstances, consistent with the recommended decision framework described in Figure 1 of U.S. EPA (2007b), and considering the following additional information:

1. Quality assurance. The attachment describes *in vivo* and *in vitro* approaches for predicting soil lead RBA that have undergone extensive testing and evaluation. Detailed protocols for the assays and results of inter-laboratory comparisons of the data are available (U.S. EPA, 2007a, Casteel *et al.*, 2006, Drexler and Brattin, 2006). These protocols have been reviewed by the Agency for site-specific application and serve as the basis for inter-laboratory comparisons and quality assurance evaluations of results obtained with the assay that are submitted to the Agency in support of site-specific risk assessments.

2. Scientific validation status. As noted above, the methodologies described in the attached document generally meet the recommended criteria for acceptance of these toxicological test methods by EPA. It should be noted that an underlying assumption in the application of these assays is that the RBA predicted for juvenile swine provides an accurate estimate of the RBA in human children. Although this assumption has not been rigorously tested, extensive physiological studies support the use of swine over other potentially feasible laboratory species (*e.g.*, rodents) for studies of absorption of lead from the gastrointestinal tract (U.S. EPA, 2007a; Weis and LaVelle, 1991).

3. Application to children and extrapolation to adults. The juvenile swine model, described in the attachment, has been utilized as an experimental methodology for predicting RBA in human children; therefore, the prediction equations for estimating RBA from results of the *in vitro* assay apply to human children (but see issues raised in item #2, above). While there is evidence to indicate that absolute bioavailability of soluble lead (*e.g.*, in food or water) varies with age, the Agency is not aware at this time of information on the age-dependence (or independence) of the RBA for lead in soil. However, existing information on the development of gastric secretion in mammals indicates that gastric acid and pepsinogen production rates and acidity are lower in the neonate than in adults. A limitation in the availability of gastric acid, if it were to affect dissolution rates of soil-borne lead in the stomach at all, would be expected to lower RBA. Thus, it is conceivable that RBA for a given lead and soil matrix could be lower in children compared to adults (U.S. EPA, 2007a), introducing additional uncertainty into RBA estimates for adults that are derived from the methodology described in the attachment.

4. Sample lead concentration limits. The 19 samples tested in the *in vitro* - *in vivo* comparison described in the attached document ranged from 1,200-14,000 ppm lead. This validation range should be sufficient for most applications of the methodology. Although

there is no basis for predicting that errors would necessarily be introduced into the estimates of RBA if sample concentrations outside this range were used in the *in vitro* methodology, use of such samples without validating comparisons with results of the *in vivo* swine assay generally will introduce additional uncertainty into estimates of RBA. A further constraint on the lead concentration is noted in the attachment; sample concentrations used in the *in vitro* bioaccessibility assay should not exceed 50,000 ppm for relatively soluble forms of lead (*i.e.*, lead acetate, lead oxide, lead carbonate), in order to avoid saturation of the extraction fluid. However, applications of the *in vitro* bioaccessibility assay to such high lead concentrations is unlikely to be relevant for improving risk management decisions; thus, this limitation is not likely to be a serious constraint for use of the methodology. Should additional data become available that would suggest modification of the above limits, the Agency will issue additional guidance.

5. Particle size. All samples tested in the *in vitro* - *in vivo* comparison described in the attached document were sieved through a 60 mesh screen which excluded particles greater than 250 μm . Particle size can be expected to affect dissolution rates for lead that is embedded in particles and is known to affect absolute bioavailability of lead (U.S. EPA, 1986). Therefore, additional uncertainty typically will be associated with RBA estimates based on application of the *in vitro* assay to samples having particle sizes larger than 250 μm . In general, humans are believed to ingest particles that are predominantly smaller than 250 μm in diameter (Kissel *et al.*, 1996; Sheppard and Evenden, 1994; Driver *et al.*, 1989; Duggan and Inskip, 1985; Que Hee, *et al.*, 1985; Duggan, 1983), so measures of RBA on samples more coarse than this would usually not be considered relevant to risk assessment. Likewise, RBA estimates based on *in vitro* bioaccessibility assays of samples that have not been processed through a 60 mesh (or finer) sieve are generally not appropriate for quantitative use in site-specific risk assessments.

6. Soil mineralogy. Results of evaluations that are described in the attached document indicate that RBA of lead in soil-like materials typically can be reliably estimated using the *in vitro* assay and the associated regression equation relating *in vitro* bioaccessibility to *in vivo* RBA. At present, it appears that this equation should be widely appropriate, having been found to hold true for a wide range of different soil types and lead phases from a variety of different sites. However, most of the 19 samples included in the evaluation were collected from mining and milling sites, and it is plausible that some forms of lead that do not occur at this type of site might not follow the observed correlation. Thus, whenever a sample that contains an unusual and/or untested lead phase is evaluated by the *in vitro* bioaccessibility protocol, this should be identified as a potential source of uncertainty. In the future, as additional samples, having a wider variety of new and different lead forms, are tested by both *in vivo* and *in vitro* methods, the applicability of the method to a wider range of lead mineralogy and soil characteristics should be more clearly defined. The Agency encourages the collection and dissemination of such data as a means for further assessing uncertainties in the application of the assays for predicting site-specific RBA. Although mineralogy is among

the factors that influence RBA, soil mineralogy information alone does not provide the basis for substitution of bioavailability information for quantitative risk assessment.

7. Uncertainty in predicted RBA value. As noted above, the *in vitro* methodology for lead (U.S. EPA, 2007a) measures IVBA for a test material, and converts this to an estimate of RBA by application of a mathematical formula. The resulting prediction of RBA should be thought of as the best estimate of the true RBA associated with that IVBA, but the actual RBA (if measured *in vivo*) might be either higher or lower than the prediction, due either to authentic inter-sample variability and/or to measurement error in RBA or IVBA. In general, the best estimate of RBA is the most appropriate value for use in the IEUBK model, but risk assessors and risk managers should use their professional judgment to decide if calculations using other values from within the RBA prediction interval should also be evaluated as part of an uncertainty analysis.

OSRTI has established a “Bioavailability Committee,” which will operate under EPA’s Technical Review Work Group for Metals and Asbestos (TRW), to provide technical support to those engaged in human health risk assessment at contaminated sites. Part of the Committee’s responsibilities will be to review new methods for assessing bioavailability of inorganic soil contaminants (*i.e.*, new method validation). It is anticipated that the attached document normally will serve as a template for future submissions of methods to the Bioavailability Committee. In addition, the Bioavailability Committee of the TRW will compile and evaluate information on applications of bioavailability assessments in EPA site-specific risk assessments, with the objective of promoting consistent application of the framework described in U.S. EPA (2007b) across the EPA Regions. To facilitate collection of this information, the Regions are asked to report all site-specific risk assessment applications of the *in vitro* lead bioaccessibility methodology or *in vivo* juvenile swine model to the Bioavailability Committee. The Regions are also asked to contact Aaron Yeow (yeow.aaron@epa.gov) in OSRTI or Michael Beringer (beringer.michael@epa.gov) in Region 7 of the Bioavailability Committee for information on any other bioavailability assessment methodologies under consideration for use in site risk assessment.

References

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Attachment

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