

## Oral Bioavailability of Metals from Soil

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## ABSTRACT

Although the bioavailability of metals from contaminated soils is usually significantly less than the amount of metal that is released by acid extraction, risk assessments are often based on the acid extractable amount, unless bioavailability estimates from *in vivo* experiments with animals are available. An *in vitro* synthetic bio-fluid extraction model has been developed to preclude the requirement of conducting expensive and time-consuming *in vivo* experiments. This work addresses the evaluation of the *in vitro* bio-fluid extraction model with respect to experimentally determined oral bioavailability of the metals in animals. The bioavailability of arsenic (As), cesium (Cs), chromium (Cr), lead (Pb), and uranium (U) from contaminated soils will be estimated and compared to the bioaccessibility, estimated using an *in vitro* synthetic bio-fluid extraction model. It is anticipated that the bioaccessibility will be consistently greater than the bioavailability determined by *in vivo* experimentation, but would be significantly less than the amount released by standard EPA acid extraction methods.

## INTRODUCTION (A)

### Hypothesis

*In vivo bioavailability of heavy metals from contaminated soil is significantly less than both in vitro bioaccessibility and the amount of metal that is made available by standard EPA recommended acid extraction methodology (EPA Methods 3050 and 3051).*

### Motivation

*In vitro* methods of estimating bioavailability of toxic heavy metals in soils are less expensive and time-consuming than *in vivo* assays. The currently EPA recommended procedure for the *in vitro* estimation of bioavailability of heavy metals in soils is based on acid extraction of the heavy metals from the soil matrix (EPA Methods 3050 and 3051). However the currently recommended *in vitro* methods (EPA Methods 3050 and 3051) usually significantly overestimate the actual bioavailability.

The results of this project will be used to evaluate an *in vitro* assay for estimating bioaccessibility, and to confirm that the bioaccessible fraction is an upper bound for the bioavailable fraction. The *in vitro* assay being developed involves the sequential extraction of heavy metals from soil samples by synthetic human gastric fluids. The bioaccessible fraction is the amount of metal released into the gastric fluid from the soil, relative to the amount released by a standard acid extraction procedure.

## INTRODUCTION (B)

### Stakeholder Involvement

This project is part of an independently funded DOE project to develop alternative methods of estimating bioavailability of toxic heavy metals in soil matrices.

### Novel Aspects of Project

The project will be used to evaluate the suitability of the synthetic bio-fluid based *in vitro* assay being developed to estimate the upper bound of bioavailability of heavy metals and radionuclides.

The evaluation will be conducted using soils from a number of hazardous waste sites, including a site in Jersey City New Jersey, and sites within the DOE Savannah River Site (SRS). An evaluation will also be conducted using NIST standard soil from Montana, so that the results of the work can be compared with future experiments with the standard soil.

## METHODS (A)

### Mechanisms of Metal Uptake in the Gut

- passive and facilitated diffusion,
- active (carrier-mediated) transport, and
- pinocytosis.
- It is generally accepted that the predominant mechanisms of uptake are diffusion and active transport, which are significant for only for soluble chemical species.
- The primary site of uptake is probably through the epithelial cells that line the gut lumen, and the secondary site is probably through the gap junctions between the epithelial cell.
- The trans-epithelial transport of metals can be conceptually divided into:
  - transport into epithelial cells from gut lumen, and
  - transport out of epithelial cells, through the basement membrane, into serosal fluid which eventually merges with the blood.

## METHODS (B)

### Experimental Procedure for Test Soil Exposures

- 5 samples of soil contaminated with heavy metals was obtained from a hazardous waste site in Jersey City, New Jersey, and the soil samples were air dried, and sieved through a 75 $\mu$ m mesh.
- Male Sprague-Dawley rats (200–250 g) were housed in metabolism cages, and were fasted for 24 hours prior to exposure.
- A single dose of the soil (4 g/kg) was administered in a 5% gum arabic solution.
- Following The rats were allowed to feed *ad libitum* following the administered soil dose.
- The rats were sacrificed at 24, 48, 72, and 96 hours, and blood, liver, lung, kidney, spleen, brain, hair, bone, testes, muscle, and heart were removed and weighed.
- Samples of rat tissues were digested with nitric acid and analyzed for metal content using ICP-MS.

**RESULTS (A)**

Metal	Body Burden as a % of Total Extractable Metal [Days following Exposure]			
	1	2	3	4
As	44.18 ± 2.63	54.01 ± 5.08	64.12 ± 16.5	31.90 ± 3.33
Cr	0.18 ± 0.04	0.13 ± 0.03	0.18 ± 0.03	0.12 ± 0.02
Pb	1.59 ± 0.66	1.27 ± 0.71	0.65 ± 0.15	0.58 ± 0.17

## RESULTS (B)

### Comparison of Body Burden, Bioaccessible, and Acid Extractable Fractions

Metal	Bioaccessible Amount [ppm]	Acid Extractable Amount (EPA Method 3051) [ppm]	Bioaccessibility
As	143 ± 27.9	310 ± 78	46.1 %
Cr	1187 ± 174	11227 ± 6018	10.5 %
Pb	2383 ± 536	2688 ± 413	88.6 %

## DISCUSSION

- The results of the *in vitro* experiments indicate that the amount of Pb, Cr, and As that was made bioaccessible by sequential extraction with synthetic bio-fluids is consistently less than that extractable by EPA Method 3051.
- The bioaccessible percentage varies widely between metals, and could also vary between soil samples from different site for a given metal.
- The maximum observed body burden following an oral dose was significantly lower for As, Cr, and Pb.
- Deconvolution analysis will be performed with the tissue concentration data to calculate the bioavailability of these metals.

## FUTURE PLANS

- Bioavailability of As, Cr, and Pb will be estimated by deconvolution of the blood concentrations measured following the orally administered soil dose.
- The *in vitro* sequential bio-fluid extraction procedure will be applied to SRS soils, procured in co-operation with the CRESP/EOHHSI-Remediation Task Group.
- SRS soils with background concentrations of contaminants, and soils and sediments known to contain radioactive contaminants will be analyzed for Cs<sup>137</sup>, Sr<sup>90</sup>, and U<sup>238</sup>.
- Bioavailability of Cs<sup>137</sup>, Sr<sup>90</sup>, and U<sup>238</sup> from the various SRS soils and sediments will be estimated by deconvolution and compared with the results of the *in vitro* bioaccessibility assay.