

Final Report
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**Comparison of the Bioavailability of Waste
Laden Soils Using “In Vivo” “In Vitro” Analytical Methodology
and Bioaccessibility of Radionuclides for Refinement of Exposure/Dose Estimates**

Paul J. Liroy, Ph.D.
University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School
Environmental and Occupational Health Sciences Institute (EOHSI)
170 Frelinghuysen Road
Piscataway, NJ 08854

Michael Gallo, Ph.D. and Panos Georgopoulos, Ph.D.
UMDNJ-RWJMS and EOHSI
170 Frelinghuysen Road
Piscataway, NJ 08854

Robert Tate, Ph.D. and Brian Buckley, Ph.D.
Rutgers, The State University of New Jersey and EOHSI
New Brunswick, NJ 08901

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Project Manager, Wendy L. Huggins (Phone # 208-526-2808)
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3. EXECUTIVE SUMMARY

The bioavailability of soil contaminants can be measured using *in vitro* or *in vivo* techniques. Since there was no standard method for intercomparison among laboratories, we compared two techniques for bioavailability estimation: *in vitro* dissolution and *in vivo* rat feeding model for a NIST traceable soil material. Lead, arsenic and chromium were chosen for their range of concentration in the soil and their toxicological relevance. Bioaccessibility was measured using a sequential soil extraction in synthetic analogues of human saliva, gastric and intestinal fluids. Bioavailability was measured in Sprague Dawley rats by determining metal levels in the major organs and urine, feces and blood at 1, 2, 3 and 4 day time points. Bioaccessibility extractions yielded a gastric solubility of 76.1%, 69.4% and 3.7% respectively, while intestinal solubilities were 10.7%, 65.9% and 3.0%. The bioavailability of the NIST SRM was 0.63%, 36.5% and 4.8% for Pb, As and Cr. As the three metals are absorbed mainly in the small intestine, intestinal solubility was used for the *in vitro-in vivo* comparison. Although lead had the highest soil concentration of the selected metals, it was the least bioavailable. Arsenic, however, was highly available in both the *in vitro* and *in vivo* method. Bioaccessibility was \geq bioavailability in all cases, which would be expected due to the limitation of intestinal absorption. Bioaccessibility was found to be a good indicator of relative metal bioavailability. Furthermore an exposure analysis and risk determination for this particular soil using an assumed contact with the total metal concentration would lead to a high risk. This assessment would be misleading based upon the results from these experiments.

Bioaccessibility analyses were performed on samples from two radionuclide contaminated sites. The main purpose of the experiments was to refine our methods for the special difficulties involved in low level radiation measurement. A soil sample was obtained from the Lower Three Runs on the Savannah River Site(SRS), and had detectable levels of Cs¹³⁷. Bioaccessibility extractions were performed on this soil and included varying certain steps in the procedure. The soil was extracted at differing liquid to solid ratios, resulting in a slight increase in bioaccessibility with decreasing liquid to solid ratio (6.4% at 1080ml/g to 2.8% at 54ml/g). The highest liquid to solid ratio resulted in a large error margin, due to the extremely low levels of Cs¹³⁷ in the extract. A second experiment was conducted in order to compare centrifugation and filtration of the aqueous samples as methods for liquid and solid separation. Previous work has involved centrifugation, but the low measurement levels required a clear distinction between small particulate associated Cs¹³⁷ and dissolved Cs¹³⁷. Filtration provided a clear difference in the measured outcome, as gastric solubility was 3.3% in filtered samples and 9.1% in centrifuged

samples. The final experiment performed with Cs¹³⁷ involved the addition of various organic acids to the gastric fluid at physiological levels. The addition of organic acids to the synthetic gastric fluid did not change the resulting bioaccessibility of Cs¹³⁷.

The next step in the method development for bioaccessibility of radionuclides was to expand our work to alpha and beta emitters. Two soils (BA-002 and BA-004) were sampled from a berm located at a seepage basin area on the SRS. These soils were found to contain a mixture of gamma, alpha and beta emitting contaminants such as Cs¹³⁷, Cm²⁴³, Ra²²⁶ and Sr⁹⁰. Cs¹³⁷ had very low solubility in gastric fluid, and was below detection limits in the gastrointestinal extracts even after completing improvements in geometry by means of selective Cs¹³⁷ sorption by 3M RAD disks. After final analysis of both the BA-002 and BA-004 soils, the bioaccessibility of Cs¹³⁷ was less than 8.7%. Before chemical separation of alpha emitters, a gross alpha count was run of the gastric fluid extract. A beta count was conducted choosing energy ranges where Cs¹³⁷ and Sr⁹⁰ were detectable, and isolating these ranges to partially differentiate the Cs¹³⁷ and Sr⁹⁰ peaks. Alpha and beta activity was detected in the gastric fluid extracts of BA-002 and BA-004 at levels approximately two times higher than the method blanks. The elevated beta activity is possibly due to the presence of Cs¹³⁷, Sr⁹⁰ or other beta emitters, and some of the possible alpha emitters are Cm^{244/243}, U²³⁸, Pu^{239/240} or a thorium isotope. The results indicate that the modified methodology for bioaccessibility can be used for specific radionuclide analysis. Future work will be on specific radionuclide isolation for bioaccessibility measurements in soils.

4. RESEARCH OBJECTIVES

Hazardous waste contamination problems are prevalent in soils at DOE sites. Currently, they are prioritized by estimating the human exposure and health risks for contaminants of concern based upon the total extractable levels of contaminants present in the soil or soil/waste matrix. This is done for single and multi-route exposure conditions with exposure via ingestion of contaminants in the soil being a major pathway. Since it is anticipated that portions of some DOE sites will become parkland, commercial or residential restorations, a major concern is exposure of children to wastes. Studies have shown that children ingest between 40-200 mg of soil/day, and that children who suffer from PICA will ingest even larger quantities. The American Industrial Research Council has tabulated these data. The total acid extractable concentration of a contaminant present in the soil is not the best estimator of exposure since the human digestive system does not have the capacity to extract all of the contaminant from a soil matrix. However, the total extractable mass is the current standard used in exposure and risk assessments.

Conversely, a determination of the bioavailable or bioaccessible portion of the contaminants in the soil/waste can be used by risk assessors to more accurately estimate the risk via ingestion. Use of the concentration present in a simulated ingested bioavailable or bioaccessible fraction of soil should be a better indicator of the risk to a contaminant because it represents the portion of the mass which will yield exposure, uptake and then the internal dose to an individual or a sub-group of a population. To date the difficulty in obtaining an accurate assessment of the internal dose is caused by the lack of scientific information on the degree to which a contaminant will accumulate in bodily fluids and will be made available for migration across the membranes within the digestive system. The uncertainties associated with exposure/dose calculations derived from total elemental concentrations can be overcome by developing a procedure that accurately estimates the bioavailable fraction of the contaminant found in a particular soil or soil/waste mixture.

If it is difficult to liberate the total concentration of the elements from a soil, even with concentrated acids and high temperatures and pressures, then the bioavailability of the elements in that soil would be uncertain. The bioavailability of elemental contaminants should be estimated by extracting with biological fluids (e.g. intestinal fluids). Unfortunately, governmental agencies have routinely relied on a conventional definition of total extractable elements based on acid extractions using EPA method 3015, 3050 or 3051 when reporting a soil concentration for use in an ingestion exposure assessment. In reality, bio-fluids may only solubilize a small percentage of the total elements present in the soil, and traditional acid extraction techniques can lead to artificially high estimates of internal dose, and uncertain/unrealistic estimates of the risk to a population.

The extractability of contaminants affects its transport into various organ systems within the body. Assuming the contaminants will undergo dissolution and absorption by specific target tissues and organs, artificially high estimations of the bioavailable fraction or 100% bioavailability will alter internal dose estimates. The ratio of the total amount of material present in the soil/waste matrix to that which is available for dissolution in a bio-fluid extractant gives an indication of the bioavailability of the contaminant.

Introduction

Bioavailability is defined as the rate and extent of absorption of a contaminant at the target organ (Gibaldi, 1984). Bioavailability studies are used to gain an understanding of the availability of heavy metals and other contaminants (radionuclides, organics) within an animal or plant receptor. Such studies can be used to identify areas

on a site where heavy metal mobility is a major concern, and thus assist in the prioritization of remedial efforts. If a component is proposed to be biologically active in an organism, the first step is to measure its bioavailability at the affected site of action.

The two principal limiting factors in the assimilation of a heavy metal by a mammalian system are dissolution in the gastrointestinal tract and absorption through the intestinal wall. The dissolution of a metal depends on the characteristics of the contaminant itself and on the environmental (soil) matrix. Finally, the solubility of a metal in a soil matrix will be a function of the composition of the gastrointestinal fluids encountered in the digestive system.

Bioaccessibility is the term that defines the amount of metal that can be dissolved in the gastrointestinal fluid. Once dissolved in the gastrointestinal fluid, intestinal absorption is affected by the retention time of the metal near the site of absorption. Both of these processes can be affected by the speciation of the metal in the fluid. In contrast, it is inherent in the definition of bioavailability that some biological membrane (intestinal wall) has been crossed, therefore bioaccessibility values should be greater than bioavailability.

Studies have been conducted to measure the amount of a contaminant that reaches biological receptors, namely the bioavailable fraction. Plant bioavailability has been measured by analyzing plant tissues (Carbonell, et al. 1998), soil leach techniques (Pichtel, et al. 1997), (Novozamsky, et al. 1993), (Lebourg, et al. 1998) and earthworm uptake of metals (Brown, et al. 1995). Human bioavailability studies incorporate various leaching and dissolution techniques (Ruby, et al. 1996), (Davis, et al. 1993), studies of soil type and structure (Davis, et al. 1997), actual human feeding studies (Gargas, et al. 1994), (Maddaloni, et al. 1998), (Rieuwert and Farago, 1995) and extrapolation from animal experiments (Freeman, et al. 1996), (Freeman, et al. 1992), (Groen, et al. 1994), (Clapp, et al. 1991), (Nessel, et al. 1992). Studies have also been performed in order to compare gastrointestinal dissolution techniques with *in vivo* animal models, (Rodriguez, et al. 1999), (Sheppard, et al. 1995), (Ruby, et al. 1996) however, no clear association has been made between bioavailability and the dissolution of contaminants within the gastrointestinal system. All results vary according to the method used, soil and contaminant characteristics, and consequently the importance of a comparison with a certified soil becomes evident. Bioaccessibility and bioavailability results obtained for the same standard reference material could eventually be used by all for comparison of methods and environmental matrices. Once done, the method can then begin to be used to examine other contaminants at DOE sites, namely, radionuclides deposited in soil matrices.

The **hypotheses** tested by the research were: 1) the more closely the synthetic, *in vitro*, extractant mimics the extraction properties of the human digestive bio-fluids, the more accurate will be the estimate of an internal dose; 2) performance can be evaluated by *in vivo* studies with a rat model and quantitative examination of a mass balance, calculation and dose estimates from model simulations for the *in vitro* and *in vivo* system; and 3) the concentration of the elements and radionuclides present in the bioavailable fraction obtained with a synthetic extraction system will be a better indicator of contaminant ingestion from a contaminated soil because it represents the portion of the mass which can yield exposure, uptake and then the internal dose to an individual.

The research conducted under the auspices of the grant has benefited from two other projects that were conducted just prior to and during the first half of the grant period. The first was a grant-in-aid given to EOHSI by Merck & Company, Inc, (P.O. Box 100, WS2F45, Whitehouse Station, New Jersey 08889-0100) to develop the basic analytical procedure for “*in vitro*” bioaccessibility studies. This led to the first EOHSI manuscript on bioaccessibility (Hamel et al., 1998). The second was a grant from the Hazard Waste Management Research Center that was funded by NSF and Industry. It is located at the New Jersey Institute of Technology. The research provided the mass balance technique now employed in all DOE experiments. Partial funding for the mass balance research was derived directly from the DOE Grant. A manuscript that resulted from the combined efforts on mass balance is found in Appendix A.

5. Methods and Results

A. *In vivo* and *In vitro* Intercomparison Experiment.

1. Materials and Methods

A standard reference material, Montana Highly Elevated Levels 2710 (SRM 2710), was obtained from the National Institute of Standards and Technology (NIST). The soil material was homogenized and sieved to less than 74 μ m in diameter by NIST. For the three metals examined in the study, lead, arsenic and chromium, the NIST SRM 2710 has certified metal concentration levels of 5532 μ g/g and 627 μ g/g for lead and arsenic, respectively, and an uncertified value of 39 μ g/g for chromium.

In Vitro Test of Bioaccessibility

The *in vitro* bioaccessibility procedure was performed as a modification of a previously published by Hamel, et al. 1998 and Hamel et al. 1999. Approximately 0.5g samples of the NIST 2710 SRM were weighed and placed into

wide mouth 250 ml polyethylene bottles. Eight milliliters of artificial saliva were added to each bottle, which was followed by 100 ml of gastric fluid. The gastric fluid was 0.03M NaCl, containing 0.32%(w/v) pepsin at pH 1.47. The estimated activity of the purchased pepsin during the gastric portion of the extraction procedure, was approximately 1,920,000 units/L to 5,760,000 units/L.

The samples were then placed into a constant temperature bath set at 37°C and allowed to shake for two hours at 90 cycles per minute. After two hours the bottles were taken out of the bath, and a 10 ml aliquot was taken from each bottle. The bottles were then centrifuged at 1000 rpm(198g) for 20 minutes, and the supernate removed by pipette from the Nalgene bottle. Next, the pH was adjusted to 6.54 by adding 100 ml of intestinal fluid, a 0.2M NaHCO₃ solution. The samples were then allowed to shake at 37°C for another 2 hours. After this two hour period, the liquid portion of the extraction was reintroduced to the bottles containing soil, and allowed to shake for another 2 hours at 37°C in the water bath. After the third extraction period, another aliquot of 10 ml was taken from the bottle. This procedure was also performed without the intermediate step requiring the soil separation. The fluid aliquots were digested by adding 0.5 ml of nitric acid to 9.5 mL of sample. These samples were allowed to digest for 48 hours in capped polystyrene test tubes, and then filtered through a Puradisc Whatman syringe filter with a 0.45µm pore size.

Following the sequential gastrointestinal extraction, the residual soil mass was recaptured from the bottle. The soil remaining in the polyethylene bottles was captured on filter using a 90 mm diameter 0.45µm pore size cellulose nitrate filter. The filters and remaining soil were then digested in 10 ml of concentrated Optima nitric acid, according to the EPA method 3051 digestion series. (Kingston, 1986)

The samples were then diluted to concentrations within the linear portion of the calibration curve. The diluent used was a 2% Optima nitric acid solution. All metal analysis included the appropriate laboratory blanks plus controls and method blanks plus controls.

In Vivo Test of Bioavailability

One gram of the Montana Standard Reference Material 2710 was suspended in 5 ml of an aqueous solution which contained 5% gum Arabic to help maintain a suspension. Gum Arabic was obtained from Sigma Chemical Co. (St. Louis, MO). Optima nitric acid, 50% H₂O₂, ethyl ether and feeding needles were obtained from Fisher Scientific (Pittsburgh, PA). The suspension was stored until needed at room temperature in a dark container.

Fifteen male Sprague-Dawley rats, 180-200 g, were obtained from Hilltop Labs. The animals were housed under standard conditions in wire mesh cages prior to treatment. Twenty four hours prior to treatment, the animals were placed in plastic metabolism cages and were fasted to reduce stomach content. The animals were separated into 5 groups, 3 animals per group. On day 0, all rats were given a single injection, by oral gavage, of either the soil suspension (25 ml/kg) or an equal volume of the vehicle control. The animals were then given free access to food and water. Any excrement was discarded that was collected during the previous 24 hours. On days 1, 2, 3, and 4, three rats from each group were sacrificed and necropsies were performed on each animal. Vehicle control animals were sacrificed on day 4.

Animals were anesthetized with ether and blood was collected from the descending aorta and the volume was recorded. Other tissues, listed in table 2, were collected and their weights recorded. Large tissues were sectioned before digestion. The femurs that were collected had their marrow removed by aspiration. The bone was then dried to constant weight before digestion. All tissues were placed in 20 X 150 mm test tubes containing 2 ml of Optima HNO₃. Tissues were allowed to digest at 70°C until clear. Two milliliters of 50% H₂O₂ was added and the samples were allowed to incubate overnight at room temperature. Samples were then quantitatively transferred to volumetric flasks and brought up to volume using deionized water. Any samples that contained solids were filtered using Whatman #1 filter paper. The samples were then sent for analysis by ICP-MS.

Before ICP/MS analysis, 0.5g of feces was added to 10 ml of ultra pure nitric acid in a digestion vessel, and after capping and sealing the sample was ready for microwave digestion (CEM MDS-2000). A staged microwave digestion was conducted as follows: 40% power, 5 min; 50% power, 5 min.; 60% power, 5 min; 10% power, 5 min and 80%power, 5 min. These samples were cooled and diluted to concentrations within the linear portion of the calibration curve.

Analysis of Metals

Samples were analyzed by ICP-MS at EOHST's Chemical Analysis Facility. All samples were diluted to <5% acid content. The bioaccessibility samples were analyzed for chromium by Graphite Furnace Atomic Absorption Spectrometry at a dilution of 2% acid. The method detection limits for the bioaccessibility analyses were 0.472, 1.09 and 0.42 ppb for lead, arsenic and chromium respectively. The method detection limits for the bioavailability procedures were 2.21, 4.7 and 3.90 ppb for lead arsenic and chromium respectively.

Dose Analysis

The dosing of the rats was tested for accuracy and precision using a gravimetric method. First, a gum Arabic-soil suspension was administered to three pre-weighed and dried Whatman 90 mm paper filters using a gavage needle. The soil suspension was then dried in an oven at 105°C to constant weight (within 0.01g). The filters were then weighed on a Sartorius Analytical Microbalance, and the amount of soil dosed was calculated by subtracting the weight of the tare weight of the filter. The Sartorius Analytical Microbalance had an accuracy of 0.4% and a precision of 2.6% for the measurement of a 10 mg standard weight.

2. Results

Bioaccessibility

Bioaccessibility results for the Montana SRM 2710 are presented in Table 1. The values in the first column, the percent soluble in gastric fluid, were calculated by dividing the concentration of metal soluble in gastric fluid (μg metal from g of soil) by the certified metal concentration value. The values in the second column, the percent soluble in intestinal fluid and, is the percent metal soluble in gastric and intestinal fluids divided by the certified metal concentration. The intestinal solubility is a function of the addition of saliva, gastric and intestinal fluids. Gastric solubility, is a result of the addition of saliva and gastric fluids. The final column is an account of the total metal recovery from the system. Recoveries are calculated by the addition of the amount soluble in intestinal fluid with the concentration of metal from the soil recovered in the final step of the extraction procedure. The standard deviations associated with the bioaccessibility values were propagated according to random error calculations.

Table 1 *In Vitro* Bioaccessibility of Selected Metals in NIST SRM 2710

	% Soluble in Gastric Fluid	% Soluble in Intestinal Fluid	Recovery
Pb	76.1%±11%	10.7%±2.3%	75.5% ± 9.6%
As	69.4%±8.3%	65.9%±5.2%	94.7% ± 6.9%
Cr	3.7%±2.1%	3.0%±1.9%	46.5%±13.2%

Percent solubility values were calculated with respect to the NIST-reported values. All values represent averages of 3 replicate samples. Recoveries are calculated as the sum of the dissolved mass in the intestinal fluid and the undissolved portion in the residual soil mass. All errors were propagated according to random propagational error calculations.

Lead was the most soluble in gastric fluid, at 76.1%, where as chromium was the least at 3.7%. There was an order of magnitude drop in the solubility of lead from the gastric compartment to the intestinal compartment. Arsenic also dropped in solubility when presented to intestinal fluids, however the decrease was not as sharp, 69.4% to 46.9%, as the decrease in lead solubility. The chromium bioaccessibility was consistent between gastric and intestinal fluids where the decrease from the intestinal solubility to gastric solubility was only 3.7% to 3.0%.

Table 2. *In Vivo* Bioavailability of Selected Metals in NIST SRM 2710

Metal	Exposure	Bioavailability by tissue (%)											TOTAL	RECOVERY
		Blood	Lung	Liver	Kidneys	Spleen	Testes	Heart	Muscle	Femurs	Urine	Feces		
As	1 Day	32.9	0.1	0.7	0.1	0.1	0.0	0.1	0.0	0.0	0.3	57.2	34.0	91.5
	2 Day	36.2	0.1	0.7	0.1	0.1	0.1	0.1	0.1	0.0	0.4	29.2	37.5	67.1
	3 Day	38.6	0.1	0.7	0.1	0.1	0.0	0.1	0.1	0.0	1.4	44.5	39.8	85.7
	4 Day	33.7	0.0	0.7	0.1	0.1	0.0	0.1	0.1	0.0	2.2	17.2	34.8	54.2
Cr	1 Day	1.7	0.0	2.4	0.0	0.0	0.1	0.0	0.0	0.0	0.0	6.9	4.2	11.1
	2 Day	3.9	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	2.9	4.4	7.3
	3 Day	2.5	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.1	0.2	17.5	2.8	20.5
	4 Day	2.4	0.0	5.1	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	7.8	7.8
Pb	1 Day	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.0	68.7	0.9	69.6
	2 Day	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	44.3	0.4	44.7
	3 Day	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0	62.8	0.9	63.7
	4 Day	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	24.1	0.3	24.4

Recoveries were calculated by summing the mass soluble in the intestinal fluid with the residual mass after the final soil collection. These mass balance recoveries varied from approximately 94.7% for arsenic to 75% for lead and 46.5% for chromium. The values for arsenic and lead were good, but the recovery for chromium was quite low. Chromium recovery was also low for aggressive acid leaching procedures, such as EPA 3051, at 57% of the value provided by NIST. This value is uncertified, and therefore the provided value may have a certain degree of error surrounding it. The levels of chromium are also the lowest of the three metals of interest, and may have pushed the detection limits of the instrument.

Bioavailability

The results of the animal study are presented in Table 2. Each metal and its corresponding exposure times are represented in the horizontal rows. The various tissue levels of metal are listed in columns and are expressed as a percentage of the administered dose. Each value represents the average of concentrations obtained from 3 animals. The total value for each row is a sum of all tissues, except excreta. This value is referred to in this study as the “percent bioavailability” of a given metal.

Recoveries were calculated by adding together all tissues plus excreta and are expressed as a percentage of the administered dose. Recovery values are less than 100% due to losses, instrument variability, etc. Furthermore, the values for chromium in the various tissues are near the method detection limits. Any outliers were re-analyzed for confirmation, and no values were excluded from analysis.

A gravimetric analysis for total soil delivery using the gavage needle dosing technique was performed. The soil weights were calculated by subtracting the weight of a dried filter from the dried filter and soil weights. The dried soil weight delivered by needle gavage was 0.4957 ± 0.023 . The actual dose of soil by gavage feeding was 99.1% of the expected dose, with a relative standard deviation of approximately 2.3%.

A comparison of the bioavailability and bioaccessibility is presented in Table 3. Intestinal fluid was chosen as the appropriate fluid for comparison, as the small intestine is the main location for the uptake of the metals of interest. Bioaccessibility values for arsenic and lead were greater than bioavailability values at 65.9% vs. 36.5%, and 10.7% vs 0.63% respectively. The bioaccessibility and bioavailability values were approximately the same for chromium at 3.0% and 4.8% respectively.

Table 3. *In Vitro* - *In Vivo* Comparison

	In Vitro		In Vivo	
	Bioaccessibility	(Recovery)	Bioavailability	(Recovery)
As	65.9% \pm 5.2%	94.7% \pm 6.9%	36.52 (34.0, 39.8)	74.63 (54.2, 91.5)
Cr	3.0% \pm 1.9%	46.5% \pm 13.2%	4.8 (2.8, 7.8)	11.66 (7.3, 20.5)
Pb	10.7% \pm 2.3%	75.5% \pm 9.6%	0.63 (0.3, 0.9)	50.6 (24.4, 69.6)

Bioaccessibility values are reported as a mean of four values and a standard deviation.

Bioavailability values are reported as a mean and a range of three values.

Neither the bioaccessibility nor the bioavailability results reflected the progression in the total metal concentrations in the NIST SRM 2710, (i.e. Pb>As>Cr). Using either the measurement of bioaccessibility or

bioavailability, arsenic was determined to be most available for predicting an internal dose. The reported recoveries corresponded for the two methods, meaning that the recovery discrepancy from 100% is due either to the affects of instrumentation sensitivity or the geochemical influence of the soil matrix.

3. Discussion

Site risk characterizations can be based on one of three methods for quantitating the level of contaminant that are important for human exposure analyses. Typically, site based risk characterizations are driven by results from aggressive acid leaching methodologies on soils which were then used to estimate exposure. Although risk assessments based solely on the total metal content of a soil are considered to be generally acceptable, they are unable to account for any biological processes which may alter the actual exposure and ultimate dose and the true concern about the levels of contaminants present at a site. In an effort to improve the biological relevance of risk assessments, animal studies were incorporated to investigate the role of physiological processes, such as adsorption, distribution, metabolism and excretion, on risk outcomes. A key variable of *in vivo* studies is bioavailability. Animal studies, however, are costly and labor-intensive. In addition, large quantities of test material are needed to perform these studies. The purpose of the work was to compare an *in vitro* method that employs the ease of an acid digestion with an *in vivo* animal experiment that provides a physiological basis for the system.

The NIST reported concentrations for each of the three study metals in the soil are included in Table 3, and are separated by approximately one order of magnitude. Lead is the most abundant of the metals at 5532ppm, arsenic the second at 627ppm and chromium the lowest at 39ppm(uncertified value). Percent bioaccessible, bioavailable and recovery calculations were based on these concentrations.

From the animal experiments, bioavailability of arsenic was 36.5%, which was the highest of the three metals examined, while the lead and chromium were 0.63% and 4.8%, respectively. The results were most consistent for arsenic, and the bioavailability ranged from 34.0% to 39.8%, with the highest accumulation of arsenic in all the tissues examined occurred on day three. Most of the arsenic was deposited in the blood with 0.7% depositing in the liver. Other tissues accounted for 0.1% or less of the total administered dose. A major route of elimination appeared to be the feces, while the urine showed a steady increase to 2% at day four. Recovery of arsenic ranged from 54.2% on day four to 91% on day 1. Variations in recovery values were due to inter-animal variability, with an average of 74.6%.

Chromium levels found in all animals were very low across all time points. Average bioavailability was 4.8%, and it peaked at day 4 with levels of 7.8%. Liver accumulation occurred on day 1 and 4, but was below detection limits on days 2 and 3. Conversely, small amounts of chromium were found in the muscle on days 2 and 3, and none was found at the other time points. Chromium appears to accumulate in the bone on days 3 and 4. Recoveries were quite low, ranging from 7.3% to 20.5%. All average values carried a 50% relative error. It is clear that the low soil concentrations of chromium precluded us from obtaining accurate levels, and suggest a lower limit of detection in an animal system.

Average lead bioavailability was 0.63%, with most of the accumulation occurring in the bone at all time points. Blood levels peaked at day 3, and then were undetectable at day 4. Recoveries range from 24.4% on day 4 to 69.6% on day 1. Most of this discrepancy can be attributable to decreasing amounts in the feces over time. Day 2 feces were low due to low readings in one single animal at 18.3%. These data illustrate the importance of proper time and tissue selection. We chose to use the total of all tissues averaged over all time points as a composite indicator for biological activity of these metals in the soil.

Bioaccessibility values are reported for both gastric conditions and small intestinal conditions. The gastric solubilities of each of arsenic, chromium and lead were 69.4%, 3.7% and 76.1%. The solubility of lead markedly decreased to 10.7% in the intestinal fluid. Arsenic and chromium did not show as great a decrease, where the intestinal solubility of arsenic was 65.9% and chromium 3.0%, although the solubility of chromium was very low throughout the experiment. Recoveries were approximately 75% for lead, 94.7% for arsenic and 46.5% for chromium. Intestinal fluid was chosen as the appropriate comparison fluid, as this is the main location of absorption for the metals. Lead intestinal absorption in the rat has been found to occur mainly in the duodenum (reviewed in Mushak, 1991). The introduction of Cr salts directly into the jejunum decreased amount of chromium measured in the feces, and therefore this is the main site of absorption for this compound (Donaldson, 1966). Arsenic has also been found to be methylated in the liver, and therefore intestinal absorption may be the major site of absorption for this metal (Stevens, et al. 1977). In general, each of the metals of interest are absorbed, mainly, in the small intestine. For this reason the intestinal solubility, measured by the *in vitro* method, was used for the bioavailability-bioaccessibility comparison and should be considered the most favorable method for soil comparison of bioaccessibility. Lead was reported as having the highest absolute concentration on the soil, however its bioavailability and bioaccessibility values were demonstrably low. Conversely, arsenic was reported in rather low

concentrations but was found to be highly available. Chromium, we believe, may have been near our limits of detection and therefore the numbers are difficult to compare. It is noteworthy, however, that the bioavailability and bioaccessibility patterns seem to be in close agreement, reinforcing the notion that biological processes and the geochemical characteristics of a soil play a more important role in determining risk (Diamond, et al 1997; Labieniec, et al. 1996).

Using a traditional total metal-based approach, lead might be considered for priority remediation. Arsenic, however, exists in such low abundance that it may not be considered a candidate for remediation. Clearly, a prioritization scheme changes when based on bioaccessible or bioavailable metal rather than the total concentration in the soil. We believe biologically-based assessments of hazard levels are superior to total metal-based assessments, and that an *in vitro* extraction system can provide adequate prediction of bioavailability. The conducted work has also provided a basis for the comparison of independent results from other studies, because of the use of a standard, easily accessible soil. Lastly, *in vitro* methodologies can contribute meaningful data with reduced costs of up to 10 times, less labor and the ability to perform on-site analyses.

B. Bioaccessibility of Radionuclides

1. Methods

Radionuclide laden soils were separated into sizes appropriate for testing, but preparation did not alter bulk composition or structure of the component material. The size fraction used in the radionuclide study is \leq 250 μ m in diameter, which appears to be the most probable range for adhering to a human hand. The method provided an efficient means to fractionate soil so as not to waste the limited amount of parent material. Fractionation and sample transfer was accomplished in such a way as to minimize the risk of exposing research personnel to airborne particles. In sieving, soil fractionation was accomplished without mechanical grinding. The sieving utilized a stack of calibrated sieves that decreased in mesh size. In addition, each sieve was sprayed with water to minimize the creation of airborne particles, thereby minimizing the chance of exposure to the investigators. The soil was sieved for approximately 15 minutes. The collection jar is removed and the soil counted for radiation, and then stored until analysis. Soils were stored in labeled polypropylene bottles.

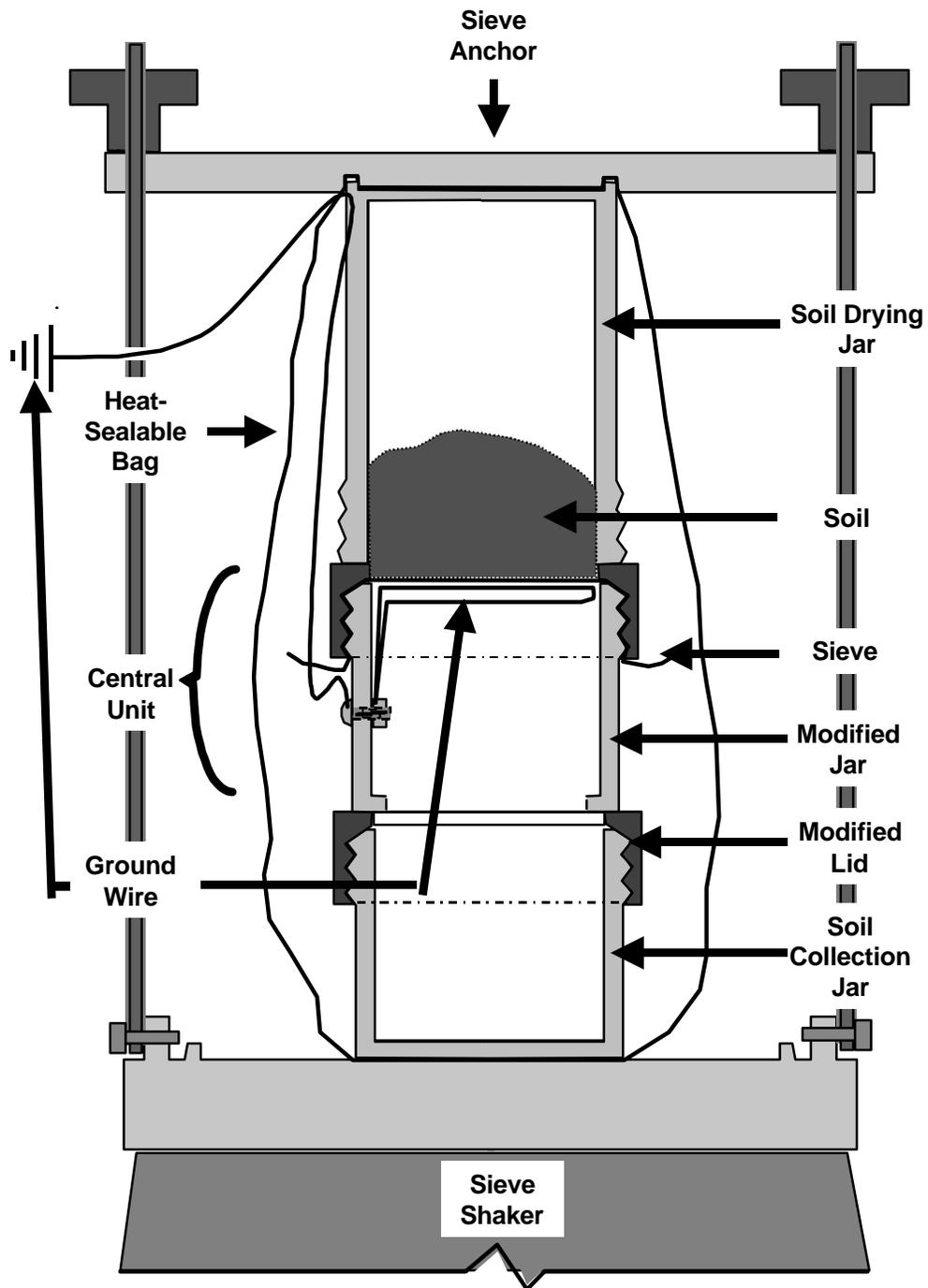
Disposal parts of the sieving apparatus are discarded as radioactive waste. Other parts were decontaminated and surveyed prior to storage.

Sample Collection, Handling, and Preservation

The radionuclide laden soils were obtained from the Savannah River Site core samples of seepage basins. These have been used to dispose of radioactive wastes over several decades. Soil samples were collected by the Department of Energy and are stored at Rutgers Environmental Health and Safety Department's Environmental Services Building. Samples are stored in 1-liter polyethylene bottles, wrapped in a polyethylene bag, and placed in styrofoam coolers/shipping boxes. A record of sample flow is recorded with a chain of custody form that was assigned to each soil sample.

Quality Control

Nylon mesh is used with the appropriate opening size. Nylon mesh is preferred over wire mesh to eliminate the possibility of metal leaching from a metal sieve. To eliminate cross contamination, the sieve mesh will not be reused rather disposed after a soil sample has been sieved. Samples are dried to the same extent, and sieved for an equal amount of time (per the timer located on the sieving device). All samples are sieved at the sieve shaker control rate of 1-3 (per the shaker control on the front of the sieve shaker), and once chosen this sieve rate remains constant for all soil samples. Samples are labeled with a batch number, the date of sieving, and the sample identification number in order to eliminate possible identification confusion. Finally, the balances are checked with a test weight prior to use.



Soil Drying Method

To transfer the soil in a closed environment, the lid of a 250ml wide mouth Nalgene™ Sample bottle was sealed to the lid of a 1000ml wide mouth Nalgene™ sample jar with an epoxy adhesive, Figure 1. The top of the two lids was bonded facing away from one another and the smaller (250ml) lid was placed in the center of the larger (1000ml) lid. Once the epoxy adhesive has bonded, a 1/2" circle was drilled into the center of the two lids. The larger lid is secured onto a 1000ml Nalgene™ sample jar. The 1000ml jar is inverted, and the lid is screwed onto the 250ml wide mouth bottle. Approximately 100 grams or less of soil is transferred into the 1000ml Wide Mouth Nalgene™ sample jar within a glove bag. This glove box must have a HEPA filter attached to capture dust. After the transfer, the HEPA vacuum was operated for 1 minute. The sample jar was then wiped with a moistened paper towel before removing it from glove box. Included was the inner accessible part of the lid. All wipes were tested for radioactivity with the appropriate survey equipment.

The 1000ml Wide Mouth Nalgene™ sample jar and soil was weighed, and the approximate soil weight was determined by subtracting the soil and jar from the initial jar weight. Once the soil had been weighed, the tops from the jars and place them in the oven at 100°C -110°C to dry. The soil was shaken occasionally back and forth in order to allow the soil on the bottom of the sample jar to dry as well. When the soil appeared dry (light in color, and a loosening of the texture), the soil was placed on a balance and its weight was monitored for stability weight. If the weight decreased over time, the soil needed further drying. If the soil was still quite humid, the soil was placed jar in the drying oven again at 100°C - 110°C. If the weight of the soil remains unstable, a vacuum oven may be used to take off the last portion of water from the soil. This final drying step was conducted using a vacuum oven with the following settings: temperature = 105°C and pressure = 15"Hg. Once the soil had a constant weight, the soil sample (with lid secured) was placed the vacuum desiccator for one day. After one day the soil was weighed and then placed in the desiccator for an equal amount of time. If the soil weight remained constant after a second incubation in the vacuum desiccator, the sample was ready for use.

2. Analysis for Radionuclides

Three radionuclides were examined for bioaccessibility: total alpha, total beta and cesium-137. Approximately 5ml of the gastric fluid extract is dispensed into a 20ml liquid scintillation vial by pipette. Next, 15ml of Packard Ultima Gold A/B liquid scintillation cocktail is dispensed into each of the vials containing extract, including the

method blanks. Three channels were set up including an alpha channel from 0-2000, and two beta channels using 15-550 and 550-2000. The samples are counted for 16 hours, along with two standards, on a Packard Liquid Scintillation Counter. One standard contains known quantities of Sr-90 and Cs-137, the other is a mixed alpha standard from previous uranium work. The detection limits for the alpha and beta analyses are 0.0033Bq and 0.038-0.04Bq respectively. The soil, collected soil mass and the gastrointestinal extracts are counted directly for Cesium 137 with an EG-G Ortec Low Background Gamma Spectrometer, using appropriate geometry calibration standards. This instrument has a minimum detectable level of 0.15Bq for a 1 hour count.

Bioaccessibility assays were performed on three soils from the Savannah River site in Aiken, South Carolina. The solubility in both synthetic gastric and intestinal fluid was measured, because gastric fluid generally provides a more conservative estimate of bioaccessibility due to the low pH. Intestinal fluid solubility, however, provides the concentration of contaminant that is available for absorption in the area where absorption is the greatest: the small intestine. The three soils are from various sampling locations on SRS. Two are from a berm between two seepage basins, and the other is from the banks of a small creek named the Lower Three Runs. Each of the soils was sieved and dried before extraction in artificial human gastrointestinal fluids, and sieved to a particle size of less than 250 μ m.

The Lower Three Runs soil, DSR-1, contained 4.83Bq/g of cesium-137, which was the only contaminant of interest in this particular sample. There was an enrichment in the lower particle size, less than 250 μ m diameter, where this portion had 8.27Bq/g \pm 0.68Bq/g of Cs¹³⁷. There were two additional soils collected in this area at other sites along the Lower Three Runs. These soils had levels of Cs¹³⁷ at 2.92Bq/g and 2.37Bq/g, and each had 4.94Bq/g and 2.56Bq/g in the smaller particle size fraction. Bioaccessibility has not yet been measured in these last two soils. Table 4 includes the results from the bioaccessibility studies performed on the Lower Three Runs DS-1 soil. In all cases a mean was supplied to a value when three replicate analyses were available, otherwise data points were listed separately. Missing data points were below detection limits of the instrumentation. Method blanks were used for blank subtraction. Blanks were not subtracted if there was larger than a 90% probability of the value (method blank value) being equal to or below 0. This was calculated using the normal distribution of the blank.

Table 4 Method Development Data for Cs¹³⁷ Bioaccessibility Measurement: Lower Three Runs Soil

Liquid to Solid Ratio Tests (N=3)		
Liquid to Solid Ratio(ml/g)	Gastric Solubility	Recoveries
2160	5.2%±11%	104%±25%
1080	6.4%±0.75%	72%±15%
540	5.5%±1.4%	99%±16%
108	3.3%±0.52%	89%±10%
54	2.8%±0.53%	72%±7.3%

Centrifuged vs- Filtered Samples (N=3)		
	Centrifuged Samples	Filtered Samples
Gastric Solubility	9.1%±1.9% •	3.3%±0.91%
Recoveries	74%±14% •	83%±3.9%
Intestinal Solubility	1.5%±0.47%	8.6%±1.3%
Recoveries	78%±7.6%	81%±5.0%

- Less than three replicates were available

Organic Acid Addition to Synthetic Gastric Fluids (N=3)

Gastric Solubility	Recoveries	Intestinal Solubility	Recoveries
6.9%±3.6%	98%±6.6%	2.9%±1.4% •	103%±12% •

- Less than three replicates were available.

For the Lower Three Runs sample,

liquid to solid ratios for gastric fluid and the amount of soil used were compared in a bioaccessibility extraction experiment. It was conducted to find a ratio that had a large enough amount of soil present for detection limit purposes, while remaining within the extraction efficiency similar to that of what is physiologically relevant for dilution in the digestion system. Default values for both of the ingestion rates of soil by children, and the amount of gastric fluid produced per day, yields a liquid to solid ratio of 1800. We choose a range of 108ml/g to 2160ml/g, for the liquid to soil ratio to encompass this value. A value of 216ml/g (0.5g soil in 100ml gastric fluid and 8ml saliva) was chosen as the upper limit of soil to be extracted by this method. This is due both to slight differences in the observed extraction efficiency and practical limitations of the addition of larger soil quantities.

The difference in measured concentrations obtained by 1) centrifuging samples to separate solid from the liquid phase, and 2) filtering samples through a 0.45µm cellulose nitrate filter were obtained in a second experiment. Method development question here was especially important for examining trace contaminants on soil matrices, since much of the trace contaminants may be contained within the colloidal portion of soil. If this is the case, simple slow centrifugation of a sample will over estimate the portion of contaminant in the aqueous phase. If filtration, however, allows for total separation of fine particulates, and provides larger liquid sample for counting purposes (as

the entire liquid sample is recoverable). We found that centrifuged samples had greater levels of Cs¹³⁷ present than filtered counterparts.

To improve the ability of the synthetic gastric fluid to more closely mimic human gastric fluid, a study was performed in which several organic acids were added to the fluids at physiological levels. The presence of organic acids is an interesting variable in stomach fluids that has not yet been adequately investigated, in prior bioaccessibility studies. Both Ca-EDTA, D-penicillamine, dimercaptopropanol and citrate have been found to increase total lead absorption in rats (Jugo, et al., 1975). Other compounds found to increase absorption of lead are several amino acids, succinic acid, fructose and ascorbic acid, suggesting that lead is maintained in solution by binding or chelating agents of the stomach after the pH increase in the small intestine (Conrad and Barton, 1978). Organic acids were found to be naturally present in human gastric fluid including: ascorbic acid, lactic acid, sialic acid, pyruvic acid, citric acid, uric acid and glucuronic acid at levels of 5.39E-5M, 3.1E-4M, 2.36E-4M, 7.49E-5M, 2.54E-4M, 6.13E-5M, 1.03E-4M respectively (Richmond, et al., 1955, Freeman et al, 1955, Rodriguez et al., 1999, Brodsky, 1986). Results using the organic acids yielded no large differences in Cs¹³⁷ bioaccessibility, but this may be due to the chemistry of Cs¹³⁷. As a +1 ion, Cs will not chelate as other heavy metals such as lead.

Bioaccessibility analyses were completed was performed on two soils (called BA-002 and BA-004) from a seepage basin in Savannah River. The soil samples were taken from a berm between the second and third seepage basin in a system of four total seepage basins. Soils were tested for their bioaccessibility in both synthetic gastric and intestinal fluids. Both of these soils were analyzed first for Cs¹³⁷, as this is a non-destructive method of analysis. The levels of Cs¹³⁷ in the whole soil were 0.326Bq/g for soil BA-002 and 0.11Bq/g for soil BA-004. The main difference between these soils is their sampling depth. Soil BA-002 was a surface soil and BA-004 was 0-12" below the surface. The Cs¹³⁷ level in the <250µm particle size for both soils was 0.4236 ± 0.0535Bq/g for BA-002 and 0.0965 ± 0.00624Bq/g in BA-004. These samples did not show as large of a <250µm particle size enrichment for Cs¹³⁷ as the Lower Three runs soil. Both of the soils had very low levels of Cs¹³⁷, however this was not the primary contaminant of interest in these soils. Both of the seepage basin soils contained measurable levels of Cm²⁴³, Ra²²⁸ and Sr⁹⁰. As gamma spectroscopy is a non-destructive, this analysis was performed first.

The Cs¹³⁷ levels present after extraction by gastric and intestinal fluids for both of the berm soils were below the limits of detection, (counting times up to 24 hours). As the residual soil mass was collected after extraction, these counts can be used to approximate the amount of Cs¹³⁷ to desorb from the soil matrix. By subtraction of the percent

of Cs¹³⁷ that was insoluble from 1 a maximum amount of solubility can be determined. As 69% of the original Cs¹³⁷ mass was recovered in the residual soil from the intestinal extraction and 77% from the gastric extraction, we can assume that the no more than 23% of Cs¹³⁷ was soluble in gastric fluid and 31% was soluble in intestinal fluid.

In order to measure the actual quantity of extracted Cs¹³⁷ in the solutions; we improved the geometry matrix for gamma counting. This was accomplished by filtering the aqueous gastric samples onto 3M Empore Cs Rad disks. This increases counting efficiency by 2.8 times, and therefore decreases our method detection limit from 0.0207Bq to 0.0074Bq (Brodsky, 1986ⁱ). With this added step, our samples remained under the detection limits. Using the detection limit calculation, the gastric fluid bioaccessibility of the first berm soil (soil BA-002) was less than 8.7%. The collected soil samples for BA-004 recovered approximately 110% of the original Cs¹³⁷ mass, therefore very little if any Cs¹³⁷ was transferred to the aqueous phase. Thus, Cs¹³⁷ in these two soils has remarkably low bioaccessibility in both the gastric and intestinal phases.

The next step in the measurement of bioaccessibility of radionuclides, and method development for this purpose, was to expand our work to alpha and beta emitters. Two soils were sampled from a berm located at a seepage basin area on the SRS. The soil samples were found to contain a mixture of gamma, alpha and beta emitting contaminants such as Cs¹³⁷, Cm²⁴³, Ra²²⁶ and Sr⁹⁰. The levels of the radionuclides are reported in Bq/g in Table 5. These levels were measured in the whole soil, before sieving to <250µm.

Table 5 Radionuclide Levels in the Seepage Basin Soil (Bq/g)

Radionuclide	Ba-002	Ba-004
Gross alpha	0.49	0.21
Non-volatile Beta	4.1	1.52
Cs ¹³⁷	0.33	0.11
Ra total, Ra ²²⁶ , Ra ²²⁸ Sr ⁹⁰		
Cm ^{243/244}	0.55	0.14
U ²³⁵ , U ²³⁸ , U ^{233/234}	ND, 0.05, 0.04	
Pu ²³⁸ , Pu ^{239/240}	ND, 0.06	
Th ²²⁸ , Th ²³⁰ , Th ²³² , Th ²³⁴	ND, ND, ND, 0.05	0.05, ND, 0.04, 0.07

A gross alpha analysis, using liquid scintillation, was completed for the gastric fluid extracts. The main objective was to ascertain that the alpha and beta emitters were soluble in gastrointestinal fluids and were at levels above method and instrumentation detection limits. A beta count was completed, but limited to energy ranges that would partially separate Cs¹³⁷ and Sr⁹⁰ peaks. Alpha and beta activity was detected in the gastric fluid extracts of BA-002 and BA-004 at levels approximately two times higher than the method blanks. The elevated beta activity is probably due to the presence of Cs¹³⁷, Sr⁹⁰ or other beta emitters, and possible alpha emitters were Cm^{243/244}, U²³⁸,

Pu^{239/240} or a thorium isotope. These results indicate that there is a possibility of radionuclide isolation and measurement, for the analysis of bioaccessibility of specific radioisotopes.

Further work with these soils, using another DOE cooperative agreement, will implement 3M Rad disks to isolate Sr⁹⁰ and Ra²²⁶ from the gastrointestinal extracts for more specific activity values. The results of these experiments demonstrated that there are differences in the levels of radionuclides extracted by our bioaccessibility assay from a typical extraction procedure that determines total radionuclide concentration. The results for the alpha and beta extraction suggest the same results, however more detailed analyses of specific alpha and beta emitting radionuclides need to be conducted on the collected samples.

6. Relevance, Impact, and Technology Transfer

a. How does this new scientific knowledge focus on critical DOE environmental problems?

The successful development and application of an “*in vitro*” technique that mimics the action of the digestive system can be used as an effective tool to prioritize radionuclide and metal laden soils for potential exposure and risk. The technique is cost effective and mimics the actual amount of a waste presented to the lining of the stomach or intestine rather than the total mass of a contaminant in a soil. The latter may contain large amounts that will not be extracted during digestion, and thus, not retained in the body.

b. How will the new scientific knowledge that is generated by this project improve technologies and cleanup approaches to significantly reduce future costs, schedules, and risks and meet DOE compliance requirements?

The methodology will provide a simple and more accurate means to examine current and residual risks to radionuclide and metals ingestion and exposure in soils. This would be done by providing an estimate of bioaccessibility of the contaminants in contrast to total mass present. The latter may be not be removed from the soil by extraction through the digestive system. The technique can be easily applied to soils at many DOE waste sites using DOE laboratories or a contractor.

c. To what extent does the new scientific knowledge bridge the gap between broad fundamental research that has wide-ranging applications and the timeliness to meet needs-driven applied technology development?

The techniques provides an inexpensive screening tool to focus the examination and prioritization of waste cleanup at DOE sites on those which can provide the greatest risk from soil exposure, based upon a biologically relevant

“indicator of human exposure”. This will improve the DOE’s ability to effectively allocate remediation resources for soil clean-up.

- d. What is the project’s impact on individuals, laboratories, departments, and institutions? Will results be used? If so, how will they be used, by whom, and when?

If the technique is seriously considered for application to DOE, it will provide a new tool for risk characterization before and after remediation. Impact is dependent upon range of application and the utilization of results in regulatory and remediation planning.

- e. Are larger scale trials warranted? What difference has the project made? Now that the project is complete, what new capacity, equipment or expertise has been developed?

Technology transfer would be the next level of application and applied research. This could involve a contract between EOHSI and DOE to examine ways of automating the analyses, and developing cost effectiveness of the technique for personal utilization, and equipment selection. Until the above is completed, which was not part of the cooperative grant activities, it is difficult to provide a thorough answer to the question.

- f. How have the scientific capabilities of collaborating scientists been improved?

The EOHSI Laboratory now has the capability to complete bioaccessibility studies on both metals and radionuclides and these studies can now reference a thorough comparison with an “*in vivo*” system.

- g. How has this research advanced our understanding in the area?

The EOHSI project has completed the first “*in vivo*” and “*in vitro*” comparison of metals bioavailability and bioaccessibility to a standard “*in vivo*” rate model, with a standard test soil. Thus, our work on bioaccessibility has provided a reference point for comparison by others, and for use in calibrating other and new “*in vitro*” systems to simulate bioaccessibility in the human digestive system.

- h. What additional scientific or other hurdles must be overcome before the results of this project can be successfully applied to DOE Environmental Management problems?

A pilot study and collaboration between DOE and EOHSI to examine a current DOE waste problem that requires a prioritization for soil clean up. This would involve a comparison of priority assigned by using the current EPA

protocol, method 3051 versus the priority that would be established based upon the EOHSI “*in vitro*” protocol. Finally as stated from question “e” there needs to be consideration of approaches for technology transfer.

i. Have any other government agencies or private enterprises expressed interest in the project? Please provide contact information.

YES. 1. Dr. Robert Hazen , New Jersey Department of Environmental Protection, 401 East State Street, (CN 409 7th FL, West Wing) Trenton, NJ 08625 Phone: 609-292-8294.

2. Dr. Edward Sargent, MERCK and Company, Inc. Director of Toxicology & Environmental Health, PO Box 100, WS 2F 45, 1 Merck Drive, Whitehouse Station, NJ 08889-0100 Phone: 732-423-7906

Both have provided funding or mechanisms for grant funding to conduct the initial development aspects of the research.

7. Project Productivity:

The goals of the project were obtained through the development of methodology and application to test soils. The project took approximately eight months longer to complete than anticipated when the proposal was submitted to DOE. We had requested and received a “no cost” extension to complete the research. The unanticipated delay was due to the length of time necessary to obtain approvals for our radiation handling procedures and for the use of soils laden with radionuclides in the “*in vitro*” simulation of the human digestive system. These included the procedures for soil drying, preparations of soil sub-fractions for analysis, the bioaccessibility analysis protocol, and the Laboratory Radiological Survey Procedures. The work plan was accordingly revised, and the final protocols were provided in a supplement to the 1997-1998 Annual Report.

8. Professional Staff Supported on Project

1. Paul J. Lioy, Ph.D., Professor Env. Med., UMDNJ
2. Michael Gallo, Ph.D., Professor, Med., UMDNJ
3. Brian Buckley, Ph.D., Laboratory Administrator, EOHSI, RU
4. Robert Tate, Ph.D., Professor, Env. Sci., RU

5. David Kosson, Ph.D. Associate Professor, Chem. Eng., RU
6. Kristie Ellickson, M.S., Ph.D. Student, UMDNJ/RU
7. Stephanie Hamel, Ph.D., Grad Student, UMDNJ/RU
8. Robert Meeker, Senior Lab Technician, UMDNJ
9. Michael Pollock, M.S., Student, RU
10. Carl Shopfer, Ph.D., Student, RU

9. Publications:

1. Hamel, S.C., Ellickson, K.M., Liroy, P.J., "The Estimation of The Bioaccessibility of Heavy Metals in Soils Using Artificial Biofluids: A Mass-Balance Technique", Science of the Total Environment, (Submitted, 12/98), Revised for reviewer comments and resent to Editor, 7/99).
2. Ellickson, K.M., Meeker, R.S., Gallo, M.A., Buckley, B.J., and Liroy, P.J., "Bioavailability of Chromium, Lead, and Arsenic from a NIST Certified Standard Reference Material Using "In Vitro" and "In Vivo" Techniques, Submitted to Fundamentals of Applied Toxicology, 7/99.
3. Ellickson, K.M., Kosson, D., Schopfer, C., and Liroy, P.J., The Bioaccessibility of Radionuclides in Contaminated Soils Using "In Vitro" Methods For The Digestive System, in preparation for Health Physics. Presented at 9th Annual Meeting of the International Society of Exposure Analysis, September 4-9, 1999 Athens, Greece.

10. Interactions

Research has been presented at the DOE-EM Workshop in Chicago, IL, July, 1998.

Subsequently, it has been presented in September 1999 at an International Conference in Greece, on Exposure Analysis. See above Reference #3. The research is presently being employed in our DOE related research funded in a Cooperative Agreement called Consortium for Risk Evaluation and Stakeholder Participation (CRESP). We are analyzing samples for the bioaccessibility of selected metals and radionuclides. This is being done in collaboration with Personnel at the SRS in South Carolina.

11. Transitions

None yet

12. Patents

None

13. Future Work

We intend for the near term to continue the project by determining the bioaccessibility of heavy metals and radionuclides from specific soils in the Savannah River site. This will be conducted under the DOE cooperative agreement that was described above as CRESP. The longer term research should include application of the bioaccessibility technique to other DOE sites for pre- and post- remediation risk characterization.

14. Literature Cited

Brodsky, A. Accuracy and detection limits for bioassay measurements in radiation protection: Statistical considerations. Washington DC: US Nuclear Regulatory Commission; NUREG-1156; 1986.

Brown, S.L., Bell, J.N.B. "Earthworms and Radionuclides with Experimental Investigation on the Uptake and Exchangeability of Radiocesium. *Environ. Pollut.* Vol. 88. No. 1. pps. 27-39. 1995.

Carbonell, A.A., Aarabi, M.A., Delaune, R.D., Gambrell, R.P., Patrick, W.H.Jr. "Bioavailability and Uptake by Wetland Vegetation: Effects on Plant Growth and Nutrition". *J. Environ. Sci. Health. Part A, Toxic/Hazardous Substances and Environmental Engineering.* Vol. A33. No. 1. pps. 45-66. January 1998.

Clapp, T.C., Umbreit, T.H., Meeker, R.J., Kosson, D.S., Gray, D., Gallo, M.A. "Bioavailability of Lead and Chromium from Encapsulated Pigment Materials". *Bull. Environ. Contam. Toxicol.* Vol. 46. No. 2. pps. 271-275. 1991.

Conrad, M.E., Barton, J.C. "Factors Affecting the Absorption and Excretion of Lead in the Rat." *Gastroenterology.* Vol. 74. No. 4. Pps. 731-740. 1978.

Davis, A., Ruby, M., Bergstrom, P.D. "Bioavailability of Arsenic and Lead In Soils from the Butte, Montana, Mining District." *Environ. Sci. Technol.* Vol. No. pps. 1993.

- Davis, A., Ruby, M.V., Goad, P., Eberle, S., Chryssoulis, S. "Mass Balance on Surface Bound, Mineralogic, and Total Lead Concentrations as Related to Industrial Aggregate Bioaccessibility". *Environ. Sci. Technol.* Vol. 31. No. 1 pps. 37-44. 1997.
- Diamond, G.L., Goodrum, P.E., Felter, S.P., Ruoff, W.L. "Gastrointestinal Absorption of Metals." *Drug Chem. Toxicol.* Vol. 20. No. 4. Pps. 345-368. 1997
- Donaldson, R.M., Barrerras, R.F. "Intestinal Absorption of Trace Quantities of Chromium." *J. Lab. Clin. Med.* Vol. 68. pps. 484-493. 1966.
- Freeman, J.T., Hafkesbring, R., Caldwell, EK. "Comparitive Study of Ascorbic Acid Levels in Gastric Secretion, Blood, Wine and Saliva." *Gastroenterology.* Volume. 18. pps. 224-229. 1951.
- Freeman, G.B., Johnson, J.M., Killinger, J.M., Liao, S.C., Feder, P.I., Davis, A.O., Ruby, M.V., Chaney, R.L., Lovre, S.C., Bergstrom, P.D. "Relative Bioavailability of Lead from mining Waste Soil in Rats." *Fundam. App. Toxicol.* Vol. 19. pps. 388-398. 1992.
- Freeman, G.B., Dill, J.A., Johnson, P.J., Kurtz, P.J., Parham, F., Matthews, H.B. "Comparative Absorption of Lead from Contaminated Soil and Lead Salts by Weanling Fisher 344 Rats." *Fundam. App. Toxicol.* Vol. 33. pps. 109-119. 1996.
- Gargas, M.L., Norton, R.L., Harris, M.A., Paustenbach, D.J., Finley, B.L. "Urinary Excretion of Chromium Following Ingestion of Chromite-Ore Processing Residues in Humans: Implications for Biomonitoring." *Risk Analysis.* Vol. 14. No. 6. pps. 1019-1024. 1994.
- Gibaldi, M. *Biopharmaceutics and Clinical Pharmacokinetics Third Edition.* pps. 131-135. Lea and Febiger, Philadelphia. 1984.
- Groen, K., Vaessen, H.A.M.G., Liest, J.J.G., de Boer, J.L.M., van Ooik, T., Timmerman, A., Vlug, R.F. "Bioavailability of Inorganic Arsenic from Bog Ore-containing Soil in the Dog." *Environ. Health Perspect.* V. 102. No. 2. 182-184. 1994.
- Hamel, S.C., Buckley, B, Liroy, P.J. "Bioaccessibility of Metals in Soils for Different Liquid to Solid Ratios in Synthetic Gastric Fluid". *Environ. Sci. Technol.* Vol. 32. pps. 358-362. 1998.
- Hamel, S.C., Ellickson, K.M., Liroy, P.J. "Bioaccessibility using a Mass Balance Model" in press.

- Jugo, S., Malikovi, T., Kostial, K. "Influence of Chelating Agents on the Gastrointestinal Absorption of Lead." *Toxicology and Applied Pharmacology*. Vol. 34. Pps. 259-263. 1975.
- Kingston, H. M., Test Methods for Evaluating Solid Waste, Physical Methods, 3rd ed; U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response. U.S. Government Printing Office: Washington, DC, SW 846-3051, (1986).
- Labieniec, P.A., Dzombak, D.A., Siegrist, R.L. "Risk Variability Due to Uniform Soil Remediation Goals. *J. Environ. Eng.* Vol. 122. No. 7. Pps. 612-621. 1996.
- Lebourg, A., Sterckman, H. Ciesielski, H. Proix, N. "Trace Metal Speciation in Three Unbuffered Salt Solutions Used to Assess their Bioavailability in Soil" *J. Environ. Qual.* Vol. 27. pps. 584-590. 1998.
- Maddaloni, M., LoIacono, N., Manton, W., Blum, C., Drexler, J., Graziano, J. "Bioavailability of Soilborne Lead in Adults, by Stable Isotope Dilution." *Environ. Health Perspect.* Vol. 106. Supplement. 6. pps. 1589-1594. 1998.
- Mushak, Paul "Gastro-Intestinal Absorption of Lead in Children and Adults: Overview of Biological and Biophysico-Chemical Aspects". *Chem. Spec. Bioavail.* Vol. 3. pps.87-104. 1991.
- Nessel, C.S., Amoruso, M.A., Umbreit, T.H., Meeker, R.J., Gallo, M.A. "Pulmonary Bioavailability and Fine Particle Enrichment of 2,3,7,8-tetrachlorodibenzo-p-dioxin in Respirable Soil Particles." *Fundam. App. Toxicol.* Vol. 19. No. 2. pps. 279-285. 1992.
- Novozamsky, I, Lexmond, Th.M., Houba, V.J.G., Rauret, G. "A Single Extraction Procedure for Evaluation of Uptake of some Heavy Metals by Plants" *Int. J. Environ. Anal. Chem.* Vol. 51. No. 1-4. pps.47-58. 1993.
- Pichtel, J., Anderson, M. "Trace Metal Bioavailability in Municipal Solid Waste and Sewage Sludge Composts". *Bioresource Technol.* Vol. 60. No. 3. pps. 223-229. June 1997.
- Piper, D.W., Fenton, B.H., Goodman, L.R. "Lactic, Pyruvic, Citric, and uric Acid levels and urea Content of Human Gastric Juice." *Gastroenterology*. Vol. 53. No. 1. Pps. 42-47. 1967.
- Richmond, V., Caputo, R., Wolf, S. "Biochemical Study of the Large Molecular Constituents of Gastric Juice." *Gastroenterology*. Vol. 29. 1017-1021. 1955.
- Rieuwerts, J.S. and Farago, M.E. "Lead Contamination in Smelting and Mining Environments and Variations in Chemical Forms and Bioavailability". *Chemical Speciation and Bioavailability*". Vol 3, pps 87-104, 1995.

Rodriguez, R.R., Basta, N.T., Casteel, S.W., Pace, L.W. "An In Vitro Gastrointestinal Method to Estimate Bioavailable Arsenic in Contaminated Soils and Solid Media." *Environ. Sci. Technol.* Vol. 33. pps. 642-649. 1999.

Ruby, M.V., Davis, A., Schoof, R., Eberle, S., Sellstone, C.M. "Estimation of Lead and Arsenic Bioavailability Using a Physiologically Based Extraction Test." *Environ. Sci. Technol.* Vol. 30. pps. 422-430. 1996.

Sheppard, S.C., Eveden, W.G., Schwartz, W.J. "Ingested Soil: Bioavailability of sorbed lead, cadmium, cesium, iodine and mercury." *J. Environ. Qual.* Vol. 24. pps. 498-505. 1995.

Stevens, J.T. Hall, L.L., Farmer, J.D., et al. "Disposition of C14 and/or As74-cacodylic acid in rats after intravenous, intratracheal or peroral administration." *Environ. Health Perspect.* Vol. 19. pps. 151-157. 1977.

Bioavailability of Chromium, Lead and Arsenic from a NIST Certified Standard Reference Material Using *In Vitro* and *In Vivo* Techniques

Ellickson, K.M.¹, Meeker, R.J.², Gallo, M.A.², Buckley, B.T.³, Lioy, P.J.^{2,4}

¹Joint Ph.D. program in Exposure Assessment of Rutgers University and the University of Medicine and Dentistry of New Jersey

²Department of Environmental and Community Medicine, Environmental and Occupational Health Sciences Institute, University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School

³Environmental and Occupational Health Sciences Institute, Rutgers University and the University of Medicine and Dentistry of New Jersey, Center for Analytical Excellence.

⁴Corresponding Author: Exposure Measurement and Assessment Division, Environmental and Occupational Health Sciences Division, 170 Frelinghuysen Road, Piscataway, New Jersey, 08855.

Phone: 732-445-0150

Fax: 732-445-0116

Email: plioy@eohsi.rutgers.edu

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Abstract

The bioavailability of soil contaminants can be measured using *in vitro* or *in vivo* techniques. Since there is no standard method for intercomparison among laboratories, we present a comparison of two techniques for bioavailability estimation: *in vitro* dissolution and *in vivo* rat feeding model for a NIST traceable soil material. Lead, arsenic and chromium were chosen for their range of concentration in the soil and their toxicological relevance. Bioaccessibility was measured using a sequential soil extraction in synthetic analogues of human saliva, gastric and intestinal fluids. Bioavailability was measured in Sprague Dawley rats by determining metal levels in the major organs and urine, feces and blood at 1, 2, 3 and 4 day time points. Bioaccessibility extractions yielded a gastric solubility of 76.1%, 69.4% and 3.7% respectively, while intestinal solubilities were 10.7%, 65.9% and 3.0%. The bioavailability of the NIST SRM was 0.63%, 36.5% and 4.8% for Pb, As and Cr. As the three metals are absorbed mainly in the small intestine, intestinal solubility was used for the *in vitro* - *in vivo* comparison. Although lead had the highest soil concentration of the selected metals, it was the least bioavailable. Arsenic, however, was highly available in both the *in vitro* and *in vivo* method. Bioaccessibility was \geq bioavailability in all cases, which would be expected due to the limitation of intestinal absorption. Bioaccessibility was found to be a good indicator of relative metal bioavailability, furthermore a risk determination for this soil would be misleading without the incorporation of these results.

Introduction

The initial step in a site risk assessment involves the measurement of the form and concentration of contaminants in the environmental media. We cannot and should not, however, assume that the total mass of the contaminant will be available within different receptors. The attenuation of the potential exposure to a contaminant is most remarkable in soil, as soils have the capacity to sorb metals into the organic or mineral phase through biological, chemical or physical processes. Further, as a contaminant ages in a soil, if it is not leached from the system, it has a chance to become assimilated further into the soil matrices. This may lead to additional decreases in the contaminant's availability to humans over time.

Bioavailability is defined as the rate and extent of absorption of a contaminant at the target organ (Gibaldi, 1984). Bioavailability studies are used to gain an understanding of the availability of heavy metals and other contaminants (radionuclides, organics) within an animal or plant receptor. Such studies can be used to identify areas on a site where heavy metal mobility is a major concern, and thus assist in the prioritization of remedial efforts. If a component is proposed to be biologically active in an organism, the first step is to measure its bioavailability at the affected site.

The two principal limiting factors in the assimilation of a heavy metal by a mammalian system are dissolution in the gastrointestinal tract and absorption through the intestinal wall. The dissolution of a metal depends on the characteristics of the contaminant itself and the environmental matrix in which it has been incorporated. Additionally, solubility of a metal in a soil matrix will be a function of the gastrointestinal fluid composition.

Bioaccessibility is the term that defines the amount of metal that is dissolved in the gastrointestinal fluid. Once dissolved in the gastrointestinal fluid, intestinal absorption is affected by the retention time of the metal near the site of absorption. Both of these processes can be affected by the speciation of the metal in the fluid. It is inherent in the definition of bioavailability that some biological membrane (intestinal wall) has been crossed, therefore bioaccessibility values should be greater than bioavailability values as this limitation is not in place.

Studies have been conducted to measure the amount of a contaminant that reaches receptors, namely the bioavailable fraction. Plant bioavailability has been measured by analyzing plant tissues (Carbonell, et al. 1998), soil leach techniques (Pichtel, et al. 1997), (Novozamsky, et al. 1993), (Lebourg, et al. 1998) and earthworm uptake of metals (Brown, et al. 1995). Human bioavailability studies incorporate various leaching and dissolution techniques (Ruby, et al. 1996), (Davis, et al. 1993), studies of soil type and structure (Davis, et al. 1997), actual human feeding studies (Gargas, et al. 1994), (Maddaloni, et al. 1998) and extrapolation from animal experiments (Freeman, et al. 1996), (Freeman, et al. 1992), (Groen, et al. 1994), (Clapp, et al. 1991), (Nessel, et al. 1992). Studies have also been performed in order to compare gastrointestinal dissolution techniques with *in vivo* animal models, (Rodriguez, et al. 1999), (Sheppard, et al. 1995), (Ruby, et al. 1996) however, no clear association has been made between bioavailability and the dissolution of contaminants within the gastrointestinal system. All results vary according to the method used, soil and contaminant characteristics, and consequently the importance of a comparison with a certified soil becomes evident. Bioaccessibility and

bioavailability results obtained for the same standard reference material could eventually be used by all for comparison of methods and environmental matrices.

Models have been developed to estimate human bioavailability of metals resulting from the ingestion of contaminated soils and solid waste materials. To provide a standard for the inter-comparison of these methods, we present the comparison an *in vitro* dissolution technique in parallel with a rat feeding model using a NIST-traceable soil. Bioaccessibility was estimated by introducing the contaminated soil to synthetic analogues of human gastrointestinal fluids. The *in vitro* dissolution technique suspended a contaminated soil in a three step sequential extraction including synthetic analogues of human saliva, gastric and intestinal fluids. The residual soil mass was collected in order to report metal recoveries. A rat feeding model was used to measure bioavailability. For this *in vivo* technique, organs and tissues were analyzed to provide a more complete mass balance of the metal in the animal. Four exposure times were chosen to examine the distribution and excretion phenomena of the selected metals. The bioavailability was then estimated by using the percentage of metal concentration measured in the target organs of the study animals with respect to the total metal concentration in the administered dose.

The three metals were chosen both for their toxicological relevance and for their relative concentrations. Lead was chosen because of its high abundance in this test soil, as well as the extensive database available in the literature. Arsenic is of interest for comparison purposes with previous work performed in our laboratory. Chromium was used as a test of the method detection limits of both the *in vitro* and *in vivo* procedures. The use of a standard reference material is particularly useful in this field as a means of comparison between methods.

Materials and Methods

The standard reference material Montana Highly Elevated Levels 2710 (SRM 2710) was obtained from the National Institute of Standards and Technology (NIST). The soil material was homogenized and sieved to less than 74 μ m in diameter by NIST before transport to the EOHSI. For the three metals examined in the study, lead, arsenic and chromium, the NIST SRM 2710 has certified metal concentration levels of 5532 μ g/g and 627 μ g/g for lead and arsenic, respectively, and an uncertified value of 39 μ g/g for chromium.

In Vitro Test of Bioaccessibility

The in vitro bioaccessibility procedure was performed as a modification of a previously published method developed in our laboratories. (Hamel, et al. 1998), (Hamel et al. 1999). Approximately 0.5g samples of the NIST 2710 SRM were weighed and placed into wide mouth 250 ml polyethylene bottles. Next, 8 ml of artificial saliva were added to each bottle, which was followed by 100 ml of gastric fluid. The gastric fluid is 0.03M NaCl, containing 0.32%(w/v) pepsin at pH 1.47. The estimated activity of the purchased pepsin during the gastric portion of the extraction procedure, was approximately 1,920,000 units/L to 5,760,000 units/L.

The samples were then placed into a constant temperature bath set at 37°C and allowed to shake for two hours at 90 cycles per minute. After two hours the bottles were taken out of the bath, and a 10 ml aliquot was taken from each bottle. The bottles were then centrifuged at 1000 rpm(198g) for 20 minutes, and the supernate removed by pipette from the Nalgene bottle. Next, the pH was adjusted to 6.54 by adding 100 ml of synthetic intestinal fluid, a 0.2M NaHCO₃ solution. The samples were then allowed to shake at

37°C for another 2 hours. After this two hour period, the liquid portion of the extraction was reintroduced to the bottles containing soil, and allowed to shake for another 2 hours at 37°C in the water bath. After the third extraction period, another aliquot of 10 ml was taken from the bottle. This procedure was also performed without the intermediate step requiring the soil separation. The fluid aliquots were digested by adding 0.5 ml of nitric acid to 9.5 mL of sample. These samples were allowed to digest for 48 hours in capped polystyrene test tubes, and then filtered through a Puradisc Whatman syringe filter with a 0.45µm pore size.

Following the sequential gastrointestinal extraction, the residual soil mass was recaptured from the bottle. The soil remaining in the polyethylene bottles was captured on filter using a 90 mm diameter 0.45µm pore size cellulose nitrate filter. The filters and remaining soil were then digested in 10 ml of concentrated Optima nitric acid, according to the EPA method 3051 digestion series. (Kingston, 1986)

The samples were then diluted to concentrations within the linear portion of the calibration curve. The diluent used was a 2% Optima nitric acid solution. All metal analysis included the appropriate laboratory blanks plus controls and method blanks plus controls.

In Vivo Test of Bioavailability

Chemicals and supplies: Gum Arabic was from Sigma Chemical Co. (St. Louis, MO). Optima nitric acid, 50% H₂O₂, ethyl ether and feeding needles were from Fisher Scientific (Pittsburgh, PA)

Soil Preparation: One gram of the Montana Standard Reference Material 2710 was suspended in 5 ml of an aqueous solution which contained 5% gum Arabic to help maintain a suspension. The suspension was stored until needed at room temperature in a dark container.

Animal Treatment: Fifteen male Sprague-Dawley rats, 180-200 g, were obtained from Hilltop Labs. The animals were housed under standard conditions in wire mesh cages prior to treatment. Twenty four hours prior to treatment, the animals were placed in plastic metabolism cages and were fasted to reduce stomach content. The animals were separated into 5 groups, 3 animals per group. On day 0, all rats were given a single injection, by oral gavage, of either the soil suspension (25 ml/kg) or an equal volume of the vehicle control. The animals were then given free access to food and water. Any excrement was discarded that was collected during the previous 24 hours. On days 1, 2, 3, and 4, three rats from each group were sacrificed and necropsies were performed. Vehicle control animals were sacrificed on day 4.

Tissue Collection and Digestion: Animals were anesthetized with ether and blood was collected from the descending aorta and the volume was recorded. Other tissues, listed in table 2, were collected and their weights recorded. Large tissues were sectioned before digestion. The femurs that were collected had their marrow removed by aspiration. The bone was then dried to constant weight before digestion. All tissues were placed in 20 X 150 mm test tubes containing 2 ml of Optima HNO₃. Tissues were allowed to digest at 70^o C until clear. Two milliliters of 50% H₂O₂ was added and the samples were allowed to incubate overnight at room temperature. Samples were then quantitatively transferred to volumetric flasks and brought up to volume using deionized water. Any

samples that contained solids were filtered using Whatman #1 filter paper. The samples were then sent for analysis by ICP-MS.

Before ICP/MS analysis, 0.5g of feces was added to 10 ml of ultra pure nitric acid in a digestion vessel, and after capping and sealing the sample was ready for microwave digestion (CEM MDS-2000). A staged microwave digestion was conducted as follows: 40% power, 5 min; 50% power, 5 min.; 60% power, 5 min; 10% power, 5 min and 80%power, 5 min. These samples were cooled and diluted to concentrations within the linear portion of the calibration curve.

Analysis of Metals

Samples were analyzed by ICP-MS at EOHSI's Chemical Analysis Facility. All samples were diluted to <5% acid content. The bioaccessibility samples were analyzed for chromium by Graphite Furnace Atomic Absorption Spectrometry (Department of Environmental Sciences, Rutgers University) at a dilution of 2% acid. The method detection limits for the bioaccessibility analyses were 0.472, 1.09 and 0.42 ppb for lead, arsenic and chromium respectively. The method detection limits for the bioavailability procedures were 2.21, 4.7 and 3.90 ppb for lead arsenic and chromium respectively.

Dose Analysis

The dosing of the rats was tested for accuracy and precision using a gravimetric method. First, a gum Arabic-soil suspension was administered to three pre-weighed and dried Whatman 90 mm paper filters using a gavage needle. The soil suspension was then dried in a oven at 105°C to constant weight (within 0.01g). The filters were then weighed on a Sartorius Analytical Microbalance, and the amount of soil dosed was calculated by

subtracting the weight of the tare weight of the filter. The Sartorius Analytical Microbalance had an accuracy of 0.4% and a precision of 2.6% for the measurement of a 10 mg standard weight.

Results

Bioaccessibility

Bioaccessibility results for the Montana SRM 2710 are presented in Table 1. The values in the first column, the percent soluble in gastric fluid, were calculated by dividing the concentration of metal soluble in gastric fluid (μg metal from g of soil) by the certified metal concentration value. The values in the second column, the percent soluble in intestinal fluid, is the percent metal soluble in gastric and intestinal fluids divided by the total certified value. The intestinal solubility is a function of the addition of saliva, gastric and intestinal fluids. Gastric solubility, is a result of the addition of saliva and gastric fluids. The final column is an account of the total metal recovery from the system. Recoveries are calculated by the addition of the amount soluble in intestinal fluid with the concentration of metal from the soil recovered in the final step of the extraction procedure. The standard deviations associated with the bioaccessibility values were propagated according to random error calculations.

Lead was the most soluble in gastric fluid, at 76.1%, where as chromium was the least at 3.7%. There was an order of magnitude drop in the solubility of lead from the gastric compartment to the intestinal compartment. Arsenic also dropped in solubility when presented to intestinal fluids, however the decrease was not as sharp, 69.4% to 46.9%, as the decrease in lead solubility. The chromium bioaccessibility was consistent

between gastric and intestinal fluids where the decrease from the intestinal solubility to gastric solubility was only 3.7% to 3.0%.

Recoveries were calculated by summing the mass soluble in the intestinal fluid with the residual mass after the final soil collection. These mass balance recoveries varied from approximately 94.7% for arsenic to 75% for lead and 46.5% for chromium. The values for arsenic and lead were good, but the recovery for chromium was quite low. Chromium recovery was also low for aggressive acid leaching procedures, such as EPA 3051, at 57% of the value provided by NIST. This value is uncertified, and therefore the provided value may have a certain degree of error surrounding it. The levels of chromium are also the lowest of the three metals of interest, and may push the detection limits of the instrument.

Bioavailability

The results of the animal study are presented in Table 2. Each metal and its corresponding exposure times are represented in the horizontal rows. The various tissue levels of metal are listed in columns and are expressed as a percentage of the administered dose. Each value represents the average of concentrations obtained from 3 animals. The total value for each row is a sum of all tissues, except excreta. This value is referred to in this study as the “percent bioavailability” of a given metal.

Recoveries were calculated by adding together all tissues plus excreta and are expressed as a percentage of the administered dose. Recovery values are less than 100% due to losses, instrument variability, etc. Furthermore, the values for chromium in the various tissues are near the method detection limits. Any outliers were re-analyzed for confirmation, and no values were excluded from analysis.

A gravimetric analysis for total soil delivery using the gavage needle dosing technique was performed. The soil weights were calculated by subtracting the weight of a dried filter from the dried filter and soil weights. The dried soil weight delivered by needle gavage was 0.4957 ± 0.023 . The actual dose of soil by gavage feeding was 99.1% of the expected dose, with a relative standard deviation of approximately 2.3%.

A comparison of the bioavailability and bioaccessibility is present in Table 3, and illustrated in Figure 3. Intestinal fluid was chosen as the appropriate fluid for comparison, as the small intestine is the main location for the uptake of the metals of interest. Bioaccessibility values for arsenic and lead were greater than bioavailability values at 65.9% vs. 36.5%, and 10.7% vs 0.63% respectively. The bioaccessibility and bioavailability values were approximately the same for chromium at 3.0% and 4.8% respectively. Neither the bioaccessibility nor the bioavailability results reflected the progression in the total metal concentrations in the NIST SRM 2710, (i.e. $Pb > As > Cr$). Using either the measurement of bioaccessibility or bioavailability, arsenic was determined to be most available for predicting an internal dose. The reported recoveries corresponded for the two methods, meaning that the recovery discrepancy from 100% is due either to the affects of instrumentation or the geochemical affects of the soil matrix.

Discussion

Site risk characterizations can be based on one of three methods for quantitating the level of contaminant that are important for human exposure analyses. Typically, site based risk characterizations are driven by results from aggressive acid leaching methodologies on soils which were then used to estimate exposure. Although risk assessments based solely on the total metal content of a soil are considered to be generally

acceptable, they are unable to account for any biological processes which may alter the actual exposure and ultimate dose and the true concern about the levels of contaminants present at a site. In an effort to improve the biological relevance of risk assessments, animal studies were incorporated to investigate the role of physiological processes, such as adsorption, distribution, metabolism and excretion, on risk outcomes. A key variable of *in vivo* studies is bioavailability. Animal studies, however, are costly and labor-intensive. In addition, large quantities of test material are needed to perform these studies. The purpose of this work was to compare an *in vitro* method that employs the ease of an acid digestion with an *in vivo* animal experiment that provides a physiological basis for the system.

The NIST reported concentrations for each of the three study metals in the soil are included in Table 3, and are approximately separated by an order of magnitude. Lead is the most abundant of the metals at 5532ppm, arsenic the second at 627ppm and chromium the lowest at 39ppm(uncertified value). Percent bioaccessible, bioavailable and recovery calculations were based on these concentrations.

From the animal experiments, bioavailability of arsenic was 36.5%, which was the highest of the three metals examined, while the lead and chromium were 0.63% and 4.8%, respectively. The results were most consistent for arsenic, and the bioavailability ranged from 34.0% to 39.8%, with the highest accumulation of arsenic in all the tissues examined occurred on day three. Most of the arsenic was deposited in the blood with 0.7% depositing in the liver. Other tissues accounted for 0.1% or less of the total administered dose. A major route of elimination appeared to be the feces, while the urine showed a steady increase to 2% at day four. Recovery of arsenic ranged from 54.2% on day four to

91% on day 1. Variations in recovery values were due to inter-animal variability, with an average of 74.6%.

Chromium levels found in all animals were very low across all time points. Average bioavailability was 4.8%, and it peaked at day 4 with levels of 7.8%. Liver accumulation occurred on day 1 and 4, but was below detection limits on days 2 and 3. Conversely, small amounts of chromium were found in the muscle on days 2 and 3, and none was found at the other time points. Chromium appears to accumulate in the bone on days 3 and 4. Recoveries were quite low, ranging from 7.3% to 20.5%. All average values carried a 50% relative error. It is clear that the low soil concentrations of chromium precluded us from obtaining accurate levels, and suggest a lower limit of detection in an animal system.

Average lead bioavailability was 0.63%, with most of the accumulation occurring in the bone at all time points. Blood levels peaked at day 3, and then were undetectable at day 4. Recoveries range from 24.4% on day 4 to 69.6% on day 1. Most of this discrepancy can be attributable to decreasing amounts in the feces over time. Day 2 feces were low due to low readings in one single animal at 18.3%. These data illustrate the importance of proper time and tissue selection. We chose to use the total of all tissues averaged over all time points as a composite indicator for biological activity of these metals in the soil.

Bioaccessibility values are reported for both gastric conditions and small intestinal conditions. The gastric solubilities of each of arsenic, chromium and lead were 69.4%, 3.7% and 76.1%. The solubility of lead markedly decreased to 10.7% in the intestinal fluid. Arsenic and chromium did not show as great a decrease, where the intestinal

solubility of arsenic was 65.9% and chromium 3.0%, although the solubility of chromium was very low throughout the experiment. Recoveries were approximately 75% for lead, 94.7% for arsenic and 46.5% for chromium. Intestinal fluid was chosen as the appropriate comparison fluid, as this is the main location of absorption for the metals discussed in this paper. Lead intestinal absorption in the rat has been found to occur mainly in the duodenum (reviewed in Mushak, 1991). The introduction of Cr salts directly into the jejunum decreased amount of chromium measured in the feces, and therefore this is the main site of absorption for this compound (Donaldson, 1966). Arsenic has also been found to be methylated in the liver, and therefore intestinal absorption may be the major site of absorption for this metal.(Stevens, et al. 1977) In general, each of the metals of interest are absorbed, mainly, in the small intestine. For this reason the intestinal solubility, measured by the *in vitro* method, was used for the bioavailability-bioaccessibility comparison and should be considered the most favorable method for soil comparison of bioaccessibility.

Figure 3 presents the trends, obtained for total metal levels with respect to bioavailability and bioaccessibility. Lead was reported as having the highest absolute concentration on the soil, however its bioavailability and bioaccessibility values were demonstrably low. Conversely, arsenic was reported in rather low concentrations but was found to be highly available.

Chromium, we believe, may have been near our limits of detection and therefore the numbers are difficult to compare. It is noteworthy, however, that the bioavailability and bioaccessibility patterns seem to be in close agreement, reinforcing the notion that

biological processes and the geochemical characteristics of a soil play a more important role in determining risk. (Diamond, et al 1997), (Labieniec, et al. 1996).

Conclusions

Using a traditional total metal-based approach, lead might be considered for priority remediation. Arsenic, however, exists in such low abundance that it may not be considered a candidate for remediation. Clearly, a prioritization scheme changes when based on bioaccessible or bioavailable metal rather than the total concentration in the soil. In the present case, resources that may have been allocated towards lead remediation could be better utilized for arsenic. We believe biologically-based assessments of hazard levels are superior to total metal-based assessments, and that an *in vitro* extraction system can provide adequate prediction of bioavailability. The conducted work has also provided a basis for the comparison of independent results from other studies, because of the use of a standard, easily accessible soil. Lastly, *in vitro* methodologies can contribute meaningful data with reduced costs of up to 10 times, less labor and the ability to perform on-site analyses.

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References

- Brown, S.L., Bell, J.N.B. "Earthworms and Radionuclides with Experimental Investigation on the Uptake and Exchangeability of Radiocesium. *Environ. Pollut.* Vol. 88. No. 1. pps. 27-39. 1995.
- Carbonell, A.A., Aarabi, M.A., Delaune, R.D., Gambrell, R.P., Patrick, W.H.Jr. "Bioavailability and Uptake by Wetland Vegetation: Effects on Plant Growth and Nutrition." *J. Environ. Sci. Health. Part A, Toxic/Hazardous Substances and Environmental Engineering.* Vol. A33. No. 1. pps. 45-66. January 1998.
- Clapp, T.C., Umbreit, T.H., Meeker, R.J., Kosson, D.S., Gray, D., Gallo, M.A. "Bioavailability of Lead and Chromium from Encapsulated Pigment Materials". *Bull. Environ. Contam. Toxicol.* Vol. 46. No. 2. pps. 271-275. 1991.
- Davis, A., Ruby, M., Bergstrom, P.D. "Bioavailability of Arsenic and Lead In Soils from the Butte, Montana, Mining District." *Environ. Sci. Technol.* Vol. No. pps. 1993.
- Davis, A., Ruby, M.V., Goad, P., Eberle, S., Chryssoulis, S. "Mass Balance on Surface Bound, Mineralogic, and Total Lead Concentrations as Related to Industrial Aggregate Bioaccessibility". *Environ. Sci. Technol.* Vol. 31. No. 1 pps. 37-44. 1997.
- Diamond, G.L., Goodrum, P.E., Felter, S.P., Ruoff, W.L. "Gastrointestinal Absorption of Metals." *Drug Chem. Toxicol.* Vol. 20. No. 4. Pps. 345-368. 1997

Donaldson, R.M., Barrerras, R.F. "Intestinal Absorption of Trace Quantities of Chromium." *J. Lab. Clin. Med.* Vol. 68. pps. 484-493. 1966.

Freeman, G.B., Johnson, J.M., Killinger, J.M., Liao, S.C., Feder, P.I., Davis, A.O., Ruby, M.V., Chaney, R.L., Lovre, S.C., Bergstrom, P.D. "Relative Bioavailability of Lead from mining Waste Soil in Rats." *Fundam. App. Toxicol.* Vol. 19. pps. 388-398. 1992.

Freeman, G.B., Dill, J.A., Johnson, P.J., Kurtz, P.J., Parham, F., Matthews, H.B. "Comparative Absorption of Lead from Contaminated Soil and Lead Salts by Weanling Fisher 344 Rats." *Fundam. App. Toxicol.* Vol. 33. pps. 109-119. 1996.

Gargas, M.L., Norton, R.L., Harris, M.A., Paustenbach, D.J., Finley, B.L. "Urinary Excretion of Chromium Following Ingestion of Chromite-Ore Processing Residues in Humans: Implications for Biomonitoring." *Risk Analysis.* Vol. 14. No. 6. pps. 1019-1024. 1994.

Gibaldi, M. *Biopharmaceutics and Clinical Pharmacokinetics Third Edition.* pps. 131-135. Lea and Febiger, Philadelphia. 1984.

Groen, K., Vaessen, H.A.M.G., Liest, J.J.G., de Boer, J.L.M., van Ooik, T., Timmerman, A., Vlug, R.F. "Bioavailability of Inorganic Arsenic from Bog Ore-containing Soil in the Dog." *Environ. Health Perspect.* V. 102. No. 2. 182-184. 1994.

Hamel, S.C., Buckley, B., Lioy, P.J. "Bioaccessibility of Metals in Soils for Different Liquid to Solid Ratios in Synthetic Gastric Fluid". *Environ. Sci. Technol.* Vol. 32. pps. 358-362. 1998.

Hamel, S.C., Ellickson, K.M., Lioy, P.J. "Bioaccessibility using a Mass Balance Model" in press.

Kingston, H. M., Test Methods for Evaluating Solid Waste, Physical Methods, 3rd ed; U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response. U.S. Government Printing Office: Washington, DC, SW 846-3051, (1986).

Labieniec, P.A., Dzombak, D.A., Siegrist, R.L. "Risk Variability Due to Uniform Soil Remediation Goals. *J. Environ. Eng.* Vol. 122. No. 7. Pps. 612-621. 1996.

Lebourg, A., Sterckman, H. Ciesielski, H. Proix, N. "Trace Metal Speciation in Three Unbuffered Salt Solutions Used to Assess their Bioavailability in Soil" *J. Environ. Qual.* Vol. 27. pps. 584-590. 1998.

Maddaloni, M., LoIacono, N., Manton, W., Blum, C., Drexler, J., Graziano, J. "Bioavailability of Soilborne Lead in Adults, by Stable Isotope Dilution." *Environ. Health Perspect.* Vol. 106. Supplement. 6. pps. 1589-1594. 1998.

Mushak, Paul "Gastro-Intestinal Absorption of Lead in Children and Adults: Overview of Biological and Biophysico-Chemical Aspects". *Chem. Spec. Bioavail.* Vol. 3. pps.87-104. 1991.

Nessel, C.S., Amoruso, M.A., Umbreit, T.H., Meeker, R.J., Gallo, M.A. "Pulmonary Bioavailability and Fine Particle Enrichment of 2,3,7,8-tetrachlorodibenzo-p-dioxin in Respirable Soil Particles." *Fundam. App. Toxicol.* Vol. 19. No. 2. pps. 279-285. 1992.

Novozamsky, I, Lexmond, Th.M., Houba, V.J.G., Rauret, G. "A Single Extraction Procedure for Evaluation of Uptake of some Heavy Metals by Plants" *Int. J. Environ. Anal. Chem.* Vol. 51. No. 1-4. pps.47-58. 1993.

Pichtel, J., Anderson, M. "Trace Metal Bioavailability in Municipal Solid Waste and Sewage Sludge Composts". *Bioresource Technol.* Vol. 60. No. 3. pps. 223-229. June 1997.

Rodriguez, R.R., Basta, N.T., Casteel, S.W., Pace, L.W. "An In Vitro Gastrointestinal Method to Estimate Bioavailable Arsenic in Contaminated Soils and Solid Media." *Environ. Sci. Technol.* Vol. 33. pps. 642-649. 1999.

Ruby, M.V., Davis, A., Schoof, R., Eberle, S., Sellstone, C.M. "Estimation of Lead and Arsenic Bioavailability Using a Physiologically Based Extraction Test." *Environ. Sci. Technol.* Vol. 30. pps. 422-430. 1996.

Sheppard, S.C., Eveden, W.G., Schwartz, W.J. "Ingested Soil: Bioavailability of sorbed lead, cadmium, cesium, iodine and mercury." *J. Environ. Qual.* Vol. 24. pps. 498-505. 1995.

Stevens, J.T. Hall, L.L., Farmer, J.D., et al. "Disposition of C14 and/or As74-cacodylic acid in rats after intravenous, intratracheal or peroral administration. *Environ. Health Perspect.* Vol. 19. pps. 151-157. 1977.

Table 1 *In Vitro* Bioaccessibility of Selected Metals in NIST SRM 2710

	% Soluble in Gastric Fluid	% Soluble in Intestinal Fluid	Recovery
Pb	76.1%±11%	10.7%±2.3%	75.5% ± 9.6%
As	69.4%±8.3%	65.9%±5.2%	94.7% ± 6.9%
Cr	3.7%±2.1%	3.0%±1.9%	46.5%±13.2%

Percent solubility values were calculated with respect to the NIST-reported values. All values represent averages of 3 replicate samples. Recoveries are calculated as the sum of the dissolved mass in the intestinal fluid and the undissolved portion in the residual soil mass. All errors were propagated according to random propagational error calculations.

Table 2. *In Vivo* Bioavailability of Selected Metals in NIST SRM 2710

		Bioavailability by tissue (%)												
Metal	Exposure	Blood	Lung	Liver	Kidneys	Spleen	Testes	Heart	Muscle	Femurs	Urine	Feces	TOTAL	RECOVERY
As	1 Day	32.9	0.1	0.7	0.1	0.1	0.0	0.1	0.0	0.0	0.3	57.2	34.0	91.5
	2 Day	36.2	0.1	0.7	0.1	0.1	0.1	0.1	0.1	0.0	0.4	29.2	37.5	67.1
	3 Day	38.6	0.1	0.7	0.1	0.1	0.0	0.1	0.1	0.0	1.4	44.5	39.8	85.7
	4 Day	33.7	0.0	0.7	0.1	0.1	0.0	0.1	0.1	0.0	2.2	17.2	34.8	54.2
Cr	1 Day	1.7	0.0	2.4	0.0	0.0	0.1	0.0	0.0	0.0	0.0	6.9	4.2	11.1
	2 Day	3.9	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	2.9	4.4	7.3
	3 Day	2.5	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.1	0.2	17.5	2.8	20.5
	4 Day	2.4	0.0	5.1	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	7.8	7.8
Pb	1 Day	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.0	68.7	0.9	69.6
	2 Day	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	44.3	0.4	44.7
	3 Day	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0	62.8	0.9	63.7
	4 Day	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	24.1	0.3	24.4

Percent bioavailability was calculated based on the administered dose. Tissue values represent the average of 3 animals. Total values are the sum of all tissues, except for excreta. The total percentage of metal from all tissues within a group is expressed as “Recovery”. The level of lead, arsenic and chromium in the brain and hair were below detection limits.

Table 3. *In Vitro* - *In Vivo* Comparison

	<i>In Vitro</i>		<i>In Vivo</i>	
	Bioaccessibility	(Recovery)	Bioavailability	(Recovery)
As	65.9%±5.2%	94.7% ± 6.9%	36.52 (34.0, 39.8)	74.63 (54.2, 91.5)
Cr	3.0%±1.9%	46.5%±13.2%	4.8 (2.8, 7.8)	11.66 (7.3, 20.5)
Pb	10.7%±2.3%	75.5% ± 9.6%	0.63 (0.3, 0.9)	50.6 (24.4, 69.6)

Bioaccessibility values are reported as a mean of four values and a standard deviation.

Bioavailability values are reported as a a mean and a range of three values.

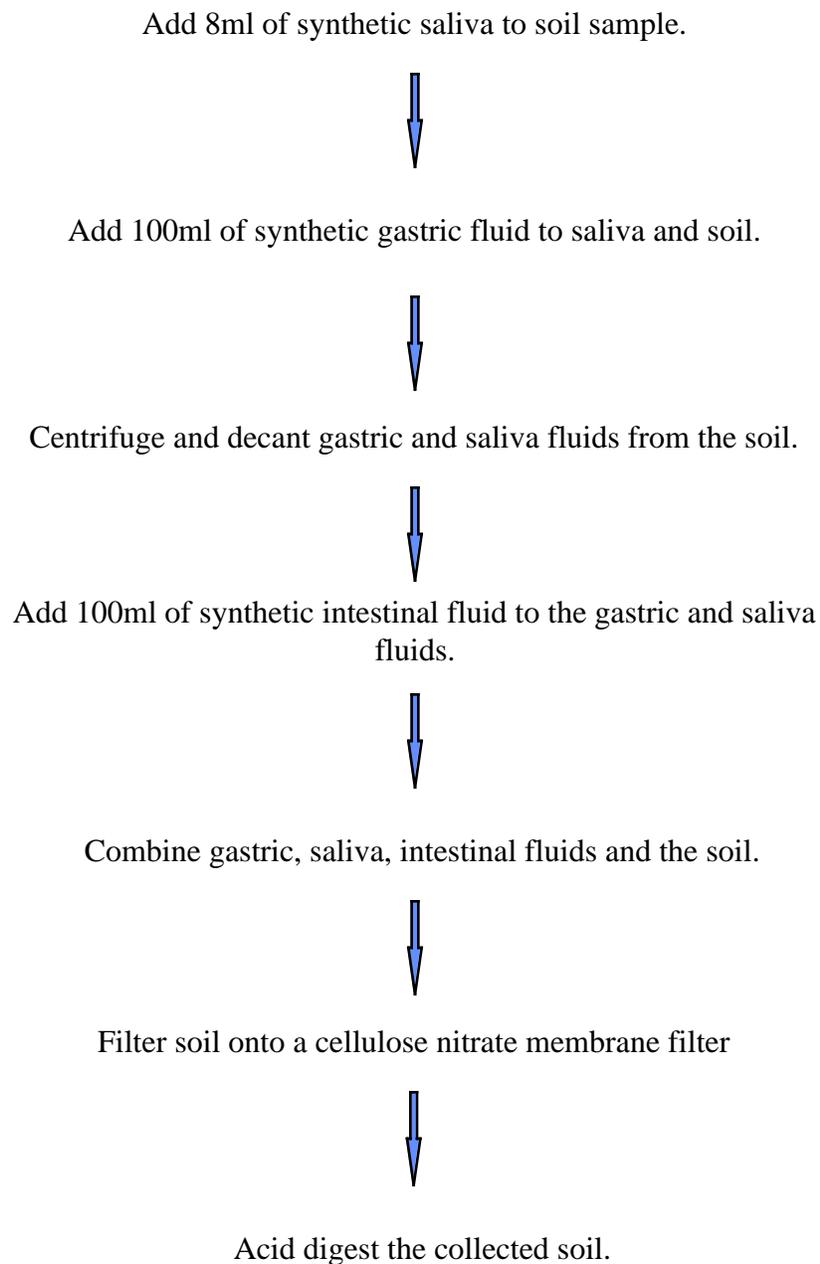


Figure 1: Schematic representation of *in vitro* bioaccessibility methods

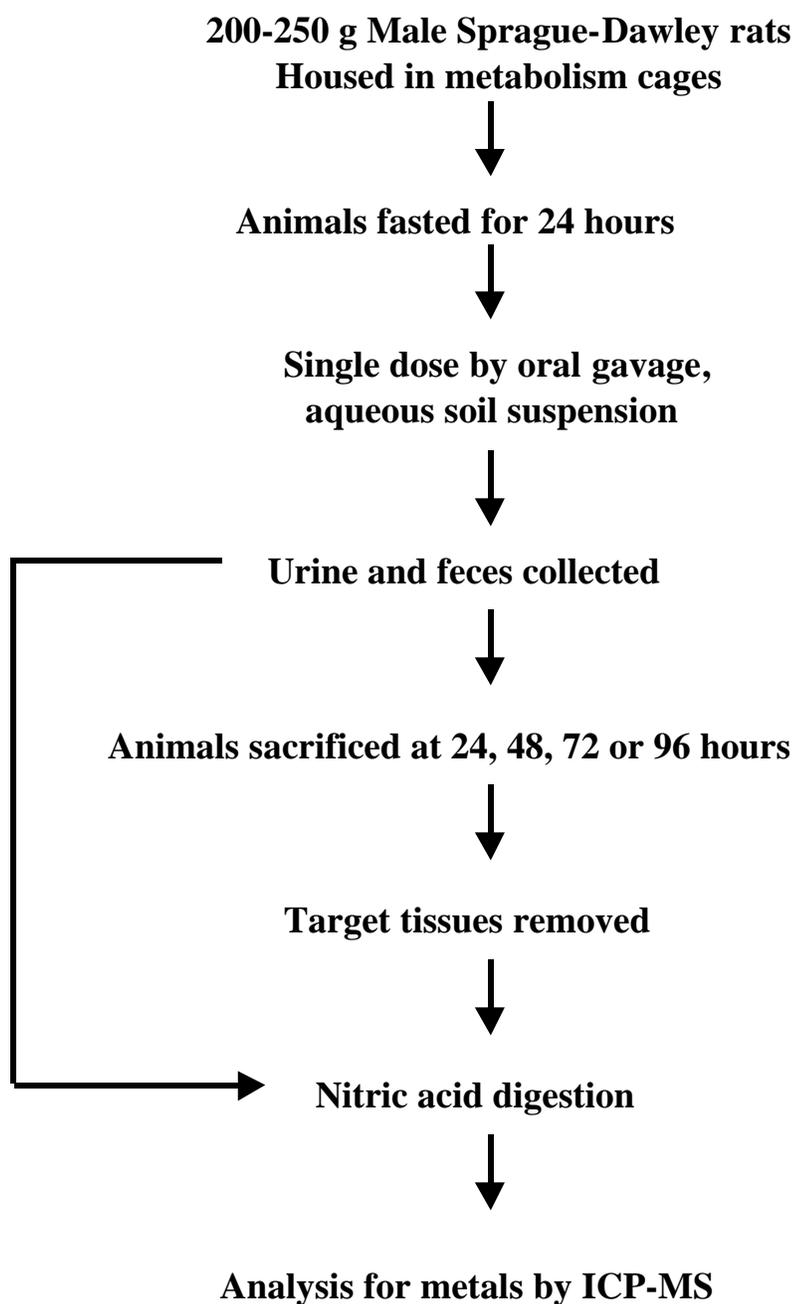


Figure 2: Schematic representation of *in vivo* bioavailability methods

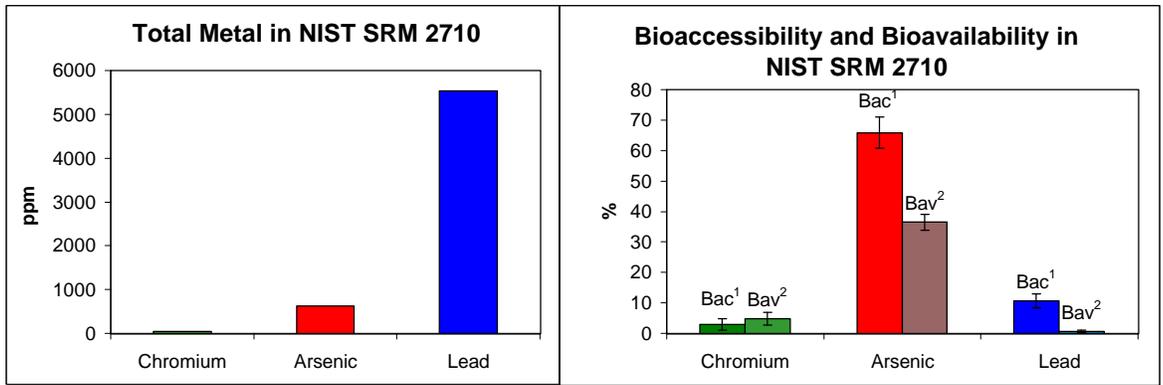


Figure 3a. NIST certified metal concentrations are plotted by metal ppm (ug/g).

Figure 3b. Bioaccessibility¹ and Bioavailability² values from Table 3 are plotted by metal.

Title: The Estimation of the Bioaccessibility of Heavy Metals in Soils Using Artificial Biofluids:
A Mass-Balance Technique

Authors: Stephanie C. Hamel, Kristie M. Ellickson¹, Paul, J. Liroy²

1 Graduate Student in the Joint Ph.D. program in Exposure Assessment of Department of Environmental Sciences, Rutgers, The State University of New Jersey and University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School

2 To whom all correspondence should be addressed.

Address:

Exposure Measurement and Assessment Division
Environmental and Occupational Health Sciences Institute
170 Frelinghuysen Road
Piscataway, NJ 08854

Telephone: (732) 445-0150
Facsimile: (732) 445-0116
E-mail: plioy@eohsi.rutgers.edu

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Abstract (word count: 246):

The possible health effects resulting from the ingestion of soil bound heavy metals can be poorly estimated if concentration data of metals in soil, rather than bioavailability data, are incorporated into dose calculations. Bioavailability data are usually obtained from animal studies, but to simulate quickly the fraction of contaminant in a soil material that is soluble in gastrointestinal tract fluids (the bioaccessible fraction), an *in vitro* sequential extraction

technique has been developed. Using a mass balance analytical approach, bioaccessibility of lead in Standard Reference Material Montana SRM 2710 was 62 ± 1 % of the total metal, while lead in contaminated soil collected from Bunker Hill, ID was 70 ± 11 %. Lead in Jersey City, NJ slag material was only 39 ± 14 % bioaccessible while lead in residential soil collected from underneath a treated wooden deck was 69 %. Arsenic, cadmium and chromium data in these soils show that each bioaccessibility was less than its total metal in soil.

Furthermore, by recovering the soil at the end of the *in vitro* extraction the insoluble fraction of the total metal was recaptured. This recaptured soil metal mass was a valuable tool since it reduced the analysis required to determine bioaccessibility, and therefore the labor and time needed for determination. It also allowed for calculation of a bioaccessibility value in a soil with very low metal mass, which would otherwise have resulted in a non-detectable concentration at the dilutions present in the synthetic human biofluid system.

Introduction:

Incidental soil ingestion is a major concern as a route of heavy metal exposure, especially for young children. Soil is a heterogeneous mixture of parent material, macro and microorganisms and decaying organic matter. Soil components have the capacity to sorb actively contaminants such as heavy metals and metal availability is controlled by the biological, chemical and physical nature of a soil. Thus, availability varies over space and time, with the degree of metal absorption onto a soil matrix affecting mobility of a metal. Because of these constraints, the total metal contaminant concentration in a soil may not be bioavailable upon ingestion.

To estimate human bioavailability of contaminant metals in soils, a laboratory protocol has been developed which employs artificial biofluids as part of a sequential extraction (Davis *et*

al., 1996; Ruby *et al.*, 1993). This method is a simulation of human gastrointestinal physiology, as a mechanism to simulate ingested dosages of heavy metals. Data from such an *in vitro* study can be used to quickly ascertain information for a human exposure assessment, allowing regulators to screen many soil samples to determine if further studies are warranted for a particular site. An advantage of adopting a laboratory procedure, rather than animal or human testing, is the relative ease of using an *in vitro* technique, in addition to cost and ethical considerations.

A similar *in vitro* protocol for estimating human exposure to heavy metals in soil was developed for this work. This synthetic system involves a sequential extraction of soil using artificial biofluids, followed by soil recapture at the end of the sequence. The artificial biofluids were analyzed for metals concentration and the recaptured soil and residual matter were acid-digested together to determine the amount of metal remaining intact in the soil following sequential extraction in the biofluids. A mass-balance scheme for analysis thus was performed to confirm that all of the separable metal within the soil matrix could be tracked during the biofluid extractions and in the recaptured soil and residual matter.

The first biofluid in the extraction sequence was artificial saliva. Evidence exists that prolonged mouthing of objects by infants and young children results in increased solubilization of contaminants from materials, with subsequent absorption of toxic metals or organic compounds (Healy, 1982). Artificial gastric fluid was placed in the protocol, as the stomach is the region of the gastrointestinal tract which is considered to have the greatest influence on metal bioavailability, due to increased solubilization associated with low pH. Once ingested materials leave the stomach, they enter the small intestine, with the primary region of heavy metal absorption in rats (Mushak, 1991), and in humans (Ashmead *et al.*, 1985), the proximal area of

the small intestine. The incorporation of a final duodenal biofluid in this *in vitro* laboratory sequence should ensure a reasonable characterization of the processes that precede *in vivo* absorption of metals.

The pharmaceutical definition of bioavailability is the rate and extent of absorption of a compound to a target organ or tissue (Gibaldi, 1984). An ingested metal contaminant must cross the gastrointestinal mucosa to become bioavailable in either humans or other mammalian systems. The maximal concentration that is soluble, and therefore available for transport across the intestinal lining (regardless of method of transport), is labeled for this work as the bioaccessible fraction. *In vivo* studies using animals can provide bioavailability data, while *in vitro* studies using laboratory extractions of soils estimate the bioaccessibility. Since the bioaccessible fraction of a contaminant does not pass the additional barrier of the mucosal wall to reach the target tissue or bloodstream, it is expected that, for a given ingested soil, the bioaccessible fraction will be greater than the bioavailable fraction.

The bioaccessible fraction is the soluble mass of metal, M_B , divided by the total mass of metal in soil, M_T . M_T can be ascertained by various analytical methodologies, however a soil value of M_T may not be consistently reported if different analytical extraction and detection methods are used for determination (Kitsa *et al.*, 1992). For example, techniques such as electron microscopy and x-ray fluorescence can locate metal particles embedded in a soil without cracking the soil matrix. The total metal mass measured from one of these procedures may be larger than the total metal mass derived from a less complete acid digestion extraction, such as Us EPA method 3051.

Bioaccessibility calculations become a function of analytical technique since values of bioaccessibility are calculated using total metal mass. A consistent method for reporting total

metal mass is a prerequisite for inter-laboratory comparison of results. For these reasons, a mass balance protocol was developed to determine the mass total of metal. It relied on the summation of the metal extractable of each artificial digestive biofluid, plus the amount of the metal that was recovered in soil and residual matter recapture, as described in the formula:

$$M_{T\Sigma} = M_B + M_R,$$

where:

$M_{T\Sigma}$ = total mass of metal in a soil material, by summation,

M_B = maximal extractable mass of metal soluble in artificial human gastrointestinal biofluids (Mass Bioaccessible), and

M_R = recaptured residual metal mass precipitated or remaining in the soil following sequential extraction (Mass Recaptured). It is determined by US EPA Method 3051.

Experiments were performed to compare the summation value for metal mass total, $M_{T\Sigma}$, to M_T values generated by other techniques, including the certified value for standard reference material, M_{Tcv} , and the results of US EPA Acid Extraction method 3051, M_{T3051} .

Bioaccessibility was determined for each heavy metal based on a ratio of $M_B/M_{T\Sigma}$. Finally, a proposed method to simplify the technique was examined, using only the recaptured residual soil metal mass, M_R , and M_{T3051} , for calculations of bioaccessibility.

Materials and Methods:

Soil Test Materials:

Four soils, or soil materials were examined. Montana Standard Reference Material (SRM) 2710 was purchased from the National Institutes of Standards and Technology (NIST), and was chosen for this study because it is useful for cross-comparison and validation of the bioaccessibility methodology.

The second test soil material was a contaminated slag material collected from Liberty State Park, Jersey City, NJ, which had been examined in earlier studies (Kitsa *et al.*, 1992; Hamel *et al.*, 1998)). It contained high levels of trace metals, particularly chromium from chromium ore processing residue. A third test soil was obtained from researchers at Columbia University School of Public Health, in New York City. It was contaminated with lead from a Superfund site, and was obtained from a residential yard in Bunker Hill, Idaho. It was an excellent candidate for an *in vitro* bioaccessibility study, since it had been fed to adult human subjects to determine its *in vivo* lead bioavailability (Nancy LoIacano, personal communication, 1997).

The final test material was a soil collected from underneath a wooden deck in a residential neighborhood, in western New Jersey. It contained a higher level of arsenic than the surrounding soil. Ostensibly, the arsenic in the soil originated in pressure treated wood containing copper-chromium-arsenate (CCA) which was subject to weathering. The deck had the characteristic green-stain of copper treated wood, though the current homeowners were not able to positively verify that CCA was used for treatment.

A summary of the test materials used for the experiments was provided in Table 1. Information was included on the collection and drying techniques, as well as details on the metals and pH.

Table 1. Summary of Test Materials Used for Bioaccessibility and Mass-Balance Experiments.

Test Material	pH	Particle size Diameter (μm)	Source	Drying	Metals of Interest (conc. in soil)
Bunker Hill, ID	4.0	< 125	US EPA Residential yard at mining impacted site.	Air, R.T.* Placed in filtered incubator for 8 days Sterilized by γ irradiation.	Pb 2924 ± 36 ppm
Califon, NJ (residential soil)	6	< 125	S. Hamel Top 1 in. of soil from under a pressure-treated wood deck.	Air-dried for 2 days at R.T. oven dried for 1 h.	As 163 ± 2 ppm Pb 68 ± 3 ppm
Liberty State Park, NJ	8.2	< 125	V. Kitsa Top 2 cm of soil.	Air-dried for 64 days at R.T.	Cr 2428 ± 357 ppm Pb 1163 ± 53 ppm
Montana SRM 2710	4.5	< 74	USGS Top 4 in. of pastureland along Silver Bow Creek in Butte, MT.	Air-drying oven for 3 days at R.T. Blended prior to shipment.	Pb 5532 ± 80 ppm As 626 ± 38 ppm Cd 21.8 ± 0.2 ppm

*R.T.= Room Temperature

Particle Size and Amount

A particle size fraction of less than 125 μm in diameter was chosen for each soil. This particle size has been shown to be the fraction that is most likely to adhere to the hands of children (Healy *et al.*, 1982; Steele *et al.*, 1990). Montana SRM 2710 was the exception, as it was shipped at less than 74 μm diameter particle size. For the experiments, the Superfund Exposure Assessment Guidance Manual value, 50 mg/day, was chosen as rate of soil ingestion; with a child's exposure of 50 mg of soil.

Preparation of Artificial Biofluids

Artificial Saliva

The formula for artificial saliva used for this work was a modification of the Fusayama protocol (1963). It included:

Mucin	4.0 g
Urea	1.0 g
Na_2HPO_4	0.6 g
$\text{CaCl}_2 \cdot 4\text{H}_2\text{O}$	0.99 g
KCl	0.4 g
NaCl	0.4 g

q.s. to 1000 ml using deionized water in a volumetric flask.
pH was approximately 5.5.

Mucin and urea were purchased from Sigma Chemical Co., St. Louis, MO. All others were purchased from VWR Scientific, St. Louis, MO.

Hypothiocyanite, used as an antimicrobial component (Tenovuo, 1992), and a preservative, sodium benzoate, were in the Fusayama formulation. They were excluded in the artificial saliva in this work because neither microbial activity nor long-term storage of the artificial saliva were of concern.

Artificial Gastric Fluid

US Pharmacopoeia Artificial Preparation of Gastric Fluid

The gastric fluid used was prepared from the US Pharmacopoeia formula (USPXII, 11990), which is usually employed to simulate drug dissolution in the stomach. Two grams of NaCl [suprapur, EM Science (7647-14-5)] were dissolved in a solution of 7 ml of ultrapure sub-boiling, distilled hydrochloric acid (High Purity Standards, Charleston, SC), and approximately 250 ml of deionized water. Pepsin, 3.2 g, [Sigma, St. Louis, MO (9001-75-61)] was added, and the solution q.s to 1000 ml, for immediate use.

Artificial Intestinal Fluid

A 0.2 M sodium bicarbonate solution prepared with sodium bicarbonate (EM Science, Gibbstown, NJ) and deionized water was used for the simulation of the duodenal fluid of the intestine.

Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS)

Samples were analyzed using a VG FISIONS PQ2+ inductively coupled plasma/ mass spectrometer (VG Elemental, Danvers, MA) for parts per billion (ppb) samples concentrations of lead, arsenic, chromium, and cadmium, using a modified US EPA method 200.8. Standards for instrumental analysis were prepared from solutions obtained from High Purity Standards, Charleston, SC. Quality assurance checks were run at intervals using National Institutes of Standards and Technology 1643d: Trace Elements in Water. For each sample, the detection limit associated with each sample was 1 ppb.

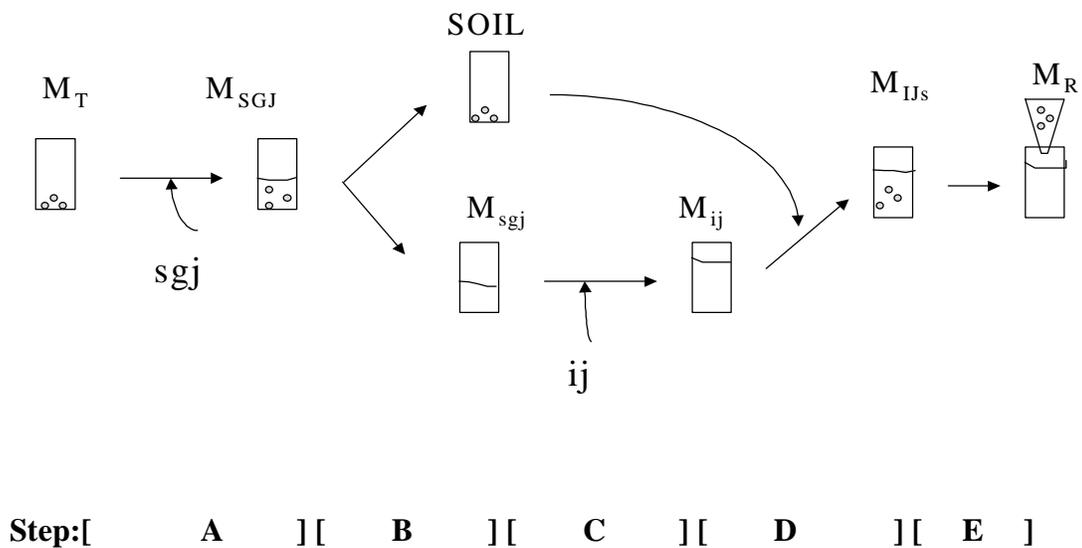
Mass Balance Experiment

Table 2 is a presentation of the individual steps of the mass-balance experiment. Data from this experiment were used to calculate bioaccessibility. Figure 1 is a schematic diagram of the same.

Table 2. Steps of Extraction

Step	Description
A	Saliva/Gastric Biofluid Extraction of Soil
B	Separation of Soil from Saliva/Gastric Biofluid
C	Intestinal Biofluid Addition to Saliva/Gastric Biofluid
D	Intestinal /Saliva/Gastric Biofluid Extraction of Soil
E	Separation of Soil from Biofluids; Recapture of Soil and Residual Matter.

Figure 1. Schematic of Mass-Balance Experiment



The soil was mixed with saliva/gastric juice, SGJ, to simulate upper gastrointestinal tract extractability, in Step A. M_{SGJ} , the mass of metal that is soluble in the saliva/gastric fluid, was a straightforward measurement of the concentration of a fluid sample. Multiplication of the concentration by the volume of fluid yielded mass of soluble metal.

Since *in vivo* gastric contents are transferred to the intestines, and the biofluid experiment was designed to attempt to mimic the system, it was not possible to analyze the metal contaminant solubility in an isolated artificial intestinal biofluid. To determine the intestinal juice contribution (M_{IJ}) to bioaccessibility, a separation step at the intestinal biofluid stage of the experiment was incorporated (See Figure 1, Step B). This was accomplished by separating the saliva/gastric juice (SGJ) from the soil, to render the saliva/gastric biofluid (sgj). (Note: Upper case initials denote that soil is interacting with biofluid; lower case soil is not in contact with the fluid.) Intestinal biofluid (ij) was then added to the saliva/gastric biofluid without soil (sgj), creating sgj/ij biofluid mixture. The soluble metal mass in sgj/ij is termed M_{ij} . When the value of M_{ij} was less than M_{sgj} , heavy metal was precipitated by the intestinal biofluid. When this occurred, the metal was becoming less soluble, likely due to the rise in pH accompanying the addition of the basic intestinal biofluid to the previously acidic biofluids.

The measurement of M_{ij} , however, neglects consideration of the soil interaction in the intestinal fluids. To remedy the situation, (See Figure 1, Step D), after determining the extent of precipitation, the sgj/ij biofluid mixture was recombined with the free soil to simulate intestinal extraction in the presence of soil. After extraction, an aliquot of biofluid was removed and analyzed for metal mass, M_{IJS} .

The soil and any precipitated residual matter were collected using a vacuum filtration system. Acid digestion and analysis of the combined soil and residual precipitated matter rendered a mass of recaptured soil metal.

A full derivation of the mass balance equation, and experimental details are included in the Appendices. The total mass of metal was determined by adding the amount of metal that was remaining in the recaptured soil and residual matter, M_R , to M_B to be equal to $M_{T\Sigma}$.

Results and Discussion

When certified values of metal in soils exist, (from sources such as the National Institutes of Standards and Technology) bioaccessibility can be reported as the bioaccessible mass, M_B , divided by the mass value derived from the certified value of total metal concentration, (M_{Tcv}). M_T also can be reported as M_{T3051} , the acid extraction mass of metal from US EPA method 3051, or by the mass total by summation, $M_{T\Sigma}$, described above.

$M_{T\Sigma}$ values were compared for individual metals in each soil to M_{Tcv} (available only for the reference material), and M_{T3051} . Table 3 is a summary of the average recoveries for each metal, reported as a fraction of $M_{T\Sigma}$ divided by M_{T3051} , or, in the case of Montana SRM, M_{Tcv} . The number of samples for each soil ranged from $n=3$ to $n=5$. The average value is shown, as is one standard deviation.

For lead in Montana SRM 2710, $[(M_{T\Sigma}/ M_{Tcv}) \times 100]$ was $120 \% \pm 15 \%$. The recovery of lead in soil collected from Bunker Hill, ID, found $[(M_{T\Sigma}/ M_{T3051}) \times 100]$ to be $161 \pm 51 \%$. Recovery varied between 117 and 219 %. The average recovery of lead in the residential soil yielded $[(M_{T\Sigma}/ M_{T3051}) \times 100]$ of $106 \% \pm 71 \%$. The spread in values was quite large, ranging from 25 to 151 %. The sources of this variation may be due to complex chemistry of the heavy metals in biofluids, analytical capabilities of the detection instruments, or lack of homogeneity of

the soil or the metal in the soil. The recovery of arsenic in the residential soil, $[(M_{T\Sigma} / M_{T3051}) \times 100]$, had two odd results: the recovery was only 61 %, and variation between samples was quite small at $\pm 1\%$.

The recovery from Jersey City slag material yielded $[(M_{T\Sigma} / M_{T3051}) \times 100]$ of 80 % \pm 28 % for lead and 85 % \pm 32 for chromium.

The mass-balance protocol accounts for within 20 % of the certified value of the lead, arsenic and cadmium in Montana SRM 2710, and lead and chromium in Jersey City slag material (using EPA method 3051 total metal as a comparison). In the residential soil and the soil collected from Bunker Hill, ID, lead calculations of $M_{T\Sigma}$ were not within the acceptable instrumental error limits (20%) of M_{T3051} . The percent recoveries/variation indicate that M_{T3051} cannot be interchanged in bioaccessibility calculations, with $M_{T\Sigma}$, for these soil materials.

The percent bioaccessible is a comparison of extraction procedures performed on two different soil samples when EPA method 3051 or a certified value were used in the denominator. Using the summation of the mass balance for calculation of total metal mass allows bioaccessibility to be determined using information collected from one soil sample. This method resulted in the highest precision (K. Ellickson, unpublished result), possibly because error was reduced when all the components of the bioaccessibility calculation were gathered on an individual soil sample. The use of $M_{T\Sigma}$ was appropriate for use in the calculation of bioaccessibility

Table 3. Comparison of Percent Total Mass Recovery $[(M_{T\Sigma}/ M_{T3051}) \times 100]$ Using Sequential Extraction Technique.

Sample	Percent Recovered (Fraction x 100) $M_{T\Sigma}/M_{T3051}$
Lead	
Montana SRM 2710*	120 ± 15
Bunker Hill	161 ± 51
Jersey City Slag	80 ± 28
Residential	106 ± 71
Chromium	
Jersey City Slag	85 ± 32
Arsenic	
Residential	61 ± 1
Montana SRM 2710*	87 ± 23
Cadmium	
Montana SRM 2710*	96 ± 6

Average fraction ± 1 standard deviation

NA= not applicable

* M_{Tcv} used instead of M_{T3051} .

The average bioaccessible percent, $[(M_B/M_{T\Sigma}) \times 100]$, for lead in each of the soils was less than 100 % of the total lead in the soil. The soil collected from Bunker Hill, ID, had the highest average bioaccessible percent, $[(M_B/M_{T\Sigma}) \times 100]$ for lead, of 70 % ± 11%, followed by

residential soil with a bioaccessible percent of 69 %. The reference soil, Montana SRM 2710 was $62\% \pm 1\%$ bioaccessible, while lead in Jersey City slag material had the lowest bioaccessibility] of $39\% \pm 14\%$.

The other heavy metals measured were also less than 100 % bioaccessible. Chromium in Jersey City slag material had the average bioaccessible percent, $[(M_B/M_{T\Sigma}) \times 100]$, of $34\% \pm 4\%$; the average bioaccessible percent of arsenic was $41\% \pm 2\%$, in residential soil. Using the certified value for arsenic, Montana SRM 2710 was $66\% \pm 8\%$.

Table 4 is a summary of the above data and of the bioaccessibility calculation using recaptured mass, M_R , described below.

Table 4. Summary of Percent Bioaccessibility as expressed using: $[(M_B/M_T) \times 100]$, and the Soil Recapture Technique: Bioaccessibility = $\{[1-(M_R/M_{T3051})] \times 100\}$.

Sample	Average \pm 1 SD $M_B/M_{T\Sigma}$	$\{[1-(M_R/M_{T3051})] \times 100\}$
Lead		
Montana SRM 2710	$62 \pm 1\%$	62 %
Bunker Hill Soil	$70 \pm 11\%$	Not determined
Jersey City Slag Material	$39 \pm 14\%$	28 %
Residential Soil*	69 %	54 %
Chromium		
Jersey City Slag Material	$34 \pm 14\%$	40 %
Arsenic		
Residential Soil	$41 \pm 2\%$	Not determined
Montana SRM 2710	$66 \pm 8\%$	Not determined
Cadmium		
Montana SRM 2710	Not determined	** $48 \pm 7\%$

n=3-5, * n=2

** certified value was used as denominator $\{[1 - (M_R/ M_{Tcv})] \times 100\}$

The data for metal bioaccessibility indicate that all samples have a bioaccessible mass, M_B lower than the total metal mass ($M_{T\Sigma}$) present in each soil examined in this study.

In human systems, metal bioavailability involves membrane transport and delivery to target organs, however bioaccessibility involves only gastrointestinal solubility. The internal dose is expected to be greater than the bioeffective dose (Lioy, 1990). In order for a metal to be bioavailable it must be soluble, therefore bioaccessibility values are expected to be greater than bioavailability values.

The Columbia School of Public Health has conducted studies on humans, to measure the amount of lead absorbed upon ingestion of a mine-tailing soil as an indication of bioavailability (Mark Maddaloni, personal communication, 1995). Comparison of *in vitro* results to data from human studies aids in providing a link between what is ingested and what is found in the blood. Data generated from this work were compared to the results of the blood lead levels of volunteers who ingested soil from Bunker Hill, ID. Blood was sampled, as it is considered to reflect environmental lead absorption (Rieuwerts *et al.*, 1995). The adult humans fed soil collected from Bunker Hill, ID had a lead bioavailability of $26.2\% \pm 8.1\%$ (Nancy LoIacano, personal communication, 1997). The results from these studies provide evidence that Human Bioavailability (26.2%) was $< In vitro$ Bioaccessibility (70% of Total Metal) which was $<$ Total Soil Metal Concentration.

Bioaccessibility Calculations using Recaptured Mass (M_R):

Collection of the soil at the end of the extraction sequence allows the measurement of recaptured metal mass (M_R). Dividing M_R by the total metal concentration M_T results in the percent of metal which was not solubilized by the biofluid extraction. The subtraction of this insoluble portion from 100% of total metal can be used to calculate bioaccessibility in percentage form: $(M_B/M_T) = \{[1 - (M_R/M_T)] \times 100\}$. For the metals where total metal mass $M_{T\Sigma} = M_{T3051}$, the two methods can be used interchangeably and $[M_B/M_{T\Sigma} = (1 - M_R/M_{T3051})]$.

Summation of values, each with a corresponding error value, results in the propagation of each individual error value, so it is likely that summation of the mass balance as a means to calculate bioaccessibility may result in high variability. This may be minimized by the calculation of bioaccessibility from the recaptured metal mass, M_R , which eliminates three biofluid component calculations.

A mass-balance protocol was used to measure the solubility of heavy metals from a soil matrix within each of the gastrointestinal fluids present in a human system and calculates a mass of potentially bioavailable metal. By recapturing the soil and residual matter at the end of the sequential *in vitro* extraction, the metal concentration not extracted by the synthetic digestive system could be measured, and translated into a mass. These two masses together yield a summation mass total, $M_{T\Sigma}$, which allowed the comparison of recoveries with certified heavy metal values M_{Tcv} and concentrations from EPA 3051 extractions, M_{T3051} .

Table 4 also contains a column of the percent bioaccessible $\{[1 - (M_R / M_{T3051})] \times 100\}$ for the metals for the soils in which $M_{T\Sigma} = M_{T3051}$, (within $100 \pm 20 \%$). Using mass recaptured method for determination of bioaccessibility, rather than the mass balance summation should provide the same information, but with considerably less effort.

The bioaccessible lead in the residential soil was 54 %, when data from the EPA method 3051 total metal data were used in the denominator of the bioaccessibility calculation: $\{[1 - (M_R / M_{T3051})] \times 100\}$. Using the same calculation, the lead bioaccessibility in Montana SRM was 54 %, where the lead in Jersey City slag material was only 28 %. Chromium bioaccessibility in Jersey City slag material was 40 %.

If soils are ranked according to their bioaccessibility for remediation purposes, the two techniques for determining bioaccessibility, $M_B / M_{T\Sigma}$, and $(1 - M_R / M_{T3051})$, should render the same

order. This occurs for lead, as seen in the Table 4. Jersey City slag soil had lowest bioaccessibility, followed by the Montana soil, then the residential soil. Since prioritization of soils for clean up is an important issue, the recaptured mass technique is extremely useful, as it can provide the same information obtained in the bioaccessibility experiments with considerably less work.

The final row in Table 4 also presents an example of results found by the mass recaptured method for a metal of very low concentration. The concentration of cadmium in the Montana SRM 2710 is only $21.8 \mu\text{g/g} \pm 0.2$, which translates to metal concentration below detection limits in the dilution of at least 2000:1 (ml/g) in synthetic gastric and intestinal biofluids. Since detectable amounts of masses are necessary to determine M_B in the formula: $M_B/M_{T\Sigma}$, using the mass recaptured method was the only viable method for determination of M_B . Using the certified value as a denominator, the cadmium bioaccessibility in this material was $48 \pm 7\%$.

The use of the recaptured metal fraction as a means of calculating bioaccessibility allows an adequate estimation of bioaccessibility. It encourages the use of more complex physiologically based artificial biofluids, while minimizing the labor of laboratory protocols for efficient processing of many soil samples. As soil and metal mobility within a soil are inherently heterogeneous, this method offers a way of assessing a contaminated site without having to rely on a limited estimate of site-wide averaged soil characteristics.

This technique used for the evaluation of the soil materials in the bioaccessibility studies was able to replace the bioaccessible data acquired from the digestive fluid extractions, when $M_{T3051} = M_{T\Sigma}$. The recapture of post extraction soil may be the most cost-effective technique, to date, to estimate metal bioavailability in soils. If the mass recaptured formula is used for the calculation of bioaccessibility, in place of the summation data, the intermediate steps of the *in*

vitro sequential extraction technique can be performed without analysis. The expediency and simplicity of determining the bioaccessibility using the mass recaptured method facilitates the study of bioaccessibility in several other ways. The ease of analysis allows more complicated fluid systems to be incorporated into the protocol without increasing the difficulty of the analysis due to matrix complications, and soil types with varying physical, chemical and biological characteristics can be studied quickly. Finally, the method allows the analysis of low level contaminants which otherwise would fall under detection limits in the synthetic biofluids, which represent human fluid concentrations.

The bioaccessible fraction, as an estimate for bioavailability, is less than 100 % of the total metal concentration in soils, for the soils and metals that were examined. *In vitro* estimation of bioaccessibility of the lead in the soil collected at Bunker Hill, ID can more accurately estimate human exposure to heavy metals than does assuming one hundred percent availability or total extractable metal mass via concentrated acid extraction. The mass-balance technique can be used routinely to determine bioaccessibility of heavy metals in contaminated soils as an estimate of human bioavailability.

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REFERENCES

- Ashmead HD, Graff DJ, and HH Ashmead, In: *Intestinal Absorption of Metal Ions and Chelates*, (Ed.) Charles C Thomas, Springfield IL, 1985, p 76-77.
- Davis A, Ruby MV, Bloom M, Schoof R, Freeman G and PD Bergstrom, Mineralogic Constraints on the Bioavailability of Arsenic in Smelter-Impacted Soils, *Environmental Science and Technology*, **30**(2): 392-399, 1996.
- Fusayama T, Katayori T and S Nomoto, Corrosion of Gold and Amalgam Placed in Contact with Each Other, *Journal of Dental Research*, **42**: 1183-1197, 1963.
- Gibaldi, Milo Biopharmaceuticals and Clinical Pharmacokinetics: Third Edition, Lea & Febiger, Philadelphia, 1984.
- Hamel SC, Buckley, B and PJ Lioy, Bioaccessibility of Metals in Soils for Different Liquid to Soil Ratios in Synthetic Gastric Fluid, *Environmental Science and Technology*, **32**(3), 358-362, 1998.
- Healy MA, Harrision PG, Aslam P, Davis SS and CG Wilson, Lead Sulfide and Traditional Preparations: Routes for Ingestion, and Solubility and Reactions in Gastric Fluid, *Journal of Clinical and Hospital Pharmacy* 7: 169-173, 1982.
- Kitsa V, Lioy PJ, Chow JC, Watson JG, Shupack S, Howell T, and P Sanders, Particle-Size Distribution of Chromium: Total and Hexavalent Chromium in Inspirable, Thoracic and Respirable Soil Particles From Contaminated Sites in New Jersey, *Aerosol Science and Technology*, **17**: 213- 229, 1992.
- Lioy PJ, Assessing Total Human Exposure to Contaminants, *Environmental Science and Technology*, **24**(7): 938-945, 1990.
- Mushak P, Gastro-Intestinal Absorption of Lead in Children and Adults: Overview of Biological and Biophysico-Chemical Aspects, *Chemical Speciation and Bioavailability*, **3**: 87-104, 1991.
- Rieuwerts JS and ME Farago, Lead Contamination in Smelting and Mining Environments and Variations in Chemical Forms and Bioavailability. *Chemical Speciation and Bioavailability*, 7: 113-123, 1995.
- Ruby MV, Davis A, Link TE, Schoof R, Chaney RL, Freeman GB and P Bergstrom, Development of an *in Vitro* Screening Test To Evaluate the *in Vivo* Bioaccessibility of Ingested Mine-Waste Lead, *Environmental Science and Technology*, **27**: 2870-2876, 1993.

Ruby MV, Davis A, Schoof R, Eberle, S and C Sellstone, Estimation of Lead and Arsenic Bioavailability Using a Physiologically Based Extraction Test, *Environmental Science and Technology*, **30**:422-430, 1996.

Steele MJ, Beck BD, Murphy BL and HS Strauss, Assessing the Contribution from Lead in Mining Wastes to Blood Lead, *Regulatory Toxicology and Pharmacology*, **11**: 158-190, 1990.

U.S. EPA Guidance manual for the integrated exposure uptake biokinetic model for lead in children. U.S. Environmental Protection Agency, Office of Emergency and Remedial Response, Washington, D.C., 1994.

U.S. EPA Test methods for evaluating solid waste. Method 3050 Volume 1A: Laboratory manual, physical/chemical methods; U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response: Washington, DC, 1986.

The United States Pharmacopoeia XXII, Inc. US Pharmacopoeial Convention, Twinbrook Parkway, Rockville, MD 1990, p 1178.

Appendix I : Calculations and Equations

The bioaccessible mass of metal is defined as:

$$M_B = M_{SGJ} + M_{IJ}$$

where:

M_B = maximal extractable mass of metal soluble in artificial human gastrointestinal biofluids,

M_{SGJ} = mass of metal extracted into artificial saliva/gastric juice, and

M_{IJ} = mass of metal affected by intestinal juice (not including SGJ that is traveling with the IJ).

Mathematically, the determination of M_{IJ} is the difference of the mass of metal extracted during the intestinal phase minus the mass of metal transferred with the sgj, at the beginning of the whole phase. Mass at end of intestinal phase is measured as M_{IIs} . Metal that is precipitated during the intestinal phase will be counted in the soil recovery phase, M_R , and needs to be removed from a calculation at the intestinal fluid phase. The mass at the beginning of the intestinal phase is the difference between the metal concentrations of the gastric fluid and any precipitated mass. The formula is expressed as:

$$M_{IJ} = M_{IIs} - [M_{SGJ} - (M_{sgj} - M_{ij})],$$

where $[M_{SGJ} - (M_{sgj} - M_{ij})]$ is the concentration of metal at the beginning of the intestinal phase of the extraction; the transferred mass from M_{sgj} minus any precipitated mass.

M_{IIs} is the concentration of metal at the end of the intestinal phase of the extraction.

Since $M_T = M_{SGJ} + M_{IJ} + M_R$,

$$M_T = M_{SGJ} + M_{IIs} - [M_{SGJ} - (M_{sgj} - M_{ij})] + M_R, \text{ which collapses to:}$$

$$M_T = M_{SGJ} + M_{IJs} - M_{ij} + M_R.$$

From the mass-balance equation, it follows that:

$$M_B = M_{SGJ} + M_{IJs} - [M_{SGJ} - (M_{sgj} - M_{ij})], \text{ which collapses to :}$$

$$M_B = M_{SGJ} + M_{IJs} - M_{ij}$$

The percent bioaccessible is determined by dividing the gastrointestinal fluid contribution, M_B , by the total metal concentration of the soil, M_T . M_T , however, may be determined via several different methods, by using the certified value, M_{Tcv} , by using an acid extraction techniques, M_{T3051} , or by using the summation data from the mass-balance protocol, $M_{T\Sigma}$.

The summation of the masses in the equation to calculate the total metal, $M_{T\Sigma}$ is: $M_{T\Sigma} = M_{SGJ} + (M_{IJs} - M_{ij}) + M_R$. By dividing M_B by this calculated $M_{T\Sigma}$, rather than M_{T3051} , the bioaccessibility is now based only on the values obtained from the bioaccessibility protocol.

$$M_B/M_{T\Sigma} = (M_{SGJ} + M_{IJs} - M_{ij}) / (M_{SGJ} + M_{IJs} - M_{ij} + M_R).$$

Appendix 2: Experimental Details

Sequential Extraction Protocol:

Step A. Eight milliliters of artificial saliva was added to 50 mg of soil material in a 250 ml Nalgene bottle. The mixture was hand-shaken for 5 seconds, then 100 ml of artificial gastric fluid was added to the sample. The bottle was capped and the cap sealed with parafilm. The bottle was re-shaken by hand for 5 seconds, then placed in a Magni Whirl® constant temperature water bath (MSB-1122A-1 General Signal, Blue Island, Illinois) at 37°C, at a speed of 90 cycles per minute, for 2 hours.

After 2 hours, the bottle was removed from the shaker bath and the contents settled for 2 minutes. Ten milliliters of sample was removed from the top of the bottle and placed in a

disposable centrifuge tube (Falcon 15ml Polystyrene screw top centrifuge tube), using a volumetric pipette equipped with a disposable plastic tip. The centrifuge tube was capped and then spun in a Metpath tabletop centrifuge (906 g) for 10 minutes. The decantate was filtered using 0.45 μm Acrodiscs attached to Luer-Lok glass syringes, and stored for analysis to give M_{sgj} .

The extractable amount of metal is determined using instrumentation which provides measurements in parts-per-billion (ppb) concentration, ($\mu\text{g/L}$). The information is converted to a mass (μg) of metal by multiplication of the concentration by the volume of the fluid:

$$(\mu\text{g metal/L fluid}) \times (\text{L fluid}) \times (\text{any dilution factors}) = \mu\text{g metal.}$$

The residue at the bottom of the centrifuge tube was saved and eventually resuspended with approximately 5 ml of intestinal fluid and returned to the extraction sequence. This last step does not occur until later in the protocol, see asterisk* in Step D.

Step B. From the top of the Nalgene bottle containing the remaining soil, saliva and gastric juice biofluid, the biofluid mixture was decanted and placed in a clean, labeled Nalgene bottle. To optimize separation of fluid from soil, it is recommended that centrifugation of the Nalgene bottle prior to decanting is incorporated into the experiment. The soil was saved in the original Nalgene bottle, for later reintroduction to the fluid (Step D).

Step C. One hundred milliliters of artificial intestinal fluid was added to the isolated biofluids of Step B to create sgj/ij, and the suspension was placed in the water bath for 2 hours at 37°C, at a shaking speed of 90 cycles per minute. A 10 ml aliquot was removed from the sample, and spun in a Metpath tabletop centrifuge (906 g) for ten minutes. The sample was decanted and placed aside for analysis. The sample provided the concentration data for the calculation of M_{ij} .

For analysis, each sgj/ij biofluid sample was digested in concentrated ultrapure nitric acid in a laboratory microwave oven (CEM Corporation, Matthews, NC). A sample of 9.5 ml was placed in a clean, acid-rinsed Teflon® vessel, and 0.5 ml of nitric acid was added to the vessel. The sample was digested open-vessel for 8 minutes at 40% power, and allowed to cool. It is recommended that the vessels be capped and closed, to prevent loss of volatile metals. The sample was diluted in acid-rinsed glass volumetric flasks, to 2% acid solutions, prior to analysis.

Step D. The portion of sgj/ij biofluid that was not microwave digested and analyzed was returned to the soil saved in the original Nalgene bottle. * The centrifuge tubes used for aliquot for analysis often contained a soil/precipitate at the bottom. These pellets (from Steps B and C) were also rinsed into the Nalgene bottle containing soil using a small volume of sgj/ij biofluid mixture. Once the rinse was completed, the bottle was hand-shaken for 5 seconds, capped, and placed in the water bath, at 37°C, at a speed of 90 cycles per minute, for 2 hours. After 2 hours, the sample was allowed to settle, (again, centrifugation is recommended) and another 10 ml aliquot was removed for analysis, providing the data for M_{IIS} .

The aliquot was prepared for analysis by immediate centrifugation in a Metpath Tabletop centrifuge for 10 minutes (906g), followed by filtration using 0.45 μm Acrodiscs attached to Luer-Lok glass syringes. Prior to Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS) analysis, 9.5 ml of sample in 0.5 ml concentrated ultrapure nitric acid was digested in a laboratory microwave in an open-vessel for 2 X 8 minutes, at 40% power, then diluted to 2% acid solutions for ICP-MS analysis.

Step E. The soil at the bottom of the Nalgene bottle was collected on cellulose nitrate (Whatman No. 7184 009, 0.45 μm , VWR Scientific) filter disc, using a vacuum filtration system. A small amount of deionized water was needed to rinse the residual soil from the bottom and

sides of the bottle, to aid in soil collection. The soil, any precipitated material, and the filter were carefully placed into a clean Teflon digestion vessel.

Ten milliliters of ultrapure concentrated nitric acid was added to the vessel. The vessel was covered with Kimwipes, and allowed to sit overnight, without a lid, at room temperature, in a laboratory hood. The filter was reduced to a pulp by the next day, and further dissolved in the acid upon gentle shaking of the vessel. The vessel was placed in a microwave, and digested open vessel for 2 X 8 minutes at 40 % power, with cooling allowed between each digestion. After the second digestion, the sample was capped and digested closed-vessel using US EPA method 3051 technique. The conditions used for the microwave extraction were 10 minutes (5 minutes, time at pressure) 60 p.s.i. and 100 % power. The sample was diluted to no greater than 2% acid concentration solution in a volumetric flask and then analyzed by ICP-MS.

The resulting concentration of extracted metal was converted to M_R by multiplying the concentration by the volume of the sample, dilution factors and by original soil mass. The final weight of soil recaptured was not used in this calculation because it contained precipitates which were not part of the original soil mass. To minimize any error in mathematical calculation of M_R , due to precipitate formation, the original soil mass (50 mg) was used for calculations of M_R .