Annual Report

Comparison of the Bioavailability of Elemental Waste Laden Soils Using In Vivo and In Vitro Analytical Methodology and Refinement of Exposure/Dose Model

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INTRODUCTION:

The bioavailability study has made significant progress in developing our “in vitro” methodology, and we completed our time course “in vivo” studies. The “in vitro” studies have been conducted to establish the major digestive variables of concern and the values to be used in application of both the saliva/gastric juice and intestinal fluid components of a synthetic digestive extraction. “In vitro” and “in vivo” experiments have been conducted on the $\leq 75 \mu\text{m}$ particle fraction of a soil sample collected in a Jersey City State Park. Five Jersey City soil samples were first characterized for physical and chemical characteristics. Based upon the composition of the five soils, one was selected for use in the first series of experiments.

The second set of “in vivo” studies are to be conducted on a standard NIST Montana soil. It has already been examined for bioaccessibility and availability with the “in vitro” methodology. A sample has been collected in Bayonne to obtain an urban background soil.

Surficial soil samples have been acquired from the Savannah River Site of the DOE. These are not radioactive but are contaminated with heavy metals, e.g. arsenic, and are being analyzed by both the “in vivo” and “in vitro” methodology.

During this past summer a second set of soil samples were collected at Savannah River Site. These contain levels of both heavy metals and radionuclides. Recently, a special extraction laboratory has been constructed at EOHSI, with resources made available from our organization. It will handle the extraction and measurement of the radioactivity of the soil, and extracts obtained by the “in vivo” techniques. It is anticipated that the SRS samples collected this summer will be available for analysis in both the “in vivo” and “in vitro” systems this fall. The initial characterization will be for soil, physical and chemical content, and microbial characteristics. The samples will be analyzed for the $\leq 75 \mu\text{m}$ particle size fraction, and the total mass $\leq 250 \mu\text{m}$ in particle size. Previous experience has suggested a preferential distribution of toxic materials in the small size

fraction of soils, and that these particles will define the majority of the potential bioavailability of the soil.

Initial mass balance experiments have been completed on the “in vitro” methodology, and we are attempting to optimize the recovery of total mass of each metal or radionuclide present in a soil. Our mass balance studies have proceeded with the use of test soils from a home in California that has known arsenic contamination, and a lead contaminated soil from Columbia University which has been used in human feeding studies.

The plans for next year intend to focus on the analysis of the SRS samples, radioactive and non-radioactive and soils from other DOE sites and pharmacokinetic modeling. Our technique development has moved to the point where the applications can be made using a reproducible protocol.

SOIL SAMPLES:

Soil, organic matter, pH, cation exchange capacity (CEC), particle size distribution, and metal loadings were analyzed in soils sampled from Liberty State Park and from residential site in Bayonne. The park site was a former dredge fill site. Thus, it represents a managed soil site with high metal levels. The latter site was selected as a potential urban control soil. Results of all these analyses (with pertinent methods citations) are presented in Table 1, and are summarized as follows:

Liberty State Park Samples: Soil samples were collected at Liberty State Park NJ on February 6, 1997. Five samples were collected and analyzed from a hazardous waste containment area, from a nearby hill, and from a nearby low-lying area. All samples contained relatively high levels of gravel or greater size particles (% waste in Table 1).

The pH values of all five samples are above 7 and would result from liming at this site. The site is a former dredge fill area and in an attempt to prevent leaching, was likely limed to increase the pH of the soil. The organic matter contents of these soils were elevated above those values generally anticipated for soils of the region. These organic

Table 1. Soil properties and metals loadings for Liberty State Park samples

Parameter	Sample #1	Sample#2	Sample#3	Sample#4	Sample #5
pH ¹	7.9	7.5	8.0	7.5	7.3
Organic Matter (%) ^{2,3}	16.3 ± 1.1	30.9 ± 0.2	34.0 ± 1.2	25.8 ± 0.9	3.9 ± 0.4
CEC (cmoles/kg) ⁴	32.16 ± 3.61	41.49 ± 2.60	32.80 ± 2.28	46.46 ± 0.70	26.73 ± 0.66
Particle Size Analysis ⁵ (<2mm)					
%Sand	79.4	82.4	76.4	88.4	77.4
%Clay	6.8	6.4	10.0	6.0	8.0
% Silt	13.8	11.2	13.6	5.6	14.6
(<1mm fraction)					
%Sand	74.8	75.8	68.4	75.8	85.2
%Clay	6.8	5.6	12.8	7.6	5.6
% Silt	18.4	18.6	18.8	16.6	9.2
% Waste (>1mm)	54.1	57.8	70.4	70.7	51.1
Metals (ppm) ⁶ ± σ					
Cd ⁷	1.5 ± 0.4	1.1 ± 0.3	1.7 ± 0.2	0.8 ± 0.1	4.2 ± 4.8
Cr ⁸	11000 ± 1915	2220 ± 177	390 ± 69	80 ± 37	50 ± 14
Pb ⁸	1640 ± 181	370 ± 37	1250 ± 37	250 ± 14	60 ± 24
As ⁷	264 ± 19	16.2 ± 0.34	118 ± 21	22.4 ± 1.0	6.23 ± 0.49
Fe ⁸	127300 ± 5704	35600 ± 3187	74000 ± 2040	47400 ± 1944	16600 ± 374

1. pH was determined using a 1:1 soil:distilled water slurry (McLean, 1986).
2. Organic matter content for samples 1-4 was measured by loss on ignition (Bear, 1955).
3. Organic matter content for sample 5 was determined by the Walkely-Black procedure (Walkely, 1947).
4. Cation exchange capacity measured by saturation with pH 7 ammonium acetate (Chapman, 1965).
5. Particle size analysis was by the hydrometer method of Bouyoucos (1986).
6. Metal loadings were measured by a modified EPA Method 3050 using 10ml of concentrated Nitric acid with digestion at 120°C.
7. Metals analysis performed using by graphite furnace atomic absorption spectroscopy (Perkin Elmer, Norwalk, CT; Model #4100ZL).
8. Metals analysis performed using by flame atomic absorption spectroscopy (Perkin Elmer, Norwalk, CT; Model 3030)

matter levels further suggest that these sites are dredge filled since elevated organic matter levels are not unusual in submerged sediments.

Samples 4 and 5 were collected from outside the containment area and contained typical background concentration of the analyzed metals. Because of the low metal loadings, these soil samples were eliminated from further study. Samples 1, 2 and 3 contained elevated levels of As, Pb, and Cr. However, they also contained unusually high contents of organic matter. Since sample 1 had the lowest organic matter content of the three contaminated samples and metal loadings within the range desirable for further study, it was chosen for further bioavailability study. The reduced content of gravel also made sample 1 a more optimal sample for study than were samples 3 through 5.

Bayonne soils: A residential site in Bayonne, NJ was chosen as a possible urban background site because of heavy industry located in surrounding areas. Three samples were taken from this site on May 8, 1997. These samples underwent the same assays as the above Liberty State Park samples. The elemental analyses are listed in Table 2; however, the results for cadmium loading is pending.

Samples 3 and 5 were taken from the same side of a dirt path approximately 10 feet from each other and are very similar in their characteristics. Sample 4 was collected from the opposite side of the path approximately 25 feet from sample 3. The sample is very different from the previous two, and may be the remnants of some type of clay liner or deposited lower horizon of the natural soil unearthed during construction of nearby homes. It contained an unusually large percentage of red and brown clays close to the soil surface, which caused clumping of the soil and necessitated, crushing prior to size separation. The physical crushing of this sample is reflected in the similarity of results across the three size ranges. All three size ranges included were somewhat homogenized by clay inclusions during crushing and sieving.

This is in contrast to samples 3 and 5, which although similar in metal content, percent organic matter, and CEC for the 2 mm and 500-75 μm sizes, showed large differences when compared to the same size fractions and the <75 μm sizes, also showed large differences when compared to the same size fractions. It is recommended that

Table 2. Soil properties and metals loadings for Bayonne samples.

Parameter	Sample #3	Sample #4	Sample#5
pH¹			
<2mm fraction	6.7	6.0	6.7
500-75µm fraction	6.7	6.0	6.8
<75µm fraction	6.6	6.3	6.5
% Organic Matter³			
<2mm fraction	2.06 ± 0.25	4.55 ± 0.03	3.15 ± 0.45
500-75µm fraction	1.95 ± 0.40	4.13 ± 0.26	1.84 ± 0.25
<75µm fraction	6.48 ± 0.58	4.68 ± 0.33	6.58 ± 0.04
CEC (cmoles/kg)⁴			
<2mm fraction			
500-75µm fraction			
<75µm fraction			
Particle Size Analysis⁵ (<2mm fraction)			
%Sand	86.4	54.0	86.0
%Clay	7.6	15.6	7.6
% Silt	6.0	40.4	6.4
(500-75µm fraction)			
%Sand	86.0	56.0	83.4
%Clay	7.6	14.0	9.6
% Silt	6.4	30.0	7.0
Metals (ppm)⁶ <2mm ± σ			
Cr ⁷	24.7 ± 0.20	123 ± 2.45	23.1 ± 0.59
Pb ⁸	160 ± 15.0	220 ± 35.4	120 ± 0.0
As ⁷	6.45 ± 0.51	8.44 ± 0.37	5.84 ± 0.56
Fe ⁸	12300 ± 424	21200 ± 787	12700 ± 374
Metals (ppm) 500-75µm ± σ			
Cr	30.2 ± 12.6	118 ± 1.73	19.3 ± 1.32
Pb	135 ± 0.0	210 ± 21.2	120 ± 0.0
As	6.45 ± 0.22	8.26 ± 0.17	5.04 ± 0.26
Fe	12200 ± 141	19100 ± 374	12400 ± 510
Metals (ppm) <75µm ± σ			
Cr	300 ± 12.6	137 ± 1.0	124 ± 9.37
Pb	450 ± 0.0	245 ± 18.7	470 ± 12.2
As	9.40 ± 0.66	8.22 ± 0.29	8.99 ± 0.40
Fe	20000 ± 283	20600 ± 374	20500 ± 283

Footnotes: See Table 1.

sample 4 not be used for further experimentation but that a composite of sites 3, and 5 can serve as an excellent urban background soil.

BIOACCESSIBILITY

INTRODUCTION:

The bioaccessibility protocol was developed to estimate the potential and internal doses of heavy metal contaminants in soils. Using this procedure, artificial saliva, gastric fluid and intestinal fluids sequentially extract the soil and the soluble metal fraction is reported as percent bioaccessible metal.

MASS-BALANCE:

A mass-balance concept for this protocol was suggested and developed for several reasons. It is desirable to account for the mass of metal at each step of the protocol for analytical verification, and also to enable the evaluation of mass recovered at the end of the procedure. With the bioaccessible amount dissolved in the sequential fluids, it follows that any mass of metal recovered at the end, M_R , is not available. When subtracted from the total mass, M_T , the inaccessible amount should reflect and provide the bioaccessibility of metals in a given soil.

The usefulness of a mass balance protocol is further understood if one considers the difficulty of analysis of the typical conditions of the artificial fluids. Neither Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) nor Atomic Adsorption Spectroscopy (AA) perform optimally with suspensions, as they distort the results. This phenomenon then disallows complicated mixtures of components for the fluids. If this constraint can be eliminated by measuring M_R (rather than M_{sgj} and/or M_{ij}) as a measure of bioaccessibility, more complex gastrointestinal fluids can be incorporated into the protocol, while lowering the number of required analyses.

Currently, the bioaccessible fraction is calculated by dividing the bioaccessible mass, M_b , by the total mass of metal, M_T , in the sample. The total mass of a soil is determined by digesting a 0.05g sample in concentrated nitric acid, under high pressure and temperature, and then analyzing the soil for metal concentration. This technique measures concentration and is used to calculate metal mass in each sample. This is not

necessarily the best procedure, especially where soil heterogeneity is prevalent. If the total mass, M_T , can be projected by the summation of the components of the mass-balance experiment, the use of Method 3051 to derive total mass of metal can be considered unnecessary.

Four different soil materials were analyzed including Montana Standard Reference Material 2710 (Montana Soil), a mine-waste Superfund soil (Columbia Soil) and a chromium-laden slag (Jersey City Soil). The same experiment was performed on a residential soil which was collected from beneath a ten-year-old deck in Califon, NJ made of pressure-treated wood. The wood exudes arsenic, copper, and chromium due to weathering processes, and the metals collect onto the ground below.

A study was performed at Columbia University in which human subjects were fed lead contaminated soil with resulting blood lead levels measured. This soil (Columbia Soil) was obtained and extracted with our protocol and our values for bioaccessibility were compiled and compared to their results. This is the only human data acquired to date.

Part 1 Mass Balance Equation:

METHODS:

A 0.05 g soil sample was suspended in 8 ml of artificial saliva and 100 ml of artificial gastric juice in a Nalgene bottle. The mixture was allowed to shake in a 37°C shaker water bath for 2 hours. A 10 ml aliquot was removed and analyzed via ICP-MS. The data were reported as $\mu\text{g/L}$, and converted to grams of metal, M_{sgj} .

The soil was removed from the remaining fluid via decantation and was set aside for later re-addition to the fluid. Then 100 ml of 0.2 M sodium bicarbonate solution was added to the fluid and the mixture was again shaken for two hours at 37°C. A 10 ml aliquot was removed, and spun in a centrifuge at 3400 rpm for 10 minutes. The supernatant (9.5 ml) was placed in a Teflon microwave vessel and 0.5 ml of ultrapure concentrated nitric acid were added. The mixtures were digested, open vessel, for 8 minutes at 40% power in a microwave, and then allowed to cool. Dilution and

analysis followed. M_{ij} , the mass of metal was calculated for the saliva/gastric/intestinal fluid combination.

The fluid not utilized for M_{ij} analysis was added to the original soil and that mixture was allowed to shake for two hours. Using the same work-up protocol as the above paragraph, the mass of the metal soluble in the intestinal/saliva/gastric juice combination was calculated after 2 hours of contact with soil, M_{IJS} .

The precipitated solids and soil residue were collected on a cellulose acetate filter and digested overnight at room temperature in a Teflon digestion vessel by 10 ml of ultrapure concentrated nitric acid. The filter paper dissolved overnight. The vessels were then capped and digested using the same vigorous conditions that were used for the determination of the total mass, M_T . M_R , the mass of metal precipitated or remaining in the soil was calculated following extraction (determined by Method 3051).

Mass Balance Derivation:

The mass-balance derivation has been completed, and the formula developed is as follows, (See Figure 1):

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

where: M_T = mass of metal in soil (determined by Method 3051)

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

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

M_R = mass of metal precipitated or remaining in the soil following extraction (determined by Method 3051).

The mass determinations are reasonably straight-forward, with the exception of mass of intestinal fluid. During the extraction sequence, the gastric fluid is added to the intestinal fluid. The mass of metal from the intestinal juice contribution, M_{IJ} , alone, therefore, is not able to be elucidated upon analysis. To determine the intestinal juice contribution, M_{IJ} , a more complex scheme was incorporated for the intestinal fluid stage, described above. (See Figure 2. Note: For clarity, lower case denotes no soil in this mass, upper case and solid blocks denote soil is contained in the mixture.)

Derivation of the formula:

As stated, M_T , M_R , and M_{SGJ} are straightforward measurements. The intestinal fluid component, M_{IJ} , is not a directly quantifiable parameter. It must be determined by subtracting the mass at the beginning of the phase from the mass at the end of the phase. $M_{IJ} = [\text{Mass at end of intestinal phase} - \text{Mass at beginning of intestinal phase}]$. Mass at end of intestinal phase is measured as M_{IJs} , while the mass at the beginning of the intestinal phase is the difference between the metal concentrations of the gastric fluid and that of the intestinal fluid. The formula (See Figure 3.) for the beginning of the phase is expressed as:

$$\begin{aligned} M_{IJ(\text{beginning})} &= M_{SGJ} - (M_{sgj} - M_{ij}) \quad (\text{original mass minus precipitated mass}) \text{ while} \\ M_{IJ(\text{end})} &= M_{IJs}, \text{ and therefore,} \\ M_{IJ} &= M_{IJs} - [M_{SGJ} - (M_{sgj} - M_{ij})]. \end{aligned}$$

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

$$\begin{aligned} M_T &= M_{SGJ} + M_{IJs} - [M_{SGJ} - (M_{sgj} - M_{ij})] + M_R, \text{ which collapses to:} \\ M_T &= M_{SGJ} + M_{IJs} - M_{ij} + M_R. \end{aligned}$$

RESULTS:

The soil is mixed with saliva/gastric juice, SGJ, and is followed by separation of soil from that fluid, The next interaction, between this fluid, SGJ, (Note: $SGJ = sgj$ and $M_{sgj} = M_{SGJ}$.) and the saliva/gastric/intestinal fluid, ij, can yield a metal mass that can be equal or less than M_{sgj} as no soil is present to release more metal mass into the solution. A value for M_{ij} , therefore, that is less than M_{sgj} is indicative of intestinal fluid precipitating heavy metals. $(M_{ij} - M_{sgj})$ can be less than or equal to $0 \mu g$, and equals the soluble mass in the intestinal fluid mixture with no effect from the soil. Montana Soil and Columbia Soil extractions have shown that lead precipitates in the gastric/intestinal fluid mixture: $(M_{ij} - M_{sgj}) < 0 \mu g$.

That step, however, does not include consideration of the interaction between soil and the fluid mixture. When the fluid mixture is recombined with the free soil, it is as

though no separation step had occurred; an additional 2 hours of mixing is undertaken to simulate intestinal absorption in the presence of soil. An aliquot from this step is removed and analyzed for metal mass, M_{IJS} . The soil contribution to metal solubility is determined by the subtraction of the “fluids only” component from the “fluids with soil” mixture: $(M_{IJS} - M_{ij})$. For those two soil extractions it was also seen that M_{IJS} was not different than M_{ij} , indicating that there was no mass of lead extracted by the intestinal fluid mixture alone.

Comparing the M_T data obtained by Method 3051 (reported as 100%, the reference value for M_T) to the mass summated by the extracted fractions of the mass-balance, the overall mass recoveries for lead were calculated with Columbia Soil $67\% \pm 20\%$ of M_T , and Montana Soil lead recovery 71% (See Figure 4.).

The concentration of arsenic in the residential soil gathered from under the treated deck was 165 ppm. Precipitation was found to occur with addition of intestinal fluid to the gastric juice; $(M_{ij} - M_{sgj}) < 0 \mu g$. Arsenic also appeared to be extracted from the soil by the intestinal fluid. The mass balance of arsenic in this soil was determined; compared to total mass of metal in soil, $82\% \pm 16\%$ of the arsenic was retrieved.

For chromium mass balance determination in the Jersey City soil using the same protocol, the recovered mass was $86\% \pm 31\%$. (See Figure 4.)

The separation of the intestinal extraction into components indicates that lead is not being extracted into the intestinal fluid for Montana and Columbia Soils. The bicarbonate may be readily precipitating lead. Arsenic in the residential soil appears to be further extractable in the intestinal fluid stage, IJs. This latter finding indicates that intestinal fluid extractability may be needed to be considered in all bioavailability experiments for metals in uncharacterized soils.

Due to difficulties including protocol implementation and the potential lack of homogeneity of the soils, the variation in the mass-balance recovery is not surprising.

Part 2 Bioaccessible Fraction:

METHODS:

Using the information from the Mass-Balance experiment in Part 1. The bioaccessible mass of metal is determined according to the following formula (Figure 5.):

$$M_{\text{bioaccessible}} = M_b = M_{\text{SGJ}} + M_{\text{IJ}}, \text{ where:}$$

$M_{\text{bioaccessible}}$ = mass of metal soluble in artificial human gastrointestinal tract,

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

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

It follows that:

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

$$M_{\text{bioaccessible}} = M_b = M_{\text{SGJ}} + M_{\text{IJs}} - M_{\text{ij}}$$

RESULTS:

If the mass bioaccessible is calculated using the formula in Part 2, the mass of the total metal is then $M_T = M_{\text{SGJ}} + (M_{\text{IJs}} - M_{\text{ij}}) + M_R$. By dividing $M_{\text{bioaccessible}}$ by this newly calculated M_T , rather than Method 3051, the bioaccessibility can now be recalculated, based only on the values obtained from the bioaccessibility protocol (See Figure 6.).

For lead in Montana Soil, this calculation yields a bioaccessibility of 69%, while Columbia Soil has 70% $\pm$ 11%. Arsenic in residential soil showed a bioaccessibility of 89% $\pm$ 23%. The chromium in the Jersey City soil revealed a bioaccessibility of 34% $\pm$ 5 (See Figure 7.).

The use of this method to calculate the total mass appears to be feasible. It compares well to Method 3051 for the arsenic and chromium soils and for lead in Montana Soil.

The method for detecting the soluble metal has been developed and expresses the maximal amount of metal that is extracted into the artificial body fluids. The results are

not surprising for the arsenic in residential soil, since the metal was delivered to the soil because of its ease of solubility in rainwater. It is expected to be quite extractable in an aqueous solution and thus highly bioaccessible. The chromium in Jersey City Soil, in contrast, is not likely to be bioaccessible, as the soil is slag in which rigorous industrial extraction of heavy metals has already been performed. Remaining chromium is likely to be tightly bound to the soil, with weathering exposing any minor extractable fraction. Lead bioaccessibility for the Montana soil was 69%, due entirely to the artificial gastric fluid.

Further, the inaccessible amount, M_R , can be derived from the mass balance equation:

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

by subtracting the bioaccessible fraction from the total:

$$M_R = M_T - (M_{SGJ} + M_{IJ}),$$

and may be an adequate replacement for the measurement of the individual fluid components.

Comparison to Columbia University Human Data

The Columbia Soil gives a bioaccessibility of 70%. This is therefore a useful method of metal determination, compared to the extra acid extraction, Method 3051, for calculation, which produced 72% bioaccessibility.

The Columbia School of Public Health study measured human absorption following ingestion of a lead contaminated soil, from the Bunker Hill Idaho Superfund site, and found blood lead levels of $26.2 \% \pm 8.1$. When we compared this to the bioaccessible percent from our sequential extraction of the Columbia Soil, our solubility was approximately 40% higher. It is expected that the bioaccessible fraction will be higher than the absorbed dose, so this is not surprising. The only human data we have, however, are on this Superfund soil for the metal lead, and a trend cannot be ascertained at this time.

The mass balance protocol will require further validation, however, undigested and precipitated amount of metal, M_R , in soil may represent the inaccessible amount. Eliminating analyses of each fluid suspensions, and, just determining percent mass recovered, M_R and the M_T by Method 3051 would be less time consuming appears to offer a sample technique for estimating bioaccessibility.

The stepwise mass-balance experiment will be performed on soil studied in year 02, ICP-MS will be operated with an internal standard to minimize analytical error.

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METHOD APPLICATION FOR “IN VITRO” EXPERIMENTS

INTRODUCTION:

One of the major exposure pathways for heavy metals to the general public is through the incidental ingestion of soil. This is of special concern for children due to their increased hand to mouth activity. Currently, a microwave digestion process involving concentrated nitric acid and high pressure, entitled Protocol 3051¹, is used as a driving force for remediation measures. It has been found, however, that one cannot assume the total metal in the soil will become a biologically effective dose in the receptor. The availability of metals to the target organ is a function of many factors, including the way in which the contaminant is held within the soil matrix and the source of the contaminant. The obvious consideration in the case of incidental soil ingestion is the way in which the human gastrointestinal system treats a contaminant.

These concerns have driven the development of a bioaccessibility assay which introduces a soil sample to an environment closer to that of the human gastro-intestinal system. The assay was developed by Stephanie Hamel² as a modified version of the Pharmacopoeia method³ for drug bioaccessibility. This method involves a sequential extraction process that includes the addition of synthetic saliva, gastric fluid, and intestinal fluid to a soil sample.

Initially, the bioaccessibility assay was also performed on a soil collected from around an apartment building in New Brunswick, New Jersey. The soils were from the soils laboratory of Dr. H. Motto at Rutgers University, and were run as a blind experiment to test a soil treatment method. Dr. Motto is interested in the procedure being developed in our research, and may incorporate the analysis into a general soil characterization protocol for the State of New Jersey. The bioaccessibility procedure is attractive as a means of correlating soil characteristics (pH, organic content, texture) with the relative bioaccessibility value.

As previously discussed, Liberty State Park was sampled in order to obtain a metal laden environmental soil. These samples were found through ICP/MS analysis to have

quite high heavy metal levels. The question as to their bioaccessibility is important due to the numerous sources of contamination around this area. This region has a history of heavy industry as well as the storage of dredge materials within the park itself. This area, therefore is in question as to the possible health risks stemming from exposure of visitors to these contaminants.

In addition to the investigation of various soil types, a study was performed in order to understand the influence of filtering on the bioaccessibility data. This became a part of the protocol to ensure clear ICP/MS results. The correct level of filtration is considerable because of the mechanism of transfer over the intestinal brush border. Another aspect which was studied more closely was the question of particle size. This is of importance, because the smaller particle sizes would be more likely to be redistributed, or mobilized in the digestive system.

Finally, a study was performed to determine the contribution of water to bioaccessibility. This experiment was carried out as a clarification of the effect of water and time on the availability of metals from within a soil matrix.

METHODS:

Liberty State Park of Jersey City, New Jersey was sampled in 5 different sites at an approximate depth of 6". This was chosen as a test soil because of the restrictive arrangements required for obtaining a soil from the Savannah River Site in South Carolina. Soils from Liberty State Park were analyzed by the soil analysis laboratory at Rutgers University for metal content, organic content, and pH. Soil sample #1 was chosen, because it had the lowest iron levels and percentage of organic material. Iron content was used as a deciding factor because this element can cause interferences with ICP/MS analysis. Low organic matter was desirable, because dredge material was a possible constituent of the soils in this area. Sample #1 was chosen, therefore because it had the closest characteristic to a true soil. Sample #1 was then air dried, sieved to $<75\ \mu\text{m}$ and between $75\ \mu\text{m}$ - $500\ \mu\text{m}$, and stored in a dark location. The New Brunswick soils were processed as received, with no prior knowledge of their characteristics.

In order to obtain a total metal value for these soil samples a microwave digestion was performed, using protocol SW846-3051. As stated in the E.P.A. protocol, 10 ml of Fisher high purity concentrated nitric acid was added to approximately 0.05g of each soil sample, in triplicate. The samples were enclosed in the vessels and processed in a CEM MIX-200 microwave at 60 PSI and 100% power for a total of 10 minutes, with 5 minutes and 30 seconds time at pressure. In preparation for ICP/MS analysis, the samples were diluted 1 ml into 10 and 1 ml into 100 ml, in 2% Fisher high purity nitric acid and deionized water. At this point the samples were analyzed with a Fisons ICP/MS for arsenic, cadmium, lead, chromium, mercury, nickel, uranium, and cesium.

Following the total metal analysis, a bioaccessibility assay was performed on the soil samples. First, the synthetic biofluids were prepared for the extraction. The saliva was prepared by adding 0.8 g $\text{CaCl}_2 \cdot 4\text{H}_2\text{O}$, 1g Urea, 0.6g Na_2HPO_4 , 0.4g KCl , 0.4g NaCl , and 4 g Mucin and diluted to 1 liter with deionized water. The synthetic gastric fluid was made by adding 7 mL HCl , 2g NaCl , 3.2g pepsin, and diluted to 1 liter. The pepsin was added directly before use. Approximately 50 mg of each soil sample was measured in triplicate into Nalgene 250 mL bottles. Into the bottles, 8mL of synthetic saliva was added, as well as 100mL of synthetic gastric fluid. These samples were then shaken in a hot water, bath set at 37°C for 2 hours. An aliquot was taken from these samples of approximately 12mL. After this, 100mL of synthetic intestinal fluid, 0.2M sodium bicarbonate solution, was added to the saliva, gastric fluid, and intestinal fluid. The saliva, gastric fluid, intestinal fluid were shaken with the soil for two hours in a water bath at 37°C for two hours. Finally, an aliquot of 12 mL was taken from the saliva/ gastric/ intestinal samples.

The preparation of the bioaccessibility samples includes centrifugation, microwave digestion, and filtration. Both the saliva/gastric and the saliva/gastric/ intestinal fluids were centrifuged at 3400 rpm for 20 minutes. In order to increase the shelf life of the samples, the bioaccessibility samples were then processed by way of a microwave digestion. The volume of 9.5 mL of the supernatant of each sample was extracted and added to 0.5 mL of Fisher high purity nitric acid. These samples were digested in closed

vessels. A ramped digestion protocol was used which had the following specifications; 40% power for 5 minutes followed by 20 minutes at 0 power. The sequence was then repeated two times.

Before diluting these samples the digested analyte was filtered through a Whatman Puradisc 25 TF 0.45 μm syringe filter. In the case of the Liberty State Park samples and the New Brunswick soils, aliquots were run without first filtering the samples. All samples were diluted so that ICP values would fall between 1 ppb and 100 ppb with Fisher high purity nitric acid and analyzed using a Fisons ICP/MS for metals analysis.

Bioaccessibility was calculated by dividing the average concentration found in synthetic biofluid by the average total metal found by the 3051 protocol. Standard deviations were calculated using propagational random error calculations. T-tests were performed on the log-transformed Liberty State Park data set using SPSS for Microsoft Windows.

Quality control measures involved the use of NIST Buffalo River 2704 SRM material, NIST Montana 2710 SRM material, NIST 1643d element in water. Two NIST quality control checks were run on the ICP/MS after every 8-10 samples. All reported data were run before a NIST standard (all previously mentioned) that fell within a 20% recovery limit in the ICP/MS analysis procedure.

An experiment was run with a protocol similar to that of the bioaccessibility assay, wherein deionized water was used as the only solvent. Approximately the same amount of fluid was added to approximately 0.05 g of NIST Montana SRM 2710, 108 ml, as would be in the bioaccessibility assay. This was allowed to shake at 37°C for two hours. At the end of this time period a 12ml aliquot was taken from each sample, and another 100 mL of deionized water was applied to the remaining suspension. This was also set to shake at 37°C for two hours. At the end of this sequence these samples were treated as a normal bioaccessibility assay wherein they were centrifuged, digested, and then filtered before analysis on ICP/MS.

RESULTS:**Liberty State Park Soil Results**

Particle Size <75 μm :

Total metal and bioaccessibility analyses were performed on the Liberty State Park soil sample #1, which had been sieved to <75 μm . The bioaccessibility samples were analyzed on the ICP/MS without prior filtration. Results from these analyses are presented in Table 1.

Table 1: Liberty State Park Soils analyzed without filtration (particle fraction <75 μm)

Element	Total Metal ($\mu\text{g/g}$)	Saliva/Gastric	Saliva/Gastric/Intestinal
		Bioaccessibility	Bioaccessibility
As	261 $\pm$ 33	47% $\pm$ 19%	43% $\pm$ 23%
Cr	15782 $\pm$ 1487	6% $\pm$ 2%	1 $\pm$ 0.4%
Pb	1154 $\pm$ 292	48% $\pm$ 11%	29% $\pm$ 16%

The above analysis was followed by an experiment which compared the bioaccessibility of filtered and unfiltered samples, within the same particle size of <75 μm . This filtration comparison experiment (Table 2a) was replicated, and these results are reported in Table 2.b

Table 2a: Filtration comparison of Liberty State Park Soils (particle fraction <75 μm)

	Total Metal ($\mu\text{g/g}$)	Saliva/Gastric Bioavailability		Saliva/Gastric/Intestinal Bioavailability	
		filtered	unfiltered	filtered	unfiltered
As	327 $\pm$ 60	48 $\pm$ 7%	42 $\pm$ 9%	48 $\pm$ 10%	37 $\pm$ 7%
Cr	5329	10 $\pm$ 3%	33 $\pm$ 7%	20 $\pm$ 6%	3 $\pm$ 0.6%
Pb	2263 $\pm$ 295	117 $\pm$ 20%	97 $\pm$ 14%	38 $\pm$ 7%	3 $\pm$ 0.5%

Table 2b: Replication of the Filtration Comparison of Liberty State Park Soils (particle size <75 μm)

	Saliva/Gastric		Saliva/Gastric/Intestinal	
	filtered	unfiltered	filtered	unfiltered
As	52 $\pm$ 9.2%	50 $\pm$ 11%	58 $\pm$ 8.6%	65 $\pm$ 9.4%
Cr	14 $\pm$ 1.2%	19 $\pm$ 8%	2 $\pm$ 0.2%	2 $\pm$ 0.1%
Pb	117 $\pm$ 29%	121 $\pm$ 43%	121 $\pm$ 111%	9 $\pm$ 7%

The bioaccessibility values from Tables 2a and 2b are referred to average total metal values, which are reported in Table 2a. These total metal values of Liberty State Park soil are a result of two digestion and analysis procedures, and are reported as being 100%. One digestion was performed in our laboratory by protocol 3051, while analysis was completed using a Fisons ICP/MS. The other procedure was run in the Environmental Sciences Department of Rutgers University, where the final analysis was performed by atomic absorption spectroscopy.

The two data sets of filtered samples at particle size <75 μm from the Liberty State Park soil and the three unfiltered data sets were compiled (Tables 1, 2a, and 2b). Presented below is the compilation of three separate experiments, comparing three metals, affects of filtration, and results from both the saliva/gastric and saliva/gastric/intestinal biofluid systems.

Table 3: Compilation of the Bioaccessibility Results for Particle Size <75 μm

	Total Metals	Saliva/Gastric		Saliva/Gastric/Intestinal	
		filtered, N=2	unfiltered, N=3	filtered, N=3	unfiltered, N=2
As	310 $\pm$ 78	59.2 $\pm$ 30.1%	76.7 $\pm$ 35.6%	80.8 $\pm$ 57.6%	93.6 $\pm$ 49.3%
Cr	11227 $\pm$ 6018	9.03 $\pm$ 5.06%	9.07 $\pm$ 4.94%	1.76 $\pm$ 1.61%	2.14 $\pm$ 1.9%
Pb	2688 $\pm$ 413	83.64 $\pm$ 42.9%	76.28 $\pm$ 67.7%	67.7 $\pm$ 54.5%	8.06 $\pm$ 8.16%

The values presented in Table 3 were calculated using the original data from Tables 1, 2a, and 2b. The concentrations for each element were averaged, and the random propogational error was calculated. The total metal values analyzed by atomic absorption were not used in this compilation in order to compare gastric fluid extractions analyzed by ICP/MS with total metal digestions analyzed by ICP/MS.

Particle Size 75-500 μm :

This same sequence of experiments and analyses was then performed on the larger particle size fraction (75-500 μm). Two experiments are presented in table 4a and 4b. Table 4a is a report of the bioaccessibility assay performed on the particle size 75-500 μm , without filtering the samples before analysis. Table 4b is a report of a comparison of the affect of filtration on bioaccessibility within the particle size 75-500 μm .

Table 4a: Bioaccessibility of Liberty State Park Soils of Particle Fraction 75-500 μm

	Total Metal ($\mu\text{g/g}$)	Saliva/Gastric Bioavailability	Saliva/Gastric/Intestinal Bioavailability
		unfiltered	unfiltered
As	378 $\pm$ 81	22 $\pm$ 9%	15 $\pm$ 4%
Cr	4396 $\pm$ 1114	13 $\pm$ 4%	1 $\pm$ 0.4%
Pb	3092 $\pm$ 163	36 $\pm$ 8%	1 $\pm$ 0.2%

Table 4b: Comparison of Filtration of Liberty State Park Soils (Particle Fraction 75-500 μm)

	Saliva/Gastric		Saliva/Gastric/Intestinal	
	filtered	unfiltered	filtered	unfiltered
As	29 $\pm$ 13%	21 $\pm$ 4.6%	28 $\pm$ 6.2%	25 $\pm$ 5.6%
Cr	23 $\pm$ 8%	24 $\pm$ 17%	4 $\pm$ 1%	4 $\pm$ 1%
Pb	79 $\pm$ 60%	32 $\pm$ 18%	46 $\pm$ 34%	2 $\pm$ 0.2%

The concentrations from each element of the values presented in Tables 4a and 4b were averaged, and the random propogational error calculated. The compilation of saliva/gastric, saliva/gastric/intestinal, and total metal values are reported in Table 5.

Table 5: Compilation of Bioaccessibility Results for Particle Fraction 75-500 μm

	Total Metal ($\mu\text{g/g}$)	Saliva/Gastric		Saliva/Gastric/Intestinal	
		filtered	unfiltered	filtered	unfiltered
As	267 $\pm$ 60	38 $\pm$ 29%	49 $\pm$ 30%	2.0 $\pm$ 2.4%	75 $\pm$ 53%
Cr	2515 $\pm$ 598	1.8 $\pm$ 1.8%	5.3 $\pm$ 2.8%	2.0 $\pm$ 2.4%	1.6 $\pm$ 1.7%
Pb	2144 $\pm$ 295	91 $\pm$ 58%	43 $\pm$ 11%	39 $\pm$ 33%	6.3 $\pm$ 6.9%

New Brunswick Soil

As stated previously, an experiment was performed with a New Brunswick, NJ soil from the laboratory of Dr. H. Motto. The results are presented below. Total metals, bioaccessibility, and an experiment comparing filtered and unfiltered samples are presented below. All of the bioaccessible values are based on the total metal amounts presented in Table 6.

Table 6: Bioaccessibility of New Brunswick, NJ Soils

Soil Name	AHT		AHC	
Total Lead	4953±187		4815±227	
	filtered	unfiltered	filtered	unfiltered
Saliva/Gastric	34±7%	19±3%	96±11%	74±16%
Saliva/Gastric/ Intestinal	19±4%	15±2%	68±7%	63±6%

Water Contribution to Bioaccessibility in NIST Montana SRM 2710

Finally, an experiment was performed in order to understand the contribution of the water component of the bioaccessible assay to the extraction of metals from a soil matrix. The bioaccessible values reported in Table 7 were based on the NIST certified metal concentrations for the SRM Montana 2710 material. The column identified Saliva/Gastric refers to the two hour extraction with water which was described in the Methods section. The column identified as Saliva/Gastric/Intestinal refers to the four hour water extraction explained in the Methods section. These results are compared to an average of the values previously obtained from the bioaccessibility assay.

Table 7: Comparison of Water Availability and Bioaccessibility in SRM Montana 2710

Element	Saliva/Gastric		Saliva/Gastric/Intestinal	
	water extraction	bioaccessibility assay	water extraction	bioaccessibility assay
As	4.0±1.1%	57%	7.0±1.2%	55%
Cr	7.0±1.2%	36%	13±3.6%	41%
Pb	4.0±1.5%	78%	6.0±0.4%	64%

DISCUSSION:

Statistical analyses were performed on the results from the Liberty State Park soil in order to answer the following questions: 1) Is there a difference between samples that have been filtered and samples that have been analyzed without filtering? 2) Is there a difference between the bioaccessibility in the soil sample of particle size $<75\ \mu\text{m}$ and $75\text{--}500\ \mu\text{m}$, and finally 3) Is there a difference between the availability of metals within the saliva/gastric and the saliva/gastric/intestinal biofluid systems? Initially the Liberty State Park data set was tested for normality, and was found to be lognormal. The data was then log-transformed and t-tests on the transformed data using Windows SPSS.

A significant difference was found between the bioaccessibility associated with filtered samples and unfiltered samples ($t\text{-value}=2.31$). The average metal concentration was higher for filtered samples than unfiltered samples. The lower ICP values found in unfiltered samples probably stem from a poor matrix match in the samples with respect to the calibration standards which are prepared in 2% acid. Differences in acid concentration and type can lead to differences in droplet size and transport properties within the ICP. Another problem with suspended solids in ICP/MS analysis is the deposition of solids onto the sampling orifices and ion optics of the mass spectrometer. The deposition of solids on these areas can result in a change in the sampling orifice⁴. Due to these types of analytical interferences, filtering at the $0.45\ \mu\text{m}$ size fraction was incorporated into our bioaccessibility procedure. This size fraction was chosen initially because of its use in the field of trace metal analysis for water, since it is the established operational definition of a soluble substance?

The question of filtering bioaccessibility samples has more than analytical significance. Intestinal absorption occurs through two possible pathways: specific and non-specific. Specific absorption can occur, for example, by the binding of a protein to a metal species in order to carry it across the brush border. The non-specific pathway depends on the fluidity of three different junctions where absorption could occur: the tight junction ($0.1\text{--}0.2\ \mu\text{m}$), the intermediate junction ($10\ \mu\text{m}$ long and $20\ \mu\text{m}$ wide), and the basolateral region which is longer and wider than the two previous junctions. Any non-

specific absorption, therefore, would need to pass through a junction of no less than approximately 0.1 μm in diameter. Below 0.2 μm is considered sterile, and furthermore it is not a physiological constant. Many compounds including citrate have the ability to open tight junctions to increase absorption⁶, and therefore filtration at 0.2 μm may result in an underestimation of bioaccessible fractions. In conclusion, the level of filtration has been limited to 0.45 μm as particles larger than this would be limited by the permeability.

Particle size of the soil sample was found to be a significant factor in bioaccessibility (t-value 2.83). The smaller particle size (<75 μm) was found to have a higher overall bioaccessibility value than the larger particle size fraction (75-500 μm). This may stem from the increased surface area to volume ratio of the smaller particle size, which would result in a larger number of metals easily available for dissolution. The larger particle fraction also may bias results due to large particle bulk, with very little metal concentration.

Within the Liberty State Park results, a significant difference was found between the saliva/gastric system and the saliva/gastric/intestinal biofluid systems for the cumulated data set. When the data was analyzed separately for each metal, no difference was found between the saliva/gastric system and the saliva/gastric/intestinal system for arsenic. A difference was found, however, between the lead and chromium extracted by the saliva/gastric and saliva/gastric/intestinal biofluid systems.

The most obvious component of the Liberty State Park data was the variability within the results. The variability between samples that were run in parallel was not great, however it was very large between replications. The variance, therefore may result largely from the inherent heterogeneity in environmental samples. This variability could be decreased by two measures: 1) homogenization of samples; and 2) comparison of the bioaccessibility value within the same soil sample. The first measure is not necessarily possible in all areas with metal laden soils. If homogenization is carried out possible hot spots could remain unidentified. The second approach, therefore may be more reasonable.

The variability within the Liberty State Park environmental samples becomes apparent when compared to the results from the soils collected around an apartment

building in New Brunswick, New Jersey. The results from the filtration experiment correspond to the results found in the Liberty State Park soils as well. Within this experiment it is also apparent that the filtering of samples affects results wherein unfiltered samples produced lower values on the ICP/MS.

The Montana SRM 2710 soil was run with DI-water under the same conditions as the bioaccessibility assay (table 7). The water availability was similar for lead, arsenic, and chromium ranging from 11% for chromium to approximately 5% for lead and arsenic. In comparison, the bioaccessibility ranges from approximately 70% for lead to 40% for chromium. This presents an interesting situation for chromium, being the most available in water and the least in the bioaccessibility assay. The results indicate there is a portion of readily available chromium in this reference material, whereas the remainder is relatively tightly bound. Time and water alone do contribute to the leaching of metals from a soil matrix. Other factors represented in total bioaccessibility values namely pH, ionic species such as sodium, and interactions therein present a conservative picture of the potential dose due to soil ingestion.

The technique will now be employed using filtering of the sample, and this will be applied on the SRS samples during the next quarter. The techniques have now been optimized for use in conjunction with the mass balance experiments and the “in vivo” experiments. During the next quarter the results of the “in vitro” experiments for the Jersey City Soils will be compared with the results that were recently obtained for bioavailability as the same soil using an “in vivo” assay. These results are described in the next section.

BIOAVAILABILITY: “IN VIVO” STUDIES

RATIONALE:

The purpose of this study is to develop a reliable method to quantify the levels of various metals in the tissues of rats orally exposed to contaminated soils. Our approach is to use readily available soils to develop an *in vivo* system and, thereby minimize the amount of test soils needed. The Liberty State Park soil used in this first series of experiments is available in sufficient quantities and contains contaminants at levels which are easily measured.

METHODS:

Soil Preparation: the soil was obtained from Liberty State Park, Jersey City, NJ and was characterized in a previous section of the report. One sample was chosen for further study and was sieved to $<75\ \mu\text{m}$ and dried, and a summary of metal analysis of this soil is shown in Table 1. One gram of this soil was suspended in 5 ml of an aqueous solution which contained 5% gum arabic to help maintain a suspension. The suspension was stored at room temperature in a dark container until needed.

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 and their excrement, that was collected during the previous 24 hours, was discarded. 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 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 HNO_3 . Tissues were allowed to digest at 70° C until clear. Two milliliters of 50% H_2O_2 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.

Analysis of Metals: Samples were analyzed by ICP-MS at EOHSI's Chemical Analysis Facility, as described in the previous sections of this report. All samples were diluted to <5% acid content.

Results:

The results of the tissue analyses are shown in tables 2-16. Every group of 5 tables represents one metal. The first and second columns describe the animal and tissue that was used. Columns three and four are taken from the ICP-MS analysis and represent the level of a given metal in the sample and blank respectively. Column five is the corrected amount of metal in the target tissue. Column six shows the final volume of digested tissue which is 25 ml for all tissues, except hair which was concentrated into 10 ml. Column seven represents the dilution factor used to reduce the amount of acid in the sample to less than 5%. Column eight is the weight or volume of the sample that was digested. Column nine shows the total weight or volume of the tissue if only an aliquot of the tissue was used. It was assumed that there is 7 ml of blood per 100 g of body weight in a normal rat. In addition, 4.3% of the body weight of a normal rat consists of bone. Column ten is the amount of metal that was measured in the tissue, corrected for the weight of the digested tissue. Column eleven is the calculated amount of metal found in the entire tissue sample. The percent administered dose is obtained by dividing the amount of metal in the whole tissue by the dose given. Percent Bioavailability is calculated by adding the percent administered dose from every tissue, except urine and feces, and multiplying by 100.

Table 1
Levels of Selected Metals Found in Liberty State Park Soil

Metal	Content (ppm)
Cr	11,000 ± 1,915
Pb	1,640± 180
As	260 ± 20

Table 2

Liberty State Park Soil Study Vehicle Control Animals-Cr

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CHROMIUM-CONTROL										
ANIMAL	TISSUE	ppb	mean blank	ppb-blank	qs	DF	sample wt	organ wt	ug metal/ g organ	ug metal/ total tissue
#		(ug/L)	(ug/L)	(ug/L)	(ml)		(gm)	(gm)		
1	BLOOD	0.55	0	0.55	25	42	6.9	18.48	0.08	1.55
2		0.19	0	0.19	25	33	2.8	17.99	0.06	1.01
3		0.95	0	0.95	25	33	7.6	17.5	0.10	1.80
1	LUNG	6.1	1.2	4.9	25	2	1.35		0.18	0.25
2		5.2	1.2	4	25	2	1.34		0.15	0.20
3		9.4	1.2	8.2	25	2	1.27		0.32	0.41
1	LIVER	12.4	1.2	11.2	25	2	2.27	10.28	0.25	2.54
2		11.5	1.2	10.3	25	2	2.42	8.96	0.21	1.91
3		23.5	1.2	22.3	25	2	2.25	6.85	0.50	3.39
1	KIDNEYS	7.8	1.2	6.6	25	2	2.33		0.14	0.33
2		9.1	1.2	7.9	25	2	2		0.20	0.40
3		18	1.2	16.8	25	2	2.1		0.40	0.84
1	SPLEEN	2.9	1.2	1.7	25	2	0.72		0.12	0.09
2		3.8	1.2	2.6	25	2	0.79		0.16	0.13
3		10.2	1.2	9	25	2	0.58		0.78	0.45
1	TESTES	6.4	1.2	5.2	25	2	2.66		0.10	0.26
2		8.2	1.2	7	25	2	3.34		0.10	0.35
3		8.2	1.2	7	25	2	2.83		0.12	0.35
1	HEART	3.7	1.2	2.5	25	2	0.99		0.13	0.13
2		4.2	1.2	3	25	2	0.9		0.17	0.15
3		5.7	1.2	4.5	25	2	0.81		0.28	0.23
1	BRAIN	8.9	1.2	7.7	25	2	1.59		0.24	0.39
2		8.7	1.2	7.5	25	2	1.64		0.23	0.38
3		9.1	1.2	7.9	25	2	1.72		0.23	0.40
1	HAIR	0.66	0.19	0.47	10	4	0.09		0.21	0.02
2		0.53	0.19	0.34	10	4	0.07		0.19	0.01
3		0.29	0.19	0.1	10	4	0.09		0.04	0.00
1	MUSCLE	13.5	1.2	12.3	25	2	2.7		0.23	0.62
2		33.1	1.2	31.9	25	2	3.42		0.47	1.60
3		48.6	1.2	47.4	25	2	3.85		0.62	2.37
1	URINE	75.7	1.2	74.5	25	2	5	27	0.75	20.12
2		77.1	1.2	75.9	25	2	5	34	0.76	25.81
3		73.8	1.2	72.6	25	2	5	29	0.73	21.05
1	FEMURS	0.2	0	0.2	25	33	0.236	11.352	0.70	7.94
2		0.24	0	0.24	25	33	0.212	11.051	0.93	10.32
3		0.41	0	0.41	25	33	0.154	10.75	2.20	23.61

Animal #	final BW (g)	Cr Dose (ug)
1	264	0
2	257	0
3	250	0

Total blood volume= 7 mls/100 g BW
Total bone weight= 4.3% BW

Table 3 Liberty State Park Soil Study 1 Day Exposure-Cr

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CHROMIUM-1-DAY											
SAMPLE	TISSUE	ppb (ug/L)	mean blank (ug/L)	ppb-blank (ug/L)	qs (ml)	DF	sample wt (gm)	organ wt (gm)	ug metal/ g organ	ug metal/ total tissue	% admin dose
1	BLOOD	0.71	0	0.71	25	33	6.2	18.41	0.09	1.74	0.014
2		0.82	0	0.82	25	33	6.4	18.55	0.11	1.96	0.016
3		0.15	0	0.15	25	33	6	18.13	0.02	0.37	0.003
1	LUNG	63.7	0.32	63.38	25	2	1.5		2.11	3.17	0.025
2		9.8	0.32	9.48	25	2	1.17		0.41	0.47	0.004
3		50.2	0.32	49.88	25	2	1.28		1.95	2.49	0.020
1	LIVER	66	0.32	65.68	25	2	3.92	11.87	0.84	9.94	0.079
2		34.9	0.32	34.58	25	2	2.93	12.18	0.59	7.19	0.057
3		30.2	0.32	29.88	25	2	3.24	12.5	0.46	5.76	0.046
1	KIDNEYS	7.6	0.32	7.28	25	2	1.92		0.19	0.36	0.003
2		6.3	0.32	5.98	25	2	2.05		0.15	0.30	0.002
3		11	0.32	10.68	25	2	2.24		0.24	0.53	0.004
1	SPLEEN	36.1	0.32	35.78	25	2	0.66		2.71	1.79	0.014
2		18.1	0.32	17.78	25	2	0.77		1.15	0.89	0.007
3		4.2	0.32	3.88	25	2	0.54		0.36	0.19	0.002
1	TESTES	24.6	0.32	24.28	25	2	3.56		0.34	1.21	0.010
2		25.3	0.32	24.98	25	2	2.8		0.45	1.25	0.010
3		8.4	0.32	8.08	25	2	2.65		0.15	0.40	0.003
1	HEART	13	0.32	12.68	25	2	0.75		0.85	0.63	0.005
2		6.3	0.32	5.98	25	2	0.85		0.35	0.30	0.002
3		4.2	0.32	3.88	25	2	0.76		0.26	0.19	0.002
1	BRAIN	103.1	0.32	102.78	25	2	1.63		3.15	5.14	0.041
2		9.3	0.32	8.98	25	2	1.75		0.26	0.45	0.004
3		15.2	0.32	14.88	25	2	1.72		0.43	0.74	0.006
1	HAIR	12.6	1.3	11.3	10	4	0.08		5.65	0.45	0.004
2		21.2	1.5	19.7	10	4	0.09		8.76	0.79	0.006
3		9.7	1.5	8.2	10	4	0.09		3.64	0.33	0.003
1	MUSCLE	45.5	0.32	45.18	25	2	3.95		0.57	2.26	0.018
2		15	0.32	14.68	25	2	3.84		0.19	0.73	0.006
3		14.7	0.32	14.38	25	2	1.88		0.38	0.72	0.006
1	URINE	14.2	0.32	13.88	25	2	5	10	0.14	1.39	0.011
2		18.9	0.32	18.58	25	2	5	11	0.19	2.04	0.016
3		23.5	0.32	23.18	25	2	5	5	0.23	1.16	0.009
1	FEMURS	0.01	0	0.01	25	33	0.147	11.309		0.63	0.005
2		0.14	0	0.14	25	33	0.208	11.395		6.33	0.050
3		0.16	0	0.16	25	33	0.166	11.137		8.86	0.071

Animal #	final BW (g)	Cr Dose (ug)	Bioavailability (%)
1	263	12,540	0.22
2	265	12,540	0.16
3	259	12,540	0.16

Total blood volume= 7 mls/100 g BW
Total bone weight= 4.3% BW

Table 4   **Liberty State Park Soil Study**
2 Day Exposure-Cr

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CHROMIUM-2-DAY											
SAMPLE	TISSUE	ppb (ug/L)	mean blank (ug/L)	ppb-blank (ug/L)	qs (ml)	DF	sample wt (gm)	organ wt (gm)	ug metal/ g organ	ug metal/ total tissue	% admin dose
1	BLOOD	0.93	0	0.93	25	33	6	17.64	0.13	2.26	0.018
2		0.8	0	0.8	25	33	6.5	18.55	0.10	1.88	0.015
3		0.78	0	0.78	25	33	5.9	19.18	0.11	2.09	0.017
1	LUNG	7.3	1.3	6	25	2	1.28		0.23	0.30	0.002
2		17.7	1.3	16.4	25	2	1.39		0.59	0.82	0.007
3		9.7	1.3	8.4	25	2	1.42		0.30	0.42	0.003
1	LIVER	27.9	1.3	26.6	25	2	3.36	11.99	0.40	4.75	0.038
2		32.9	1.3	31.6	25	2	4.24	13.01	0.37	4.85	0.039
3		24.1	1.3	22.8	25	2	3.8	12.46	0.30	3.74	0.030
1	KIDNEYS	8.7	1.3	7.4	25	2	2.07		0.18	0.37	0.003
2		14.5	1.3	13.2	25	2	2.17		0.30	0.66	0.005
3		8.1	1.3	6.8	25	2	2.34		0.15	0.34	0.003
1	SPLEEN	6.2	1.3	4.9	25	2	0.61		0.40	0.25	0.002
2		9.7	1.3	8.4	25	2	0.63		0.67	0.42	0.003
3		4.9	1.3	3.6	25	2	0.64		0.28	0.18	0.001
1	TESTES	9.5	1.3	8.2	25	2	2.98		0.14	0.41	0.003
2		11.7	1.3	10.4	25	2	2.72		0.19	0.52	0.004
3		8.2	1.3	6.9	25	2	3.36		0.10	0.35	0.003
1	HEART	6	1.3	4.7	25	2	0.78		0.30	0.24	0.002
2		8.9	1.3	7.6	25	2	0.86		0.44	0.38	0.003
3		7.2	1.3	5.9	25	2	0.93		0.32	0.30	0.002
1	BRAIN	11.3	1.3	10	25	2	1.43		0.35	0.50	0.004
2		8.7	1.3	7.4	25	2	1.67		0.22	0.37	0.003
3		9.6	1.3	8.3	25	2	1.7		0.24	0.42	0.003
1	HAIR	12.7	1.3	11.4	10	4	0.07		6.51	0.46	0.004
2		13.3	1.3	12	10	4	0.11		4.36	0.48	0.004
3		13.7	1.3	12.4	10	4	0.09		5.51	0.50	0.004
1	MUSCLE	25.7	1.3	24.4	25	2	2.38		0.51	1.22	0.010
2		54.4	1.3	53.1	25	2	3.51		0.76	2.66	0.021
3		11.6	1.3	10.3	25	2	3.99		0.13	0.52	0.004
1	URINE	28.9	1.3	27.6	25	2	5	15	0.28	4.14	0.033
2		20.6	1.3	19.3	25	2	5	17	0.19	3.28	0.026
3		49.3	1.3	48	25	2	5	13	0.48	6.24	0.050
1	FEMURS	0.18	0	0.18	25	33	0.203	10.836		7.93	0.063
2		0	0	0	25	33	0.198	11.395		0.00	0.000
3		0.18	0	0.18	25	33	0.159	11.782		11.00	0.088

Animal #	final BW (g)	Cr Dose (ug)	Bioavailability (%)
1	252	11,726	0.15
2	265	11,880	0.10
3	274	12,606	0.14

Total blood volume= 7 mls/100 g BW
Total bone weight= 4.3% BW

Table 5   **Liberty State Park Soil Study**
3 Day Exposure-Cr

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CHROMIUM-3-DAY											
SAMPLE	TISSUE	ppb (ug/L)	mean blank (ug/L)	ppb-blank (ug/L)	qs (ml)	DF	sample wt (gm)	organ wt (gm)	ug metal/ g organ	ug metal/ total tissue	% admin dose
1	BLOOD	0.69	0	0.69	25	33	5.7	19.04	0.10	1.90	0.015
2		1.9	0	1.9	25	33	6	17.36	0.26	4.54	0.036
3		0.71	0	0.71	25	33	6	18.13	0.10	1.77	0.014
1	LUNG	13.4	1.53	11.87	25	2	1.28		0.46	0.59	0.005
2		15.2	1.53	13.67	25	2	1.14		0.60	0.68	0.005
3		18.9	1.53	17.37	25	2	1.46		0.59	0.87	0.007
1	LIVER	27.2	0.32	26.88	25	2	3.37	11.6	0.40	4.63	0.037
2		48.8	1.53	47.27	25	2	2.65	10.42	0.89	9.29	0.074
3		30.4	0.32	30.08	25	2	3.29	12.34	0.46	5.64	0.045
1	KIDNEYS	35.8	1.53	34.27	25	2	2.17		0.79	1.71	0.014
2		35.6	1.53	34.07	25	2	1.88		0.91	1.70	0.014
3		32.3	1.53	30.77	25	2	1.82		0.85	1.54	0.012
1	SPLEEN	11.3	1.53	9.77	25	2	0.76		0.64	0.49	0.004
2		18.5	1.53	16.97	25	2	0.58		1.46	0.85	0.007
3		25	1.53	23.47	25	2	0.78		1.50	1.17	0.009
1	TESTES	31.8	1.53	30.27	25	2	2.87		0.53	1.51	0.012
2		8.9	1.53	7.37	25	2	2.93		0.13	0.37	0.003
3		80.1	1.53	78.57	25	2	3.08		1.28	3.93	0.031
1	HEART	9.4	1.53	7.87	25	2	0.89		0.44	0.39	0.003
2		33.3	1.53	31.77	25	2	0.77		2.06	1.59	0.013
3		10.5	1.53	8.97	25	2	0.95		0.47	0.45	0.004
1	BRAIN	5.2	1.53	3.67	25	2	1.46		0.13	0.18	0.001
2		16.8	1.53	15.27	25	2	1.31		0.58	0.76	0.006
3		10.5	1.53	8.97	25	2	1.42		0.32	0.45	0.004
1	HAIR	0	0	0	10	4	0.06		0.00	0.00	0.000
2		0	0	0	10	4	0.05		0.00	0.00	0.000
3		0	0	0	10	4	0.06		0.00	0.00	0.000
1	MUSCLE	11.4	1.53	9.87	25	2	1.45		0.34	0.49	0.004
2		36.2	1.53	34.67	25	2	2.49		0.70	1.73	0.014
3		44.4	1.53	42.87	25	2	2.48		0.86	2.14	0.017
1	URINE	23.9	1.53	22.37	25	2	5	35	0.22	7.83	0.062
2		32.6	1.53	31.07	25	2	5	17	0.31	5.28	0.042
3		26.4	1.53	24.87	25	2	5	31	0.25	7.71	0.061
1	FEMURS	0.14	0	0.14	25	33	0.225	11.696		6.00	0.048
2		0.06	0	0.06	25	33	0.171	10.664		3.09	0.025
3		0.16	0	0.16	25	33	0.208	11.137		7.07	0.056

Animal #	final BW (g)	Cr Dose (ug)	Bioavailability (%)
1	272	12,540	0.14
2	248	11,550	0.20
3	259	11,990	0.19

Total blood volume= 7 mls/100 g BW
Total bone weight= 4.3% BW

Table 6 Liberty State Park Soil Study 4 Day Exposure-Cr

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CHROMIUM-4-DAY											
SAMPLE	TISSUE	ppb (ug/L)	mean blank (ug/L)	ppb-blank (ug/L)	qs (ml)	DF	sample wt (gm)	organ wt (gm)	ug metal/ g organ	ug metal/ total tissue	% admin dose
1	BLOOD	0.23	0	0.23	25	33	3.4	17.22	0.06	0.96	0.008
2		0.74	0	0.74	25	33	7.6	20.51	0.08	1.65	0.013
3		0.78	0	0.78	25	33	6.8	19.81	0.09	1.87	0.015
1	LUNG	3.7	1.5	2.2	25	2	1.34		0.08	0.11	0.001
2		16	1.5	14.5	25	2	1.48		0.49	0.73	0.006
3		6.2	1.5	4.7	25	2	1.26		0.19	0.24	0.002
1	LIVER	14.1	1.5	12.6	25	2	2.46	8.12	0.26	2.08	0.017
2		15.5	1.5	14	25	2	2.42	11.85	0.29	3.43	0.027
3		27.4	1.5	25.9	25	2	3.01	11.51	0.43	4.95	0.039
1	KIDNEYS	8.2	1.5	6.7	25	2	2.26		0.15	0.34	0.003
2		9.6	1.5	8.1	25	2	2.35		0.17	0.41	0.003
3		7.7	1.5	6.2	25	2	2.07		0.15	0.31	0.002
1	SPLEEN	3.9	1.5	2.4	25	2	0.49		0.24	0.12	0.001
2		4.8	1.5	3.3	25	2	0.81		0.20	0.17	0.001
3		5.3	1.5	3.8	25	2	0.74		0.26	0.19	0.002
1	TESTES	7.9	1.5	6.4	25	2	2.84		0.11	0.32	0.003
2		6.9	1.5	5.4	25	2	3.23		0.08	0.27	0.002
3		9.7	1.5	8.2	25	2	2.89		0.14	0.41	0.003
1	HEART	5.2	1.5	3.7	25	2	0.87		0.21	0.19	0.001
2		4.9	1.5	3.4	25	2	1.04		0.16	0.17	0.001
3		9.1	1.5	7.6	25	2	0.77		0.49	0.38	0.003
1	BRAIN	7.6	1.5	6.1	25	2	1.74		0.18	0.31	0.002
2		6.4	1.5	4.9	25	2	1.87		0.13	0.25	0.002
3		6.4	1.5	4.9	25	2	1.7		0.14	0.25	0.002
1	HAIR	15.2	1.5	13.7	10	4	0.12		4.57	0.55	0.004
2		8.7	1.5	7.2	10	4	0.13		2.22	0.29	0.002
3		37.9	1.5	36.4	10	4	0.14		10.40	1.46	0.012
1	MUSCLE	31.3	1.5	29.8	25	2	3.53		0.42	1.49	0.012
2		30.2	1.5	28.7	25	2	3.8		0.38	1.44	0.011
3		36.2	1.5	34.7	25	2	3.5		0.50	1.74	0.014
1	URINE	36.2	1.5	34.7	25	2	5	23	0.35	7.98	0.064
2		44.2	1.5	42.7	25	2	5	23	0.43	9.82	0.078
3		22.8	1.5	21.3	25	2	5	50	0.21	10.65	0.085
1	FEMURS	0.14	0	0.14	25	33	0.123	10.578		9.93	0.079
2		0.16	0	0.16	25	33	0.218	12.599		7.63	0.061
3		0.01	0	0.01	25	33	0.131	12.169		0.77	0.006

Animal #	final BW (g)	Cr Dose (ug)	Bioavailability (%)
1	246	12,980	0.13
2	293	13,090	0.13
3	283	13,750	0.09

Total blood volume= 7 mls/100 g BW
Total bone weight= 4.3% BW

Table 7 Liberty State Park Soil Study Vehicle Control Animals-Pb

35

LEAD-CONTROL										
ANIMAL	TISSUE	ppb	mean blank	ppb-blank	qs	DF	sample wt	organ wt	ug metal/ g organ	ug metal/ total tissue
#		(ug/L)	(ug/L)	(ug/L)	(ml)		(gm)	(gm)		
1	BLOOD	0.44	0.03	0.41	25	42	6.9	18.48	0.06	1.15
2		0.24	0.03	0.21	25	42	2.8	17.99	0.08	1.42
3		0.3	0.03	0.27	25	42	7.6	17.5	0.04	0.65
1	LUNG	21.2	0.26	20.94	25	2	1.35		0.78	1.05
2		5.2	0.26	4.94	25	2	1.34		0.18	0.25
3		6.1	0.26	5.84	25	2	1.27		0.23	0.29
1	LIVER	9	0.26	8.74	25	2	2.27	10.28	0.19	1.98
2		9.1	0.26	8.84	25	2	2.42	8.96	0.18	1.64
3		6.4	0.26	6.14	25	2	2.25	6.85	0.14	0.93
1	KIDNEYS	7.9	0.26	7.64	25	2	2.33		0.16	0.38
2		12.4	0.26	12.14	25	2	2		0.30	0.61
3		11.5	0.26	11.24	25	2	2.1		0.27	0.56
1	SPLEEN	3.9	0.26	3.64	25	2	0.72		0.25	0.18
2		9.3	0.26	9.04	25	2	0.79		0.57	0.45
3		5.9	0.26	5.64	25	2	0.58		0.49	0.28
1	TESTES	15.3	0.26	15.04	25	2	2.66		0.28	0.75
2		5.1	0.26	4.84	25	2	3.34		0.07	0.24
3		16.9	0.26	16.64	25	2	2.83		0.29	0.83
1	HEART	5.3	0.26	5.04	25	2	0.99		0.25	0.25
2		16.6	0.26	16.34	25	2	0.9		0.91	0.82
3		10.1	0.26	9.84	25	2	0.81		0.61	0.49
1	BRAIN	9.3	0.26	9.04	25	2	1.59		0.28	0.45
2		6.7	0.26	6.44	25	2	1.64		0.20	0.32
3		7.7	0.26	7.44	25	2	1.72		0.22	0.37
1	HAIR	18.2	0.27	17.93	10	4	0.09		7.97	0.72
2		13.5	0.17	13.33	10	4	0.07		7.62	0.53
3		65.5	0.17	65.33	10	4	0.09		29.04	2.61
1	MUSCLE	10.2	0.26	9.94	25	2	2.7		0.18	0.50
2		17.7	0.26	17.44	25	2	3.42		0.25	0.87
3		7.1	0.26	6.84	25	2	3.85		0.09	0.34
1	URINE	7.5	0.26	7.24	25	2	5	27	0.07	1.95
2		16.2	0.26	15.94	25	2	5	34	0.16	5.42
3		11.4	0.26	11.14	25	2	5	29	0.11	3.23
1	FEMURS	0.23	0.03	0.2	25	33	0.236	11.352	0.70	7.94
2		0.21	0.03	0.18	25	33	0.212	11.051	0.70	7.74
3		0.12	0.03	0.09	25	33	0.154	10.75	0.48	5.18

Animal # final BW Pb Dose
(g) (ug)
1 264 0
2 257 0
3 250 0

Total blood volume= 7 mls/100 g BW
Total bone weight= 4.3% BW

Table 8 Liberty State Park Soil Study 1 Day Exposure-Pb

36

LEAD-1-DAY											
SAMPLE	TISSUE	ppb (ug/L)	mean blank (ug/L)	ppb-blank (ug/L)	qs (ml)	DF	sample wt (gm)	organ wt (gm)	ug metal/ g organ	ug metal/ total tissue	% admin dose
1	BLOOD	0.56	0.03	0.53	25	33	6.2	18.41	0.07	1.30	0.069
2		1.3	0.03	1.27	25	33	6.4	18.55	0.16	3.04	0.162
3		0.42	0.03	0.39	25	33	6	18.13	0.05	0.97	0.052
1	LUNG	6.3	0	6.3	25	2	1.5		0.21	0.32	0.017
2		4.3	0	4.3	25	2	1.17		0.18	0.22	0.011
3		5.7	0	5.7	25	2	1.28		0.22	0.29	0.015
1	LIVER	6.1	0	6.1	25	2	3.92	11.87	0.08	0.92	0.049
2		4	0	4	25	2	2.93	12.18	0.07	0.83	0.044
3		16.1	0	16.1	25	2	3.24	12.5	0.25	3.11	0.166
1	KIDNEYS	14.2	0	14.2	25	2	1.92		0.37	0.71	0.038
2		16.3	0	16.3	25	2	2.05		0.40	0.82	0.044
3		9.5	0	9.5	25	2	2.24		0.21	0.48	0.025
1	SPLEEN	2.2	0	2.2	25	2	0.66		0.17	0.11	0.006
2		81.7	0	81.7	25	2	0.77		5.31	4.09	0.218
3		2.5	0	2.5	25	2	0.54		0.23	0.13	0.007
1	TESTES	12.2	0	12.2	25	2	3.56		0.17	0.61	0.033
2		19.4	0	19.4	25	2	2.8		0.35	0.97	0.052
3		4.3	0	4.3	25	2	2.65		0.08	0.22	0.011
1	HEART	4.6	0	4.6	25	2	0.75		0.31	0.23	0.012
2		8.8	0	8.8	25	2	0.85		0.52	0.44	0.024
3		2	0	2	25	2	0.76		0.13	0.10	0.005
1	BRAIN	15.7	0	15.7	25	2	1.63		0.48	0.79	0.042
2		4	0	4	25	2	1.75		0.11	0.20	0.011
3		3.2	0	3.2	25	2	1.72		0.09	0.16	0.009
1	HAIR	50.7	0.27	50.43	10	4	0.08		25.22	2.02	0.108
2		7.5	0.27	7.23	10	4	0.09		3.21	0.29	0.015
3		9.3	0.17	9.13	10	4	0.09		4.06	0.37	0.020
1	MUSCLE	3.7	0	3.7	25	2	3.95		0.05	0.19	0.010
2		5.3	0	5.3	25	2	3.84		0.07	0.27	0.014
3		6.1	0	6.1	25	2	1.88		0.16	0.31	0.016
1	URINE	5.3	0	5.3	25	2	5	10	0.05	0.53	0.028
2		6.9	0	6.9	25	2	5	11	0.07	0.76	0.041
3		8.8	0	8.8	25	2	5	5	0.09	0.44	0.024
1	FEMURS	0.5	0.03	0.47	25	33	0.147	11.309		29.83	1.596
2		0.6	0.03	0.57	25	33	0.208	11.395		25.76	1.378
3		0.2	0.03	0.17	25	33	0.166	11.137		9.41	0.503

Animal #	final BW (g)	Pb Dose (ug)	Bioavailability (%)
1	263	1869.6	1.98
2	265	1869.6	1.97
3	259	1869.6	0.83

Total blood volume= 7 mls/100 g BW
Total bone weight= 4.3% BW

Table 9 Liberty State Park Soil Study 2 Day Exposure-Pb

37

LEAD-2-DAY											
SAMPLE	TISSUE	ppb (ug/L)	mean blan (ug/L)	ppb-blank (ug/L)	qs (ml)	DF	sample wt (gm)	organ wt (gm)	ug metal/ g organ	ug metal/ total tissue	% admin dose
1	BLOOD	0.47	0.03	0.44	25	33	6	17.64	0.06	1.07	0.061
2		0.5	0.03	0.47	25	33	6.5	18.55	0.06	1.11	0.062
3		0.58	0.03	0.55	25	33	5.9	19.18	0.08	1.48	0.078
1	LUNG	11.1	0.17	10.93	25	2	1.28		0.43	0.55	0.031
2		5.9	0.17	5.73	25	2	1.39		0.21	0.29	0.016
3		4.2	0.17	4.03	25	2	1.42		0.14	0.20	0.011
1	LIVER	11.6	0.17	11.43	25	2	3.36	11.99	0.17	2.04	0.117
2		16.8	0.17	16.63	25	2	4.24	13.01	0.20	2.55	0.144
3		8.7	0.17	8.53	25	2	3.8	12.46	0.11	1.40	0.074
1	KIDNEYS	12.7	0.17	12.53	25	2	2.07		0.30	0.63	0.036
2		15	0.17	14.83	25	2	2.17		0.34	0.74	0.042
3		8.8	0.17	8.63	25	2	2.34		0.18	0.43	0.023
1	SPLEEN	13.7	0.17	13.53	25	2	0.61		1.11	0.68	0.039
2		5.6	0.17	5.43	25	2	0.63		0.43	0.27	0.015
3		4.6	0.17	4.43	25	2	0.64		0.35	0.22	0.012
1	TESTES	5.6	0.17	5.43	25	2	2.98		0.09	0.27	0.016
2		3.7	0.17	3.53	25	2	2.72		0.06	0.18	0.010
3		4.1	0.17	3.93	25	2	3.36		0.06	0.20	0.010
1	HEART	28.1	0.17	27.93	25	2	0.78		1.79	1.40	0.080
2		13.9	0.17	13.73	25	2	0.86		0.80	0.69	0.039
3				0	25	2	0.93		0.00	0.00	0.000
1	BRAIN	15.8	0.17	15.63	25	2	1.43		0.55	0.78	0.045
2		8.1	0.17	7.93	25	2	1.67		0.24	0.40	0.022
3				0	25	2	1.7		0.00	0.00	0.000
1	HAIR	9.6	0.17	9.43	10	4	0.07		5.39	0.38	0.022
2		11.5	0.17	11.33	10	4	0.11		4.12	0.45	0.026
3		9.3	0.17	9.13	10	4	0.09		4.06	0.37	0.019
1	MUSCLE	13.1	0.17	12.93	25	2	2.38		0.27	0.65	0.037
2		4.9	0.17	4.73	25	2	3.51		0.07	0.24	0.013
3		49	0.17	48.83	25	2	3.99		0.61	2.44	0.130
1	URINE	13.9	0.17	13.73	25	2	5	15	0.14	2.06	0.118
2		16.1	0.17	15.93	25	2	5	17	0.16	2.71	0.153
3		11.4	0.26	11.14	25	2	5	13	0.11	1.45	0.077
1	FEMURS	0.24	0.03	0.21	25	33	0.203	10.836		9.25	0.529
2		0.66	0.03	0.63	25	33	0.198	11.395		29.91	1.689
3		0.2	0.03	0.17	25	22	0.159	11.782		6.93	0.369

Animal #	final BW (g)	Cr Dose (ug)	Bioavailability (%)
1	252	1748.2	1.01
2	265	1771.2	2.08
3	274	1879.4	0.73

Total blood volume= 7 ml/100 g BW
Total bone weight= 4.3% BW

Table 10 Liberty State Park Soil Study 3 Day Exposure-Pb

38

LEAD-3-DAY											
SAMPLE	TISSUE	ppb (ug/L)	mean blank (ug/L)	ppb-blank (ug/L)	qs (ml)	DF	sample wt (gm)	organ wt (gm)	ug metal/ g organ	ug metal/ total tissue	% admin dose
1	BLOOD	0.27	0.03	0.24	25	33	5.7	19.04	0.03	0.66	0.035
2		0.32	0.03	0.29	25	33	6	17.36	0.04	0.69	0.040
3		0.45	0.03	0.42	25	33	6	18.13	0.06	1.05	0.059
1	LUNG	7	0.22	6.78	25	2	1.28		0.26	0.34	0.018
2		4.7	0.22	4.48	25	2	1.14		0.20	0.22	0.013
3		20.8	0.22	20.58	25	2	1.46		0.70	1.03	0.058
1	LIVER	4.5	0	4.5	25	2	3.37	11.6	0.07	0.77	0.041
2		5.2	0	5.2	25	2	2.65	10.42	0.10	1.02	0.059
3		5	0	5	25	2	3.29	12.34	0.08	0.94	0.052
1	KIDNEYS	36.4	0.22	36.18	25	2	2.17		0.83	1.81	0.097
2		36.9	0.22	36.68	25	2	1.88		0.98	1.83	0.107
3		17.5	0.22	17.28	25	2	1.82		0.47	0.86	0.048
1	SPLEEN	10.8	0.22	10.58	25	2	0.76		0.70	0.53	0.028
2		7.6	0.22	7.38	25	2	0.58		0.64	0.37	0.021
3		4.3	0.22	4.08	25	2	0.78		0.26	0.20	0.011
1	TESTES	16.1	0.22	15.88	25	2	2.87		0.28	0.79	0.042
2		3.3	0.22	3.08	25	2	2.93		0.05	0.15	0.009
3		6.2	0.22	5.98	25	2	3.08		0.10	0.30	0.017
1	HEART	18.3	0.22	18.08	25	2	0.89		1.02	0.90	0.048
2		7.3	0.22	7.08	25	2	0.77		0.46	0.35	0.021
3		11.9	0.22	11.68	25	2	0.95		0.61	0.58	0.033
1	BRAIN	22.5	0.22	22.28	25	2	1.46		0.76	1.11	0.060
2		8.6	0.22	8.38	25	2	1.31		0.32	0.42	0.153
3		11.6	0.22	11.38	25	2	1.42		0.40	0.57	0.032
1	HAIR	0.12	0.03	0.09	10	4	0.06		0.06	0.00	0.000
2		0.15	0.03	0.12	10	4	0.05		0.10	0.00	0.000
3		0.17	0.03	0.14	10	4	0.06		0.09	0.01	0.000
1	MUSCLE	6.3	0.22	6.08	25	2	1.45		0.21	0.30	0.016
2		5.1	0.22	4.88	25	2	2.49		0.10	0.24	0.014
3		5.7	0.22	5.48	25	2	2.48		0.11	0.27	0.015
1	URINE	17.3	0.22	17.08	25	2	5	35	0.17	5.98	0.320
2		15.7	0.22	15.48	25	2	5	17	0.15	2.63	0.153
3		19.7	0.22	19.48	25	2	5	31	0.19	6.04	0.338
1	FEMURS	0.18	0.03	0.15	25	33	0.225	11.696		6.43	0.344
2		0.13	0.03	0.1	25	33	0.171	10.664		5.14	0.299
3		0.09	0.03	0.06	25	33	0.208	11.137		2.65	0.148

Animal #	final BW (g)	Pb Dose (ug)	Bioavailability (%)
1	272	1869.6	0.73
2	248	1722.0	0.74
3	259	1787.6	0.47

Total blood volume= 7 mls/100 g BW
Total bone weight= 4.3% BW

Table 11 Liberty State Park Soil Study 4 Day Exposure-Pb

39

LEAD-4-DAY											
SAMPLE	TISSUE	ppb (ug/L)	mean blank (ug/L)	ppb-blank (ug/L)	qs (ml)	DF	sample wt (gm)	organ wt (gm)	ug metal/ g organ	ug metal/ total tissue	% admin dose
1	BLOOD	0.24	0.03	0.21	25	33	3.4	17.22	0.05	0.88	0.045
2		0.39	0.03	0.36	25	33	7.6	20.51	0.04	0.80	0.041
3		0.57	0.03	0.54	25	33	6.8	19.81	0.07	1.30	0.063
1	LUNG	5.4	0.22	5.18	25	2	1.34		0.19	0.26	0.013
2		36.3	0.22	36.08	25	2	1.48		1.22	1.80	0.092
3		3.9	0.27	3.63	25	2	1.26		0.14	0.18	0.009
1	LIVER	6.7	0.27	6.43	25	2	2.46	8.12	0.13	1.06	0.055
2		8.6	0.27	8.33	25	2	2.42	11.85	0.17	2.04	0.105
3		7.8	0.27	7.53	25	2	3.01	11.51	0.13	1.44	0.070
1	KIDNEYS	5.7	0.22	5.48	25	2	2.26		0.12	0.27	0.014
2		13.6	0.22	13.38	25	2	2.35		0.28	0.67	0.034
3		6.1	0.22	5.88	25	2	2.07		0.14	0.29	0.014
1	SPLEEN	17.9	0.27	17.63	25	2	0.49		1.80	0.88	0.046
2		10.7	0.27	10.43	25	2	0.81		0.64	0.52	0.027
3		8.1	0.27	7.83	25	2	0.74		0.53	0.39	0.019
1	TESTES	7.3	0.27	7.03	25	2	2.84		0.12	0.35	0.018
2		4.5	0.27	4.23	25	2	3.23		0.07	0.21	0.011
3		3.5	0.27	3.23	25	2	2.89		0.06	0.16	0.008
1	HEART	9.9	0.27	9.63	25	2	0.87		0.55	0.48	0.025
2		20.4	0.27	20.13	25	2	1.04		0.97	1.01	0.052
3		11.9	0.27	11.63	25	2	0.77		0.76	0.58	0.028
1	BRAIN	5.4	0.27	5.13	25	2	1.74		0.15	0.26	0.013
2		14.9	0.27	14.63	25	2	1.87		0.39	0.73	0.037
3		6.1	0.27	5.83	25	2	1.7		0.17	0.29	0.014
1	HAIR	15.2	0.27	14.93	10	4	0.12		4.98	0.60	0.031
2		31.6	0.27	31.33	10	4	0.13		9.64	1.25	0.064
3		31.2	0.27	30.93	10	4	0.14		8.84	1.24	0.060
1	MUSCLE	5.6	0.27	5.33	25	2	3.53		0.08	0.27	0.014
2		26.5	0.27	26.23	25	2	3.8		0.35	1.31	0.067
3		6.8	0.27	6.53	25	2	3.5		0.09	0.33	0.016
1	URINE	20.6	0.27	20.33	25	2	5	23	0.20	4.68	0.242
2		20.1	0.27	19.83	25	2	5	23	0.20	4.56	0.234
3		5.8	0.27	5.53	25	2	5	50	0.06	2.77	0.135
1	FEMURS	0.1	0.03	0.07	25	33	0.123	10.578		4.97	0.257
2		0.13	0.03	0.1	25	33	0.218	12.599		4.77	0.244
3		0.07	0.03	0.04	25	33	0.131	12.169		3.07	0.150

Animal #	final BW (g)	Pb Dose (ug)	Bioavailability (%)
1	246	1935.2	0.53
2	293	1951.6	0.77
3	283	2050.0	0.45

Total blood volume= 7 mls/100 g BW
Total bone weight= 4.3% BW

Table 12 Liberty State Park Soil Study Vehicle Control Animals-As

40

ARSENIC-CONTROL										
ANIMAL	TISSUE	ppb	mean blank	ppb-blank	qs	DF	sample wt	organ wt	ug metal/ g organ	ug metal/ total tissue
#		(ug/L)	(ug/L)	(ug/L)	(ml)		(gm)	(gm)		
1	BLOOD	32.9	0.41	32.49	25	42	6.9	18.48	4.94	91.37
2		15.7	0.41	15.29	25	33	2.8	17.99	4.51	81.05
3		38.4	0.41	37.99	25	33	7.6	17.5	4.12	72.17
1	LUNG	47.3	13.8	33.5	25	2	1.35		1.24	1.68
2		50.6	13.8	36.8	25	2	1.34		1.37	1.84
3		44.5	13.8	30.7	25	2	1.27		1.21	1.54
1	LIVER	38.7	13.8	24.9	25	2	2.27	10.28	0.55	5.64
2		29.5	13.8	15.7	25	2	2.42	8.96	0.32	2.91
3		53.5	13.8	39.7	25	2	2.25	6.85	0.88	6.04
1	KIDNEYS	47.6	13.8	33.8	25	2	2.33		0.73	1.69
2		23.7	13.8	9.9	25	2	2		0.25	0.50
3		38	13.8	24.2	25	2	2.1		0.58	1.21
1	SPLEEN	45.7	13.8	31.9	25	2	0.72		2.22	1.60
2		29.5	13.8	15.7	25	2	0.79		0.99	0.79
3		23.7	13.8	9.9	25	2	0.58		0.85	0.50
1	TESTES	16.4	13.8	2.6	25	2	2.66		0.05	0.13
2		15.2	13.8	1.4	25	2	3.34		0.02	0.07
3		12.1	13.8	0	25	2	2.83		0.00	0.00
1	HEART	16.1	13.8	2.3	25	2	0.99		0.12	0.12
2		45	13.8	31.2	25	2	0.9		1.73	1.56
3		17.3	13.8	3.5	25	2	0.81		0.22	0.18
1	BRAIN	8.2	13.8	0	25	2	1.59		0.00	0.00
2		5.7	13.8	0	25	2	1.64		0.00	0.00
3		9.9	13.8	0	25	2	1.72		0.00	0.00
1	HAIR	5.8	11.4	0	10	4	0.09		0.00	0.00
2		1.1	11.4	0	10	4	0.07		0.00	0.00
3		2.7	11.4	0	10	4	0.09		0.00	0.00
1	MUSCLE	11.1	13.8	0	25	2	2.7		0.00	0.00
2		18.3	13.8	4.5	25	2	3.42		0.07	0.23
3		29.2	13.8	15.4	25	2	3.85		0.20	0.77
1	URINE	56	13.8	42.2	25	2	5	27	0.42	11.39
2		70.6	13.8	56.8	25	2	5	34	0.57	19.31
3		70.1	13.8	56.3	25	2	5	29	0.56	16.33
1	FEMURS	0.29	0.41	0	25	33	0.236	11.352	0.00	0.00
2		0.29	0.41	0	25	33	0.212	11.051	0.00	0.00
3		0.6	0.41	0	25	33	0.154	10.75	0.00	0.00

Animal # final BW As Dose
(g) (ug)
1 264 0
2 257 0
3 250 0

Total blood volume= 7 mls/100 g BW
Total bone weight= 4.3% BW

Table 13 Liberty State Park Soil Study 1 Day Exposure-As

41

ARSENIC-1-DAY											
SAMPLE	TISSUE	ppb (ug/L)	mean blan (ug/L)	ppb-blank (ug/L)	qs (ml)	DF	sample wt (gm)	organ wt (gm)	ug metal/ g organ	ug metal/ total tissue	% admin dose
1	BLOOD	49.1	0.41	48.69	25	33	6.2	18.41	6.48	119.28	40.242
2		50.2	0.41	49.79	25	33	6.4	18.55	6.42	119.06	40.168
3		42.3	0.41	41.89	25	33	6	18.13	5.76	104.43	35.232
1	LUNG	40.8	6.6	34.2	25	2	1.5		1.14	1.71	0.577
2		31.4	6.6	24.8	25	2	1.17		1.06	1.24	0.418
3		35.2	6.6	28.6	25	2	1.28		1.12	1.43	0.482
1	LIVER	43.7	6.6	37.1	25	2	3.92	11.87	0.47	5.62	1.895
2		71.5	6.6	64.9	25	2	2.93	12.18	1.11	13.49	4.551
3		69.8	6.6	63.2	25	2	3.24	12.5	0.98	12.19	4.113
1	KIDNEYS	40.6	6.6	34	25	2	1.92		0.89	1.70	0.574
2		35.2	6.6	28.6	25	2	2.05		0.70	1.43	0.482
3		66.3	6.6	59.7	25	2	2.24		1.33	2.99	1.007
1	SPLEEN	30	6.6	23.4	25	2	0.66		1.77	1.17	0.395
2		32.8	6.6	26.2	25	2	0.77		1.70	1.31	0.442
3		33.1	6.6	26.5	25	2	0.54		2.45	1.33	0.447
1	TESTES	14.7	6.6	8.1	25	2	3.56		0.11	0.41	0.137
2		48.7	6.6	42.1	25	2	2.8		0.75	2.11	0.710
3		5.2	6.6	0	25	2	2.65		0.00	0.00	0.000
1	HEART	12.9	6.6	6.3	25	2	0.75		0.42	0.32	0.106
2		8.8	6.6	2.2	25	2	0.85		0.13	0.11	0.037
3		9.3	6.6	2.7	25	2	0.76		0.18	0.14	0.046
1	BRAIN	0.15	6.6	0	25	2	1.63		0.00	0.00	0.000
2		2	6.6	0	25	2	1.75		0.00	0.00	0.000
3		27.4	6.6	20.8	25	2	1.72		0.60	1.04	0.351
1	HAIR	3.2	11.4	0	10	4	0.08		0.00	0.00	0.000
2		3.3	11.4	0	10	4	0.09		0.00	0.00	0.000
3		3	11.4	0	10	4	0.09		0.00	0.00	0.000
1	MUSCLE	4.4	6.6	0	25	2	3.95		0.00	0.00	0.000
2		13.2	6.6	6.6	25	2	3.84		0.09	0.33	0.111
3		6.7	6.6	0.1	25	2	1.88		0.00	0.01	0.002
1	URINE	66.1	6.6	59.5	25	2	5	10	0.60	5.95	2.007
2		64.7	6.6	58.1	25	2	5	11	0.58	6.39	2.156
3		107.8	6.6	101.2	25	2	5	5	1.01	5.06	1.707
1	FEMURS	0	0.09	0	25	33	0.147	11.309		0.00	0.000
2		0	0.09	0	25	33	0.208	11.395		0.00	0.000
3		0	0.09	0	25	33	0.166	11.137		0.00	0.000

Animal #	final BW (g)	As Dose (ug)	Bioavailability (%)
1	263	296.4	43.93
2	265	296.4	46.92
3	259	296.4	41.68

Total blood volume= 7 mls/100 g BW
Total bone weight= 4.3% BW

Table 14 Liberty State Park Soil Study 2 Day Exposure-As

42

ARESENIC-2-DAY											
SAMPLE	TISSUE	ppb (ug/L)	mean blan (ug/L)	ppb-blank (ug/L)	qs (ml)	DF	sample wt (gm)	organ wt (gm)	ug metal/ g organ	ug metal/ total tissue	% admin dose
1	BLOOD	60.7	0.41	60.29	25	33	6	17.64	8.29	146.23	52.754
2		59.1	0.41	58.69	25	33	6.5	18.55	7.45	138.18	49.210
3		50.3	0.41	49.89	25	33	5.9	19.18	6.98	133.80	44.900
1	LUNG	56.7	12.4	44.3	25	2	1.28		1.73	2.22	0.799
2		45.8	12.4	33.4	25	2	1.39		1.20	1.67	0.595
3		42.3	12.4	29.9	25	2	1.42		1.05	1.50	0.502
1	LIVER	63.9	12.4	51.5	25	2	3.36	11.99	0.77	9.19	3.315
2		38.8	12.4	26.4	25	2	4.24	13.01	0.31	4.05	1.442
3		60.1	12.4	47.7	25	2	3.8	12.46	0.63	7.82	2.624
1	KIDNEYS	63.9	12.4	51.5	25	2	2.07		1.24	2.58	0.929
2		50.1	12.4	37.7	25	2	2.17		0.87	1.89	0.671
3		32.8	12.4	20.4	25	2	2.34		0.44	1.02	0.342
1	SPLEEN	38.4	12.4	26	25	2	0.61		2.13	1.30	0.469
2		46.9	12.4	34.5	25	2	0.63		2.74	1.73	0.614
3		27.4	12.4	15	25	2	0.64		1.17	0.75	0.252
1	TESTES	23.3	12.4	10.9	25	2	2.98		0.18	0.55	0.197
2		17.3	12.4	4.9	25	2	2.72		0.09	0.25	0.087
3		12.1	12.4	0	25	2	3.36		0.00	0.00	0.000
1	HEART	18.5	12.4	6.1	25	2	0.78		0.39	0.31	0.110
2		19.5	12.4	7.1	25	2	0.86		0.41	0.36	0.126
3		18	12.4	5.6	25	2	0.93		0.30	0.28	0.094
1	BRAIN	5	12.4	0	25	2	1.43		0.00	0.00	0.000
2		6.3	12.4	0	25	2	1.67		0.00	0.00	0.000
3		5.5	12.4	0	25	2	1.7		0.00	0.00	0.000
1	HAIR	1.9	12.4	0	10	4	0.07		0.00	0.00	0.000
2		0.88	12.4	0	10	4	0.11		0.00	0.00	0.000
3		8.1	12.4	0	10	4	0.09		0.00	0.00	0.000
1	MUSCLE	26.8	12.4	14.4	25	2	2.38		0.30	0.72	0.260
2		33.9	12.4	21.5	25	2	3.51		0.31	1.08	0.383
3		9.8	12.4	0	25	2	3.99		0.00	0.00	0.000
1	URINE	112.3	12.4	99.9	25	2	5	15	1.00	14.99	5.406
2		104.7	12.4	92.3	25	2	5	17	0.92	15.69	5.588
3		101.6	13.8	87.8	25	2	5	13	0.88	11.41	3.830
1	FEMURS	0.03	0.09	0	25	33	0.203	10.836		0.00	0.000
2		0.17	0.09	0.08	25	33	0.198	11.395		3.80	1.353
3		0	0.09	0	25	33	0.159	11.782		0.00	0.000

Animal #	final BW (g)	Cr Dose (ug)	Bioavailability (%)
1	252	277.2	58.83
2	265	280.8	54.48
3	274	298.0	48.71

Total blood volume= 7 ml/100 g BW
Total bone weight= 4.3% BW

Table 15 Liberty State Park Soil Study 3 Day Exposure-As

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ARSENIC-3-DAY											
SAMPLE	TISSUE	ppb (ug/L)	mean blan (ug/L)	ppb-blank (ug/L)	qs (ml)	DF	sample wt (gm)	organ wt (gm)	ug metal/ g organ	ug metal/ total tissue	% admin dose
1	BLOOD	72.5	0.41	72.09	25	33	5.7	19.04	10.43	198.66	67.026
2		79.4	0.41	78.99	25	33	6	17.36	10.86	188.55	69.066
3		44.8	0.41	44.39	25	33	6	18.13	6.10	110.66	39.047
1	LUNG	58.4	11.9	46.5	25	2	1.28		1.82	2.33	0.784
2		16.8	11.9	4.9	25	2	1.14		0.21	0.25	0.090
3		42.6	11.9	30.7	25	2	1.46		1.05	1.54	0.542
1	LIVER	51.1	6.6	44.5	25	2	3.37	11.6	0.66	7.66	2.584
2		62	6.6	55.4	25	2	2.65	10.42	1.05	10.89	3.990
3		66.9	6.6	60.3	25	2	3.29	12.34	0.92	11.31	3.990
1	KIDNEYS	64.1	11.9	52.2	25	2	2.17		1.20	2.61	0.881
2		51.4	11.9	39.5	25	2	1.88		1.05	1.98	0.723
3		40	11.9	28.1	25	2	1.82		0.77	1.41	0.496
1	SPLEEN	53.8	11.9	41.9	25	2	0.76		2.76	2.10	0.707
2		35.2	11.9	23.3	25	2	0.58		2.01	1.17	0.427
3		41.8	11.9	29.9	25	2	0.78		1.92	1.50	0.528
1	TESTES	13	11.9	1.1	25	2	2.87		0.02	0.06	0.019
2		19.4	11.9	7.5	25	2	2.93		0.13	0.38	0.137
3		26.4	11.9	14.5	25	2	3.08		0.24	0.73	0.256
1	HEART	37.3	11.9	25.4	25	2	0.89		1.43	1.27	0.428
2		21.3	11.9	9.4	25	2	0.77		0.61	0.47	0.172
3		22.8	11.9	10.9	25	2	0.95		0.57	0.55	0.192
1	BRAIN	7.3	11.9	0	25	2	1.46		0.00	0.00	0.000
2		9.9	11.9	0	25	2	1.31		0.00	0.00	0.000
3		7.5	11.9	0	25	2	1.42		0.00	0.00	0.000
1	HAIR	0.41	0.09	0.32	10	4	0.06		0.21	0.01	0.004
2		0.05	0.09	0	10	4	0.05		0.00	0.00	0.000
3		0.02	0.09	0	10	4	0.06		0.00	0.00	0.000
1	MUSCLE	10.3	11.9	0	25	2	1.45		0.00	0.00	0.000
2		23.3	11.9	11.4	25	2	2.49		0.23	0.57	0.209
3		15.9	11.9	4	25	2	2.48		0.08	0.20	0.071
1	URINE	78	11.9	66.1	25	2	5	35	0.66	23.14	7.805
2		147.1	11.9	135.2	25	2	5	17	1.35	22.98	8.419
3		76.8	11.9	64.9	25	2	5	31	0.65	20.12	7.099
1	FEMURS	0	0.091	0	25	33	0.225	11.696		0.00	0.000
2		0	0.091	0	25	33	0.171	10.664		0.00	0.000
3		0	0.091	0	25	33	0.208	11.137		0.00	0.000

Animal #	final BW (g)	As Dose (ug)	Bioavailability (%)
1	272	296.4	72.43
2	248	273.0	74.81
3	259	283.4	45.12

Total blood volume= 7 mls/100 g BW
Total bone weight= 4.3% BW

Table 16 Liberty State Park Soil Study 4 Day Exposure-As

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ARSENIC-4-DAY											
SAMPLE	TISSUE	ppb (ug/L)	mean blank (ug/L)	ppb-blank (ug/L)	qs (ml)	DF	sample wt (gm)	organ wt (gm)	ug metal/ g organ	ug metal/ total tissue	% admin dose
1	BLOOD	19.2	0.41	18.79	25	33	3.4	17.22	4.56	78.51	25.591
2		44.8	0.41	44.39	25	33	7.6	20.51	4.82	98.83	31.943
3		38.1	0.41	37.69	25	33	6.8	19.81	4.57	90.58	27.872
1	LUNG	21.7	11.9	9.8	25	2	1.34		0.37	0.49	0.160
2		45.2	11.9	33.3	25	2	1.48		1.13	1.67	0.538
3		39.3	11.4	27.9	25	2	1.26		1.11	1.40	0.429
1	LIVER	46.4	11.4	35	25	2	2.46	8.12	0.71	5.78	1.883
2		33.9	11.4	22.5	25	2	2.42	11.85	0.46	5.51	1.780
3		55.1	11.4	43.7	25	2	3.01	11.51	0.73	8.36	2.571
1	KIDNEYS	27.5	11.9	15.6	25	2	2.26		0.35	0.78	0.254
2		32.5	11.9	20.6	25	2	2.35		0.44	1.03	0.333
3		37	11.9	25.1	25	2	2.07		0.61	1.26	0.386
1	SPLEEN	33.7	11.4	22.3	25	2	0.49		2.28	1.12	0.363
2		28.7	11.4	17.3	25	2	0.81		1.07	0.87	0.280
3		38.3	11.4	26.9	25	2	0.74		1.82	1.35	0.414
1	TESTES	16.3	11.4	4.9	25	2	2.84		0.09	0.25	0.080
2		14.5	11.4	3.1	25	2	3.23		0.05	0.16	0.050
3		16.7	11.4	5.3	25	2	2.89		0.09	0.27	0.082
1	HEART	17.1	11.4	5.7	25	2	0.87		0.33	0.29	0.093
2		20.3	11.4	8.9	25	2	1.04		0.43	0.45	0.144
3		18.1	11.4	6.7	25	2	0.77		0.44	0.34	0.103
1	BRAIN	12.6	11.4	1.2	25	2	1.74		0.03	0.06	0.020
2		10.4	11.4	0	25	2	1.87		0.00	0.00	0.000
3		6.4	11.4	0	25	2	1.7		0.00	0.00	0.000
1	HAIR	7.6	11.4	0	10	4	0.12		0.00	0.00	0.000
2		5.7	11.4	0	10	4	0.13		0.00	0.00	0.000
3		7	11.4	0	10	4	0.14		0.00	0.00	0.000
1	MUSCLE	15.4	11.4	4	25	2	3.53		0.06	0.20	0.065
2		17.3	11.4	5.9	25	2	3.8		0.08	0.30	0.095
3		22.1	11.4	10.7	25	2	3.5		0.15	0.54	0.165
1	URINE	109	11.4	97.6	25	2	5	23	0.98	22.45	7.317
2		194.1	11.4	182.7	25	2	5	23	1.83	42.02	13.581
3		58	11.4	46.6	25	2	5	50	0.47	23.30	7.169
1	FEMURS	0	0.09	0	25	33	0.123	10.578		0.00	0.000
2		0	0.09	0	25	33	0.218	12.599		0.00	0.000
3		0	0.09	0	25	33	0.131	12.169		0.00	0.000

Animal #	final BW (g)	As Dose (ug)	Bioavailability (%)
1	246	306.8	28.51
2	293	309.4	35.16
3	283	325.0	32.02

Total blood volume= 7 mls/100 g BW
Total bone weight= 4.3% BW

DISCUSSION:

Chromium bioavailability in rats averaged around 15% for all four time points tested. The range of values was from 18%-12% from one day to four days of exposure to the soil. The highest average levels of chromium in the blood (0.022% of the administered dose) occurred after 3 days of exposure to the soil. If the animals were allowed to survive until day 4, the levels dropped to approximately 0.011% of the dose. This trend demonstrates how chromium is being absorbed from the gastrointestinal tract into the blood within the first 72 hours and is then eliminated from the blood at a rate greater than that which it is being absorbed. This is also demonstrated with increasing amounts of chromium in the urine that occurs as a function of time. Urine levels start at 0.012% of the dose at day 1 and increase steadily to an average of 0.076% at day 4. We attempted to examine the levels chromium in the feces, but we encountered problems trying to solubilize the samples. We are developing a different method which we believe will improve the results. It is difficult to establish any trends concerning the chromium burden in the various tissues over time. This is mainly the result of the variability that sometimes occurs with this type of analysis.

When lead concentrations were examined in the blood, we saw elevated amounts after one day of exposure. At days 2, 3, and 4 the levels decrease to control values. This trend is evident in most of the other tissues examined and accounts for the steady decrease in bioavailability values from day 1 to day 4 (1.6% to 0.58%, respectively). As expected, we saw a steady increase in lead concentrations in urine from day 1 up to day 3 (0.03 1% to 0.22%, respectively), then slightly lower levels at day 4 (0.22% of the dose). The percent of the administered dose that was measured in bone decreased over time from an average of 1.2% at day 1 to a low of 0.22% after 4 days of exposure. Other tissues are difficult to interpret because of the high variability and the lack of any obvious trends. It is evident that lead is not bioavailable from this soil. The metal concentration reached its peak at day 1 in most tissues that were examined, and was eliminated rapidly in the urine. What is interesting is that the calculated dose of lead was higher on a microgram basis than the doses of chromium or arsenic, yet its tissue burdens were the lowest of all groups. This illustrates how important bioavailability is for determining risks associated with contaminated soils.

The bioavailability values for arsenic were the highest for any metal examined. The average values ranged from 44% at day one to 64% after 3 days. At day 4 we see a drop to 32% of the administered dose. The largest amounts of arsenic were found in the blood for all time points, contributing roughly 90% of the total burden. The pattern of accumulation in the heart followed that of the blood where the highest levels were seen through the first three days, then a drop at day 4. Amounts of metal in the liver are highest at day 1 (3.5% of the dose) and decrease through 4 days of exposure to 2.1% of the administered dose. Kidney levels remain steady at 0.6-0.7% throughout the first three days, then decrease to 0.32% at day 4. As seen with the other metals in this study, the levels of arsenic in the urine increased over time from 2% of the dose at day 1 to 9.4% at day 4. The control values for blood are very high and represent about 25-30% of the dose given to the treated animals. If we subtract the control values from the treated values, the bioavailability drops to a range of 10-30%. We need to re-analyze the blood samples for arsenic to see if the control values are accurate. In either case, this soil should be considered bioavailable with respect to arsenic based on the sustained levels in the blood and other tissues. As Results will be compared to the values obtained in the “in vitro” studies during the first quarter of year 02.

This preliminary study was designed to develop methods to reliably measure the oral bioavailability of heavy metals from contaminated soils. We have determined from this study that 3-4 days of exposure following a single dose is necessary to compensate for most physiological processes. We have further refined our tissue preparation scheme for the next experiment to try and reduce the variability and increase the accuracy of our analyses. In addition, we will eliminate some tissues from analysis, such as hair and brain, which do not give us any more information and slow down analyses. Lastly, we will use these results to compare with an in vitro bioaccessibility system currently in development. It is hoped that both systems can provide a better picture of how contaminants are released from these complex matrices. During year 02 we will be completing the analyses of “in vivo” experiments on the Montanan soil, and on the two Savannah River soils. Other studies will be designed as new soils are obtained for analysis by both systems.