

BIOAVAILABILITY OF ARSENIC AND LEAD IN ENVIRONMENTAL SUBSTRATES

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EXECUTIVE SUMMARY

A study using immature swine as test animals was performed to determine if arsenic and lead were absorbed from the gastrointestinal tract into the bloodstream following oral dosing of soil or slag from the Ruston/North Tacoma Superfund site located in Tacoma, Washington, or following oral dosing of tailings or dust from the Triumph Mine Tailings site located in Triumph, Idaho.

A data evaluation methodology was developed to estimate the extent of arsenic absorption using the results from multiple dose groups. The methodology provided an estimate of the 95% confidence limits for the calculated mean absolute and relative bioavailability of arsenic in the soil and slag samples from the Ruston/North Tacoma Superfund site. Relative bioavailability was calculated using the oral control group data and absolute bioavailability was calculated using the intravenous control group data. These values are shown below:

Bioavailability (arsenic)	Test Material	
	Soil	Slag
Relative (mean)	78%	42%
95% confidence limit	56-111%	27-63%
Absolute (mean)	52%	28%
95% confidence limit	44-61%	20-37%

Because the toxicity criteria for arsenic were developed from oral ingestion studies, the relative bioavailability estimates would be appropriate for use, if desired, in adjusting arsenic exposure estimates.

Significant increases in blood lead concentrations were observed following oral dosing of the soil and slag samples from the Ruston/North Tacoma Superfund site, and following oral dosing of the tailings or dust from the Triumph Mine Tailings site. However, this experiment did not provide reliable bioavailability estimates for lead from any of these test materials.

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INTRODUCTION

Background

This study was initiated in late 1990 by U.S.EPA Region 10 to provide site-specific empirical data to improve the certainty of exposure estimates for the assessment of risks due to arsenic and lead contaminated residential yard and driveway materials (soil and slag) at a hazardous waste site in Tacoma, WA.

A smelter operated at the site from 1890 to 1986, first as a lead smelter, and after 1912 as a copper smelter that specialized in the processing of ores with high arsenic concentrations. Air emissions resulted in contaminated soils in adjacent residential areas, and smelter slag had been used for landscaping and top grade on gravel driveways and roads. The Ruston-Vashon Island Arsenic Exposure Pathways Study indicated an association between human residents' proximity to the hazardous waste site and urinary arsenic (Polissar, 1987; Kalman et al., 1990; Polissar et al., 1990).

As a result of a screening evaluation, two smelter-related contaminants were identified for detailed evaluation in the risk assessment: arsenic and lead (U.S.EPA, 1992). In early 1992 when site cleanup options were developed, there were few literature reports of arsenic and lead bioavailability from solid matrices such as food or soil. Studies in which animals were orally dosed with arsenic contaminated soils were difficult to interpret due to inter-animal or inter-group variation, or questionable representativeness for soils at the Tacoma study area (Freeman et al., 1993; Griffin & Turck, 1991; Boyajian, 1987). However, information from these studies supported a reasonable assumption that bioavailability of arsenic in soils was reduced as compared to drinking water. Based on best professional judgement and public health protectiveness, a relative bioavailability factor of 0.8 was assumed for soil and 0.4 for slag. Due to the widespread nature of contamination in the surrounding residential area and the potential impacts of cleanup activities on the community, confirmation of the literature based bioavailability estimates was needed prior to finalizing cleanup decisions.

Both arsenic and lead were present in elevated concentrations in the environmental matrices (soil and slag). Because these contaminants could not be separated, the uptake of both was evaluated. No other reports were identified in which the bioavailability of co-contaminants were simultaneously studied. Since this approach was developed in late 1990, other investigators have continued development of the immature swine model and further demonstrated its value in bioavailability studies (LaVelle et al., 1991; Weis et al., 1994; DuPont, 1993).

Chemical and physical characteristics of the soil and slag as well as the chemical form of the contaminants can have significant effects on the bioavailability of arsenic and lead (Chaney et al., 1989). Whereas bulk analyses provide total concentrations of arsenic or lead, physical/chemical characterization provides information about the three dimensional arrangement of elements in the soil, slag, tailing or dust matrices. Following ingestion, this composition influences the ability of arsenic and lead to move from the matrix to gastrointestinal fluids and then to body tissues.

Objectives

Objectives of the study were to identify physical and/or chemical indicators of the environmental matrices' (soil, slag, tailings) potential to release biologically available forms of arsenic and lead, and to obtain tissue data indicating whether arsenic and lead are absorbed into the body following oral exposure.

The first objective was to examine physical and chemical characteristics and identify which of these may be important determinants of the materials' potential to release arsenic and lead when orally ingested by human beings. The results of these physical and chemical analyses will be included in a subsequent report.

The second objective was to utilize the immature swine model to examine urine and blood concentrations of arsenic and lead, respectively, as evidence of their gastrointestinal absorption, and, if possible, to determine estimates of the extent of absorption. Samples of soil or slag from highly contaminated lots in the vicinity of the smelter in Tacoma were evaluated in replicate at multiple dose levels. Single samples of soil or tailings from a former mining site in Idaho provided preliminary information useful for designing a bioavailability study, if needed, for that site. No smelter was in the area of the former mining site. The results of the animal studies are described in this report.

The physical and chemical characteristics of the smelter site and mining site samples will be provided in a subsequent report.

MATERIALS

Environmental Test Materials

A composite of soils was collected in the residential area surrounding the former Asarco smelter in Tacoma, Washington. Decontaminated stainless steel hand auger, mixing bowl, spoon and sample containers were used to collect fifteen surface (zero - 3 inch depth) soil samples.

Sampling and decontamination procedures were carried out according to a standard operating protocol developed to ensure the use of safe methods as well as careful technique as part of the quality assurance plan. Briefly, equipment was decontaminated by washing with detergent and water, rinsing with distilled and reagent-grade water, rinsed with pesticide-grade methanol and allowed to air dry.

Samples were collected from two residential properties (vacant lots) within one to two blocks of the smelter stack where previous investigation indicated elevated arsenic concentrations and access was granted. Five random samples were collected from one site and ten from the other.

A composite of slag was collected in the residential area surrounding the former Asarco smelter in Tacoma, Washington. Decontaminated equipment was used to collect five surface (zero - 3 inch depth) samples from residential driveways known to contain smelter slag and where access was granted.

A composite of soil was collected from a residential location in Triumph, Idaho which provided access to EPA. Decontaminated equipment was used to collect twelve randomly chosen surface (zero to 3 inch depth) soil samples from a residence within 200 feet adjacent to and downwind of the mine tailings. The individual samples were thoroughly mixed together then placed in a sample container.

A sample of surface (zero to 3 inch depth) tailings from the upper eastward tailings pile in Triumph, Idaho was collected using decontaminated equipment.

A sample of subsurface (ten - eleven feet below surface) tailings from the same borehole as the surface tailings piles in Triumph, Idaho was collected using decontaminated equipment.

A composite sample of vacuum cleaner dust was collected from residences in Triumph, Idaho. Decontaminated equipment was used to collect and mix together three individual dust samples obtained from vacuum cleaner bags at three separate residences. Chosen residences were those who agreed to EPA's access. Vacuum dust contents were a result of residents' routine cleaning practices. This composite was included in the physical-chemical analyses but was not used in the animal dosing.

Technical Grade Test Materials

Sodium arsenate heptahydrate ($\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$, Sigma Chemical No. A756). Lead acetate trihydrate ($\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 3\text{H}_2\text{O}$, Aldrich Chemical No. 31651-2). Radiogenic lead (92% ^{206}Pb , National Institute of Standards and Technology no. 983).

Standard reference materials used as quality control specimens were obtained from the Center for Disease Control and Kaulson Laboratory (blood lead and blood arsenic, Kaulson #0141), from the

National Institute of Standards and Technology and the Centre de Toxicologie du Quebec (urinary arsenic NIST2670, Centre#S-9206, #S-200, #S-189, urinary lead, NIST2670 and vegetable matter arsenic and lead, NIST1547).

Test Facilities and Animals

The swine study was conducted from April through September 1992 at the Michigan State University Pesticide Research Center, East Lansing, MI under the direction of Drs. Robert Poppenga and Brad Thacker. Cages, containment areas and study equipment were isolated from other equipment and animals both prior and during the course of this study.

Thirty-nine female crossbred swine approximately 15 kilograms (approximately 40-50 days old) were obtained from a commercial producer. The sires were a Hampshire hybrid and the dams were crossbred Landrace/Large White/Duroc. Following preconditioning, animals were acclimated for at least seven days prior to the study. Swine were randomly assigned to experimental groups with stratification for litter of origin (to control for age, genetic background and environmental factors prior to weaning) and body weight.

Animals, individually identified by plastic ear tags, were fed a standard swine corn and soybean ration diet equal to 2% of body weight twice a day for the duration of the study except for fasting prior to dosing. This quantity was sufficient for normal growth of swine this age and size. Water was provided *ad libitum* in stainless containers. Multi-element analyses were conducted on both feed and water samples, and analyzed using plasma mass spectrometry as described below. The animals' drinking water and water samples for analyses were obtained following flushing the system for several minutes.

Immature swine were preferred as the test animal for this study because of characteristics comparable to young children (the age group at greatest risk of ingesting soil or other material containing contaminants). These included similar body size, weight, bone-to-body weight ratio and gastrointestinal anatomy and physiology. In addition, unlike other species such as rats or rabbits, the rate of growth and maturation is slower (a smaller portion of the prepubertal period will occur during the experiment), the cecum (a diverticulum of the large intestine where prolonged exposure to digestive enzymes and fluids occurs) is small, and coprophagia (reingestion of feces) is not required to maintain normal nutritional status. Like humans, swine are monogastric omnivores (stomach and intestinal fluid and bacterial composition are different than herbivores or carnivores), are adaptable to a periodic feeding schedule and have a gall bladder which excretes bile into the small intestine when food is present (some contaminants, such as lead are excreted in bile). Unlike the rat, metabolism and excretion of arsenic in swine is similar to humans. The results of pharmacokinetic studies of lead in immature swine and humans are similar (Weis et al., 1994).

Analytical Reagents and Standards

Trace metal grade concentrated nitric acid and hydrochloric acid (Baker Instra-Analyzed) were used without further purification. The sodium borohydride was 98% pure (J.T. Baker Inc.) All other common chemicals were of reagent grade purity. Fresh distilled, deionized water was used as the solvent for all solutions.

Element stock solutions were obtained from either PlasmaChem Associates or Spex Industries. Calibration working standards were prepared daily from these standards and were matrix matched

to the digestion or fusion solutions. Calibration verification standards were prepared from a second source (Environmental Protection Agency, Environmental Resources Associates, PlasmaChem or Spex Industries).

A stock solution of lead (1000 mg/L) was prepared by dissolving a portion of National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 981 Common Lead Isotopic Standard wire in 1 N nitric acid. This stock was used to prepare the primary calibration working standards in a 10% v/v nitric acid aqueous matrix for lead isotope measurements. Calibration verification standards (0.002 mg/L) were prepared from a PlasmaChem Associates stock solution (10 mg/L).

NIST SRM 2670 Toxic Metals in Freeze-Dried Urine which is provided at normal and elevated levels was reconstituted before use. The other reference materials were used as purchased including NIST SRM 955 Lead in Bovine Serum; NIST SRM 1645, River Sediment from Indiana Harbor near Gary, Indiana; NIST SRM 2704, River Sediment from Buffalo, New York; PACS-1 a marine sediment obtained from the National Research Council of Canada, Marine Analytical Chemistry Standards Program, Ottawa, Ontario, Canada; and United States Geological Survey Geochemical Exploration Reference Samples GXR-1 (Jasperite, Drum Mountains, Utah), GXR-2 (Soil, Park City, Utah), GXR-3 (Hot Springs Deposit, Humboldt County, Nevada), and GXR-4 (Porphyry Copper Mill Heads, Utah).

METHODS

Study Protocol

Preliminary Study. A preliminary range finding experiment utilizing three swine was performed to assess the proposed animal handling methodology, the analytical methodology and to determine appropriate doses.

Animal No.	Body Wt	mg soil/kg	Dose		ug ²⁰⁶ Pb/kg
	kg		mg Pb/kg	mg As/kg	
34	8.2	18.3	0.025	0.029	4.46
35	10	20.0	0.027	0.032	4.46
36	8.6	41.9	0.056	0.067	5.65

Final Study Protocol. The final study included thirty-six swine in treatment groups consisting of positive control groups, a negative control group and groups receiving environmental media. The positive control group (8 animals) received a single intravenous or gavage administration of sodium arsenate and lead acetate. The negative control group (4 animals) received only the aqueous vehicle with no arsenic or lead included. Data for baseline blood arsenic from two animals in the positive control group that received intravenous lead acetate but not sodium arsenate were included in the negative control group. Therefore, there were four animals in the negative or untreated group for lead and six animals in the untreated group for arsenic. The group receiving environmental media (21 animals) received a single oral administration of one of four quantities of soil at 25, 60, 100 or 150 milligram (mg) soil per kilogram (kg) of body weight (BW) (0.04, 0.10, 0.16 or 0.24 mg As per kg BW and 0.03, 0.08, 0.14 or 0.20 mg Pb per kg BW), or a single oral administration of one of three quantities of slag at 60, 100 or 150 mg slag per kg BW (0.61, 1.01 or 1.52 mg As per kg BW and 0.23, 0.38 or 0.57 mg Pb per kg BW). Three swine were also included to provide preliminary data from mine waste contaminated samples. Each animal received either 100 mg/kg of residential soil (1 animal), or surface tailings (1 animal), or subsurface tailings (1 animal). This was equivalent to 0.15, 1.11 or 0.18 mg As per kg BW and 0.22, 0.43 or 0.43 mg Pb per kg BW, respectively. The final study design is shown in Table 1.

Doses of soil administered to the swine were in the high end of the range expected for normal children (i.e. 0.8 grams per day) up to the range depicting a child with pica (i.e. 10,000 grams per day) for soil (U.S.EPA, 1989).

Animals in all treatment groups except the untreated controls and the intravenous controls received a single intravenous dose of ²⁰⁶Pb in an aqueous solution immediately prior (within minutes) to receiving test material described above. This intravenous administration of the ²⁰⁶Pb-enriched solution and gavage administration of test materials enabled the comparison of intravenous and oral dose responses within the same individual animal. This approach has the advantage of identical clearance and other physiological factors influencing the elimination of lead. The dose of ²⁰⁶Pb was based on results of the preliminary study and intended to add an insignificant amount (<10%) to the total dose of lead.

Intravenous or gavage doses of sodium arsenate equivalent to the highest doses of arsenic in the environmental material (0.61-1.52 mg As/kg BW, slag) were not administered. Acute toxicity has been reported in swine and in humans exposed to highly bioavailable forms of arsenic in this dose range (Osweiler et al., 1985; ATSDR, 1993). Therefore, intravenous and oral sodium arsenate doses greater than 1 mg/kg were not administered.

Twenty-four hours prior to dosing, the animals' weights were obtained and pre-dosing blood samples were collected. Animals were preanesthetized and while under general anesthesia a self-retaining catheter was placed in the urinary bladder. The catheter balloon was filled with a saline solution and the catheter was connected to an empty, sterile urine collection bag. The bags were attached to the back of the animal with adhesive tape. Animals fully recovered in the 24 hours prior to the experiment. Following a 12 hour overnight fast, a second predose blood sample was collected. All urine and feces were collected during the 24 hours prior to dosing.

At the beginning of the experiment following a 12 hour overnight fast (time 0), each animal was given a single administration of the appropriate test material. Solutions of sodium arsenate and lead acetate were administered separately and not mixed together prior to administration. Intravenous doses were administered over a 2-3 minute period into the jugular vein using an 18 gauge butterfly infusion set attached to a disposable syringe. Frequent withdrawals of blood into the infusion tubing confirmed needle placement in the vein. Gavage arsenic solutions, lead solutions or environmental media were mixed in a total of 40-ml of sterile double distilled water and administered directly into the stomach via a lubricated tube passed through a mouth gag. An additional 20-ml of water was used to flush the tube after initial dosing to assure that all material had reached the stomach. The animals were cradled in the handler's arms while another person placed the gastric tube for dose administration. The animals' behavior indicated that this handling technique minimized stress.

Clinical observations were recorded. Animals were observed at short (~15 minute) intervals following dosing for signs of toxicity or emesis (vomiting). And, at 3-4 times during each of the subsequent days. Food and water were provided four hours after dosing. At the end of the study, urinary catheters were removed and the animals were returned to a swine confinement facility prior to being sold or used for other research.

A maximum of eighteen animals could be efficiently handled for dosing and sample collection. The experiment was conducted in two portions as indicated below. Control group animals (intravenous, oral and untreated) were included in each portion. The dates were as follows: Range finding - dose administered on 2/9/92; soil treatment group - dose administered on 7/24/92; slag treatment group - dose administered on 9/11/92.

Table 1. Study Design

Treatment Group	Test Material	Dosing Frequency	Dose Rate				No. of Animals
			mg soil/kg BW	mg As/kg BW	ug Pb/kg BW	ug ²⁰⁶ Pb/kg BW	
Untreated	Deionized Water	Single	Water, only	Water, only	Water, only	Water, only	4
Control - Oral Gavage	Aqueous Lead Acetate	Single	ND	ND	656	1.68	1
Control - Oral Gavage	Aqueous Sodium Arsenate; Aqueous Lead Acetate	Single	ND	0.01	18	1.68	1
Control - Oral Gavage	Aqueous Sodium Arsenate; Aqueous Lead Acetate	Single	ND	0.11	214	1.68	1
Control - Oral Gavage	Aqueous Sodium Arsenate; Aqueous Lead Acetate	Single	ND	0.31	263	1.68	1
Control - Intravenous	Aqueous Lead Acetate	Single	ND	ND	656	ND	1
Control - Intravenous	Aqueous Sodium Arsenate; Aqueous Lead Acetate	Single	ND	0.01	18	ND	1
Control - Intravenous	Aqueous Sodium Arsenate; Aqueous Lead Acetate	Single	ND	0.11	214	ND	1
Control - Intravenous	Aqueous Sodium Arsenate; Aqueous Lead Acetate	Single	ND	0.31	263	ND	1
Oral Test Soil	Aqueous Suspension	Single	25	0.04	34	1.68	3
Oral Test Soil	Aqueous Suspension	Single	60	0.10	81	1.68	3
Oral Test Soil	Aqueous Suspension	Single	100	0.16	135	1.68	3
Oral Test Soil	Aqueous Suspension	Single	150	0.24	202	1.68	3
Oral Test Slag	Aqueous Suspension	Single	60	0.61	227	1.68	3
Oral Test Slag	Aqueous Suspension	Single	100	1.01	378	1.68	3
Oral Test Slag	Aqueous Suspension	Single	150	1.52	567	1.68	3
Oral Test Mine Soil	Aqueous Suspension	Single	100	0.15	217	1.68	1
Oral Test Mine Tailings	Aqueous Suspension	Single	100	1.11	425	1.68	1
Oral Test Subsurface Tailings	Aqueous Suspension	Single	100	0.18	2464	1.68	1

ND = not dosed with this material

Dose Preparation

Intravenous and oral technical grade dose materials were prepared the day prior to use. Sodium arsenate heptahydrate or lead acetate trihydrate were weighed on a Mettler Haining balance, and mixed with deionized, distilled water in a volumetric flask. Arsenic or lead solutions were administered separately to avoid chemical interactions. Solutions were submitted to the laboratory for confirmation of arsenic and lead concentrations.

Approximately 0.25 grams of NIST SRM 983 Radiogenic Lead Isotopic Standard lead wire was digested in a polytetrafluoroethylene centrifuge tube with 5 milliliters (ml) of glacial acetic acid and 2 ml of 30% hydrogen peroxide. The tube was heated, the digest diluted with deionized water and filtered using a 45 micron membrane filter. The solution was further diluted in sterile saline solution to give the final concentration. The isotopic composition of NIST SRM 983 Radiogenic Lead solution was reported by NIST as: 92.1497% ²⁰⁶Pb, 6.5611% ²⁰⁷Pb, 1.2550% ²⁰⁸Pb, and 0.0342% ²⁰⁴Pb.

Five samples of driveway slag collected from the vicinity of the Asarco/Ruston site were dried in a circulating air oven at 80 degrees centigrade for 48 hours. Each of the five materials were pre-screened using 1 millimeter (mm) plastic sieve, and the oversize material (greater than 1 mm) was set aside. The less than 1 mm material was sieved using a 100 mesh stainless steel sieve (Tyler). The less than 100 mesh material from each sample was analyzed and used in blending.

Preliminary analyses of each <100 mesh slag sample was conducted using microwave aqua regia digestion. Approximately 0.5 grams of samples were accurately weighed into a fluorinated ethylene propylene microwave digestion vessel: 2.5 ml of nitric acid and 7.5 ml of hydrochloric acid were added, and the mixtures were heated for about 15 minutes while maintaining a stable pressure of about 160 psig. The contents were diluted with distilled, deionized water, filtered through a 0.45 micron cellulose nitrate filter, and the volume of each solution was adjusted to 100 ml using distilled, deionized water. Arsenic and lead were determined in the individual digests by inductively coupled plasma atomic emission spectroscopy and inductively coupled plasma mass spectrometry; the latter techniques employed rhodium and iridium as internal standards. The slag blend was produced by combining a 400 gram subsample of each slag sample in a 2 liter plastic jar, which was then tumbled for about 2 hours on a Norton ball mill tumbler.

The blended slag material was tested for homogeneity by spreading the entire lot of material onto a large piece of paper and removing ten individual increments of about 0.05 grams. Each individual increment was digested using 5 ml of nitric acid in a polytetrafluoroethylene test tube at 95 degrees centigrade for about 16 hours, followed by filtration (0.45 micron cellulose nitrate) and dilution to 100 ml with distilled, deionized water. Arsenic and lead were determined in the digest using plasma mass spectrometry with germanium and platinum as internal standards.

Sixteen individual soil samples were dried in a circulating air oven at 80 degrees centigrade for about 48 hours. Each dried sample was sieved using a 100 mesh stainless steel sieve and less than 100 mesh fractions were analyzed and used in blending. Individual samples were analyzed following digestion of a 0.25 gram subsample using 5 ml of nitric acid in a polytetrafluoroethylene test tube at 85 degrees centigrade for about 2 hours, followed by filtration (0.45 micron cellulose nitrate filter) and dilution to 100 ml with distilled, deionized water. Arsenic and lead were determined in the individual digests using plasma mass spectrometry with rhodium and iridium as internal standards. Based upon lead results exceeding 500 milligrams per kilogram, a blend was produced from nine of the sixteen samples. Blending was conducted as described above for the slag blend. The blend was tested for homogeneity at the 0.05 gram level as described above for the slag material.

Mining site materials were adequately homogeneous at the 0.25 gram level. Vacuum cleaner dust was dried at 80 degrees centigrade for about 36 hours, then sieved with a 100 mesh stainless steel sieve. The less than 100 mesh fraction was analyzed. The surface soil composite, subsurface tailings composite and surface tailings composite were individually dried, sieved and analyzed as described above for dust.

Biological Sample Collection and Handling Protocol

Whole blood was collected from the jugular vein in sterile EDTA-treated containers using a new 20 gauge needle for each animal. For blood collection, animals were restrained by leaning the animal's back against the handler's legs in a nose down position. This handling technique enabled animals to rest quietly with minimum of restraint during blood collection. Blood was collected from all animals prior to dosing and at the intervals shown:

Prior to dosing: 24 and 12 hours

After dosing: 15, 30, 60 minutes, 1.5, 3, 6, 12, 24, 48, 72, 96, 144 hours

Urine volume was recorded and acidified urine samples were collected from the urine collection bags. Samples were grouped in the intervals shown.

Composite Intervals:

Before dosing: 24 hrs - 12 hrs, and 12 hrs through dosing (time 0)

After dosing: time 0-12 hrs, 12-24 hrs, 24-48 hrs, 48-72 hrs, 72-96 hrs, 96-144 hrs

All feces produced during the experiment were collected and frozen. Samples were grouped in the intervals are shown below:

Before dosing: 24 hrs - time 0

After dosing: time 0 through 3 days, 3 days through 7 days

All urine and blood samples were stored at 0-5°C in secured facilities prior to shipment to the analytical lab. Standard chain-of-custody procedures were followed. Holding times for blood, urine and feces have not been officially established by EPA; however, for water samples, the standard holding time is 6 months. In this study, blood and urine samples were analyzed within 30 days of collection. Some blood arsenic samples were reanalyzed within 120 days. Feces samples were analyzed within 90 days of collection.

Samples were prepared as follows. Feces were mixed together using an electric drill and auger adding only enough distilled, deionized water to form a thick paste. The homogenized composite for each time interval was weighed, a portion was removed and dried at 50 degrees centigrade. Percent dry weight was determined by the weight difference before and after drying. Following collection, blood and urine samples were divided at the animal testing facility. Blood and urine samples were maintained at 4 degrees centigrade at all times during preparation and analysis. A portion of each blood, urine and fecal sample was prepared for each of the testing laboratories and one archive portion was retained.

Quality Assurance

Quality Assurance (QA) Project Plans were developed by the analytical laboratory, ESA Laboratories (43 Wiggins Avenue, Bedford, MA 01730) and the animal facility, Michigan State University (MSU, E. Lansing, MI) and submitted to Region 10 EPA. QA Coordinators were designated at each laboratory and were responsible to assure procedures described in the Plans were followed. Reports were submitted to Region 10 EPA for quality assurance verification and audit of the raw data.

Split samples of a minimum of 10% of the blood and urine samples were independently analyzed by the U.S.EPA National Enforcement and Investigations Center (Denver, CO) utilizing different sample preparation and analytical techniques. Independently acquired quality control blood, urine and fecal samples were included in batches of samples sent to both labs at the rate of 7-13%. The samples' appearance was similar to the biological specimens and labeling was not distinguishable from other samples except for a unique sample number.

Characterization of Arsenic and Lead

Environmental Samples. Characterization of the environmental dose materials included multi-element determinations, homogeneity testing, particle size analysis, moisture content, organic matter content, pH, lead isotopic analysis and mineralogical evaluation.

To analyze the soil, slag, tailings and dust and verify concentrations of lead and arsenic in the positive control dosing solutions, a Jarrell-Ash model 61 inductively coupled argon plasma atomic emission spectrometer was used with a fixed cross-flow nebulizer, mass flow meters for all gas streams, and a peristaltic sample delivery pump. Spectral background and inter-element interference corrections were applied. The Jarrell-Ash model 61 inductively coupled argon plasma atomic emission spectrometer was also used with a hydride generation system and mass flow meters for all gas streams. Spectral background and inter-element interference corrections were not necessary.

To determine the lead isotope ratios, the lead and arsenic in animal food and water, and for the analyses of lead in urine and blood, a Sciex Elan model 250 inductively coupled argon plasma mass spectrometer, equipped with mass flow meters for all gas streams and a peristaltic sample delivery pump was used. A refrigerated circulating bath was used to maintain the nebulizer spray chamber at a temperature of 10 °C. Meinhard TR-C concentric glass nebulizers (J.E. Meinhard Associates, Santa Ana, CA) were used. The ion optics of the spectrometer are the updated version: voltage adjustments consist of a barrel lens setting of 11, the plate lens at 2, the Einzel at 95, and the photon stop at 37. The instrument was operated in the multichannel (peak hopping) mode, with single measurements being taken at the nominal mass value of each peak. The low resolution mode was used, producing peak widths of 1.0 - 1.1 m/z at 10% height. The program "Spectrum Display" was used to collect data, which were directed to a personal computer for storage and manipulation using Statgraphics software.

The following digestion and extraction sample preparation procedures were used. Samples of soil, slag, tailings and dust for multi-element analyses were prepared by a potassium hydroxide fusion method. The fusion consisted of mixing 0.25 grams of sample with 2.0 grams of potassium hydroxide in a 10 mL pyrolytic graphite crucible. The sample-potassium hydroxide mixture was placed in an electrically heated muffle furnace and heated for one hour at each of the following temperatures: 150, 300, and 450 °C. Following the last heating step, the fused mixtures were removed from the furnace, allowed to cool, and carefully immersed in a plastic beaker containing 15 mL of distilled, deionized water, 5 mL hydrochloric acid, and 5 mL nitric acid. After an hour of agitation on a rotary shaker, 0.5 mL of 30% hydrogen peroxide was added. The beakers were capped (a small slit was made in the plastic cap so gases could vent) and then agitated overnight on an oscillating shaker, followed by filtering through 0.45 micron cellulose nitrate membrane disposable filter units. The fusates were diluted to 100 mL with deionized water and then transferred to high-density polyethylene bottles for storage. Each fusate contained 5 mL of concentrated hydrochloric acid and 5 mL of concentrated nitric acid per 100 mL total volume.

Samples of soil, slag, tailings, animal food and water for lead isotopic analyses were prepared by a nitric acid digestion method. The digestions were performed in capped 30 mL polytetrafluoroethylene centrifuge tubes which were heated to approximately 90 °C for 18 hours in an air convection oven. The digestates consisted of a 0.25 gram portion of a sample in 5 mL concentrated nitric acid. Following heating, the digestates were allowed to cool, and 20 mL of distilled, deionized water added. The digestates were filtered through 0.45 micron cellulose nitrate membrane disposable filter units, diluted to 100 mL with distilled, deionized water, and then transferred to high density polyethylene bottles for storage.

Lead isotopic analyses were conducted. Lead has four stable isotopes ^{204}Pb , ^{206}Pb , ^{207}Pb , and ^{208}Pb of which the three higher mass isotopes are the daughter nuclides of radioactive decay from uranium and thorium. The isotope, ^{204}Pb , has no known radioactive parent of significance. As a result the absolute abundance of ^{204}Pb remains constant, while the absolute abundance of the other isotopes increases systematically with time. Consequently the isotopic composition of a given sample will be dependent on its age and the relative proportions of thorium and uranium in the parent strata. Isotope abundances of lead are commonly expressed in a relative fashion. For example, the abundance of ^{208}Pb is typically about 50% and the ^{206}Pb is approximately 25% therefore the $^{208}\text{Pb}:^{206}\text{Pb}$ ratio is 2. Each lead ore body has its own specific set of isotope ratios (i.e. its "isotopic fingerprint").

Lead isotopic ratios were determined by plasma mass spectrometry, using pneumatic nebulization, in the nitric acid digestates. Thallium was added to the digestates and served as an internal standard for mass discrimination correction. Long counting times of 100 seconds were used and the mass spectrometer was operated in a peak hopping mode with a dwell time of 50 milliseconds and the low resolution mode. Single measurements were made for each preparation (three preparations per sample). NBS SRM 981 (Common Lead) was used as the control sample from which bias is inferred.

Arsenic and Lead Aqueous Dosing Solutions. Arsenic and lead aqueous dosing solutions were analyzed as follows. Three levels of dilution of each solution of sodium arsenate or lead acetate were analyzed by plasma emission spectroscopy. Each dilution was analyzed in triplicate and one dilution for each of the solutions was spiked appropriately with arsenic or lead. Each dilution was matrix matched to the calibration standards. In addition, three second source reference standards were analyzed to verify the calibration standards. The filtered radiogenic lead isotopic standard solution was analyzed seven times by plasma mass spectrometry. The isotopes ^{206}Pb , ^{207}Pb , and ^{208}Pb were measured with ^{203}Tl and ^{205}Tl as internal standards.

Water and Animal Feed. Water and animal feed were analyzed as follows. A composite of three drinking water samples was collected from the animal watering system, approximately 3 days prior to the first animal dosing, day 3 of the first animal treatment group and at the first dosing of the second animal treatment group. A composite feed sample was prepared from five grab samples from the feed batch consumed by the swine during the study. Multi-element analyses were conducted on both feed and water samples. About 0.5 grams of pulverized feed was digested with 5 mL nitric acid in polytetrafluoroethylene 30 mL screw cap centrifuge tubes. Each vessel was heated with microwave energy in a CEM Model 205 digestion system at 400 watts. Ten mL purified water was added to each cooled sample. Solutions were filtered through 0.45 micron filters and then diluted to 100 mL. Water was analyzed after adding 5 mL nitric acid per 95 mL. Solutions, blanks and spiked samples were analyzed using plasma mass spectrometry. Rhodium and iridium were used as internal standards.

Biological Samples. Biological samples were analyzed as follows. Blood arsenic concentrations were analyzed by atomic absorption spectrophotometry (Hitachi Z-6100) and gaseous hydride generation (HFS-2 Hydride Generation). Duplicate 1.0 ml aliquots of sample are pipetted into acid washed glass tubes. A standard acid mixture of 30:10:1 (HNO₃:HClO₄:H₂SO₄) is added to each sample then heated at 128 degrees centigrade for 10-15 minutes, increased to 150 degrees for 15 minutes, increased to 220-250 degrees and digested to white HClO₄ fume for 5-10 minutes. Then cooled. Aliquots of the following were added sequentially with mixing: 2N HCl, 20% urea, 20% KI and 10% ascorbic acid.

Blood lead concentrations were determined as follows. Plasma source mass spectrometry measurements were made at ²⁰³Tl, ²⁰⁵Tl, ²⁰⁶Pb, ²⁰⁷Pb, and ²⁰⁸Pb. For all scans, an equal measurement time of thirty seconds was used for each isotope. Each digestion batch represented an instrumental analysis run along with calibration standards of 0.0005, 0.002, 0.005, and 0.010 mg L⁻¹ lead, calibration blanks, and continuing calibration standards all containing 0.100 mg L⁻¹ thallium as an internal standard. For a number of batches the calibration curve range was extended by analyzing standards to bracket the highest specimen concentration. For total lead, the signals for the three lead isotopes were summed and ratioed to the sum of the signals for the two thallium isotopes. For the individual lead isotope calibration the signal for each isotope was ratioed to the sum of the signals for the two thallium isotopes. The calibration blank average lead/thallium signal ratio was subtracted and calibration curves were fitted by regression (y=mx). For the digests, the average batch digestion blank value was subtracted.

Blood digestion procedures prior to lead analyses involved the following. Polytetrafluoroethylene 30 mL screw-cap centrifuge tubes were used as the digestion vessels. Each vessel was rinsed twice with water and 3.0 mL nitric acid was added and the tubes were filled with water. The filled tube was placed in a preheated 95 °C convection oven for at least four hours and usually overnight. The nitric acid leach was then repeated with fresh solution. For each tube prior to use, 1.0 mL of concentrated nitric acid was added to an empty tube and the tube heated for one hour at 130 °C in a forty place block digester (Techne Model DG-1). The tube was cooled, 9.0 mL water was added and the contents were shaken. This solution was analyzed by plasma source mass spectrometry for lead. If the analysis indicated a count rate of less than 50 counts per second (about 50,000 counts per second for 0.100 mg L⁻¹ lead and less than 20 counts per second for a calibration blank at ²⁰⁸Pb), then the tube was deemed clean. If the count rate was greater than 50 counts per second, the tube was rejected and taken through another cleaning and analysis sequence. Tubes were subjected to the cleaning and analysis acceptance procedure between use. Generally for the early batches the tubes had a repeat cleaning rate of about 30%. For the later batches, few of the tubes required repeated cleaning.

Each specimen vial was shaken and 0.5 gram of a specimen was weighed into a clean digestion vessel. A new disposable pipet tip was used to transfer each aliquot. The internal standard of 0.010 mL of 100 mg L⁻¹ thallium was added followed by 1.0 mL of nitric acid. The vessel was capped and placed in the preheated 130 °C block digester. After five to seven minutes each tube was vented. Each tube was vented two or three more times over the one hour digestion period. After cooling, 8.5 mL of water was added to each vessel. The specimens for two swine were digested as a batch along with six blanks and two different levels of NIST SRM 955 lead in bovine blood. One specimen for each swine was also digested in duplicate for each batch. A total of eighteen digestion batches were processed.

Urinary arsenic concentrations were determined using a Jarrell-Ash Model 61 inductively coupled argon plasma atomic emission spectrometer was used with a hydride generation system and mass flow meters for all gas streams. The incident power was 1.25 kilowatts and the reflected power was less than five watts. Argon flows of 20 and 0.625 L min⁻¹ for the torch and sample gas, respectively, were used. The observation height was set at 13 mm. Spectral background and inter-element interference corrections were not necessary. A glass hydride gas stripping cell provided mixing of the continuously introduced digested solution and reducing agents, separation of the hydrides and hydrogen from the spent liquid, and mixing of the gaseous products with the argon carrier gas for transmission to the injector of the plasma torch. The digestates were introduced into the hydride generator at a flow rate of 3.0 mL min⁻¹. The reducing agents were a potassium iodide solution (8% w/v) pumped at a flow rate of 1.2 mL min⁻¹ followed by an alkaline tetrahydroborate solution (2.4% w/v sodium tetrahydroborate in 0.1 N sodium hydroxide) pumped at a flowrate of 1.2 mL min⁻¹. Each digestion batch represented an instrumental analysis run along with calibration standards, calibration blanks, and continuing calibration standards. The known addition for each specimen was used to correct for calibration slope rotational error. For the digests, the average batch digestion blank value was subtracted.

Urine digestion procedures prior to arsenic analyses involved the following. Each specimen tube was shaken and 2.0 mL of a specimen was transferred into a clean Corex 30 mL centrifuge tube. Two small glass beads and 0.5 mL of concentrated sulfuric acid was added. The vessels were heated for approximately one hour at 160 °C in a forty place block digester (Techne Model DG-1). Completion of this step was indicated by viscous black appearance of the digestates. The temperature of the block digester was then adjusted to 300 °C. Fuming occurs when digestion is complete. Approximately 30 minutes was required to produce white sulfur trioxide fumes. After 10 minutes of fuming, the tubes were removed from the block digester, and then 0.2 mL of concentrated nitric acid was added dropwise to each vessel. The tubes were then returned to the block digester for approximately two minutes, whereupon most digestates were colorless or slightly yellow. For those vessels containing a dark colored solution, the heated nitric acid treatment was repeated until the solution cleared. After the vessels cooled, 7.8 mL distilled, deionized water and 2 mL concentrated hydrochloric acid was added to each vessel. Each vessel was mixed on a vortex mixer. Two separate 5 mL aliquots of the digestate were transferred to two 10 mL polypropylene screw cap test tubes. Ten to 100 microliters of a 5 mg L⁻¹ arsenic as monosodium methylarsonate standard (Diamond Shamrock, Houston, Texas) was added to one of the tubes to formulate a known addition. The remainder of each digestate was transferred to another test tube for storage. The specimens for four swine were digested as a batch along with six blanks and the two levels of NIST SRM 2607. Two specimens for each batch were also digested in duplicate and one was spiked with known amount of arsenic. A total of nine digestion batches were processed.

Urinary lead concentrations were determined using plasma source mass spectrometry measurements made at ²⁰³Tl, ²⁰⁵Tl, ²⁰⁶Pb, ²⁰⁷Pb, and ²⁰⁸Pb. For all scans, an equal measurement time of thirty seconds was used for each isotope. Each digestion batch represented an instrumental analysis run along with calibration standards of 0.0005, 0.002, 0.005, and 0.010 mg L⁻¹ lead, calibration blanks, and continuing calibration standards all containing 0.100 mg L⁻¹ thallium as an internal standard. For a number of batches the calibration curve range was extended by analyzing standards to bracket the highest specimen concentration. For total lead, the signals for the three lead isotopes were summed and ratioed to the sum of the signals for the two thallium isotopes. The calibration blank average lead/thallium signal ratio was subtracted and calibration curves were fitted by regression (y=mx). For the digests the average batch digestion blank value was subtracted.

Urine digestion procedures prior to lead analyses involved the following. Each specimen tube was shaken and 5.0 mL of each was transferred into a clean digestion vessel (see section 4.b.ii, above, for the cleaning verification procedure). A new disposable pipet tip was used to transfer each aliquot. The internal standard of 0.01 mL of 100 mg L⁻¹ thallium was added followed by 1.0 mL of nitric acid. The vessel was capped and placed in the preheated 130 °C block digester. After five to seven minutes each tube was vented. Each tube was vented once over the one hour digestion period. After cooling, 4.0 mL of water was added to each vessel. The specimens for four swine were digested as a batch along with six blanks and two different levels of NIST SRM 2670 reconstituted freeze dried urine. Two specimen per batch were digested in duplicate and one specimen was spiked with a known amount of lead. A total of nine digestion batches were processed.

Data Evaluation Methodology

Methodology was developed to estimate the extent of arsenic and lead absorption following oral exposure to environmental materials. Objectives were (a) To address the presence of ubiquitous pre-existing background concentrations in biological samples, (b) To enable comparisons of experimental groups of swine receiving high intakes of environmental toxicants with groups receiving low, non-toxic doses of equivalent technical grade chemicals, and (c) To provide an estimate of variability of the calculated biological availability of the metal/metalloid.

Area-under-the-curve (AUC) for blood concentration versus time was determined from zero to 144 hours for individual test animals. The time series of observations for each individual was corrected for background by subtracting each animal's average pre-experiment blood arsenic or lead concentration from the concentrations observed after dosing.

The Student's *t*-test was used to evaluate the difference between the mean pre-experiment blood arsenic concentration of the twenty-seven treated animals and the mean blood arsenic concentration of the six untreated animals. In each of the treated groups, a regression model was determined which described the relationship (with subtraction of endogenous background) between AUC and the dose. Dose was expressed as micrograms (µg) of arsenic (As) per kilogram (kg) of body weight (BW) and the regression model passed through the origin.

Widely differing predose values and postdose variances in blood lead data negated standard analyses of variance methods. In Table 10, total blood lead, the percent change between the two pre-dose baseline concentrations in an individual animal ranged between minus 34% to plus 24%. Within a treatment group, the coefficient of variation in the pre-dose baseline blood lead concentrations ranged from 17 to 65%. These widely differing pre-dose baseline values limit the sensitivity of the experiment. A treatment group must demonstrate a uniformly large change in blood lead concentrations in order to conclude statistical significance by standard analysis of variance methods.

However, within a treatment group the coefficient of variation in the calculated area-under-the-curve (see Table 20) ranged from 50-133%. This degree of variance within a group precluded detection of significant differences.

Inspection of the blood lead concentration results indicated increases in concentrations. Therefore, as an alternative approach, each animal in the untreated and treated groups was tested for significant upward or downward trend in blood lead concentration. The trends were assessed for significance by both Pearson's *r* and Kendall's tau correlation analyses, with the time sequence of successive observations serving as the independent variate. In this approach, all animals were individually tested against the hypothesis that treatment had no nonrandom effects upon blood lead concentration. Inter-group differences were judged by comparing the intra-group tests. In this way, for example, soil-dosed animals all showing a significant increase are clearly responding differently than a population of untreated animals all showing either no change or the opposite change in the same parameter. A value of *P* less than 0.05 was considered statistically significant. This statistical approach is not standard but has been previously applied to the evaluation of blood parameters in immature swine studies when unexplained pattern of variances was observed (Lorenzana et al., 1985a; Lorenzana et al. 1985b).

In each of the treated groups, a regression model was determined which described the relationship (with subtraction of pre-existing background) between AUC and the dose. Dose was expressed as micrograms (μg) of lead (Pb) per kilogram (kg) of body weight (BW). Only the regression model for the intravenously dosed animals passed through the origin. The intravenous lead infusion was introduced directly into the bloodstream; therefore, concentration responses in the blood are expected to be linear. Linear intravenous responses have been previously reported (Freeman et al., 1994; Weis et al., 1993; Aungst et al., 1981).

Non-linear responses at low oral exposures have been previously reported (Freeman et al., 1992; Weis et al., 1993; Aungst et al., 1981). Because a non-linear range was not identified in this study, regression models for slag and soil-exposed groups were not forced through the origin. The regression model describing the relationship between area-under-the-curve (AUC) and dose therefore is not adequate to describe the relationship between lead intake and blood concentrations at intake doses below the experimental range.

For each regression, the 95% confidence intervals for the regression coefficient (slope) were determined. These confidence limits were used in Monte Carlo analyses to determine confidence limits for bioavailability.

RESULTS

Range Finding Experiment

Results indicated that larger doses of the soil (higher blood lead concentrations) were needed in order to obtain greater precision in the lead isotope ratio data, and to determine concentration responses versus time. It was also determined that it was less stressful for the pigs to have urinary collection bags than to obtain urine directly from the catheter. Water consumption, appetite and clinical appearance were normal, and urinary output was adequate. No gross lesions, including bladder lesions, were observed at necropsy. Information from the range finding experiment was used to refine the final design of the experiment which is reported in this study.

Quality Assurance Review of Final Study

The quality assurance review is summarized in Appendix A. Data utilized for bioavailability estimates met quality assurance criteria developed for this study and were verified by two different laboratories.

Grain and Water Analyses

Metal residue results are shown in Table 2, and indicate that nominal concentrations of arsenic and lead were present in the feed. No arsenic was detected in drinking water. Approximately two parts per billion lead were detected in drinking water.

Element	Grain 1 (mg/kg)	Grain 2 (mg/kg)	Grain 3 (mg/kg)	Tap Water (mg/L)
Antimony	0.127	0.096	0.135	
Arsenic	0.462	0.382	0.592	< 0.004
Cadmium	0.188	0.216	0.217	< 0.0005
Cobalt	0.191	0.188	0.210	
Copper	249.	167.	189.	0.120
Iron	169.	136.	203.	0.180
Lead	1.30	1.09	1.29	0.0015
Manganese	43.	42.	44.4	0.035
Molybdenum	2.48	2.58	2.48	0.002
Nickel	2.74	2.67	2.64	
Thallium	< 0.05	< 0.05	< 0.05	< 0.0002
Selenium	1.93	0.927	2.04	< 0.010
Zinc	63.5	51.2	77.2	0.380

Dose Analyses

The arsenic and lead concentrations determined by chemical analyses were used for bioavailability calculations. The concentrations of arsenic in the control gavage and intravenous solutions were, respectively, 143 (sd=3.0) or 837 (sd=14.5) milligrams arsenic per liter (sodium arsenate solutions). The concentrations of lead in the control gavage and intravenous solutions were, respectively, 177 (sd=6.8) or 1034 (sd=26) milligrams lead per liter. The concentration of 206Pb in the dosing solution was 2.97 milligrams lead per liter with a standard deviation of 0.013 mg/L.

The elemental composition of soil, slag and mining samples are shown in Table 3. These environmental substrates contained arsenic concentrations ranging from approximately 1500 milligrams arsenic per kilogram of soil (mg/kg) to approximately 11,000 mg/kg. Lead concentrations

ranged from approximately 1,300 mg Pb/kg to 25,000 mg Pb/kg. Other metal compounds were also present in the substrates.

The isotopic composition of soil, slag and mining samples are present in Table 4. No significant bias was indicated by the NBS 981 determinations and imprecisions were within acceptable limits. The similarities of the ratios for the mining site samples strongly suggest the same lead parent strata for these environmental substrates. The same can be concluded for the two smelter site samples. The source discrimination power of the isotope technique is illustrated by the differences detected between the mining site samples, the smelter site samples and the standard reference materials.

Element	Smelter Site Soil Composite Sample				Smelter Site Slag Composite Sample			
	Average mg/Kg	Std Dev mg/Kg	LCL mg/Kg	UCL mg/Kg	Average mg/Kg	Std Dev mg/Kg	LCL mg/Kg	UCL mg/Kg
Aluminum	65000	1160	62100	67900	18200	520	16900	19500
Antimony	145	4.6	134	156	3350	309	2580	4120
Arsenic	1600	31	1530	1680	10100	407	9100	11100
Barium	526	7.0	509	544	274	8.1	254	294
Beryllium	ND	2.2	0	8	6.9	0.3	6.0	7.7
Boron	26	0.7	24	28	18	1.8	14	23
Cadmium	7	1.1	4	10	16	2.9	8.4	23
Calcium	16500	273	15800	17100	41700	1180	38700	44600
Chromium	91	0.4	89	92	401	23	343	459
Cobalt	17	0.4	16	18	269	9.1	246	291
Copper	2670	50	2540	2790	5220	169	4800	5640
Iron	34800	660	33200	36500	224000	4310	214000	235000
Lead	1350	31	1270	1430	3780	118	3480	4070
Lithium	17	1.2	14	20	8.0	0.9	5.7	10.3
Magnesium	8630	114	8350	8920	6910	163	6500	7310
Manganese	768	14	733	803	850	22	796	904
Molybdenum	15	1.2	12	18	1770	67	1600	1930
Nickel	59	3.3	50	67	93	2.3	88	99
Phosphorus	935	19	887	983	527.2	30.2	452.2	602.2
Selenium	ND	19	0	52	ND	15	0	24
Silicon	274000	12800	242000	306000	180000	11100	152000	207000
Silver	15	0.2	14	15	18.2	1.4	14.8	21.6
Sodium	16500	560	15100	17900	5000	320	4210	5790
Strontium	240	3.6	231	249	133	4.7	121	144
Sulfur	510	23	453	568	3570	128	3260	3890
Thallium	ND	6.5	0	14	ND	26	0	75
Tin	53	5.2	40	66	367	14.8	331	404
Titanium	4850	91	4630	5080	1590	62	1440	1750
Vanadium	99	2.3	93	105	58	2.6	52	65
Yttrium	15.8	0.1	15.4	16.1	11	0.3	10.7	12.1
Zinc	332	8.5	311	353	11400	458	10200	12500

Table 3. Elemental Concentrations in Environmental Substrates

Element	Mine Site Surface Tailings Sample				Mine Site Subsurface Tailings Sample			
	Average mg/Kg	Std Dev mg/Kg	LCL mg/Kg	UCL mg/Kg	Average mg/Kg	Std Dev mg/Kg	LCL mg/Kg	UCL mg/Kg
Aluminum	20400	822	18400	22500	20200	433	19100	21200
Antimony	646	21	595	697	1720	10	1700	1740
Arsenic	11100	379	10200	12000	1810	34	1730	1900
Barium	571	24	512	629	2160	64	2000	2310
Beryllium	6.2	0.4	5.3	7.1	1.7	0.1	1.4	2.0
Boron	144	6.8	127	161	116	3.6	107	125
Cadmium	76	3.4	68	85	6.1	1.8	56	65
Calcium	38800	1030	36200	41300	36100	378	35100	37000
Chromium	62	3.6	53	71	32.5	0.1	32.2	32.8
Cobalt	3.0	0.9	0.8	5.2	10.5	0.7	8.8	12.2
Copper	270	6.3	254	285	504	11.6	475	532
Iron	52100	1863	47500	56700	51600	809	49600	53600
Lead	4250	177	3810	4690	24600	511	23400	25900
Lithium	11	1.2	8	14	13.1	0.3	12.4	13.8
Magnesium	14137.0	430.6	13067.2	15206.9	11200	265	10600	11900
Manganese	5840	176	5410	6280	1360	34	1270	1450
Molybdenum	36	2.4	30	42	16.7	0.8	14.8	18.6
Nickel	80	7.5	61	98	61	2.5	54	67
Phosphorus	1980	87	1760	2200	885	32.4	805	966
Selenium	ND	16	0	54	ND	8.5	3.2	45.5
Silicon	172000	17600	129000	216000	223000	22700	166000	279000
Silver	30	1.5	26	34	303	5.3	290	316
Sodium	953	139	607	1299	1110	79	918	1310
Strontium	60	1.8	56	65	57	1.2	54	60
Sulfur	34500	1360	31100	37900	43900	291	43200	44600
Thallium	ND	15	0	48	ND	15	0	58
Tin	74	5.1	62	87	ND	4.2	0	13
Titanium	1510	65	1350	1670	1520	9.8	1500	1540
Vanadium	526	21	472	579	144	2.4	138	149
Yttrium	26.1	0.3	25.3	26.9	11.7	0.2	11.1	12.2
Zinc	11000	410	9980	12000	5410	86	5200	5630

Table 3. Elemental Concentrations in Environmental Substrates

Element	Mine Site House Dust Sample				Mine Site Surface Soil Sample			
	Average mg/Kg	Std Dev mg/Kg	LCL mg/Kg	UCL mg/Kg	Average mg/Kg	Std Dev mg/Kg	LCL mg/Kg	UCL mg/Kg
Aluminum	14500	770	12600	16400	38800	1620	34800	42900
Antimony	54	10	29	80	183	6.0	168	198
Arsenic	713	43	606	819	1540	23	1490	1600
Barium	477	24	416	538	1180	33	1100	1270
Beryllium	ND	1.2	0	3	3.8	0.2	3.3	4.2
Boron	95	10	70	121	129	3.9	119	139
Cadmium	14	0.9	11	16	32.4	0.5	31.3	33.6
Calcium	19900	1150	17000	22700	31600	490	30400	32800
Chromium	44	1.6	40	47	82	0.5	80	83
Cobalt	5	1.0	2	7	7	0.2	6	7
Copper	99	5.9	84	114	130	5.0	117	142
Iron	12500	606	11000	14000	31300	595	29800	32800
Lead	665	32	587	744	2170	14	2140	2210
Lithium	8	0.7	6.5	10.1	23.5	0.9	21.2	25.8
Magnesium	7300	375	6370	8230	19700	523	18400	2100
Manganese	841	44	731	951	2290	27	2220	2350
Molybdenum	7	1.9	2.2	11.7	27	3.3	18	35
Nickel	39	3.0	31	46	70	3.7	60	79
Phosphorus	1060	48	943	1180	1620	51	1490	1750
Selenium	ND	14	0	45	ND	14	0	50
Silicon	104000	4530	93100	116000	309000	587	307000	310000
Silver	6	1.8	2	11	17	1.5	13	21
Sodium	127000	4040	117000	137000	4070	63	3910	4220
Strontium	79	4.9	67	91	130	2.3	125	136
Sulfur	6400	356	5520	7290	6070	44	5960	6180
Thallium	ND	15	0.0	42	22.8	0.8	20.8	24.8
Tin	19	7.5	0.9	38	30	5.2	17	43
Titanium	1360	70	1190	1530	2590	67	2420	2750
Vanadium	62	2.5	56	68	276	3.4	267	284
Yttrium	7.6	0.4	6.5	8.7	25	.4	21	29
Zinc	1510	78	1310	1700	4500	55	4360	4630

Std Dev = Standard Deviation LCL = Lower confidence level at 0.05 significance level
 ND = Confidence interval included zero UCL = Upper confidence level at 0.05 significance level

Table 4. Lead Isotope Ratios

	Pb-204 to Pb-206			Pb-207 to Pb-206			Pb-208 to Pb-206		
	Average	Std Dev	%RSD	Average	Std Dev	%RSD	Average	Std Dev	%RSD
Mine Site Surface Soil	0.05063	0.00051	1.02	0.79935	0.00269	0.34	1.98702	0.01537	0.77
Mine Site Surface Tailings	0.04996	0.00041	0.82	0.79167	0.00282	0.36	1.96605	0.01825	0.93
Mine Site Subsurface Tailings	0.05053	0.00062	1.23	0.79862	0.00257	0.32	1.95291	0.01141	0.58
Mine Site House Dust	0.05062	0.00082	1.62	0.79480	0.00284	0.36	1.99505	0.00772	0.39
Smelter Site Soil	0.05566	0.00135	2.43	0.86104	0.00378	0.44	2.10150	0.01145	0.54
Smelter Site Slag	0.05491	0.00121	2.20	0.85301	0.00258	0.30	2.09250	0.01415	0.68
NBS SRM 981	0.05905	0.00088	1.50	0.91105	0.00774	0.85	2.15507	0.03490	1.62
Certified Value	0.05904			0.91464			2.16810		
Deviation	-0.00001			0.00359			0.01303		
% Deviation	-0.013			0.393			0.601		

n = 3 for all samples
 n = 27 for the SRM

Clinical Observations

All animals appeared clinically normal during the course of the study. One animal in the untreated group was found dead five days into the study. Necropsy did not reveal the cause of death. Animals were not sacrificed at the end of the study.

Urine Samples

Individual animal urine volumes are provided in Table 5. Several urine samples were lost due to disconnection of the catheter with the urine collection bag, some urine leaked around the catheters and several animals lost their catheters during the study. Consequently, analytical results do not represent all of the arsenic or lead which may have been excreted in urine

Animal# (group #)	Hour Intervals After Dosing						
	pre-24	0-12	12-24	24-48	48-72	72-96	96-144
126 (1)	370	310	610	1070	790	1050	160
137 (1)	550	510	650	530	810	1190	3340
195 (1)	770	630	750	1250	950	2110	4310
190 (1)	2290	410	870	1070	2050	1290	3750
133 (2)	270	190	310	410	900	610	1570
199 (2)		310	90	90	270	690	730
140 (3)	290	140	140	430	810	1070	1570
188 (3)	370	90	130	110	170	90	570
141 (8)	310	190	390	650	530	650	1440
182 (8)	840	580	580	980	1670	920	1080
138 (9)	330	630	710	1330	1230	880	915
196 (9)	890	350	350	1150	1210	1070	2710
130 (4)	330	170	290	570	580	800	390
131 (4)	580	610	450	790	1710	770	1490
136 (4)							
139 (5)	560	440	570	950	930	950	3340
145 (5)	125	530		1350	1170	1040	2875
148 (5)	530	570	610	1070	610	1300	1575
129 (6)	430	210	490	990	900	1330	2810
144 (6)	210	190	190	750	870	540	870
150 (6)	300			350	650	580	2940
142 (7)	440	220	280	650	470	215	875
147 (7)	540	370	390	1310	900	690	1670
149 (7)	490	180	230	630	570	780	450
192 (10)	2210	470	890	410	390	590	690
194 (10)	1130	430	830	690	1110	730	2390
189 (10)	310	210	350	870	1690	970	3270
180 (11)	280	410	750	1370	2110	1550	3630
197 (11)	250	650	730	2130	1490	282	4290
179 (11)	870	230	170	1230	510	450	470
186 (12)	1090	650	790	1270	2050	2630	4750
191 (12)	1070	570	750	110	350	190	1090
184 (12)	590	310	80	1490	2310	1390	4190
177 (13)	1440	390	550	1510	2990	770	4710
181 (14)	2790	910	1150	2530	2890	2690	5290
187 (15)	430	170	190	190	210	80	990

Empty cell indicates no sample was obtained.

Arsenic Concentrations in Blood

Arsenic concentrations in blood are shown in Table 6. The detection limit was 1 ug/L and a value of one-half the detection limit was utilized when no arsenic was detected. No significant difference ($p < 0.5$) was found between the mean pre-experiment blood arsenic concentration of the twenty-seven treated animals and the mean blood arsenic concentration of the six untreated animals. Subtraction of the pre-experiment blood arsenic concentrations from each experimental observation eliminates the effect of background concentrations on the determination of bioavailability. Elevated blood

arsenic concentrations were not prolonged indicating that gastric emptying was not delayed. Blood arsenic concentrations returned to pre-dosing levels by the last sampling at 144 hours.

Area-under-the-curve results of time versus blood arsenic concentrations are shown in Table 7.

Arsenic and Lead Concentrations in Urine

Arsenic and lead concentrations in urine are shown in Table 8 and Table 9. The detection limits were 2 ug/L for arsenic and 0.8 ug/L for lead.

Table 6. Arsenic Concentrations in Blood (micrograms per liter; ug/L)

Pig ID#	Treatment Group	Pre-Dosing		Hour Intervals After Dosing												SRM¶
		pre-1	pre-2	0.25	0.5	1.0	1.5	3.0	6.0	12	24	48	72	96	144	
#126	1	0.5	0.5	0.5	0.5	0.5	0.5	1	1	1	1	1	1	0.5	*	3
#137	1	1	1	2	2	1	1	0.5	3	2	2	2	2	1	1	0.5
#195	1	4	3	3	2	3	3	3	4	4	2	2	2	1	2	0.5
#190	1	1	0.5	0.5	0.5	0.5	1	0.5	2	2	2	2	2	1	0.5	0.5
#133	2	1	1	0.5	5	6	7	8	5	3	2	1	0.5	1	0.5	0.5
#199	2	134	0.5	25	86	89	90	99	137	44	9	3	2	1	1	121
#140	3	1	2	0.5	32	45	47	62	55	27	6	2	3	1	3	0.5
#188	3	1	0.5	0.5	1	1	0.5	1	1	2	2	1	0.5	2	1	1
#141	8	0.5	1	14	12	*	7	6	4	2	1	1	1	1	0.5	0.5
#182	8	1	0.5	496	369	318	254	183	88	42	12	6	3	3	3	4
#138	9	2	2	111	89	97	94	76	50	28	9	4	2	1	2	107, 0.5
#196	9	1	0.5	2	125	0.5	0.5	0.5	2	1	0.5	1	0.5	1	*	148
#130	4	0.5	0.5	0.5	0.5	1	2	9	12	4	1	0.5	0.5	0.5	0.5	147
#131	4	0.5	0.5	0.5	2	5	7	15	14	6	2	0.5	1	0.5	1	0.5
#136	4	0.5	0.5	0.5	0.5	0.5	1	4	16	4	0.5	0.5	0.5	0.5	0.5	10
#139	5	2	1	1	5	9	21	52	34	12	3	2	2	2	1	120, 108
#145	5	0.5	1	0.5	1	3	16	39	37	*	5	1	0.5	0.5	0.5	144
#148	5	1	1	2	5	9	16	37	37	13	4	2	3	1	2	0.5
#129	6	1	1	0.5	5	22	32	57	58	15	3	0.5	0.5	0.5	0.5	148
#144	6	1	0.5	2	5	9	14	40	50	12	4	1	1	1	1	0.5
#150	6	1	3	3	3	8	36	80	80	24	5	4	3	3	5	120, 1
#142	7	1	2	1	1	11	19	50	93	46	8	3	0.5	1	0.5	0.5
#147	7	0.5	1	2	4	13	19	55	84	37	7	2	1	1	1	0.5
#149	7	2	3	7	10	43	51	56	83	36	10	5	3	2	3	0.5
#192	10	5	2	2	0.5	3	17	78	136	52	10	4	3	3	2	0.5
#194	10	2	1	2	9	15	28	83	125	53	11	3	1	2	1	148
#189	10	*	1	3	10	24	53	87	49	22	8	3	3	4	1	147
#180	11	1	0.5	3	20	48	100	231	290	109	16	4	4	2	2	0.5
#197	11	1	1	3	13	48	60	180	211	111	20	5	4	3	2	0.5
#179	11	2	2	3	7	28	67	11	249	49	14	6	2	12	3	0.5
#186	12	1	0.5	7	35	77	118	177	243	90	20	4	3	2	2	0.5
#191	12	0.5	1	*	23	37	125	224	423	142	22	6	3	3	1	160
#184	12	*	1	2	8	20	44	58	196	78	14	4	4	4	2	2
#177	13	2	2	2	7	32	49	83	82	26	5	2	2	2	0.5	0.5
#181	14	1	2	2	2	2	6	10	10	8	4	2	2	2	1	0.5
#187	15	2	2	3	*	4	5	10	11	9	3	2	2	4	1	140

Group #s: 1 = Neg Control; 2 = Low oral sol salt; 3 = High oral sol salt; 4 = Low oral soil; 5 = Med/Low oral soil; 6 = Med/hi oral soil; 7 = High oral soil; 8 = Pos control, low IV; 9 = Pos control, high IV; 10 = Low oral slag; 11 = Med oral slag; 12 = High oral slag; 13 = Surface tailings; 14 = Subsurface tailings; 15 = Residential soil; * = Missing data;

¶ = Standard Reference Material (SRM) samples which were blinded and placed among the test blood samples

**Table 7. Arsenic Blood Area-Under-The-Curve
Results**

Pig No.	Trmt Grp	Treatment	Pig Wt (kg)	Total Dose (ug)	Total Dose (mg/kg)	AUC ug/ml/hr
141	8	IV/Control	18	163	0.01	18
182			14	4302	0.31	1971
138	9	IV/Control	15	1657	0.11	784
133	2	PO/Control	15.5	129	0.01	59
199			11	3381	0.31	1310
140	3	PO/Control	15.5	1707	0.11	625
130	4	Soil	17	681	0.04	85
131		25	18.5	741	0.04	146
136		mg/kg	16	641	0.04	82
139		Soil	17	1634	0.10	338
145	5	60	15.5	1490	0.10	291
148		mg/kg	16.5	1586	0.10	400
129	6	Soil	16	2563	0.16	440
144		100	15.5	2483	0.16	385
150		mg/kg	18.5	2964	0.16	709
142	7	Soil	16	3845	0.24	846
147		150	17	4085	0.24	773
149		mg/kg	19.5	4686	0.24	837
192	10	Slag	13.5	8192	0.61	1017
194		60	13	7889	0.61	1117
189		mg/kg	14	8496	0.61	144
180	11	Slag	15	15171	1.01	2656
197		100	11	11125	1.01	2365
179		mg/kg	14	14160	1.01	2348
186	12	Slag	12.5	18964	1.52	2332
191		150	16.5	25032	1.52	3421
184		mg/kg	14.5	21998	1.52	1752
177	13	SurfTail	11	12212	1.11	655
181	14	SubTail	14.5	2630	0.18	36
187	15	Soil	14.5	2239	0.15	144

Table 8. Total Arsenic Eliminated in Urine (micrograms)								
Animal# (Group #)	Hour Intervals After Dosing							Total (ug)
	pre-24	0-12	12-24	24-48	48-72	72-96	96-144	
126 (1)	29.6	31.0	44.5	92.0	102.7	73.5	9.1	352.9
137 (1)	26.4	32.6	46.8	89.0	75.3	89.3	187.0	520.1
195 (1)	67.8	39.1	42.0	80.0	53.2	69.6	94.8	378.7
190 (1)	57.3	31.6	57.4	77.0	67.7	60.6	142.5	436.8
133 (2)	40.5	90.6	64.8	68.9	44.1	68.9	94.2	431.5
199 (2)		1221.4	186.3	59.3	83.4	70.4	48.2	1669.0
140 (3)	29.0	905.8	425.6	226.2	115.8	117.7	171.1	1962.2
188 (3)	67.7	19.2	14.2	22.4	27.0	11.1	49.6	143.5
141 (8)	36.9	119.1	63.2	89.1	84.3	75.4	122.4	553.4
182 (8)	84.0	3306.0	1090.4	480.2	202.1	163.8	125.3	5367.7
138 (9)	25.7	882.0	323.1	244.7	98.4	90.6	40.3	1679.1
196 (9)	72.1	36.1	45.2	64.4	65.3	77.0	140.9	428.9
130 (4)	87.5	340.0	121.2	124.3	96.9	81.6	25.7	789.7
131 (4)	52.2	242.8	224.6	122.5	155.6	101.6	174.3	1021.4
136 (4)								
139 (5)	43.1	849.2	223.4	128.3	122.8	95.0	163.7	1582.3
145 (5)	10.5	588.3		108.0	124.0	75.9	123.6	1019.9
148 (5)	32.3	723.9	122.6	159.4	95.8	80.6	45.7	1228.0
129 (6)	37.4	1455.3	442.0	182.2	114.3	103.7	188.3	2485.8
144 (6)	21.4	1305.3	418.0	108.8	101.8	39.4	107.9	2081.1
150 (6)	40.8			195.3	120.9	133.4	129.4	579.0
142 (7)	26.8	1386.0	462.0	110.5	65.8	31.4	81.4	2137.1
147 (7)	33.5	1517.0	585.0	200.4	108.9	114.5	160.3	2686.2
149 (7)	38.7	2304.0	680.8	192.8	127.7	148.2	85.5	3539.0
192 (10)	75.1	2453.4	717.3	283.7	153.7	113.3	75.9	3797.3
194 (10)	80.2	2279.0	515.4	172.5	46.6	32.9	112.3	3158.7
189 (10)	7.1	1142.4	451.5	244.5	113.2	95.1	160.2	2206.9
180 (11)	44.8	4510.0	1500.0	413.7	158.3	125.6	199.7	6907.2
197 (11)	30.0	3152.5	1423.5	383.4	117.7	10.2	124.4	5211.7
179 (11)	83.5	3335.0	1042.1	365.3	101.0	72.5	64.4	4980.2
186 (12)	73.0	5050.5	1706.4	508.0	135.3	84.2	171.0	7655.4
191 (12)	89.9	4902.0	2049.8	163.9	92.1	46.7	130.8	7385.2
184 (12)	3.5	2759.0	632.8	892.5	184.8	77.8	209.5	4756.5
177 (13)	60.5	1216.8	313.0	146.5	80.7	87.8	155.4	2000.2
181 (14)	53.0	180.2	89.7	101.2	54.9	40.4	100.5	566.9
187 (15)	63.2	164.6	149.2	61.8	46.0	15.0	78.2	514.6

Empty cell indicates no sample was obtained.

Table 9. Total Lead Eliminated in Urine (micrograms)								
Animal# (Group #)	Hour Intervals After Dosing							Total ug in urine
	pre-24	0-12	12-24	24-48	48-72	72-96	96-144	
126 (1)	2.48	2.42	1.65	5.24	2.84	1.79	0.26	14.19
137 (1)	3.30	2.40	1.76	3.60	2.43	2.86	6.68	19.72
195 (1)	1.62	1.07	0.90	2.13	1.90	3.59	4.31	13.89
190 (1)	2.52	1.27	1.57	1.39	1.44	2.58	0.75	8.99
133 (2)	0.54	0.15	1.61	2.21	1.26	1.77	1.88	8.89
199 (2)	2.48	26.51	4.86	4.23	4.27	6.49	8.03	54.38
140 (3)	1.65	6.33	4.42	5.46	3.56	1.18	2.04	23.00
188 (3)	0.22	0.06	0.60	0.15	0.92	0.35	1.82	3.91
141 (8)	1.61	12.33	8.11	11.05	5.57	2.93	2.02	42.00
182 (8)	3.19	85.72		34.30	41.42	28.06	33.26	
138 (9)	2.28	57.52	29.47	44.29	21.77	23.67	12.35	189.07
196 (9)	2.31	95.59	56.11	64.40	49.25	49.22	64.23	378.78
130 (4)	0.73	1.94	1.51	2.51	2.32	1.84	0.51	10.62
131 (4)	3.07	1.89	3.24	4.11	4.79	2.39	3.13	19.54
136 (4)								
139 (5)	2.86	14.61	5.99	7.22	4.56	1.81	5.01	39.19
145 (5)	0.63	3.13	2.16	2.57	3.28	1.56	4.89	17.57
148 (5)	1.01	3.02	0.49	2.35	1.77	1.04	1.58	10.25
129 (6)	1.46	4.98	3.68	3.56	1.53	1.86	3.65	19.26
144 (6)	0.78	18.62	13.09	9.30	8.61	3.02	4.61	57.26
150 (6)	1.20	11.80	8.38	3.89	2.28	2.73	3.53	32.60
142 (7)	3.26	7.83	5.54	4.23	2.82	0.47	1.93	22.82
147 (7)	1.57	3.77	2.89	2.88	1.89	1.45	2.34	15.22
149 (7)	3.48	7.52	5.61	6.05	3.31	1.87	1.26	25.62
192 (10)	2.21	3.53	1.42	1.76	1.76	1.83	1.38	11.68
194 (10)	3.16	7.40	2.49	3.73	1.67	1.53	4.06	20.87
189 (10)	1.21	14.07	6.44	7.05	1.71	4.46	6.87	40.60
180 (11)	1.12	8.53	5.03	4.25	4.43	4.34	3.04	29.61
197 (11)	1.83	4.62	3.36	2.34	3.13	0.68	4.29	18.41
179 (11)	1.91	7.38	2.89	3.32	3.62	2.43	1.79	21.43
186 (12)	2.40	8.65	4.27	4.06	2.05	2.10	4.28	25.40
191 (12)	2.03	6.27	4.73	0.65	14.35	0.86	3.05	29.90
184 (12)	0.53	6.98	2.10	8.34	4.39	3.20	7.54	32.55
177 (13)	3.60	12.87	5.67	6.80	3.59	4.24	6.59	39.75
181 (14)	1.40	22.75	8.40	13.92	8.67	4.30	7.41	65.44
187 (15)	1.03	2.77	2.20	1.14	0.71	0.16	3.47	10.45

Empty cell indicates no sample was obtained.

Lead Concentrations in Blood

Lead concentrations in blood are shown in Table 10. In animals orally exposed to smelter soil or slag, maximum blood lead concentrations were detected at 6 or at 12 hours (T_{\max} mean=8.5; sd=3.4; n=20). In animals orally exposed to mine site substrates T_{\max} was at 6 hours except the animal which received mine site soil. In this animal, T_{\max} occurred at 24 hours. The detection limits were 0.19 ug/dL, 0.04 ug/dL, 0.05 ug/dL and 0.09 ug/dL for total lead, ^{206}Pb , ^{207}Pb and ^{208}Pb , respectively. Elevated blood lead concentrations were not prolonged indicating that gastric emptying was not delayed.

Analytically measured total blood lead concentrations are the sum of intravenously administered ^{206}Pb , background lead and lead absorbed from the environmental substrates. Because animals were randomly assigned to treatment groups and dose substrates were administered on the basis of body weight, the concentration of blood lead can be described by:

$$C_{\text{Pb,t}} = C_{\text{Pb,bk}} + C_{\text{Pb,206}} + C_{\text{pb,po}}$$

where

$C_{\text{Pb,t}}$ = total lead analytically measured, ug/dL

$C_{\text{Pb,bk}}$ = background lead concentration; average of pre-dosing concentrations, ug/dL

$C_{\text{Pb,206}}$ = intravenously administered ^{206}Pb , ug/dL

$C_{\text{pb,po}}$ = perorally absorbed lead, ug/dL

The average abundance of ^{208}Pb in the environmental substrates was 51.9% of all lead isotopes with a standard deviation of 0.37%. The ^{208}Pb abundance in blood lead concentrations of the four untreated experimental animals averaged 50.7% of all lead isotopes with a standard deviation of 1.67%. Variability in the ^{208}Pb concentrations was due to the low concentration of lead in these blood samples. The ^{208}Pb abundance in the background blood lead concentrations approximated that of the environmental substrates. Intravenous ^{206}Pb was administered with the peroral materials at one tenth or less of the estimated total lead dose. Negligible quantities of ^{208}Pb were contributed by the ^{206}Pb -enriched dose because of the low abundance of ^{208}Pb in the ^{206}Pb solution and the low intravenous dose.

Therefore, analytically measured blood ^{208}Pb concentrations were attributable to absorption of ^{208}Pb from background sources and orally administered lead-containing substrates. Total lead contributed by background sources and the perorally administered substrates can be calculated as follows:

$$C_{\text{Pb,bk}} + C_{\text{Pb,po}} = 1.927 * C_{\text{Pb208}}$$

C_{Pb208} is the analytically measured ^{208}Pb concentrations and the constant of 1.927 is the inverse of the abundance of ^{208}Pb in the environmental substrates (51.9%). Even though the 208/206 isotope ratios differ for the environmental substrates (see Table 4), the use of an average percent abundance of ^{208}Pb for all substrates does not significantly influence the outcome of the calculation because of the relatively low standard deviation of 0.37% as compared to the standard deviations and coefficients of variation in the blood lead concentrations. The intravenous ^{206}Pb concentration is calculated as follows:

$$C_{\text{Pb},206} = C_{\text{Pb},t} - [1.927 * C_{\text{Pb}208}]$$

The calculated total blood lead concentration ($C_{\text{Pb,calc}}$) which includes lead from background sources and lead from perorally administered substrates but not lead from intravenously administered ^{206}Pb is determined by subtraction:

$$C_{\text{Pb,calc}} = C_{\text{Pb},t} - C_{\text{Pb},206}$$

Calculated total blood lead concentrations ($C_{\text{Pb,calc}}$) minus background are shown in Table 11. Calculated intravenously administered ^{206}Pb ($C_{\text{Pb},206}$) concentrations are shown in Table 12. The coefficient of variation in area-under-the-curve of intravenously administered ^{206}Pb was approximately 34% (mean = 25.6; sd=7.9; n=28) as shown in Table 21.

The relative contributions to variation in blood lead concentrations were evaluated. Analytical measurement precision was evaluated by thirty-seven pairs of duplicate analyses. The average standard deviation was 0.185 ug/dL, 0.057 ug/dL, 0.050 ug/dL and 0.101 ug/dL for total lead, ^{206}Pb , ^{207}Pb and ^{208}Pb , respectively. The coefficients of variation were 3.6%, 3.2%, 5.3% and 5.0%, respectively. Replicate analyses of calibration standards prepared from NIST SRM 981 Common Lead with certified isotopic compositions were also performed. In accordance with counting statistics, the isotope ratios for the lower concentration standard were more variable than those for the higher concentrations. For analyses of the low standard (0.0005 mg/L; n=71), the coefficient of variation was 6.24%.

Background blood lead concentration may contribute to variation. The variability of background lead concentrations can be estimated from the blood lead results of the untreated animals. Table 13 presents a summary of these results. The average coefficient of variation of the background lead is approximately 11.4%. For example, for a background lead of 2 ug/dL the standard deviation would be 0.23 ug/dL.

Additional discussion of variation is contained in Appendix B, Evaluation of Sources of Variation.

**Table 10. Blood Lead Concentrations (total analytically measured;
µg/dl)**

Pig ID#	Grp#	Trmt	Hour Intervals After Dosing													
			Pre #1	Pre #2	0.25	0.5	1	1.5	3	6	12	24	48	72	96	144
126	1	Neg	1.37	1.49	1.43	1.35	1.36	1.17	1.31	1.13	1.18	1.20	1.09	1.05	1.10	
137		Neg	1.87	2.20	2.09	2.10	2.52	2.41	1.95	2.03	2.10	1.93	1.88	2.32	1.70	1.68
195		Neg	1.48	1.35	1.28	1.65	1.30	1.40	1.40	1.59	1.21	1.05	0.92	1.13	1.26	1.46
190		Neg	1.02	0.90	0.97	1.04	1.01	0.93	1.01	0.86	0.96	0.97	0.86	0.94	0.84	0.93
133	2	PO Lo	1.46	1.62	2.35	2.22	2.61	2.20	2.29	2.11	2.24	1.80	1.44	1.19	1.02	1.04
199		PO Lo		1.00	2.14	6.62	12.95	15.47	19.12	22.50	14.26	10.67	6.71	4.27	3.25	3.25
140	3	POHi	1.41	1.31	2.37	2.61	2.87	2.78	3.06	3.51	3.95	3.01	2.11	1.71	1.47	1.24
188		POHi	0.50	0.55	1.08	1.18	1.34	1.61	4.06	8.77	5.67	3.54	2.05	1.52	1.23	1.08
141	8	IV Lo	1.32	1.34	12.32	12.48	12.46	11.90	10.10	10.00	7.80	5.23	3.41	2.44	2.03	1.70
182		IV Lo	1.69	1.57	103.50	80.88	63.43	51.05	37.38	26.59	19.99	15.80	12.00	9.06	7.97	7.88
138	9	IV Hi	1.47	1.49	87.95	68.09	55.07	41.55	46.45	22.92	14.67	10.33	7.11	6.60	4.75	4.04
196		IV Hi	1.23	1.22	292.26		154.77	117.72	74.46	45.18	30.18	23.79	15.28	11.34	9.81	
130	4	Soil	0.97	0.97	1.49		1.69	1.72	1.73	1.86	1.96	1.67	1.40	1.48	1.34	1.18
131		25	2.23	1.67	2.76	2.51	2.75	2.60	2.79	2.85	3.10	2.65	2.32	2.07	1.71	1.60
136		mg/kg	1.92	2.11	2.50	2.49	2.35	2.58	2.45	2.43	2.30	2.16	1.66	1.60	1.29	1.79
139	5	Soil	1.71	2.34	2.78	2.47	2.37	2.51	3.89	7.15	6.42	4.90	3.47	3.00	2.49	2.30
145		60	1.87	1.87	2.22	2.47	2.32	2.45	2.42	2.61	2.67	2.17	1.83	1.39	1.31	1.71
148		mg/kg	1.22	1.38	2.29	2.57	2.28	2.24	2.40	2.88	2.66	2.44	1.69	1.73	1.04	1.00
129	6	Soil	2.31	1.86	2.74	3.35	3.48	3.27	3.47	5.19	5.63	4.26	3.13	2.51	1.97	2.05
144		100	0.75	0.90	1.42	1.71	1.81	2.16	3.94	8.59	6.49	4.35	2.65	1.91	1.54	1.27
150		mg/kg	1.55	1.60	2.31	3.14	2.23	2.33	2.62	3.67	3.92	2.78	2.09	1.69	1.47	1.52
142	7	Soil	1.57	1.73	2.17	2.60	2.55	2.69	2.76	4.25	3.61	2.85	2.22	1.76	1.49	1.52
147		150	1.35	1.53	2.13	2.15		1.87	2.39	2.65	2.81	2.44	1.79	1.38	1.77	1.27
149		mg/kg	2.13	1.97	2.80	2.76	2.67	3.09	3.53	4.00	5.34	3.82	2.71	2.16	1.91	1.56
192	10	Slag	1.76	1.38	1.88	1.86	1.74	2.05	1.86	2.50	2.86	2.12	1.63	1.21	1.42	1.52
194		60	2.05	1.58	2.04	2.09	2.10	2.09	2.65	3.99	3.92	3.41	2.39	2.21	1.97	1.79
189		mg/kg		1.13	1.65	2.21	3.03	5.45	7.88	8.80	6.60	4.51	2.63	1.78	1.48	1.62
180	11	Slag	1.26	1.16	1.47	1.42	1.53	3.05	2.26	3.08	2.54	2.13	1.31	1.24	0.90	1.10
197		100	2.09	2.02	2.39	2.47	2.53	2.61	3.02	3.94	3.03	2.60	1.89	1.51	1.64	1.49
179		mg/kg	1.19	1.43	2.00	2.54	2.32	2.18	2.63	4.11	3.70	3.42	2.82	2.53	2.07	1.99
186	12	Slag	0.96	0.82	1.46	1.51	1.72	1.75	2.74	3.78	3.02	2.50	1.51	1.20	1.09	0.99
191		150	0.71	0.73		1.31	1.37	1.63	1.88	3.33	2.37	2.20	1.41	1.22	1.05	1.08
184		mg/kg		1.04	1.90	2.27	3.09	4.24	6.10	7.41	6.22	4.97	2.34	2.06	1.63	1.94
177	13	SurfTail	2.14	1.51	2.19	2.35	2.35	2.75	7.62	8.51	6.62	5.30	2.68	2.18	2.01	1.90
181	14	SubTail	1.15	1.11	1.67	1.78	1.76	2.92	8.99	16.04	12.56	10.21	5.27	4.33	3.19	2.49
187	15	Soil	0.92	0.88	1.13		1.17	1.25	1.40	1.84	1.96	1.88	1.52	1.30	1.28	1.49

Empty cell indicates no sample.

Table 11. Blood Lead Concentrations (Pb,calc) µg/dl

PIG ID	Grp	Trmt	Hour Intervals After Dosing													
			Pre 1	Pre 2	0.25	0.5	1	1.5	3	6	12	24	48	72	96	144
126	1	Neg	1.37	1.49	1.43	1.35	1.36	1.17	1.31	1.13	1.18	1.20	1.09	1.05	1.10	
137	1	Neg	1.87	2.20	2.09	2.10	2.52	2.41	1.95	2.03	2.10	1.93	1.88	2.32	1.70	1.68
195	1	Neg	1.48	1.35	1.28	1.65	1.30	1.40	1.40	1.59	1.21	1.05	0.92	1.13	1.26	1.46
190	1	Neg	1.02	0.90	0.97	1.04	1.01	0.93	1.01	0.86	0.96	0.97	0.86	0.94	0.84	0.93
133	2	PO Lo	1.39	1.50	1.37	1.07	1.43	1.09	1.18	1.15	1.36	1.09	1.03	0.93	0.85	0.88
199	2	PO Lo		0.96	1.39	5.75	12.33	14.91	18.81	22.08	14.26	10.53	5.61	4.16	3.25	3.25
140	3	POHi	1.26	1.17	1.32	1.51	1.62	1.75	1.95	2.45	3.21	2.53	1.78	1.46	1.31	1.13
188	3	POHi	0.43	0.53	0.50	0.53	0.76	1.03	3.54	8.45	5.32	3.29	1.96	1.37	1.10	1.03
141	8	IV Lo	1.32	1.34	12.32	12.48	12.46	11.90	10.10	10.00	7.80	5.23	3.41	2.44	2.03	1.70
182	8	IV Lo	1.69	1.57	103.50	80.88	63.43	51.05	37.38	26.59	19.99	15.80	12.00	9.06	7.97	7.88
138	9	IV Hi	1.47	1.49	87.95	68.09	55.07	41.55	46.45	22.92	14.67	10.33	7.11	6.60	4.75	4.04
196	9	IV Hi	1.23	1.22	292.26		154.77	117.72	74.46	45.18	30.18	23.79	15.28	11.34	9.81	
130	4	Soil	0.93	0.97	0.76		1.02	1.05	1.04	1.18	1.33	1.24	1.13	1.27	1.16	1.09
131	4	25	2.23	1.64	1.84	1.75	1.98	1.92	1.94	2.19	2.38	2.09	2.04	1.83	1.55	1.53
136	4	mg/kg	1.84	2.08	1.84	1.77	1.65	1.72	1.60	1.64	1.55	1.60	1.29	1.27	1.05	1.62
139	5	Soil	1.61	2.24	1.90	1.85	1.83	1.74	3.15	6.56	5.78	4.27	3.19	2.71	2.30	2.14
145	5	60	1.86	1.86	1.63	1.68	1.70	1.78	1.70	2.09	2.05	1.74	1.62	1.24	1.11	1.49
148	5	mg/kg	1.14	1.29	1.31	1.49	1.26	1.21	1.25	1.88	1.96	1.84	1.34	1.49	0.92	0.88
129	6	Soil	2.02	1.70	1.80	2.19	2.30	1.99	2.48	4.31	5.00	3.70	2.79	2.24	1.80	1.79
144	6	100	0.75	0.90	1.02	1.16	1.31	1.73	3.52	8.21	6.24	4.00	2.46	1.80	1.45	1.25
150	6	mg/kg	1.49	1.60	1.64	2.38	1.46	1.53	1.80	2.98	3.37	2.44	1.89	1.52	1.38	1.46
142	7	Soil	1.50	1.70	1.34	1.64	1.39	1.78	1.97	3.69	3.11	2.75	2.17	1.61	1.37	1.40
147	7	150	1.35	1.51	1.18	1.20		1.02	1.37	1.79	2.13	1.94	1.49	1.20	1.62	1.17
149	7	mg/kg	2.03	1.94	2.04	1.96	1.92	2.14	2.78	3.29	4.60	3.42	2.41	1.99	1.71	1.42
192	10	Slag	1.68	1.26	1.31	1.30	1.15	1.39	1.30	1.93	2.32	1.73	1.35	1.01	1.28	1.39
194	10	60	1.98	1.51	1.41	1.46	1.41	1.42	1.92	3.36	3.46	3.03	2.14	1.98	1.78	1.62
189	10	mg/kg		1.10	1.05	1.64	2.48	4.81	7.19	8.16	6.24	4.22	2.47	1.66	1.35	1.53
180	11	Slag	1.18	1.08	0.79	0.82	0.86	2.33	1.60	2.50	2.13	1.81	1.05	1.08	0.79	0.98
197	11	100	2.09	1.94	1.85	1.95	1.93	2.00	2.47	3.34	2.67	2.28	1.76	1.43	1.62	1.39
179	11	mg/kg	1.17	1.36	1.29	1.78	1.55	1.45	1.80	3.49	3.22	3.09	2.55	2.35	1.88	1.85
186	12	Slag	0.88	0.79	0.82	0.83	0.98	1.08	2.04	3.22	2.66	2.20	1.33	1.07	0.96	0.91
191	12	150	0.68	0.68		0.72	0.85	1.02	1.31	2.78	2.02	1.91	1.24	1.09	0.95	0.94
184	12	mg/kg		0.98	1.27	1.69	2.41	3.55	5.40	6.80	5.81	4.63	2.18	1.88	1.47	1.79
177	13	SurfTail	2.11	1.40	1.35	1.50	1.45	1.94	6.87	7.88	6.19	4.95	2.53	2.18	1.87	1.85
181	14	SubTail	1.06	1.10	1.02	1.07	1.06	2.19	8.34	15.35	12.15	9.85	5.13	4.14	3.07	2.41
187	15	Soil	0.82	0.80	0.90		0.83	0.83	0.90	1.32	1.49	1.52	1.24	1.11	1.11	1.37

Empty cell indicates no sample.

Table 12. Blood Lead-206 Concentration (206Pb) µg/dl																
PIG ID	Grp	Trmt	Hour Intervals After Dosing													
			Pre 1	Pre 2	0.25	0.5	1	1.5	3	6	12	24	48	72	96	144
126	1	Neg														
137	1	Neg														
195	1	Neg														
190	1	Neg														
133	2	POLo	0.1	0.12	0.98	1.15	1.18	1.11	1.11	0.96	0.88	0.71	0.41	0.26	0.17	0.16
199	2	POLo		0.04	0.75	0.87	0.62	0.56	0.31	0.42	0	0.14	1.1	0.11	0	0
140	3	POHi	0.15	0.14	1.05	1.1	1.25	1.03	1.11	1.06	0.74	0.48	0.33	0.25	0.16	0.11
188	3	POHi	0.1	0.02	0.58	0.65	0.58	0.58	0.52	0.32	0.35	0.25	0.09	0.15	0.13	0.05
141	8	IV Lo														
182	8	IV Lo														
138	9	IV Hi														
196	9	IV Hi														
130	4	Soil	0	0	0.73		0.67	0.67	0.69	0.68	0.63	0.43	0.27	0.21	0.18	0.09
131	4	25	0	0.03	0.92	0.76	0.77	0.68	0.85	0.66	0.72	0.56	0.28	0.24	0.16	0.07
136	4	mg/kg	0.1	0.03	0.66	0.72	0.7	0.86	0.85	0.79	0.75	0.56	0.37	0.33	0.24	0.17
139	5	Soil	0.1	0.1	0.88	0.62	0.54	0.77	0.74	0.59	0.64	0.63	0.28	0.29	0.19	0.16
145	5	60	0	0.01	0.59	0.79	0.62	0.67	0.72	0.52	0.62	0.43	0.21	0.15	0.2	0.22
148	5	mg/kg	0.1	0.09	0.98	1.08	1.02	1.03	1.15	1	0.7	0.6	0.35	0.24	0.12	0.12
129	6	Soil	0.29	0.16	0.94	1.16	1.18	1.28	0.99	0.88	0.63	0.56	0.34	0.27	0.17	0.26
144	6	100	0	0	0.4	0.55	0.5	0.43	0.42	0.38	0.25	0.35	0.19	0.11	0.09	0.02
150	6	mg/kg	0.1	0	0.67	0.76	0.77	0.8	0.82	0.69	0.55	0.34	0.2	0.17	0.09	0.06
142	7	Soil	0.1	0.03	0.53	0.83	0.96	1.16	0.91	0.79	0.56	0.5	0.1	0.05	0.15	0.12
147	7	150	0	0.02	0.95	0.95		0.85	1.02	0.86	0.68	0.5	0.3	0.18	0.15	0.1
149	7	mg/kg	0.1	0.03	0.76	0.8	0.75	0.95	0.75	0.71	0.74	0.4	0.3	0.17	0.2	0.14
192	10	Slag	0.1	0.12	0.57	0.56	0.59	0.66	0.56	0.57	0.54	0.39	0.28	0.2	0.14	0.13
194	10	60	0.1	0.07	0.63	0.63	0.69	0.67	0.73	0.63	0.46	0.38	0.25	0.23	0.19	0.17
189	10	mg/kg		0.03	0.6	0.57	0.55	0.64	0.69	0.64	0.36	0.29	0.16	0.12	0.13	0.09
180	11	Slag	0.1	0.08	0.68	0.6	0.67	0.72	0.66	0.58	0.41	0.32	0.26	0.16	0.11	0.12
197	11	100	0	0.08	0.54	0.52	0.6	0.61	0.55	0.6	0.36	0.32	0.13	0.08	0.02	0.1
179	11	mg/kg	0	0.07	0.71	0.76	0.77	0.73	0.83	0.62	0.48	0.33	0.27	0.18	0.19	0.14
186	12	Slag	0.1	0.03	0.64	0.68	0.74	0.67	0.7	0.56	0.36	0.3	0.18	0.13	0.13	0.08
191	12	150	0	0.05		0.59	0.52	0.61	0.57	0.55	0.35	0.29	0.17	0.13	0.1	0.14
184	12	mg/kg		0.06	0.63	0.58	0.68	0.69	0.7	0.61	0.41	0.34	0.16	0.18	0.16	0.15
177	13	SurfTail	0	0.11	0.84	0.85	0.9	0.81	0.75	0.63	0.43	0.35	0.15	0	0.14	0.05
181	14	SubTail	0.1	0.01	0.65	0.71	0.7	0.73	0.65	0.69	0.41	0.36	0.14	0.19	0.12	0.08
187	15	Soil	0.1	0.08	0.23		0.34	0.42	0.5	0.52	0.47	0.36	0.28	0.19	0.17	0.12

Empty cell indicates no sample.

Table 13. Summary of Background Lead Concentrations in the Four Untreated Animals			
Number of Sample Analyses	C _{Pb,bk} ug/dL	C _{Pb,bk} ug/dL	Coefficient of Variation - %
13	1.25	0.144	11.5
14	2.05	0.249	12.1
14	0.95	0.064	6.73
14	1.32	0.202	

Percent Recovery of Orally Administered Arsenic and Lead

Mass balance estimates could not be accurately calculated due to the loss of significant volumes of urine, and due to the failure of fecal analytical results to meet the quality assurance criteria for bias (see Appendix A, Quality Assurance Audit Report).

Estimates of Bioavailability

Bioavailability of arsenic could be estimated using a linear regression model passing through the origin described the relationship between AUC and the dose (mg As/kg BW). Figures 1A, 1B, 1C and 1D illustrate the relationships, and Table 14 shows the results of the regression analyses.

The relationships are expressed as

$$AUC_c = m_c * Dose_c \quad (1)$$

and

$$AUC_s = m_s * Dose_s \quad (2)$$

where m is the regression coefficient (slope), c the control value to which others are compared, and s the soil or other environmental media for which bioavailability (**F**) is being estimated. Rearranging equations (1) and (2) provides

$$m_c = AUC_c \div Dose_c \quad (3)$$

and

$$m_s = AUC_s \div Dose_s \quad (4)$$

The conventional bioavailability expression (Gibaldi and Perrier, 1982) is

$$F = \frac{Dose_c * AUC_s}{Dose_s * AUC_c} \quad (5)$$

Rearranging (5) produces

$$F = \frac{AUC_s \div Dose_s}{AUC_c \div Dose_c} \quad (6)$$

By substituting (3) and (4) in equation (6), **F** can be expressed as the ratio of slopes

$$F = \frac{m_s}{m_c} \quad (7)$$

For each regression line, the 95% confidence limits for the slopes were determined as

$$m \pm t_{(.05, n-1)} S_m \quad (8)$$

Where m is the slope, $t_{(.05, n-1)}$ is the two-tailed critical value for $n-1$ degrees of freedom, and S_m is the standard error of m . The confidence intervals are shown in Table 15.

The confidence limits for each group were used in Monte Carlo analyses of equation (7) to determine confidence limits for F . The results of the Monte Carlo analyses are shown in Table 16.

Because multiple dose levels and replicates were not evaluated for the mining site environmental substrates, bioavailability estimates would be highly uncertain. Area-under-the-curve results for the individual animals are reported in Table 7.

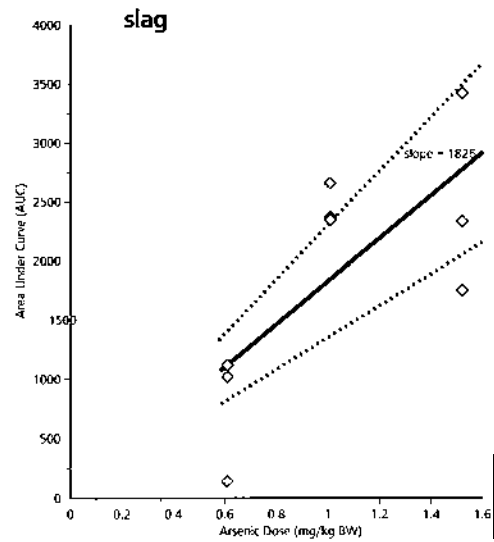
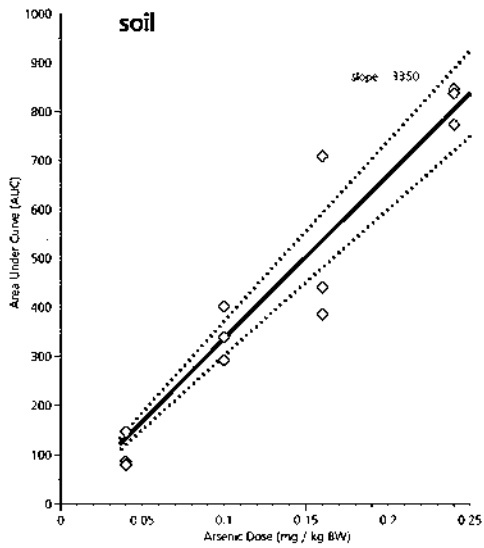
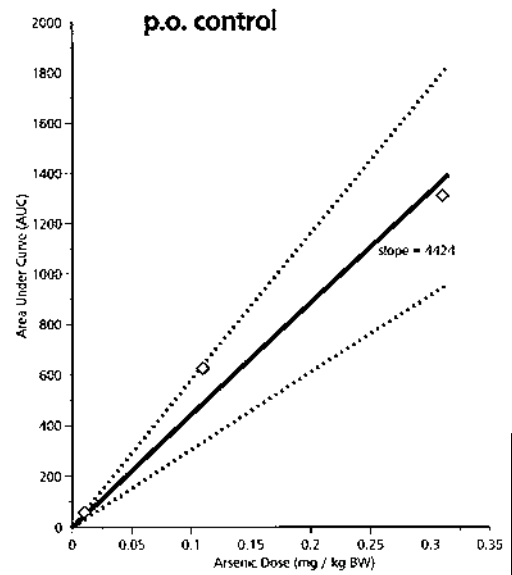
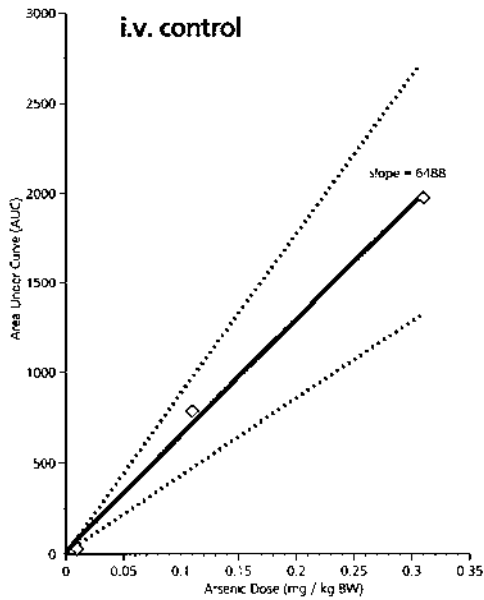
Treatment Group	m (slope)	r ²	df	p-level
Positive Control (i.v.)	6489	0.999	2	.00074
Positive Control (p.o.)	4424	0.984	2	.00521
Oral Soil	3351	0.976	11	<.00001
Oral Slag	1826	0.907	8	.000021

Treatment Group	m (slope)	± 95%
Positive Control (i.v.)	6489	762
Positive Control (p.o.)	4424	1380
Oral Soil	3351	349
Oral Slag	1825	475

F = m _s /m _c	Mean	95% Limits		Median
		Lower	Upper	
Control (p.o.)/Control (i.v.)	0.68	0.47	0.92	0.68
Soil/Control (p.o.)	0.78	0.56	1.11	0.76
Soil/Control (i.v.)	0.52	0.44	0.61	0.53
Slag/Control (p.o.)	0.42	0.27	0.63	0.41
Slag/Control (i.v.)	0.28	0.20	0.37	0.28

Figure 1. Dose response relationship between area-under-the-curve and arsenic dose ($\mu\text{g As/kg BW}$). Regression line and 95% confidence intervals of the regression are also shown.

- A. Intravenous sodium arsenate (i.v. control)
- B. Oral sodium arsenate (p.o. control)
- C. Oral smelter site soil (soil)
- D. Oral smelter site slag (slag)



This experiment did not provide reliable bioavailability estimates for lead. However, all animals receiving an oral dose of an environmental substrate (n = 24) had a positive correlation coefficient whereas the untreated animals (n = 4) had negative but nonsignificant coefficients at $p < 0.05$. The positive but nonsignificant correlation coefficients in a few treated animals may have resulted from elevated background blood lead concentrations relative to the increase in blood lead due to experimental exposures. Except for the instances noted above, oral exposures to the lead-containing environmental substrates resulted in significant increases in blood lead concentrations.

Increases due to intravenous exposures to soluble lead acetate were significant. With subtraction of background, a linear regression model passing through the origin described the relationship between AUC and intravenously administered lead acetate (ug Pb/kg BW). Figure 2A illustrates the relationship and Table 17 shows the results of the regression analysis. The use of data transformations or alternate regression models did not result in improved correlation indices.

Treatment Group	m (slope)	r ²	df	p-level
Positive Control (i.v.)	3.329	0.916	3	.005
Oral Soil	0.2152	0.05	11	0.4
Oral Slag	0.1092	0.04	8	0.5

Figures 2B and 2C show the relationships between blood lead AUC and orally administered soil or slag, respectively. Three of the four animals receiving oral doses of aqueous lead acetate had significant increases in blood lead concentrations. However, Figure 2D shows that no systematic relationship between dose and blood lead concentrations could be identified.

The confidence limits shown in Table 18 for each environmental substrate group were used in Monte Carlo analyses of equation (7) to determine confidence limits for **F** of lead. The results of the Monte Carlo analyses are shown in Table 19. Bioavailability estimates shown in Table 19 do not include values of m less than or equal to zero. These estimates are included to demonstrate the analytical method; however, the unreliability of these results is indicated by the inclusion of both zero and one hundred percent in the 95% confidence interval.

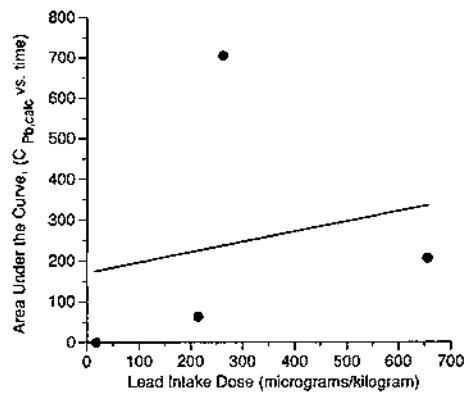
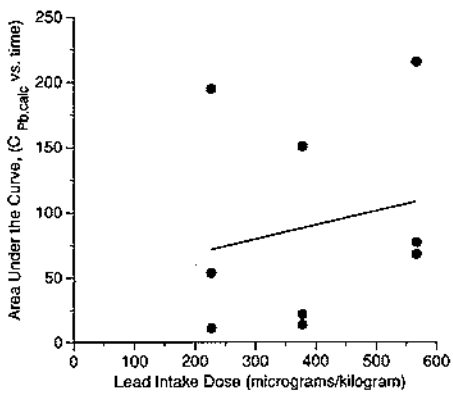
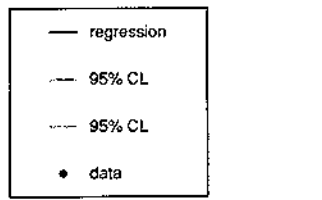
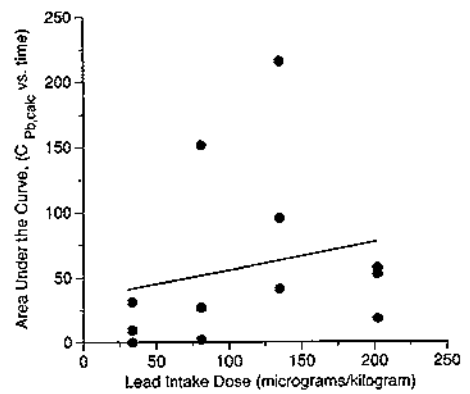
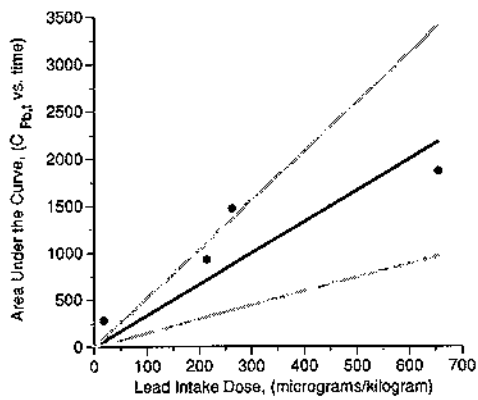
Treatment Group	m (slope)	± 95%
Positive Control (i.v.)	3.329	1.995
Oral Soil	0.2152	0.8608
Oral Slag	0.1092	0.5468

F = m_s/m_c	Mean	95% Limits		Median
		Lower	Upper	
Soil/Control (i.v.)	0.10§	0	1.25	0.11§
Slag/Control (i.v.)	0.04§	0	0.82	0.04§

§ Unreliable estimates which include both zero & 100% in the confidence interval.

Figure 2. Dose response relationship between area-under-the-curve and lead dose ($\mu\text{g Pb/kg BW}$). Regression line and 95% confidence intervals of the regression line are also shown.

- A. Intravenous lead acetate (upper left)
- B. Oral smelter site soil (upper right)
- C. Oral smelter site slag (lower left)
- D. Oral lead acetate (lower right)



Because multiple dose levels and replicates were not evaluated for the mining site environmental substrates, bioavailability estimates for lead and arsenic would be highly uncertain and were therefore not determined. Area-under-the-curve results for the individual animals are reported in Table 20.

Table 20. Area-Under-the-Curve Results for Calculated Blood Lead (Pb, calc) and Lead-206 (Pb, 206) Concentrations												
PIG ID#	Grp#	Trmt	Dose ug/kg	AUC (Pb,calc)	Tmax (hr) (Pb,calc)	Cmax (ug/dL) (Pb,calc)	T ½ (hr) (Pb,calc)	AUC (Pb,206)	Tmax (hr) (Pb,206)	Cmax (ug/dL) (Pb,206)	T ½ (hr) (Pb,206)	Cl (dL/hr) (Pb,206)
130	4	Soil	34	31.2	12	0.38		32.75	0.25	0.71	54	0.97
131	4	25	34	9.32	12	0.44		36.07	0.25	0.91	0.41	0.89
136	4	mg/kg	34	0				41.63	1.5	0.81	54	0.72
139	5	Soil	81	151.75	6	4.63	28	29.15	0.25	0.78	32	1.03
145	5	60	81	2.14	6	0.24		35.98	0.5	0.79		0.9
148	5	mg/kg	81	26.77	12	0.74		32.14	1.5	1.06	18	0.88
129	6	Soil	135	95.67	12	3.14	21	18.63	1.5	1.05	16	1.4
144	6	100	135	215.98	6	7.35	30	20.56	0.5	0.58	43	1.17
150	6	mg/kg	135	41.08	12	1.82		23.79	3	0.79	33	1.28
142	7	Soil	202	52.48	6	1.87		21.28	1.5	1.11		1.22
147	7	150	202	18.12	12	0.7		37.1	3	1.01	39	0.86
149	7	mg/kg	202	57.42	12	2.62		29.5	1.5	0.88		1.26
192	10	Slag	227	10.73	12	0.85		20.4	1.5	0.56	25	1.1
194	10	60	227	53.69	12	1.71	14	26.97	3	0.66	56	0.96
189	10	mg/kg	227	194.88	6	7.06	21	20.62	3	0.66		0.96
180	11	Slag	378	21.7	6	1.37		19.22	1.5	0.64	24	1.37
197	11	100	378	13.39	6	1.32		16.03	1.5	0.58	19	1.12
179	11	mg/kg	378	150.9	6	2.23	49	28.48	3	0.78	63	0.96
186	12	Slag	567	68.45	6	2.38	21	17.56	1	0.69		1.39
191	12	150	567	77.6	6	2.09	34	20.71	1.5	0.57	36	1.61
184	12	mg/kg	567	215.83	6	5.82	26	21.85	3	0.64		1.25
177	13	SurfTail	425	145.3	6	6.13	16	14.47	1	0.83		1.12
181	14	SubTail	2464	553.52	6	14.27	35	19.74	1.5	0.68		1.04
187	15	TSoil	217	66.11	24	0.71	56	20.12	3	0.43	39	1.25
133	2	PO PbAc	17.47	0				39	0.5	1.06	25	0.69
140	3	PO PbAc	214	64	12	2	35	9	0.5	1.1	15	1.04
199	2	PO PbAc	263	704	6	21.13	28	26	1	0.83		2.11
188	3	PO PbAc	656	207	6	7.97	33	14	0.5	0.6		1.86

Empty cell indicates no value could be determined.

Semi-simultaneous intravenous administration of ²⁰⁶Pb-enriched solution and gavage administration of test substrates also provides a means to estimate lead bioavailability as well as kinetic parameters. Each animal orally exposed to smelter site or mining site environmental substrates also received a simultaneous intravenous dose of ²⁰⁶Pb (1.68 micrograms of lead per kilogram of body weight). Area-under-the-curve, the time of maximum concentration (T_{max}) and maximum concentration (C_{max}) obtained by inspection of the data for blood ²⁰⁶Pb concentrations and are shown in Table 20. For each animal, the pharmacokinetic parameter describing the rate of elimination, K_{el}, was obtained

from the slope of the blood concentration (expressed as the natural logarithm, ln) versus time elimination curve. Half-life, $T_{1/2}$, was obtained by application of equation (9).

$$T_{1/2} = \ln^2/K_{el} \quad (9)$$

Clearance was obtained by application of equation (10)

$$Cl = \text{Dose}/\text{AUC} \quad (10)$$

Representative blood concentration (Pb_{calc}) versus time and blood concentration (^{206}Pb) curves are shown in Figure 3. The average AUC(0-96) was 24.3 ug.h/dL, the average C_{max} was 0.8 ug/dL and the average clearance was 1.1 dL/hr (Table 21). Average half-life of ^{206}Pb was estimated to be 33 hours and the average ^{206}Pb T_{max} was 1.5 hr (Table 21). The delay in T_{max} was observed consistently in all animals.

	Oral		Intravenous				
	Tmax (hr) (Pb,calc)	T 1/2 (hr) (Pb,calc)	AUC (Pb,206)	Tmax (hr) (Pb,206)	Cmax (ug/dL) (Pb,206)	T 1/2 (hr) (Pb,206)	Cl (dL/hr) (Pb,206)
Mean	9	30	25	1.51	0.78	33	1.16
SD	4	11	8	0.98	0.19	17	0.32
%CV	47%	38%	34%	65%	24%	51%	27%
n	26	15	28	28	28	18	28

The mean clearance of intravenous lead acetate was 3.5 dL/hr (sd=0.5; n=4) and the mean T_{max} was 0.3 hr (sd=0.1; n=4) (Table 22). Half-life of intravenous lead acetate was estimated using the terminal slopes from 24-96 hours, 48-144 hours and 72-144 hours (Table 23). Figures 4A, 4B, 4C and 4D show the lead elimination curves for individual animals receiving intravenous lead acetate.

Estimates of lead bioavailability from smelter site and mining site substrates shown in Table 24 were obtained by application of equation (11)

$$\text{Bioavailability (F)} = \frac{\text{AUC (oral)} \cdot \text{Dose (iv)}}{\text{AUC (iv)} \cdot \text{Dose (po)}} \quad (11)$$

PIG ID#	Grp#	Trmt	Dose ug/kg	AUC (Pb,t)	Tmax (hr) (Pb,t)	Cmax (ug/dL) (Pb,t)	Cl (dL/hr) (Pb,t)
141	8	IV PbAc	18	282	0.50	12	3.21
182	8	IV PbAc	263	1473	0.25	104	2.94
138	9	IV PbAc	214	932	0.25	88	3.61
196	9	IV PbAc	656	1870	0.25	293	4.15
Mean					0.31		3.48
SD					0.13		0.53
%CV					40%		15%
n					4		4

Table 23. Half-Life Estimates for Intravenous Lead Acetate					
PIG ID	Grp	Trmt	T _{1/2} (hr) Calculated for the time period shown		
			24-96 Hr	48-144 Hr	72-144 Hr
141	8	IV PbAc	29	41	46
182	8	IV PbAc	63	138	347
138	9	IV PbAc	53	77	77
196	9	IV PbAc	53	144	99
Mean			50	100	142
SD			15	50	138
%CV			29%	50%	97%
n			4	4	4

Table 24: Bioavailability Estimates of Lead Based on Stable Isotope Method				
PIG ID	Grp	Trmt	Dose ug/kg	Absolute Bioavailability
130	4	Soil	34	4
131	4	25	34	1
136	4	mg/kg	34	0
139	5	Soil	81	12
145	5	60	81	0
148	5	mg/kg	81	2
129	6	Soil	135	7
144	6	100	135	12
150	6	mg/kg	135	3
142	7	Soil	202	2
147	7	150	202	1
149	7	mg/kg	202	2
192	10	Slag	227	1
194	10	60	227	2
189	10	mg/kg	227	6
180	11	Slag	378	1
197	11	100	378	1
179	11	mg/kg	378	2
186	12	Slag	567	1
191	12	150	567	1
184	12	mg/kg	567	4

Figure 3. Blood Lead (Pb_{calc}) versus time and blood ^{206}Pb versus time relationship for an animal simultaneously administered an oral dose of 100 mg soil/kg BW smelter site soil and intravenous ^{206}Pb (animal #129).

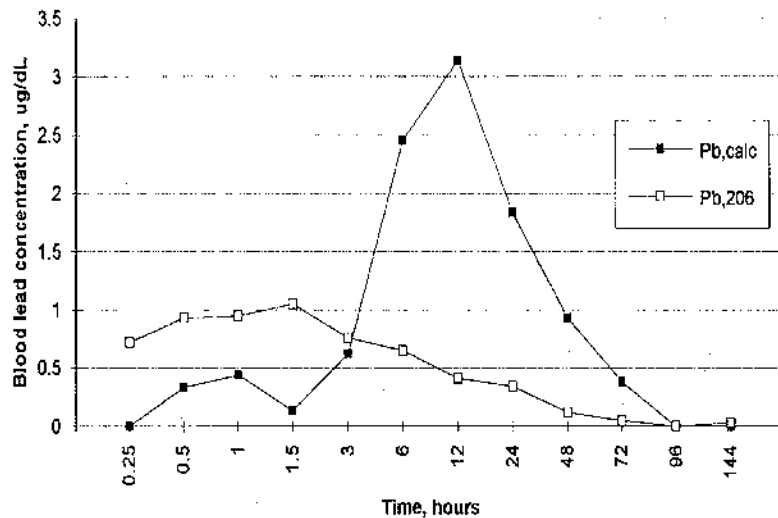
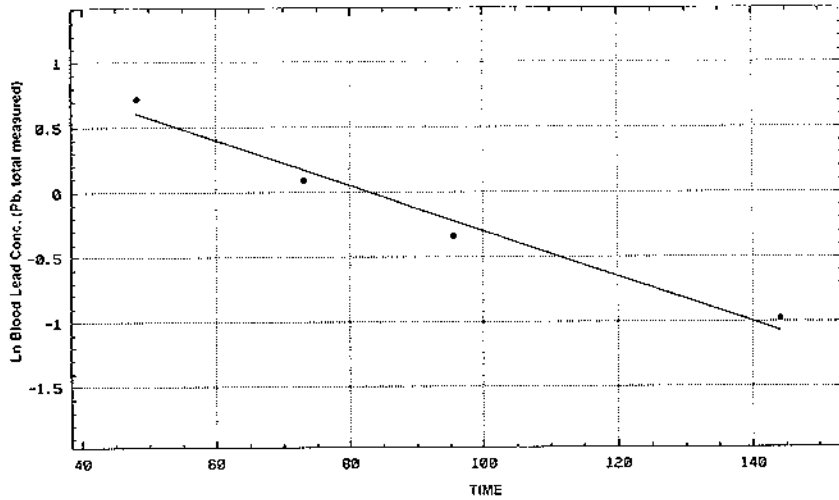


Figure 4. <next page>

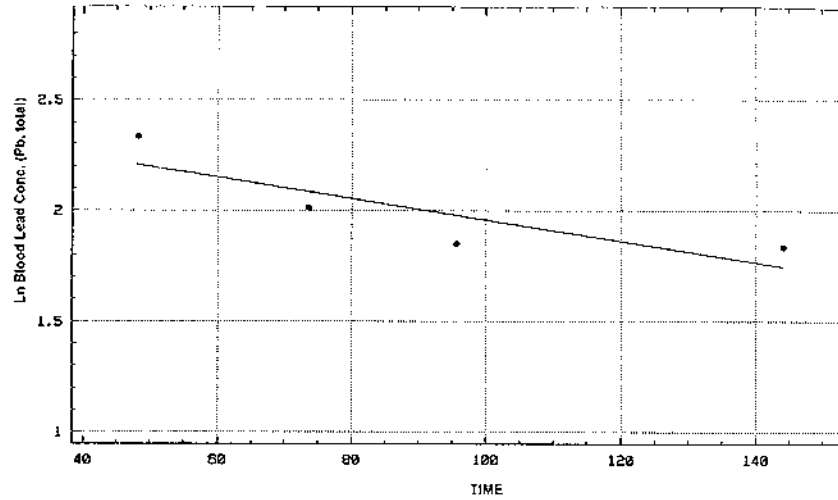
Blood lead elimination curves for individual animals receiving intravenous lead acetate. Half-life of lead acetate was estimated using the terminal slopes from 24-96 hour, 48-144 hour and 72-144 hour time periods.

- | | |
|--|---------------------------------------|
| A. Animal #141 (17.5 μg Pb/kg BW) | C. Animal #138 (214 μg Pb/kg BW) |
| B. Animal #182 (263 μg Pb/kg BW) | D. Animal #196 (657 μg Pb/kg BW) |

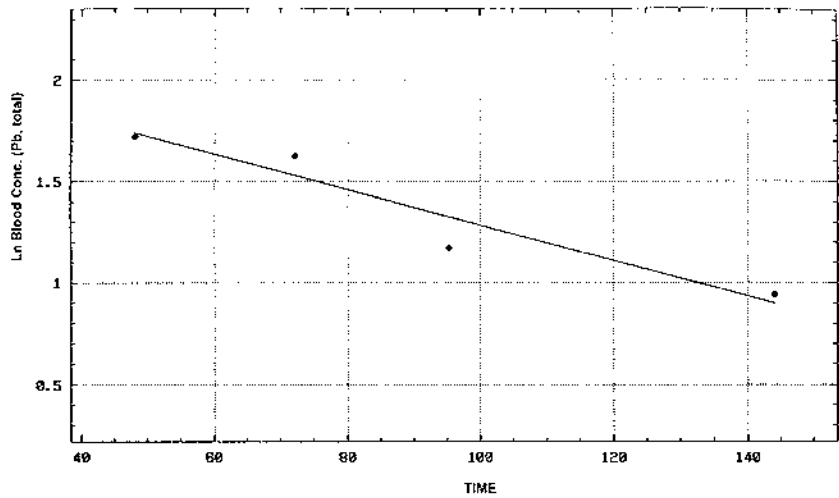
Half-Life Determination
 4 cases; $r = -.985$; $p = .015$
 $PIG_{141} = 1.444 \cdot TIME$
 Est. $t_{1/2} = 40.8$ hr
 (48 - 144 hours)



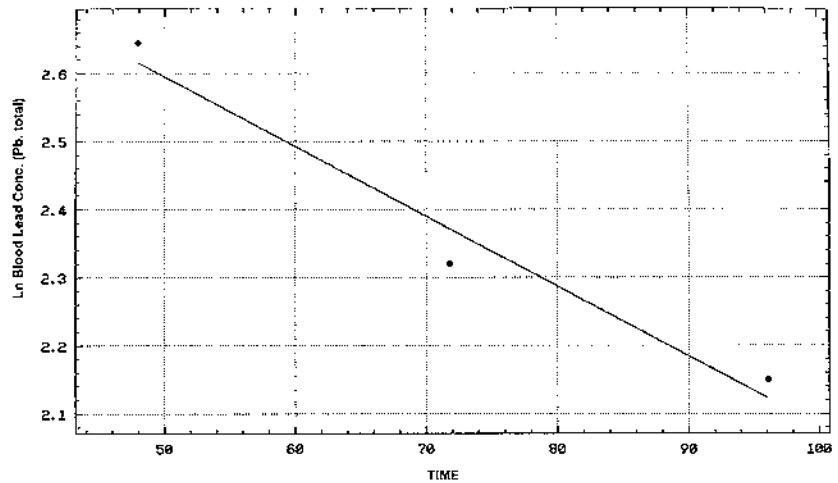
Half-Life Determination
 4 cases; $r = -.839$; $p = .161$
 $PIG_{182} = 2.440 \cdot TIME$
 Est. $t_{1/2} = 138$ hours
 (48 - 144 hours)



Half-Life Determination
 4 cases; $r = -.962$; $p = .038$
 $PIG_{138} = 2.159 \cdot TIME$
 Est. $t_{1/2} = 77$ hours
 (48 - 144 hours)



Half-Life Determination
 3 cases (MD pairwise deleted); $r = -.982$; $p = .122$
 $PIG_{196} = 3.108 \cdot TIME$
 Est. $t_{1/2} = 69.3$
 (48 - 96 hr; missing data @ 144 hr)



DISCUSSION

This study provided site specific information useful for evaluating human exposures to arsenic and lead contaminated soil, slag and tailings. Although this study demonstrated the challenges posed in a simultaneous evaluation of two contaminants, it is not uncommon for these contaminants to occur together in the environment. Arsenic and lead were absorbed into the blood following oral exposure to the environmental substrates. The methods and results of the physical and chemical studies will be discussed in a forthcoming report.

Dose rates were selected that would attain detectable blood lead concentrations. However, this resulted in doses of arsenic greater than 1 mg/kg when slag and surface tailings were administered. Due to potential toxicity, equivalent reference oral or intravenous arsenic dose could not be administered. Therefore, a data evaluation methodology was developed to address these dose differences.

The conventional bioavailability calculation method assumes a linear dose response that passes through the origin. The data evaluation methodology developed for this study utilized the linear dose response relationship observed between arsenic intake and blood concentrations. When multiple dose levels are included in the study design, bioavailability may be estimated by this method when either the dose (milligrams arsenic per kilogram body weight) or the response (area-under-the-curve) of the group receiving the environmental media are within the experimentally observed range of the control group. Although arsenic bioavailability has been previously studied, no studies have demonstrated a linear relationship prior to applying the conventional calculation methodology.

Arsenic metabolism studies have commonly measured urinary concentrations due to the ease of specimen collection, availability of analytical methods and observation that urine is the predominant excretion route (for review see ATSDR 1993). Absolute bioavailability estimates for oral sodium arsenate (mean=68%, CI=47-92%) derived from this study are comparable with estimates from studies utilizing urinary data in humans and rabbits (Buchet et al. 1981a,b; Freeman et al., 1993).

It is readily observed that a linear dose response relationship passing through the origin assumes that blood concentrations are nonexistent when substrate intake is zero. Therefore, the experimental design and data evaluation methodology addressed the presence of endogenous background concentrations in the blood samples. Bioavailability is overestimated if background concentrations are not considered. If the origin is omitted from the regression model, the influence of background concentrations are mistakenly double-counted, the regression coefficient is reduced and estimates of bioavailability are overstated.

The experimental design and data evaluation methodology provided information to characterize natural variability and uncertainty in the bioavailability estimates. In previous studies of arsenic bioavailability from environmental substrates, confidence intervals were not evaluated (Freeman et al., 1993).

Lead was bioavailable from all substrates studied. There was a higher degree of variability and uncertainty in the bioavailability estimates for lead as compared to arsenic. The variability and uncertainty of **F** is influenced by how well the regression model describes the dose-response relationship, whether background concentrations had been adequately characterized, and if there were an adequate number of experimental observations.

In this study, differing predose blood lead concentrations and postdose variances in animals orally exposed to environmental substrates resulted in low correlation indices. Although analytical measurement variability is incorporated in the coefficient of variation for background lead and ²⁰⁶Pb, the data suggest that differences between individual animals in blood clearance of lead (as indicated by ²⁰⁶Pb) and in background concentrations had more influence on variation in blood lead concentration than did the analytical measurements.

When high variance is anticipated, a greater number of experimental animals per treatment group are required. In this study, preliminary results did not indicate the magnitude of the variance eventually observed in the lead results of the final study. Therefore, a significant limitation in this study for estimating bioavailability of lead was the number of animals per treatment group in both the reference and environmental substrate (soil and slag) groups. The results from the mine site soil or tailings exposures suggested the need for a sensitive study design and protocol in the event of a future bioavailability study.

It's interesting to note that arsenic concentrations measured in the same animals did not show the same degree of variance. The variance in blood lead concentrations in this study could not be explained with the available data.

To date, no other complete lead bioavailability study utilizing the immature swine model has been published in the open literature. However, a high inter-animal variability was documented in a project report. Although consistent increasing concentrations in blood lead were identified in animals following dosing with lead-contaminated soil, investigators were unable to demonstrate statistical significance using standard analysis of variance methods (Dupont, 1993). Like the present study, the variance was high and the groups not large enough. Establishing treatment groups with minimal baseline blood lead concentrations and small variance, increasing group sizes, and switching to a sub-chronic dosing protocol have been approaches taken by one group currently active in this area of research (Chris Weis, U.S.EPA Region 8, personal communication, 1995).

Confidence in the blood lead bioavailability estimates were low and were not recommended for use in regulatory decision-making. The dose interval for lead ranged from approximately 600 to 4,000 micrograms (total dose) for soil, and from approximately 3,000 to 9,000 micrograms for slag. A non-linear relationship between lead intake and blood concentrations has been described for children's intake of less than approximately 1,000 micrograms per day (U.S.EPA, 1994; Sherlock and Quinn, 1986). The dose range and increments in this study were not sufficient to characterize a similar non-linear relationship at low doses.

Bioavailability of lead was shown to be greater than zero percent by the significant increases in individual animals' blood lead concentrations. However, the confidence intervals of the regression coefficients (slope) included zero slope. A zero slope indicates that the same amount (total mass) of lead was absorbed at each intake dose and that the absorbed amount was not dependent on intake dose. That is, the percent of the total mass of lead absorbed from the dosed materials increases with decreasing intake dose. Therefore, a slope (m) less than or equal to zero is considered an artifact of the evaluation methodology and the variability observed in this study.

Bioavailability of lead is not a constant; therefore, steady-state blood concentration would not be expected to be directly proportional to the amount of lead intake (Aungst et al., 1981). Even with the modifications utilized in this study, the conventional calculation is inadequate to describe bioavailability that varies with dose. Further refinement of the bioavailability algorithm is needed.

The wide degree of natural intersubject variability is a well recognized phenomenon in human studies of pharmaceuticals' bioavailability. Crossover study designs are preferable to concurrent controls to address this issue (Tse et al., 1991). However, variation in kinetic parameters can occur over time, even within the same subject, primarily due to changes in clearance. The stable isotope technique where isotopically characterized materials are simultaneously administered by different routes almost completely eliminates the influence of intraindividual variability (Wolen, 1986).

Simultaneous administration of the intravenous stable isotope, ^{206}Pb , and oral substrate permits study of absorption characteristics isolated from elimination and distribution kinetics when the latter are assumed to be identical for the stable isotope and the oral substrate. The statistical power of using stable isotopes for evaluation of differences in absorption is superior to conventional crossover or concurrent control designs. With only 4-6 subjects in a treatment group, it's possible to detect a 20% difference with a probability of 0.8, whereas other designs would require 8 or more animals. (Wolan, 1986). The number of animals per treatment group limited the power of the present study. Estimates of F based on the stable isotope method ranged from 0 to 12% for soil and from 0.5% to 6% for slag. These estimates are comparable to the mean estimates derived from the modified bioavailability calculations.

In animal studies, half-life in blood of 8-25 days has been reported. In those studies, it was demonstrated that bioavailability and kinetic parameters can be reliably determined from truncated blood-time data (Aungst et al., 1981; Castellino & Aloj, 1964; Weis et al., 1993). Half-life estimates developed from this study demonstrate the sensitivity of the estimate to the frequency of observations during the terminal phase. Half-life estimates based on 72-144 hour data are comparable to previous reports.

Findings of this study may challenge the assumption that the elimination and distribution of trace doses of lead are identical to larger doses. The C_{max} of intravenous lead acetate (dose range approximately 300 to 8000 micrograms) occurred at the first sampling. In contrast, C_{max} of intravenous ^{206}Pb (dose range approximately 20 to 30 micrograms) occurred at 1.5 hours. On a per microgram basis, the apparent AUC for low doses (approximately 30 micrograms) is greater than for higher doses (>300 micrograms). Delayed intravascular C_{max} has been reported following intravenous administration of tracer doses of radiogenic lead to humans (Chamberlain et al. 1978). And, a secondary peak in plasma concentrations 2-4 hours following intravenous lead administration to human volunteers has been observed (De Silva, 1981). Differential tissue affinities for lead have been described where a greater percentage of the dose is present in extravascular tissues than in plasma minutes (<1 hr) following intravenous administration (Bornemann and Colburn, 1985).

From the perspective of low-dose risk assessments for lead, similarities in tissue lead binding would strengthen the extrapolation of laboratory animal/human dose-response relationships. An hypothesis for the delayed C_{max} could be high affinity saturable binding of lead in an extravascular tissue. Since the intravenous dose was administered via the jugular vein, sites in the lungs may provide such binding. Research on lead disposition and kinetics has not suggested a special affinity for lead in pulmonary tissue. However, only the work cited above was identified as reporting blood concentration profiles of trace doses. Tissue disposition studies have typically been conducted several hours up to days or weeks following exposure. Saturable binding effects have been observed in pharmaceuticals studies utilizing the stable isotope method (Schmid et al., 1980). In pharmaceuticals, the presence of saturable binding results in decreased drug concentration at the therapeutic target tissue. Lead binding proteins have been isolated from target tissues and may play a role in lead

toxicity (Fowler et al., 1993). The toxicological significance of saturable binding of lead in extravascular tissue remains to be investigated.

The relative insensitivity of swine to clinical lead intoxication may suggest dissimilarities between swine and other laboratory animals in the affinity of lead to blood components and the critical tissues (Lassen & Buck, 1979; Osweiler et al., 1985). However, the time course of blood ²⁰⁶Pb concentrations in this study may suggest similarities of extravascular tissue affinities for tracer-level lead doses in immature swine and humans.

Blood clearance of intravenously administered lead acetate observed in this study was comparable with previously published animal studies (Aungst et al., 1981). It is noteworthy that clearance of lead from blood following intravenous administration increased as the dose increased. Further study is required to determine if single dose kinetics are predictive of steady state and to verify dose dependent clearance of low doses of lead. The disposition and clearance of lead at low intravenous doses and comparison with disposition and clearance of low oral doses warrants further investigation. Enhancing the scientific understanding of the behavior of low doses of lead in the body gains importance as the blood lead concentration of concern becomes lower and the efficacy of abatement of different exposure sources is debated.

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APPENDIX A.

QUALITY ASSURANCE AUDIT REPORT

Chain of Custody records were adequately maintained.

1. Results for Standard Reference Materials (SRM). SRM samples indistinguishable from experimental samples were included at an overall frequency of approximately 5%.
 - a. Blood Arsenic
 - i. Corrective action (repeated digestion and analysis) was initiated due to low recoveries for arsenic in blood.
 - ii. Reanalyses indicated low to acceptable (within SRM control limits or 75-125% where no limits exist) bias.
 - b. Blood Lead: Acceptable (within SRM control limits or 75-125% where no limits exist) recoveries were observed.
 - c. Urinary Arsenic: Potential high bias for arsenic was indicated.
 - d. Urinary Lead: Acceptable (within SRM control limits or 75-125% where no limits exist) recoveries were observed. A 0% recovery was reported for a single SRM with a true value that was equal to the detection limit. This recovery was therefore not evaluated. The frequency of SRM analyses was significantly low. As a result, a representative analysis of bias could not be performed based upon SRM performance.
 - e. Fecal Analyses: Potential high bias for both arsenic and lead was indicated by the finding of concentrations higher than the verified SRM concentrations.
2. RESULTS FOR INSTRUMENT CALIBRATION VERIFICATION STANDARDS.
 - a. All Arsenic Data: Inadequate frequency of standard analysis (a single standard was analyzed at the beginning of each analytical run without subsequent standard analysis during or at the end of each run). Acceptable frequency: The beginning and end of the analytical sequence and 1 per 10 samples in between.
 - b. Blood Lead: Recovery acceptable (90-110%) only for mid-range (24-25 ug/dl) standards. Greater recovery ranges were observed for lead standards at concentrations of ≤ 5 ug/dl.
 - c. Urinary Lead: Biased low in some animals and high in others.
 - d. Fecal Lead Analyses: Not Analyzed

3. Results for Blank Analyses. In all matrices, for those blank samples which were analyzed, no significant indications of lead or arsenic contamination were observed (i.e., sample concentrations were > 5 times the amount observed in any blank).
 - a. The frequency of blank analyses made assessment of false positive or negative errors near the detection limits difficult. Therefore, certainty near the detection limits is unknown in many instances.
4. Results for Duplicate Sample Analyses. Duplicate sample analyses were conducted at a frequency of 6-10%.
 - a. Blood Arsenic: Acceptable (\pm 20% RPD) duplicate results were obtained for concentrations at \geq 5-10 times the reported detection limit. Quantitative certainty near the detection limit is indeterminate due to the inherent increase in analytical variability. Below 5-10 times the detection limit, the %RPD criterion was not evaluated.
 - b. Blood Lead: Acceptable (\pm 20% RPD) duplicate results were obtained for concentrations at \geq 5-10 times the reported detection limit. Quantitative certainty near the detection limit is indeterminate due to the inherent increase in analytical variability. Below 5-10 times the detection limit, the %RPD criterion was not evaluated.
 - c. Urinary Arsenic: Acceptable (within \pm 20% RPD).
 - d. Urinary Lead: Overall acceptable (within \pm 20% RPD). RPDs of 21% and 27% were not believed to be significantly exceed the criterion for this matrix. As a result, data was not qualified based upon these RPDs.
 - e. Fecal Analyses: Acceptable (within \pm 20% RPD) for all arsenic analysis. Overall acceptability was observed for lead analysis with three observed exceedances (21%, 23%, 56% RPD). Because only one exceedance was significantly above the criterion and all matrix spike recoveries for these samples were within the acceptance range, data qualification was not required.
5. RESULTS FOR MATRIX SPIKE ANALYSES. Matrix spike sample analyses were conducted at a frequency of 6-10%.
 - a. Blood Arsenic: Acceptable (within 75-125%). A matrix spike was performed twice on one sample resulting in recoveries of 136% and 93%. As a result, data qualification was not recommended.
 - b. Blood Lead: Not Analyzed.
 - c. Urinary Arsenic: Unacceptable recoveries occurred when indigenous urinary arsenic concentrations were greater than five times the amount spiked. As a result, recoveries for these samples were not applicable to this analysis.
 - d. Urinary Lead: Potential high bias was indicated.

e. Fecal Analyses: Acceptable (within 75-125%).

6. Summary.

Potential bias may exist in the approximately 30% of the urinary lead data. Variable instrument verification standard recoveries were observed for blood lead results at or below 5 ug/dl. Sample quantitation below this concentration may be uncertain. Instrument performance with regard to fecal lead analysis is unknown. A relatively low frequency of instrument verification standard and blank analyses represent deficiencies in the information needed to support the useability of all arsenic data. The overall useability of ESA laboratory data is recommended for preliminary decision making purposes only until confirmatory (verification) analyses become available.

True Values for Blind Standard Reference Materials

Standard Concentration	Standard Type
4.6 ug/dl	Blood-Pb
10.7 ug/dl	Blood-Pb
18 ± 6 (33%) ug/dl	Blood-Pb
30 ± 4 (8%) ug/dl	Blood-Pb
200 ± 50 (25%) ug/l	Blood-As
150 ± 30 (20%) ug/l	Blood-As
109 ± 4 (4%) ug/l	Urine-Pb
10 ug/l	Urine-Pb
37.5 ug/l	Urine-As
60 ug/l	Urine-As
82.4 ug/l	Urine-As
60 ug/l	Urine-As
480 ± 100 (21%) ug/l	Urine-As
0.87 ± 0.03 (3%) ug/g	Feces-Pb
0.060 ± 0.018 (30%) ug/g	Feces-As

APPENDIX B.

EVALUATION OF SOURCES OF VARIATION

The error associated with the isotope ratios at low concentrations is important because the intravenously administered lead and the background lead are at these levels. The calculation of the intravenously administered lead concentration relies upon the isotope ratio. The average relative standard deviation of 3.6% total lead and 5.3% for ^{208}Pb when propagated as the sum of squares would indicate the average relative standard for the isotope ratio would be 6.2% which is consistent with that observed for the lower concentration standards of 6.3%. This agreement indicates that these uncertainties have been adequately defined. The observed 6.3% relative standard deviation for the ratio must be propagated with those for total lead and ^{208}Pb to estimate the uncertainty associated with the calculated intravenously administered lead values. This propagated uncertainty then must be propagated with the total lead uncertainty to derive that of the sum of the background and the perorally absorbed lead. Finally the uncertainty of the background lead must be propagated with this later uncertainty to calculate the uncertainty of the perorally absorbed lead.

The variability of the background lead for the duration of an experiment can be estimated from the negative control experiments. The negative control experiments did not, however, capture all the variability of the background lead. A number of substrate dose experiments indicate systematic sloping of the background lead. This phenomenon is indicated by the difference in the "pre 1" and "pre 2" values compared to the specimen values near the end of an experiment. Experiment 197 is an example of this phenomenon where the "pre 1" and "pre 2" values were 2.11 and 1.93 ug/dL, respectively, while the 144 hour specimen was 1.39 ug/dL. Subtraction of the average of the "pre" values of 2.02 ug/dL could result in a systematic error of as much 0.63 ug/dL. In summary, the subtraction of the background could contribute from about 0.10 to as much as 0.25 ug/dL random error (standard deviation) and on a number of experiments perhaps as much as an additional 0.63 ug/dL systematic error. Interpolation of the sloping background might reduce the systematic error component.

Now that the uncertainties have been estimated, propagation of these errors for the maxima values for the high oral slag experiments will be used to examine the analytical contribution to the total error. It should be noted that this example did not have sloping backgrounds as the uncertainty or perhaps bias associated with this phenomenon is not really an analytical uncertainty. Table 3-7 contains data extracted from Table 3-4 for the high oral slag experiments maximums.

Table 3-7 High Oral Slag Lead in Blood Maxima Data

Parameter	Specimen	Specimen	Specimen
	184-B8 ug/dL	186-B8 ug/dL	191-B8 ug/dL
Measured Pb	7.41	3.78	3.33
Measured Pb ²⁰⁸	3.53	1.67	1.44
Calculated Pb _{iv}	0.61	0.56	0.55
Calculated Pb _{po + bk}	6.80	3.22	2.77
Measured Pb _{bk}	0.98	0.835	0.68
Calculated Pb _{po}	5.82	2.38	2.09

The total uncertainty of concern is calculated from the three perorally absorbed values. The average value for the three specimens was 3.43 ug/dL with a standard deviation of 2.07 ug/dL. The variance is 4.3. The variance associated with each measured and calculated parameter for each specimen is given in the following table.

Table 3-8 Analysis of Variance for Each High Oral Slag Lead in Blood Maximum

	Specimen	Specimen	Specimen
Parameter	184-B8	186-B8	191-B8
Parameter	(ug/dL) ²	(ug/dL) ²	(ug/dL) ²
Measured Pb	0.0712	0.0185	0.0144
Measured Pb ²⁰⁸	0.0350	0.0078	0.0058
Calculated Pb _{iv}	0.3848	0.0887	0.0666
Calculated Pb _{po + bk}	0.4559	0.1072	0.0809
Measured Pb _{bk}	0.0125	0.0091	0.0060
Calculated Pb _{po}	0.4684	0.1163	0.0869

Summing the variances for each parameter for the three specimens provides the variance for the average. Table 3-9 presents these summed variances for each parameter and gives the percentage of the total variance represented by each parameter.

Table 3-9 Analysis of Variance for the Average High Oral Slag Lead in Blood Maxima

Parameter	Variance (ug/dL) ²	Percent of Observed Variance
Measured Pb	0.1040	2.42
Measured Pb ²⁰⁸	0.0487	
Calculated Pb _{iv}	0.5401	12.5
Calculated Pb _{po + bk}	0.6441	
Measured Pb _{bk}	0.0276	0.64
Calculated Pb _{po}	0.6717	15.6
Observed Pb _{po}	4.305	

This analysis indicates that the analytical error might represent about 16% of the total uncertainty. The analysis of variance indicates a large percentage of the analytical variance is associated with the derivation of the intravenous tracer concentration. However, the standard deviation observed between the three swine for the tracer maxima was only 0.032 ug/dL which gives a variance of only 0.0010. This observed variance is much less than that calculated from the propagation of errors given above. The propagation of error technique assumes the measurements are independent while the measured total lead and measured Pb²⁰⁸ are not independent and were determined at the same time. Using the observed variance, the

analytical variance for the perorally adsorbed lead changes from 0.6717 to 0.1327 which represents just 3.1% of the overall variance observed. Furthermore, if the uncertainty associated with the isotope ratios is that of the substrates of 1.67% rather than the 6.3% relative standard deviation used, analytical uncertainty would be an even smaller component of the total experimental uncertainty.

It should be noted that a few groups of other substrate dose experiments showed considerable more variability for the tracer. For example, the medium high soil experiments had tracer maxima of 1.28, 0.55, and 0.82 ug/dL. The variance was 0.6075 which is similar to that calculated by the propagation of error. However, the lead/time curve for the tracer for each experiment takes on a shape of a peak, indicating that the observed variance of the maxima is not due to analytical variance. If the variance was analytical in nature than one would expect a single point spike as opposed to a multiple point peak.

Finally, for the example selected, the total uncertainty for the perorally absorbed lead expressed as a standard deviation was about equal to the average. For many of the other substrate dose experiments the standard deviation was greater than the average. Although it is evident, both intuitively and statistically, that lead was perorally absorbed from the substrates, this type of variability limits the ability to distinguish differences in the amount of lead perorally absorbed at different dose levels. Distinction of these differences would be aided by a greater number of swine per substrate dose level. Moreover, if swine could be selected that had lower and less variable background lead, similar to negative control swine 190, then fewer number of swine per dose level would be needed to discern a difference.