

Draft manuscript

Measurement of Arsenic Bioavailability in Soil Using a Primate Model

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Abstract

Several studies have shown limited absorption of arsenic from soils. This has led to increased interest in including measurements of arsenic relative bioavailability from soils in the calculation of risks to human health posed by arsenic contaminated sites. Most of the information in the literature regarding arsenic bioavailability from soils comes from studies of mining and smelter sites in the Western United States. It is unclear whether these observations are relevant to other types of arsenic contaminated sites. In order to obtain information regarding arsenic bioavailability for other types of sites, relative bioavailability of arsenic from selected soil samples was measured in a primate model. Sodium arsenate was administered to five male *Cebus apella* monkeys by the intravenous and oral routes, and blood, urine, and feces were collected. Pharmacokinetic behavior of arsenic after intravenous administration and the fractions of dose excreted in urine and feces after both intravenous and oral doses were consistent with previous observations in humans. Soil samples from five waste sites in Florida (one from an electrical substation, one from a wood preservative treatment site, two from pesticide sites, and one from a cattle dip vat site) were dried and sieved. Soil doses were prepared from these samples and administered orally to the monkeys. Relative bioavailability was assessed based on urinary excretion of arsenic following the soil dose compared with excretion following an oral dose of arsenic in solution. Differences in bioavailability were observed for different sites, with relative bioavailability ranging from $10.7 \pm 4.9\%$ (mean \pm SD) to $24.7 \pm 3.2\%$ for the five soil samples. These observations, coupled with data in the literature, suggest limited oral bioavailability of arsenic in soils from a variety of types of arsenic contaminated sites.

Key words: arsenic, bioavailability, intestinal absorption, primate model, soil

Introduction

Arsenic is both a naturally occurring substance and a common contaminant at hazardous waste sites in the United States. Mining and manufacturing activities, as well as the use of arsenic-containing pesticides, have resulted in a wide array of types of contaminated sites, including mine tailings, smelter facilities, cattle dip sites, electric substations, wood treatment (chromated copper arsenate) sites, pesticide treatment areas, railroad right-of-ways, golf courses, and dumps. Collectively, these sites number in the tens of thousands or more, and the management of these sites is a significant public health and economic problem.

Arsenic is classified by the U.S. Environmental Protection Agency (USEPA) as a Group A carcinogen; that is, it is known to produce cancer in humans. Workers exposed to arsenic by inhalation have been found to be at increased risk of lung cancer, and a number of studies have indicated that ingestion of inorganic arsenic is associated with increased risk of cancer of the skin, bladder, and lung (NAS, 1999; Morales et al., 2000). The risk of cancer from arsenic is calculated using an estimate of arsenic exposure and an arsenic cancer slope factor. The cancer slope factor for arsenic ingestion developed by the USEPA is based on a study of Taiwanese who were exposed to elevated arsenic concentrations in drinking water (Tseng et al., 1968; 1977). This cancer slope factor is used to estimate cancer risk from arsenic ingestion, not only from drinking water, but also from other environmental media including soils. The cancer risk that results from arsenic ingestion is dependent upon the dose of arsenic that is absorbed. Arsenic in drinking water is in a water-soluble form, and its absorption from the gastrointestinal tract is thought to be extensive. Arsenic contaminants in soils, however, may be incompletely absorbed because they are present in water-insoluble forms or they interact with other soil constituents. Logically, the diminished absorption of arsenic from soil relative to water should be taken into consideration when evaluating the cancer risk posed by arsenic exposure. The problem lies in determining, for a given situation, the degree of reduction of arsenic absorption.

A number of studies have attempted to measure the relative bioavailability of arsenic from soils compared with water using animal models (see Table 1). An important limitation of these studies, from the perspective of regulating arsenic-contaminated sites throughout the United States, is that most involve soil samples from mining and smelter sites. While the data indicate diminished bioavailability from these soils, generalizing these results to other types of contaminated sites is questionable, as there is reason to suspect that differences in the type of arsenic contamination, as well as perhaps soil type, may influence arsenic bioavailability.

The need for arsenic soil bioavailability information for a broader array of contaminated sites prompted this study. A primate model was used to measure arsenic bioavailability in soil samples from sites with differing sources of arsenic contamination. The *Cebus* monkey was selected for this study because of our extensive experience with this species for GI absorption studies and its demonstrated value as a model for humans in preclinical pharmacokinetic studies (Bergeron et al., 1990; 2000). Initial experiments were directed to characterizing absorption and excretion of sodium arsenate. The objective of these experiments was to ensure that the absorption and excretion of arsenic in the *Cebus* monkey is sufficiently similar to humans that these monkeys can serve as an effective model for bioavailability studies. Subsequent experiments used urinary excretion data to measure the oral bioavailability of arsenic in five soil samples relative to sodium arsenate in water.

Materials and Methods

Animals and animal care. Five adult male *Cebus* (*Cebus apella*) monkeys, 2.5 to 3.0 kg body weight, were purchased from Osage Regional Primates, Inc. (Osage Beach, MO). Between experiments, they were housed individually in metal cages in a climate-controlled room with a population of other monkeys. During these periods, the animals were fed standard monkey chow. During the experimental period, the animals were transferred to non-metal metabolic

cages in another environmentally-controlled room. While in the metabolic cages, the monkeys were fed a low-arsenic liquid diet (see Table 2 for composition). Solids and oils for the liquid diet were obtained from Bio-Serv (Frenchtown, NJ), while minerals (manganese sulfate, calcium carbonate, potassium dihydrogen phosphate, and magnesium sulfate) were obtained from Fisher Scientific (Norcross, GA). The liquid diet presented to the animals was replaced daily from stocks kept refrigerated for up to 7 days after preparation. The arsenic concentration of each batch of liquid diet was measured as described below and confirmed to be below detection limits (< 1 ppb). The housing conditions, environmental enrichment program, and all procedures involving the animals were approved by the Institutional Animal Care and Use Committee.

Drugs and chemicals. Sodium arsenate heptahydrate was purchased from Sigma Chemical Co. (St. Louis, MO). Telazol was purchased from Fort Dodge Animal Health (Fort Dodge, IA). Atropine for injection (Fugisawa USA, Deerfield, IL), Zofran (GlaxoWellcome, Inc., Research Triangle Park, NC), Ketamine (Elkins Simm, Inc., Cherry Hill, NJ), and isoflurane (Abbott Labs, Abbott Park, IL) were obtained through the University of Florida Animal Resources Stores.

Soil Samples. Surface soil samples from selected contaminated sites were collected and provided by the Florida Department of Environmental Protection (FDEP). The top six inches of ground was excavated from areas known through previous sampling to contain substantial arsenic contamination (i.e., > 100 mg As /kg soil). Soil samples were either shipped to the laboratory at the University of Florida by commercial carrier or delivered directly by FDEP personnel. They were delivered in 5 x 5 gallon buckets and stored in an air-conditioned room until processing. For processing, the soils were dried for at least three days at 30 to 38° C, sieved through a 2 mm screen, and then thoroughly mixed. A 250 ml aliquot of this soil was retained for future reference, and the remainder was sieved to 250 µm or less using a Number 6 screen and pan sieve shaker apparatus (Meinzer Sieve Shaker, Fisher Scientific, Norcross, GA).

The 250 μm sieved soil was stored in sealed containers at room temperature until utilized. The total arsenic concentration in an aliquot of the 250 μm sieved soil was measured by the Central Chemistry Laboratory, Florida Department of Environmental Protection (Tallahassee, FL) using EPA Method 6010.

Animal Dosing and Sampling. At the beginning of each experiment, monkeys were sedated with Ketamine (10 mg/kg, i.m.) combined with atropine (0.01 mg/kg, i.m.), weighed, and a blood sample was taken for standard health assessment. The animals were then transferred to metal-free metabolic cages and fed a low-arsenic liquid diet (Table 2) beginning 48 hours prior to the arsenic dose. Each monkey was fasted overnight before dosing, but the liquid diet was restored 6 hours after the animal was dosed and returned to its metabolic cage. Feces produced during the 24 hours prior to the dose were collected and designated as baseline samples.

For experiments involving oral dosing, the animal was first pretreated with the anti-emetic Zofran (0.15 mg/kg, i.m.), and then 30 minutes later given a dose of the short-term anesthetic Telazol (2 mg/kg, i.m.). While the monkey was sedated, a bladder catheter was placed, and the contents of the bladder were collected, including a 5 ml rinse with sterile saline. This was designated as the baseline, or “0 time” urine sample. Also, a baseline blood sample (2 ml) was taken from the saphenous vein. Blood samples were collected in a 2 ml Vacutainer tube containing 0.2 ml buffered citrate (Becton Dickinson and Company, Rutherford, NJ). A gastric tube was placed, and a measured dose of sodium arsenate solution or soil was introduced into the stomach. Soil doses were administered as a slurry in metal-free, deionized water. Sodium arsenate was administered as a 1.0 mg As/ml solution in de-ionized water, and the volume was adjusted to provide a dose of 1.0 mg arsenic per kg body weight. The gastric tube was flushed with metal-free, deionized water to insure complete transfer of the dose to the stomach. Both the gastric tube and bladder catheters were then removed, and the animal was returned to its metabolic cage for one hour. During this period, the animal was allowed to recover from the

sedative. Any urine or feces produced during this period were collected from the metabolic cage. After removal of urine and feces from the metabolic cage, urine collection surfaces of the cage were rinsed with metal-free water to remove any residual excreted arsenic. Cage rinsate was recovered and analyzed separately from urine and feces. For purposes of assessing arsenic excretion, the arsenic present in cage rinsate was considered to have come from urine produced during the collection interval.

One hour after the oral dose, the animal was again sedated with Telazol and intubated. Anesthesia was maintained using isoflurane gas at 1.5%. An intravenous line was placed in the lower leg, and 100 ml of sterile saline was introduced by slow infusion for hydration. A bladder catheter was placed again by standard technique, and urine samples were collected initially and every hour for the next five hours. Blood samples (2 ml in Vacutainers with 0.2 ml buffered citrate) were collected 1, 1.5, 2, 2.5, 3, 4, 5, and 6 hours after the dose. While anesthetized, supplemental heat was provided in the form of warm water blankets, and body temperature was closely monitored. At six hours after the dose, the animal was allowed to regain consciousness and was returned to its metabolic cage where urine and feces were collected daily over the next four days. As part of the collection procedure, the cage was rinsed with metal-free water as described above to insure complete recovery of arsenic excreted in urine. At the end of the collection period, the monkey was returned to its “home” cage for a minimum of two weeks before another dosing experiment was conducted.

Initially, an experiment was conducted in which the monkeys were administered a single intravenous dose of arsenic (1 mg As [as sodium arsenate] per kg body weight in sterile saline). For this experiment, each monkey was sedated with Telazol and placed under isoflurane anesthesia as described above. Intravenous and urinary catheters were then placed. The bladder was rinsed prior to administration of the intravenous dose. The arsenic dose was introduced through the intravenous line over a period of about 5 minutes. Blood and urine samples were taken at 30 minutes and 1, 1.5, 2, and 2.5 hours. Two ml of blood were taken at each time point,

and all urine produced was collected via catheter. After six hours, the anesthetic was withdrawn, intravenous and bladder catheters were removed, and the animals were returned to their metabolic cages after regaining consciousness. Urine and feces were then collected for four days.

Sample preparation. Urine samples were collected in 1 L polycarbonate bottles containing 10 ml of 65% nitric acid and then stored in smaller, sealed polycarbonate bottles at room temperature until processed for analysis. A 1.0 ml aliquot of urine was added to 1.0 ml 65% nitric acid in a 15 ml pressure tube. The tube was sealed, placed in a 140° C oil bath for three hours to digest, and then allowed to cool. Hydrogen peroxide (0.75 ml of a 30% solution) was then added to the tube, which was placed in a 100° C oil bath for 45 to 60 minutes while loosely capped. After cooling, the contents of the tube and five successive washes with metal-free water were transferred to a 5 ml volumetric flask for analysis.

Feces were collected and weighed. Nitric acid (65%) was then added in an amount equal to 10% of the fecal weight and the samples were homogenized. A 3 g sample of homogenate (actual weight was recorded to the nearest 0.01 g) was added to 20 ml of 65% nitric acid. The mixture was refluxed at 100° C in an extractor for 24 to 48 hours until the entire sample was dissolved. The sample was allowed to cool and 5 ml of hydrogen peroxide (30%) was added. Heat was reapplied, and the mixture was allowed to reflux for one hour. After cooling, the contents of the flask and five successive washes with metal-free water were transferred to a 50 ml volumetric flask for analysis. Samples of each batch of liquid diet were processed using the same procedure as for feces.

Whole blood was centrifuged just after collection to separate plasma. The plasma layer was stored at -80° C until analyzed. A 0.25 ml aliquot of plasma was added to 1.0 ml of 65% nitric acid in a 15 ml pressure tube. As with the urine assay described above, the sample was digested in a 140° C oil bath for three hours and allowed to cool. Hydrogen peroxide (0.75 ml)

was then added to the acidified plasma, and the mixture was heated at 100° C for 45 to 60 minutes. The contents of the tube and five successive washes with metal-free water were transferred to a 5 ml volumetric flask for analysis.

Quantification of arsenic in plasma, urine and feces. All samples were analyzed with a Perkin-Elmer Model 5000 atomic absorption spectrophotometer using a modification of Method 7060A, Revision A, September 1994. The instrument used was a graphite furnace unit with Zeeman background correction. A sample was placed into the L'vov platform of a graphite tube via an auto sampler. Matrix modifiers and diluents were placed into the graphite tube. For urine samples, the following additions were made to the tube: 20 µl of sample/standard, 5 µl of diluent (6.5% HNO₃), and 10 µl of palladium nitrate working solution (500 ppm). For fecal samples, the additions were: 20 µl of sample/standard, 5 µl of diluent, 10 µl of palladium nitrate working solution and 5 µl of magnesium nitrate/ascorbic acid solution (0.5%/1.0%). All samples were ashed at 1200 – 1300° C and atomized at 2200 – 2300°C. Feces/liquid diet samples required preinjection of the matrix modifiers and ashing to 1200° C. Blanks, duplicates, spikes and lab control samples were run at a rate of 5%, or minimally one, per batch. The blank was run with all components minus the sample matrix. Metal-free water was added at the same volume as the sample matrix. Spikes were added at 10 ppb for urine and plasma samples and at 20 ppb for feces or food samples. Laboratory control samples were prepared in plasma, urine or feces samples collected prior to dosing (T0 time point). Plasma and urine samples had 5 ppb arsenic added; feces samples had 25 ppb added. Spikes and lab control samples were prepared using commercially obtained arsenic standards (SPEX CertiPrep, Inc, Metuchen, NJ).

Standard curves were run at the start of each analytical batch. At least 5 standards from 2.5 ug/l to 100 ug/L were analyzed for the calibration, requiring a minimal correlation coefficient of 0.995. Samples outside the range were diluted and rerun. The mean detection limits (MDL)

for each matrix was determined as the lowest concentration that falls within 2 to 5 times the calculated MDL ($3.14 \times$ standard deviation, $n=7$).

Derivation of descriptive pharmacokinetic parameters. Nonlinear regression (PCNONLIN, V4.2, Pharsight, Mountain View, CA) was used to estimate the terminal elimination rate constant () for disappearance of arsenic from plasma over time. The elimination half-life ($t_{1/2}$) was calculated from $0.693/$. The area under the plasma concentration-versus-time curve (AUC) between 0 time and the last data point was derived using the linear trapezoidal rule. In order to estimate the AUC from time 0 to infinity, the area beyond the last time point in the study was estimated by dividing the last measurable plasma concentration by . Mean residence time (MRT) was calculated by dividing the first moment of the plasma concentration versus time profile (AUMC) by the AUC. The volume of distribution at steady state (VD_{ss}) was calculated as $\text{Dose} \times \text{AUMC}/(\text{AUC})^2$. Clearance (Cl) was derived from the Dose/AUC .

Urinary excretion data following intravenous and oral administration of sodium arsenate were used to calculate the absolute bioavailability (F_A) of the oral dose using the following relationship:

$$F_A = \frac{U_{As,oral} \times D_{As,iv}}{U_{As,iv} \times D_{As,oral}}$$

where U refers to the amount of arsenic excreted in urine over the observation period and D is the dose of arsenic. Urinary excretion data were also used to calculate the relative bioavailability of orally administered arsenic in soil versus sodium arsenate in solution. Relative bioavailability (F_R) was calculated as:

$$F_R = \frac{U_{As,soil} \times D_{As,water}}{U_{As,water} \times D_{As,soil}}$$

Statistical Analyses. An analysis of variance was used to analyze the relationship between soil sample, animal, and relative bioavailability. Main effects for soil sample and animal were

included in the model. Statistical significance was determined if p-values were < 0.05 . In the case of multiple comparisons for differences in relative bioavailability, ten comparisons were calculated. Ten comparisons were also calculated for animal effect. So as to preserve the overall Type I error rate for the entire comparison, the Bonferroni adjustment was used and significance was determined for p-values < 0.0005 (resulting in an overall Type I error rate of 0.05).

Results

Blood samples were collected following intravenous administration of arsenic permitting pharmacokinetic analysis. Intravenous dose data were available for four monkeys.¹ Figure 1 shows the disappearance of arsenic from plasma following the 1 mg As/kg body weight dose. Plasma concentrations were remarkably consistent among the animals, with almost superimposable plasma concentration versus time profiles. Descriptive pharmacokinetic parameters were derived from the plasma concentration data for each monkey and are shown in Table 3. Mean values are also presented.

Urinary and fecal excretion were also measured after the intravenous arsenic dose. Urinary excretion of arsenic was rapid, with nearly half of the arsenic dose appearing in the urine within a few hours after the dose (time course not shown). Collection of urine over four days recovered, on average, 66.8% ($\pm 6.5\%$ standard deviation) of the arsenic dose (Table 4). As expected, only a very small fraction of the intravenous arsenic dose appeared in feces over four days of collection (0.5 to 0.6%; see Table 4), indicating little biliary excretion.

Following oral [intra-gastric] administration of the same dose of sodium arsenate, approximately 50% of the dose was recovered in urine within the four-day collection period (Table 5). Most of the dose was recovered within the first 24 hours after the dose (time course not shown). Consistent with extensive gastrointestinal absorption of sodium arsenate in solution, fecal excretion was low (0.4 to 3.3% of the dose; Table 5). Fecal excretion was, however, generally higher than that observed with intravenous administration, indicating that absorption

was not complete. An estimate of the absolute oral bioavailability of sodium arsenate solution in the monkey can be obtained by comparing the urinary excretion (cumulative percent of dose excreted over the collection period) following the oral dose with that obtained in the same animal following administration of the same dose intravenously. The absolute bioavailability estimate from these data was $74.4 \pm 4.7\%$ (mean \pm standard deviation).

Funding was available for measurement of arsenic bioavailability from five soil samples. Each soil sample included in the study was obtained from a different arsenic contaminated site, and the arsenic concentrations in the samples ranged from 101 to 743 mg/kg. For purposes of assessing relative bioavailability, an attempt was made to use an arsenic dose in soil as close as possible to the comparison dose of sodium arsenate in solution (1 mg As/kg body weight). However, it was also considered important to use soil doses that were not excessive in volume. In order to keep the dose of soil itself to 12 g or less, the arsenic in the soil doses ranged from 0.3 to 1.0 mg As/kg body weight (see Figure 6 for the arsenic dose for each soil sample).

Only a small fraction of the dose, generally less than 15%, of the dose was recovered in urine when arsenic was administered in soil (Table 6). Consistent with incomplete gastrointestinal absorption, the vast majority of the dose in nearly all cases was eliminated with the feces. The urinary excretion of arsenic from soil (data from Table 6) was compared with excretion following an oral dose of sodium arsenate in solution (data from Table 5) to generate relative bioavailability estimates for each soil sample in each animal (Table 7). Mean relative bioavailability values for the five soil samples ranged from $10.7 \pm 4.9\%$, (mean \pm standard deviation) to $24.7 \pm 3.2\%$. As expected, some variability in relative bioavailability was observed among subjects within each soil treatment group, and the average coefficient of variation was about 39%. Although the results suggested that some of the animal subjects tended to have higher arsenic bioavailability from soils than others (e.g., animal 721), differences among animals were not statistically significant as determined through an analysis of variance. Relative bioavailabilities from the highest sample (from the Cattle Dip Site) and lowest sample (from

Pesticide Site #1) were significantly different from each other. No statistically significant differences in relative bioavailability were detected between other samples.

Few of the blood samples collected after administration of arsenic in soil had concentrations above the minimum quantitation limit. Arsenic could be quantified in only one or two samples per soil per animal (data not shown), making it impossible to calculate a meaningful area under the concentration versus time curve for bioavailability measurement. Consequently, relative bioavailability was assessed based on urinary excretion only.

Discussion

In developing bioavailability information relevant to human health risk assessment, it is important to assess, to the extent possible, the degree to which absorption and elimination behavior in animal models resembles humans. Arsenic administered intravenously to the *Cebus apella* monkey disappeared rapidly from blood, with an apparent half-life of about one hour (Figure 1). This is consistent with the initial, very rapid disappearance of arsenic from blood observed in four human subjects administered an intravenous dose of As⁷⁴ (Mealey et al., 1959). By using a radiolabeled arsenic dose, plasma arsenic concentrations in the human subjects could be followed for 10 days, revealing a much slower rate of disappearance after the first few hours. The terminal elimination rate, which appeared after about 7 days, corresponded to a half-life of approximately 86 hours. Collection of urine from these subjects over nine days recovered 57 to 90% of the intravenous dose. The urinary recovery of intravenous arsenic in the *Cebus* monkey (66.8% on average) falls within this range. Ducoff et al. (1949) administered sodium arsenite intravenously to two subjects and collected urine and feces over the next seven days. Their

recovery of 65.7 and 59.1% of the dose in urine for the two subjects, and 0.9 and 0.5% of the dose from feces, matches quite closely the urinary and fecal recoveries of arsenic in the *Cebus* monkeys (see Table 4). Urinary and fecal recoveries were also consistent with previous observations in other non-human primates. Vahter et al. (1995) administered arsenic intravenously to two chimps and recovered 52 and 63% of the dose in urine and 1.2% and 1.4% of the dose in feces over four days. Another 5.1 and 5.4% of the dose was recovered in cage wash, and was presumed to reflect primarily urinary excretion. Freeman et al. (1995) administered arsenic intravenously to three cynomolgus monkeys, and recovered $76.5 \pm 2.5\%$ of the dose in urine and $3.2 \pm 1.9\%$ in feces.

Urinary and fecal excretion of sodium arsenate after oral administration in the *Cebus* monkey was also consistent with previous observations in humans and other primates. Table 8 summarizes observations from several such studies in humans. The study by Bettley and O'Shea (1975) is one of the few to present data for both urinary and fecal excretion of arsenic after oral administration in human volunteers, and the results are nearly identical to those observed here with the *Cebus* monkey. These comparisons, along with those following intravenous administration of sodium arsenate, strongly suggest that the fundamental absorption and excretion of arsenic are the same in the *Cebus* monkey and humans.

The incomplete recovery of the arsenic dose in urine and feces during the experiment indicated that a substantial fraction of the dose was being retained in the body. Under such circumstances, care must be taken that the chemical does not accumulate to toxic levels with repeated dosing, and that residual chemical in the body does not affect interpretation of absorption data from a subsequent dose. With a two-week minimum washout period between doses, we did not observe significant residual arsenic in blood, urine, or feces. That is, despite the fact that each monkey had received over time several doses of arsenic, baseline blood, urine, and fecal measurements taken before dosing were consistently below detection levels. This is not surprising. Assuming that the terminal elimination rate in the monkeys is similar to that

observed in humans (ca. 86 hours, as discussed above), the three-week minimum collection and washout period allowed six elimination half lives to pass between doses. Typically, the dosing interval was a month or more, corresponding to 8 or more half-lives. Under these circumstances, significant accumulation would not be expected.

To minimize stress to the animal subjects, it was necessary to anesthetize them during the period of most frequent blood and urine sample collection. It was also considered important to include in the experimental protocol a period without anesthesia immediately after the dose to allow normal gastric emptying. Extensive preliminary experimentation [not presented in Results] established one hour as the optimum interval between the dose and the beginning of sampling. When sampling was initiated more than an hour after the dose, peak blood concentrations and urinary excretion rate were missed, and shorter intervals resulted in reduced recovery of arsenic dose, perhaps due to interference by the anesthetic with normal gastric contractions and emptying. A half-life for gastric emptying of 30 minutes has been reported for fasted male Red Patas monkeys (Franklin, 1977). If this emptying rate is applicable also to the *Cebus* monkeys in this study, it would suggest that most of the oral dose was emptied from the stomach before induction of anesthesia with the one-hour interval protocol. Perhaps the best argument for the acceptability of this protocol is empirical; that is, that the absorption and excretion of arsenic appear to match closely that observed in unanesthetized human volunteers, as discussed above.

Relative oral bioavailability can be measured using either blood concentration or urinary excretion data, and it was the original intent of this study to use both approaches. However, with the analytical methods employed, it was very difficult to develop an accurate characterization of the blood concentration versus time profile after oral dosing, particularly for arsenic in soils. The amount absorbed was simply too small to produce blood concentrations that could be measured at more than one or two time points. A previous study of arsenic bioavailability from soil in cynomolgus monkeys also measured arsenic in blood and urine, and reported estimates of

relative bioavailability based on both approaches (Freeman et al., 1996). However, as we observed with *Cebus* monkeys, blood arsenic concentrations after the soil doses were only marginally above quantitation limits at a few time points. In this situation, the ability to capture the complete arsenic blood concentration versus time profile from the soil dose is compromised, resulting in an underestimation of absorption. This would explain why these investigators obtained a relative bioavailability estimate from blood data that was only about one-half that based on urinary excretion data. For the purposes of assessing arsenic bioavailability from soils, the use of urinary excretion data would appear to be much more reliable, at least in non-human primates.

The objective of this study was to begin to generate data on bioavailability of arsenic from soils covering a broader range of types of arsenic-contaminated sites than currently exists in the literature. This study offers some of the first measurements of the relative bioavailability of arsenic in soil at cattle dip, wood treatment, electrical substation, and pesticide sites. The limitations in generalizing these results are obvious. It would be inappropriate, for example, to use the single value reported here from a wood treatment facility as representative of all wood treatment sites. There are a variety of factors (e.g., soil characteristics, arsenic formulation, manner of release of arsenic to the soil) that could conceivably affect arsenic soil bioavailability from site to site, and even different areas within a site. This is illustrated well by the recent report (Casteel et al., 2001) of the relative bioavailability of arsenic from five samples of soil contaminated with the arsenical herbicide, PAX. All five samples were taken from residential yards at the Vasquez Boulevard and I-70 Superfund site and all were presumably contaminated with arsenic from the same product. The relative arsenic bioavailability among the five samples, as measured in a swine model, ranged from 18 to 45%.

Despite the expectation of some variability in arsenic bioavailability from specific types of sites, the results of this study are nonetheless consistent with the concept that arsenic bioavailability from soils is generally reduced compared with that from water. For the sites

included in this study, the extent of reduction ranged from 4- to 10-fold (based on relative bioavailabilities ranging, on average, from about 25 to 10%). Typically, when evaluating risks from arsenic contaminated soils, the predominant route of arsenic intake is assumed to be through incidental ingestion of soil, and the relative bioavailability of arsenic is assumed to be 100%. That is, the bioavailability of arsenic from soil is assumed to be equivalent to the bioavailability of soluble arsenic in water. Because incidental ingestion is the dominant route of exposure, any adjustment in the oral relative bioavailability from the default 100% assumption will have an essentially proportional effect on the overall dose [and risk] estimate. For example, a relative bioavailability adjustment of 4-fold (i.e., incorporation of a relative bioavailability of 0.25), reduces the estimated risk for a given concentration of arsenic in soil 4-fold. At some sites, a correction of this magnitude in the risk estimate to improve accuracy can have important economic consequences in terms of the resources required for cleanup. Consequently, even though the reduction in relative bioavailability measured in this study and others is not large, usually an order of magnitude or less, its recognition can be of enormous practical value in some situations.

There are uncertainties in the measurement of bioavailability as conducted in this and similar studies that should be acknowledged. One such uncertainty is whether the animal model used in the study serves as a sufficiently valid predictor of human response. We attempted to address this by comparing pharmacokinetic and excretion behavior of sodium arsenate in the *Cebus* monkey with previous studies in human volunteers. Although the results were quite similar, it is always possible that there could be species differences when arsenic is present in a soil matrix. Unfortunately, there are no reliable measurements of arsenic bioavailability from soil samples in human subjects to serve as a basis for comparison, making true validation of animal models difficult. A second uncertainty relates to the possible effect of arsenic concentration in soil on bioavailability. Existing measurements of relative bioavailability of arsenic from soils, including those presented here, use soil samples with arsenic concentrations

in the hundreds of ppm. These concentrations are needed in order to present to the experimental animal an arsenic dose sufficient for detection in a reasonable soil volume. This raises the question of whether bioavailability measurements at these concentrations are predictive of the bioavailability in lesser-contaminated soils (i.e., with arsenic concentrations < 100 ppm). Conceivably, arsenic bioavailability from soils could be influenced by concentration if critical interactions with soil constituents are capacity-limited, but this issue has not been well studied. Another area of uncertainty pertains to the feeding status of the animals during bioavailability measurement. In this study, monkeys received a soil dose after an overnight fast. The previous bioavailability study using monkeys (Freeman et al., 1996) and two studies using swine (Lorenzana et al., 1996; Battelle, 1996) also fasted the animals overnight before the dose. Other studies using the swine model (Casteel et al., 1997;2001) did not fast the animals *per se*, but timed the presentation of food to the animals such that the dose was always given on an empty stomach. Administering the dose on an empty stomach no doubt aids in reducing variability associated with the bioavailability measurements, but probably does not mimic well the circumstances under which humans ingest soil. If the presence of food diminishes the bioavailability of arsenic from soil, as might be expected, then the measurements as conducted in these studies are upper-bound estimates and therefore useful for regulatory purposes. However, this is speculation, and basic information on the effects of food on bioavailability from soils is lacking.

The results presented here extend considerably the types of arsenic contaminated sites for which quantitative soil bioavailability data are available. As with soils from other types of sites, those investigated in this study showed substantially diminished arsenic bioavailability. The consistency of this observation highlights the importance of explicit, quantitative consideration of bioavailability when assessing the risks from arsenic contaminated soils.

Footnotes:

¹ One of the original five monkeys purchased for the project was not in good health, and was therefore not included in the study. By the time a replacement animal was obtained, the intravenous dosing portion of the study was completed. Since obtaining intravenous data from each animal was not essential to the objectives of the study, an intravenous-dose experiment was not conducted using this animal.

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TABLE 1. Literature Reports of Arsenic Relative Bioavailability in Soils^a

<i>Study</i>	<i>Animal</i>	<i>Soil Type</i>	<i>Relative Bioavailability</i>
Freeman et al., 1993	Rabbit	Smelter area soils	48% ^b
Lorenzana et al., 1996	Swine	Mining area soil	78%
		Mining area slag	42%
Battelle, 1996	Swine	Mining area slag	ND ^c
Casteel et al., 1997	Swine	Soils and mining area wastes	0-50% ^d
Casteel et al., 2001	Swine	Residential soils	18-45% ^e
Freeman et al., 1995	Monkey	Mining area soils	20% ^f
		Mining area dusts	28% ^f

^a Note: A few reports in the literature have developed estimates of absolute bioavailability of arsenic from soils (e.g., Groen et al., 1994; Ng et al., 1998; Ellickson et al., 2001). The relative bioavailability of arsenic was not measured in these studies and cannot be reliably inferred from the data. These studies are not included in this table.

^b Average of six observations using differing doses (mg soil/kg body weight) of the soil sample in male and female rabbits.

^c ND = not detectable.

^d Two soil samples had relative bioavailability > 90%. The arsenic concentrations in soil in these samples were low, and the authors considered these measurements to be unreliable.

^e Values shown are best estimates from six soil samples. The 90% confidence intervals for the soil samples with the lowest and highest relative bioavailability values were 15-21% and 38-52%, respectively.

^f As determined from urinary excretion data.

TABLE 2. Composition of the Low Arsenic Liquid Diet

	Ingredient	Amount
Solids	Casein	180 g.
	Sucrose	194 g.
	Dextrin	194 g.
	Dextrose	194 g.
	Fiber (cellulose)	90 g.
	Vitamin mix	5 g.
	Methionine	5 g.
	Banana flavor	2 g.
	Choline chloride	2 g.
	Cholesterol	1 g.
	Oils	Corn oil
Coconut oil		45 g.
Soy lecithin		20 g.
Minerals	Manganese sulfate	0.1 g.
	Calcium carbonate	25.4 g.
	Potassium dihydrogen phosphate	35.8 g.
	Magnesium sulfate	5.2 g.
Water		1,350 ml

TABLE 3. Descriptive Pharmacokinetics After Intravenous Administration of Sodium Arsenate

<i>Parameters*</i>	<i>Animal ID</i>				<i>Mean</i>
	<i>403</i>	<i>721</i>	<i>E60</i>	<i>DOB</i>	
AUC ($\mu\text{g/L-h}$)	3260	3926	3247	3943	3594
AUMC ($\mu\text{g/L-h}^2$)	3541	4703	5068	5175	4622
VD _{ss} (L/kg)	0.333	0.305	0.481	0.333	0.363
MRT (h)	1.086	1.198	1.313	1.313	1.228
t _{1/2} (h)	0.935	0.906	1.042	1.071	0.989
Cl (L/kg-hr)	0.307	0.255	0.308	0.254	0.278

A single intravenous dose of sodium arsenate (1 mg As/kg body weight) was administered and blood samples were collected over time.

* AUC is the area under the plasma concentration versus time curve; AUMC is the first moment of the plasma concentration-time profile; VD_{ss} is the volume of distribution at steady state; MRT is the mean residence time; t_{1/2} is the terminal elimination half-life; and Cl is the clearance.

TABLE 4. Urinary and Fecal Recovery of Arsenic After an Intravenous Dose of Sodium Arsenate

<i>Animal ID</i>	<i>Excretion (% of dose)</i>		<i>Total</i>
	<i>Urine</i>	<i>Feces</i>	
721	72.5	0.6	73.1
DOB	67.4	0.5	67.9
403	69.8	0.6	70.4
E60	57.5	0.6	58.1
Mean \pm SD	66.8 \pm 6.5	0.6 \pm 0.1	67.4 \pm 6.5

Each monkey was administered a single intravenous dose (1 mg As/kg body weight) of sodium arsenate. Urine and feces were collected for 4 days, and cumulative excretion of the dose by the urinary and fecal routes was measured.

**TABLE 5. Urinary and Fecal Recovery of Arsenic
After an Oral Dose of Sodium Arsenate**

<i>Animal ID</i>	<i>Excretion (% of dose)</i>			<i>F(%)</i>
	<i>Urine</i>	<i>Feces</i>	<i>Total</i>	
721	51.5	1.2	52.7	71.0
DOB	50.1	3.2	53.3	74.3
2C6F	46.1	1.4	47.5	NA*
403	49.7	3.3	53.0	71.2
E60	46.7	0.4	47.1	81.2
Mean ± SD	48.8 ± 2.3	1.9 ± 1.3	50.7 ± 3.1	74.4 ± 4.8

Each monkey was administered a single oral dose (1 mg As/kg body weight) of sodium arsenate. Urine and feces were collected for 4 days, and cumulative excretion of the dose by the urinary and fecal routes was measured. Absolute bioavailability of sodium arsenate solution (F) was calculated by dividing urinary recovery following the oral dose by urinary recovery after the same dose administered intravenously (see Table 4 for recoveries after intravenous administration).

*NA – Absolute bioavailability could not be calculated for 2C6F because data for urinary recovery after intravenous administration were not obtained.

TABLE 6. Urinary and Fecal Excretion of Arsenic Dose After Oral Administration of Contaminated Soil

<i>Site Type</i>	<i>Animal ID</i>	<i>Excretion (% of dose)</i>		
		<i>Urine</i>	<i>Feces</i>	<i>Total</i>
Electrical Substation	721	8.26	57.3	65.6
312 mg As/kg soil	DOB	9.95	38.1	48.1
0.5 mg As/kg b.w.	2C6F	5.03	46.5	51.5
	403	3.91	71.5	75.4
	E60	8.57	45.4	54.0
Cattle Dip Site	721	14.6	66.8	81.4
189 mg As/kg soil	DOB	10.7	59.7	70.4
0.5 mg As/kg b.w.	2C6F	11.1	64.2	75.3
	403	11.0	51.4	62.4
	E60	12.9	76.8	89.7
Pesticide Site #1	721	9.08	68.1	77.2
743 mg As/kg soil	DOB	3.63	67.6	71.2
1.0 mg As/kg b.w.	2C6F	4.15	85.4	89.6
	403	6.27	77.3	83.6
	E60	2.80	65.2	68.0
Wood Treatment Site	721	7.89	92.4	100.3
101 mg As/kg soil	DOB	4.51	54.5	59.0
0.3 mg As/kg b.w.	2C6F	9.01	69.9	78.9
	403	6.78	85.7	92.5
	E60	12.20	57.1	69.3
Pesticide Site #2	721	16.7	59.8	76.5
329 mg As/kg soil	DOB	7.93	73.1	81.0
0.5 mg As/kg b.w.	2C6F	9.12	54.7	63.8
	403	3.11	60.5	63.6
	E60	5.04	94.4	99.4

Data represent the percent of the arsenic dose in soil recovered in urine and feces over a 4-day period. For each soil sample, the concentration of arsenic in soil and the arsenic dose as administered (in mg As/kg body weight) are shown.

TABLE 7. Arsenic Bioavailability Based upon Urinary Excretion Data

<i>Site Type</i>	<i>Animal ID</i>	<i>Relative Bioavailability</i> (%)	<i>Mean</i> (\pm <i>SD</i>)
Electrical Substation	721	16.0	14.6 \pm 5.1
	DOB	19.9	
	2C6F	10.9	
	403	7.9	
	E60	18.4	
Cattle Dip Site	721	28.3	24.7 \pm 3.2
	DOB	21.3	
	2C6F	24.1	
	403	22.1	
	E60	27.6	
Pesticide Site #1	721	18.3	10.7 \pm 4.9
	DOB	7.8	
	2C6F	9.0	
	403	12.6	
	E60	6.0	
Wood Treatment Site	721	15.3	16.3 \pm 6.5
	DOB	9.0	
	2C6F	19.5	
	403	13.6	
	E60	26.2	
Pesticide Site #2	721	32.4	17.0 \pm 10.0
	DOB	15.8	
	2C6F	19.8	
	403	6.3	
	E60	10.8	

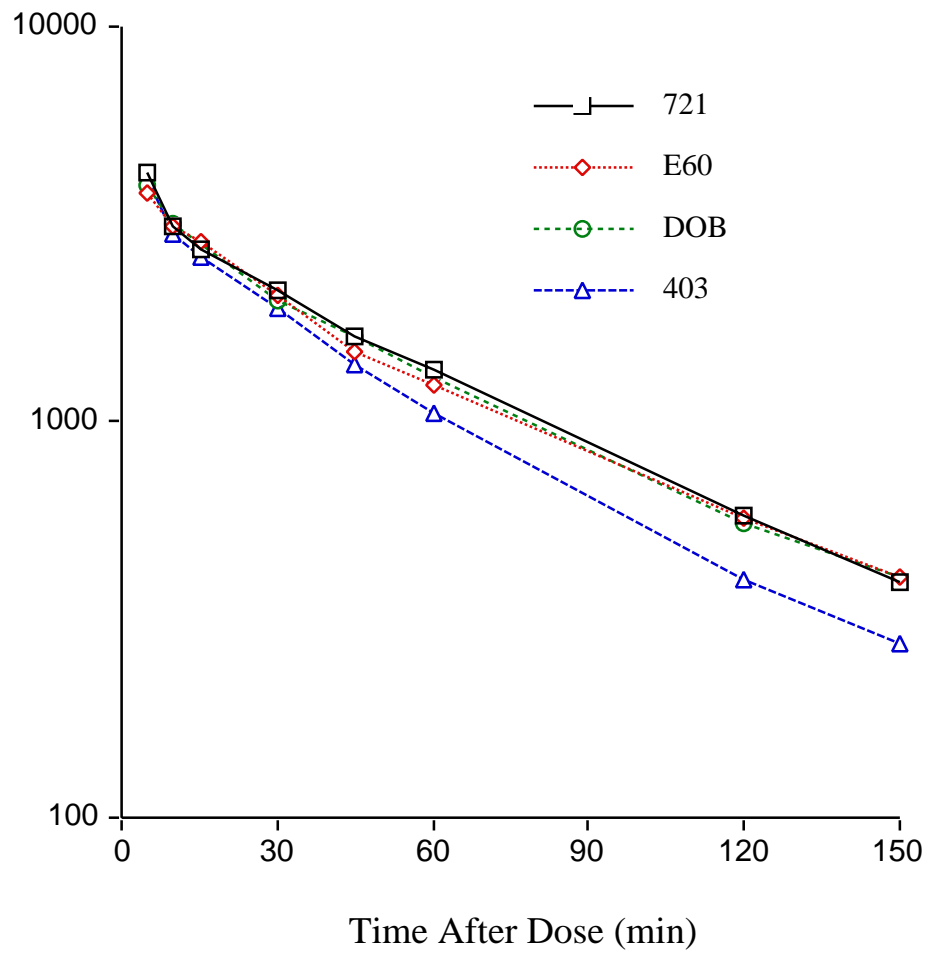
Soil arsenic concentrations and arsenic doses are shown in Table 6.

TABLE 8. Excretion of Arsenic after an Oral Dose of Arsenic Solution in Human Volunteers

<i>Study</i>	<i>No. Subjects</i>	<i>Excretion (% of dose)</i>	
		<i>Urine</i>	<i>Feces</i>
Pomroy et al., 1980	6	62.3 ± 4.0	6.1±2.8
Buchet et al., 1981	4	66	--
Tam et al., 1979	6	58	--
Crececius, 1977	1	< 50	--
Bettley and O'Shea, 1975	3	52	≤ 3.5
Mappes, 1977	1	69-72	--

Figure Legends

Figure 1. Plasma concentrations over time after a single intravenous dose of sodium arsenate. Each monkey received a single intravenous dose of sodium arsenate (1 mg As/kg body weight). Plasma concentrations for each animal are shown. Descriptive pharmacokinetic parameters derived from these data are presented in Table 3.



Draft manuscript

Index terms

arsenic; bioavailability from soil; gastrointestinal absorption; urinary excretion