TOXICOLOGICAL SCIENCES **95(1)**, 281–288 (2007)
doi:10.1093/toxsci/kfl117
Advance Access publication September 27, 2006

Relative Oral Bioavailability of Arsenic from Contaminated Soils Measured in the Cynomolgus Monkey

Stephen M. Roberts,*[,1] John W. Munson,* Yvette W. Lowney,† and Michael V. Ruby†

*Center for Environmental and Human Toxicology, University of Florida, Gainesville, Florida 32611; and †Exponent, Boulder, Colorado 80301

Received July 6, 2006; accepted September 18, 2006

A number of studies have found that gastrointestinal absorption of arsenic from soil is limited, indicating that a relative oral bioavailability (RBA) adjustment is warranted when calculating risks from exposure to arsenic-contaminated soil. However, few studies of arsenic bioavailability from soil have been conducted in animal models with phylogenetic similarity to humans, such as nonhuman primates. We report here the results of a study in which the RBA of arsenic in soil from a variety of types of contaminated sites was measured in male cynomolgus monkeys. A single oral dose of each contaminated soil was administered to five adult male cynomolgus monkeys by gavage, and the extent of oral absorption was evaluated through measurement of arsenic recovery in urine and feces. Urinary recovery of arsenic following doses of contaminated soil was compared with urinary recovery following oral administration of sodium arsenate in water in order to determine the RBA of each soil. RBA of arsenic in 14 soil samples from 12 different sites ranged from 0.05 to 0.31 (5–31%), with most RBA values in the 0.1–0.2 (10–20%) range. The RBA values were found to be inversely related to the amount of arsenic present with iron sulfate. No other significant correlations were observed between RBA and arsenic mineralogic phases in the test soils. The lack of clear relationships between arsenic mineralogy and RBA measured *in vivo* suggests that gastrointestinal absorption of arsenic from soil may be more complex than originally thought, and subject to factors other than simple dissolution behavior.

Key Words: **arsenic; oral bioavailability; contaminated soil; nonhuman primates.**

The use of arsenic as an herbicide and an insecticide, as well as its occurrence naturally in mineral deposits subject to mining, has led to the creation of numerous arsenic-contaminated sites in the United States. When assessing potential risks from arsenic contamination in soil, contemporary models and assumptions generally regard incidental soil ingestion as the dominant route of exposure. The process of estimating arsenic doses resulting from incidental soil ingestion requires an assumption on the extent to which arsenic in soil is absorbed from the gastrointestinal tract. The default assumption typically used in risk assessments is that the extent of gastrointestinal absorption of arsenic from soil is equivalent to its absorption under the conditions in which the toxicity value was derived (NRC, 2003), which in the case of arsenic is from water. Absorption from water is the relevant comparison for arsenic because the cancer slope factor used to estimate excess cancer risks was developed from studies of individuals exposed to arsenic in drinking water. Assuming equivalent absorption is the same as stating that the relative oral bioavailability (RBA) of arsenic from soil (compared to water) is 1.0, or 100%.

A variety of animal models have been used to assess arsenic bioavailability from soil, including rats and rabbits (e.g., Freeman *et al.*, 1993, Ng *et al.*, 1998). However, the principal animal models used to measure arsenic bioavailability from soils are swine and monkeys. The swine model has been used in studies of soils at a variety of contaminated sites in the western United States, principally in mining areas (Casteel *et al.*, 1997, 2001; Lorenzana *et al.*, 1996). The monkey model has been used to measure arsenic bioavailability in soils from a variety of types of sites, including soils from a mining area, electrical substation, cattle dip vat site, a wood treatment site, and pesticide sites (Freeman *et al.*, 1995; Roberts *et al.*, 2002). In general, RBA values for arsenic in soils range from 0 to about 50% in these two models (Roberts *et al.*, 2002; Ruby *et al.*, 1999).

Although the principle of reduced bioavailability of arsenic from soils is well established, understanding of the factors that dictate bioavailability is limited. One of the obstacles in conducting research on factors influencing arsenic bioavailability is the limited number of soil samples available for which bioavailability has been measured. Many of the soil samples for which bioavailability data have been published are no longer available or are inaccessible for research for other reasons. Consequently, there is a need for characterization of additional soils in terms of arsenic bioavailability, not only to support additional research on this topic but also to better define the range of arsenic bioavailabilities that may exist in contaminated soils. For this project, arsenic RBA values for 14 soil samples obtained from 12 different contaminated sites were measured in

[1] To whom correspondence should be addressed at Center for Environmental and Human Toxicology, University of Florida, Building 471, Mowry Road, Gainesville, FL 32611. Fax: (352) 392-4707. E-mail: smr@ufl.edu.

cynomolgus monkeys, expanding considerably the range of sites from which arsenic bioavailability has been measured. Correlations between the RBA of arsenic in soil and soil mineralogy were obtained to provide a preliminary evaluation of potential soil characteristics influencing bioavailability.

MATERIALS AND METHODS

Animals and animal care. Seven young adult male cynomolgus (*Macacus cynomolgus*) monkeys, 4–5 kg bw, were purchased from Primate Products, Inc (Miami, FL). Between experiments, the monkeys 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. The animals were observed daily for normal appearance and behavior, and comprehensive health assessments by a veterinarian were completed every 6 months. During the experimental period, the animals were transferred to nonmetal metabolic cages in another environmentally controlled room. While in the metabolic cages, the monkeys were fed a low-arsenic pelletized diet (Bio-Serv, Frenchtown, NJ). This diet consisted of (g/kg basis): cornstarch, 361 g; casein, 266 g; dextrin, 155 g; oils (corn, olive, and safflower) 96 g; fiber, 52 g; mineral mix, 40 g; vitamin mix, 20 g; DL-methionine, 1.2 g; L-cystine, 2.0 g; choline chloride, 2 g; and banana flavor, 4.0 g. 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). Atropine for injection (Fujisawa USA, Deerfield, IL) and ketamine (Elkins Simm, Inc, Cherry Hill, NJ) were purchased from Webster Veterinary Supply (Alachua, FL).

Soil samples. Soil samples were obtained from selected arsenic-contaminated sites. Samples were sought from sites that varied in arsenic contamination source (e.g., wood treatment, herbicide use, mining) and in geographic region. Only samples with arsenic concentration of at least 100 mg As/kg soil were accepted for study. Each soil sample was dried and sieved to 250 μm. This was selected as the particle size fraction believed to adhere to skin and to result in incidental ingestion exposures (U.S. EPA, 2000). Use of this particle size fraction is also consistent with other research regarding the RBA of metals from soils (Casteel *et al.*, 2001; Ruby *et al.*, 2002; Schroder *et al.*, 2003), and existing and proposed guidance (Kelly *et al.*, 2002; U.S. EPA, 2004). 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 using EPA Method 6010.

Animal dosing and sampling. At the beginning of each experiment, monkeys were fed a low-arsenic diet beginning 5 days prior to the arsenic dose. Three days after initiating the diet, the animals were sedated with ketamine (10 mg/kg bw, im) combined with atropine (0.01 mg/kg bw, im), a health assessment was performed, and the animals were weighed. (Note that atropine was administered to reduce intraoral secretions produced by ketamine. Although atropine can suppress gastrointestinal motility, its potential impact on measurement of arsenic absorption was considered negligible because it was administered 2 days before the arsenic dose.) The animals were then transferred to metal-free metabolic cages where urine was collected for baseline arsenic levels prior to dose. Each monkey was fasted overnight before dosing, but the low-arsenic diet was restored 4 h after the animal was dosed and continued while the animal remained in the metabolism cage.

Dosing was accomplished by transferring the animal with the use of a pole and collar arrangement to a chair designed to comfortably restrain the animal so that its hands could not contact its mouth. A gastric tube consisting of a 40 cm length of 3/16″ ID × ¼″ OD Tygon tubing 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 from a 60 ml irrigating syringe attached to the gastric tube. The mass of soil administered did not exceed 1 g per kg bw. Sodium arsenate was administered from a 1.0 mg As/ml stock solution in deionized water, and the volume was adjusted to provide a dose no greater than 1.0 mg As/kg bw. The syringe and gastric tube were flushed twice with metal-free, deionized water to ensure complete transfer of the dose to the stomach. After dosing, the tube was removed, and the animal allowed to ingest a few drops of flavored Gatorade to overcome any unpleasant taste from the gastric intubation. The animal was then walked via pole and collar back to its metabolism cage. Urine and feces were subsequently collected for 4 days. After collection of urine and feces was complete, each animal was returned to its home cage for a period of at least 3 weeks before the next dosing period. This ''wash out'' period allowed urinary and fecal arsenic concentrations to return to baseline levels. Evaluation of predosing urine samples collected over the course of the study confirmed no carryover of arsenic from one dose to the next under these conditions. Typical baseline concentrations of arsenic in urine were about 6 μg/l.

In one experiment, each animal was administered iv a single dose of sodium arsenate (1 mg As, as sodium arsenate, per kg bw in sterile saline). Animals were placed in a metal-free metabolism cage and fed a low-arsenic diet as detailed above. At the time of dosing, an iv line was placed in the leg via the saphenous vein. The arsenic dose was introduced through the iv line over a period of about 5 min. The animal was returned to the metabolism cage where urine and feces were collected as described for the gavage experiments.

Sample preparation. Urine samples were collected in 1-l polycarbonate bottles containing 10 ml of 65% nitric acid and then stored at room temperature until processing for analysis. For collection of urine, the metabolic cage was brushed and rinsed with 800 ml of deionized water. Preliminary studies were conducted in which monkeys were placed in the metabolism cage and arsenic-spiked blank urine was added beneath the animal. All conditions were the same as a standard experiment except no arsenic dose was administered. The cage-rinsing procedure was found to recover 87.2 ± 2.3% (mean ± SD, $n = 3$) of arsenic added to the cage. Feces samples were collected in tared 7 × 7 cm polypropylene cups (Nalge Co., Rochester, NY). Nitric acid (65%) was added at 30% of the feces weight, and the feces were homogenized. One gram of sample (urine or feces) was placed in a digestion vessel, and 5 ml of concentrated nitric acid was added. The sample was then heated on a digestion block for at least 2 h at 100°C. If the sample was still dark in color after 2 h, the sample was heated for an additional 30 min. One milliliter of 30% hydrogen peroxide was added, and the sample was heated for 30 min. The samples were clear and completely dissolved. The digested samples were then diluted to 100 ml with deionized water.

Quantification of arsenic in urine and feces. Baseline urine samples were analyzed by inductively coupled plasma-mass spectrometry by the Battelle Pacific Northwest Laboratory (Richland, WA). The limit of quantification for arsenic in urine was 0.3 μg/l. Urine samples collected after the dose, and all fecal samples, were analyzed by inductively coupled plasma-atomic emission spectrometry by ABC Laboratories (Gainesville, FL). The limits of quantification for urine and feces using this method were 2.3 μg/l and 0.5 μg/g, respectively.

Calculation of bioavailability. RBA of arsenic from each test soil was measured in five individual animals using urinary excretion data. Each animal received, on separate occasions, three doses of sodium arsenate by gavage—0.25, 0.5, and 1.0 mg As/kg bw (as arsenic). Measurement of arsenic in urine over 2 days prior to the dose was used to establish the baseline arsenic excretion rate due to diet for each subject in each experiment. The baseline excretion rate (in μg/day) was used to calculate the contribution of dietary arsenic to total excretion after a sodium arsenate or soil dose, and this was subtracted in order to obtain the amount excreted in urine attributable to the dose (U_{As}). The percent of arsenic dose recovered in urine ($U_{As,arsenate}/Dose_{As,arsenate}$) following each of the sodium arsenate doses was averaged for each animal. This average recovery, as a percent of dose, was used as the reference value for comparison with urinary recovery following administration of arsenic in soil.

The use of urinary recovery of arsenic as a means of comparing absorption of arsenic under different conditions (in this case, administered in water vs. soil) is valid only if the urinary excretion kinetics are identical or the urinary excretion of dose is substantially complete within the collection period.

In a previous study using Cebus monkeys (Roberts *et al.*, 2002), urinary excretion was evaluated during discrete intervals over a 4-day period after administration of sodium arsenate in water or arsenic-contaminated soil. Nearly half of the administered dose appeared in the urine within a few hours, and most of the recovered dose was collected in the first 24 h. An arsenic study in cynomolgus monkeys (Freeman *et al.*, 1995) similarly found peak excretion of arsenic within the first 24 h regardless of whether the arsenic was in water or soil. In view of these observations, a single 4-day collection of urine was considered adequate to provide comparable and essentially complete recovery of absorbed arsenic from both water and soil in this study.

For each soil sample, five animals were randomly selected, and a dose of the test soil was administered by gavage. An RBA was calculated for each subject by dividing the percent of arsenic dose in soil recovered in urine ($U_{As,soil}/Dose_{As,soil}$) by the sodium arsenate reference value for that animal. Thus, an RBA measurement was available for each of the five subjects for all the soil samples tested. Occasionally, the total arsenic recovery was less than 70% after a soil dose in a subject. When this occurred, the RBA value was flagged and the soil sample was re-administered. In all such instances, total recovery from the subsequent dose was greater than 70%, and the resultant RBA replaced the original, low-recovery value.

Soil mineralogy. Arsenic speciation on a subsample of all substrates dosed to the monkeys was evaluated by Dr John Drexler at the Laboratory for Environmental and Geological Studies at the University of Colorado, Boulder. Speciation was conducted as described previously (Davis *et al.*, 1993) using standard procedures (Drexler, 2006). The chemistry of individual arsenic-bearing grains in the sample was determined using an electron microprobe (JEOL 8600). Individual grains were evaluated until a representative number had been analyzed (generally 100–200), and the distribution of arsenic among the different arsenic forms in the soil was established.

Statistical analysis. The percentages of arsenic dose recovered in urine and feces after differing doses of sodium arsenate were compared by both parametric and nonparametric tests. A randomized complete block design ANOVA-based *F*-test was conducted, along with a test for linear trend in dose and checking the residuals for normality (Neterm *et al.*, 1989). Data were also evaluated using a nonparametric, distribution-free test for ordered alternative in a randomized complete block design (Page, 1963).

Mineralogy data were evaluated to determine whether they were useful in predicting oral RBA as measured in the cynomolgus monkey. Both backward and forward stepwise analysis evaluated the best fitting model of each size, i.e., including one variable up to including all 10 variables, based on the smallest residual sum of squares. The 10 variables used in the analysis were iron oxides, number of particles counted, arsenic concentration, iron sulfate, lead arsenate, manganese oxides, arsenic (metals) oxide, iron arsenic oxides, lead (metal) oxide, and phosphate. Analysis of the stepwise models resulted in a final model that included only variables significant at a 0.05 level.

RESULTS

To provide perspective on the recovery of arsenic in urine and feces expected following systemic absorption, each monkey in the study population was administered a single iv dose of sodium arsenate (1.0 mg As/kg bw). Urine and feces were collected over a 4-day period following the dose. Among the seven animals, urinary recovery of arsenic ranged from approximately 80 to 90%, with the exception of one subject from which only 53% was recovered (Table 1). Recovery of dose from feces was uniformly low (0.6% or less). Because of the striking difference in urinary recovery of arsenic in one animal, the iv dose was repeated in this subject. The second experiment yielded almost identical results—urinary recovery of 59% and fecal recovery of 0.5%.

TABLE 1
Urinary and Fecal Recovery of Arsenic after an iv Dose

Subject	% dose in urine	% dose in feces	% total recovery
7490	83.8	0.6	84.4
7630	84.9	0.4	85.3
7773	90.1	0.5	90.6
7597	86.4	0.1	86.5
7516	53.4[a]	0.3[a]	53.7
7499	80.4	0.5	81.0
7515	78.9	0.1	79.0
Mean ± SD	80.5 ± 10.2	0.4 ± 0.2	80.9 ± 10.2

Note. Each animal received a single iv dose of sodium arsenate (1 mg As/kg bw). The results reflect cumulative excretion in urine and feces over 4 days, expressed as a percent of administered dose.

[a]Months later a second dose was administered iv to this subject. Recovery was 58.9% of the dose in urine and 0.5% of the dose in feces.

Each monkey also received, on separate occasions, three differing doses of sodium arsenate in water by gavage. The arsenic doses were 0.25, 0.50, and 1.0 mg As/kg bw, spanning the range of doses anticipated to occur during dosing of the soil samples. The percent of arsenic dose recovered in urine was substantially lower after gavage administration than after iv injection (Table 2), indicating incomplete oral absorption of arsenic from the oral dose in water. Excretion of arsenic in feces in gavage-treated animals was correspondingly higher, and the total arsenic recovery (urine and feces combined) was essentially equivalent for the oral and iv routes. Although there was a tendency for the percent of arsenic recovered in urine to increase with increasing dose (Table 2), the differences in recovery among doses and the trend were not statistically significant. Consequently, the urinary recovery was treated as being unrelated to dose, and the recoveries from the three doses for each animal were averaged. To preserve for analysis potential differences in bioavailability among different experiment subjects, separate recoveries from sodium arsenate were calculated for each animal.

TABLE 2
Urinary and Fecal Recovery of Arsenic after a Gavage Dose of Sodium Arsenate

	Sodium arsenate dose (as As)			
	0.25 mg As/kg bw	0.50 mg As/kg bw	1.0 mg As/kg bw	Mean ± SD
% dose in urine	35.6 ± 8.6	40.9 ± 6.0	45.3 ± 16.7	40.6 ± 10.1
% dose in feces	45.9 ± 12.3	40.0 ± 9.2	40.5 ± 8.9	42.1 ± 9.1
% total recovery	79.5 ± 5.1	80.9 ± 9.0	81.5 ± 6.2	80.7 ± 4.2

Note. Each animal ($n = 7$) received, on separate experimental days, single doses of 0.25, 0.50, and 1.0 mg As/kg bw by gavage. The results reflect cumulative excretion in urine and feces over 4 days after the dose. There was no significant difference in the % of dose recovered in urine from the three sodium arsenate doses, nor was there a significant trend.

TABLE 3
Soil Arsenic Mineralogy Data—Arsenic Mass Distribution (%)

	MTSS	WISS	FLCDV	CAMT	WAOS	NYOS	COSCS	CORS	COSS	FLCPS	NYPF1	NYPF2	NYPF3	HIVS
As bromide	—	—	—	—	—	—	35.8	—	—	—	—	—	—	—
Arsenopyrite	—	—	—	70.4	—	—	—	—	—	—	—	—	—	—
Arsenic oxide (As_2O_3)	—	—	—	—	—	—	—	87.3	—	—	—	—	—	—
As (metals) oxide	6.4	—	—	—	—	—	30.0	0.2	—	—	—	—	—	—
As (metals) sulfate	—	7.5	—	—	—	—	—	—	—	—	—	—	—	—
Calcium arsenate ($CaAsO_4$)	—	—	—	—	—	—	—	—	—	—	—	—	1.7	—
Clay	—	—	85.5	—	—	—	—	—	—	—	—	—	—	—
Iron aluminum silicate	—	—	—	—	—	—	—	—	—	—	—	—	—	71.8
Fe As oxides (AsFeOOH)	12.3	10.6	—	—	—	—	3.0	—	—	—	—	—	54.5	
Iron oxides (FeOOH)	55.9	3.5	14.4	27.2	1.3	6.9	1.5	1.7	22.2	35.2	100	99.9 (37.5)	32.1	22.9
Iron sulfate ($FeSO_4$)	23.1	9.3	—	2.3	—	—	1.4	0.1	76.7	64.8	—	—	0.5	—
Lead arsenate ($PbAsO_4$)	—	66.4	—	—	98.6	37.2	24.7	10.3	—	—	—	—	8.1	2.3
Lead (metal) oxide	—	2.5	—	—	—	1.4	3.3	—	—	—	—	—	—	—
Manganese oxides (MnOOH)	0.4	—	—	—	0.04	54.5	—	0.3	—	—	—	0.1 (8.8)	3.0	3.0
Phosphate	—	0.02	—	—	—	—	—	0.2	—	—	—	—	0.1	—
Pyrite	—	0.3	—	—	—	—	—	—	—	—	—	—	—	—
Slag	1.9	—	—	—	—	—	—	—	—	—	—	—	—	—
Zinc (metal) oxide	—	0.1	—	—	—	—	—	—	—	—	—	—	—	—
No. of particles counted	130	130	147	109	215	112	105	163	183	153	88	104	118	132
Arsenic concentration (mg As/kg soil)	650	1412	189	300	301	125	394	1230	1492	268	339	546	1000	724

Note. Soil ID: CAMT, California mine tailings; WAOS, Washington orchard soil; NYOS, New York orchard soil; COSCS, Colorado smelter composite soil; COSS, Colorado smelter soil; FLCPS, Florida chemical plant soil; NYPF, New York Pesticide Facility soil; HIVS, Hawaiian volcanic soil.

From these, a measurement of RBA for each soil sample in each experimental subject could be made.

Samples of arsenic-contaminated soil were obtained from 12 different sites. As described in the ''Materials and Methods'' section, all soils were sieved to remove constituents greater than 250 μm. Total arsenic content was measured for each sample, and concentrations ranged from 125 to 1492 mg As/kg soil (Table 3). Each soil sample was administered to five randomly selected experimental subjects by gavage, and urine and feces were collected for 4 days (Table 4). For 11 out of 14 soil samples, the arsenic dose administered to the monkeys was within the range of doses used to establish absorption of sodium arsenate in water (i.e., 0.25–1.0 mg As/kg bw). For two soil samples with the lowest arsenic content, arsenic doses of 0.18 and 0.12 mg As/kg bw were administered in order to keep the total mass of the soil dose within protocol limits of $\leq$1 g soil/kg bw. The administered arsenic dose for the soil sample with the highest arsenic concentration was 1.33 mg As/kg bw. The percentages of the arsenic dose excreted in urine from soil doses were generally much less than observed after gavage doses of sodium arsenate in water, while recovery of the dose in feces was higher. This is consistent with reduced gastrointestinal absorption of the arsenic from soil relative to water. Total recovery of arsenic following the soil doses was similar to, and some instances higher than, total recovery of arsenic after gavage with sodium arsenate in water (Tables 2 and 4).

The RBA of arsenic in the soil sample was calculated for each subject. Mean (± SD) values obtained for each soil are presented in Table 4. The mean RBA values for the 14 soil samples varied from 0.05 to 0.31 (i.e., 5–31%). The coefficients of variation (COVs) were less than about 50%, except for the soil with the lowest RBA, which had a COV of 81% (Note that the RBA for this soil sample ranged from 0 to 11%). Results were calculated with and without inclusion of Subject #7516, which had unusually low-arsenic excretion after an iv dose (Table 1). Surprisingly, there was no apparent difference in the excretion of arsenic in urine between this subject and others after oral doses of sodium arsenate in water- or arsenic-contaminated soil. Consequently, data from this subject were included when calculating the RBA estimates for soils.

Because the RBA values for the various soil samples tested were all relatively low, an additional experiment was conducted to verify that the monkey model is in fact capable of measuring oral bioavailability over a wide range. For this experiment, a high bioavailability soil was created artificially by spiking a naturally low arsenic–content soil (3.6 mg As/kg soil) with sodium arsenate 3 h before the dose. The spiked soil was administered to seven animals by gavage in the same manner as the test soils. For the opposite extreme in bioavailability, six subjects were given a dose of soil spiked with arsenopyrite. In arsenopyrite, the arsenic is bound tightly and oral bioavailability is expected to be very low (Ruby *et al.*, 1999). RBA measurements from both types of spiked soil samples are shown in

TABLE 4
Relative Bioavailability (RBA) of Arsenic from Contaminated Soils

Soil sample	Arsenic dose (mg As/kg bw)	% dose in urine	% dose in feces	% total recovery	RBA
MTSS	0.65	5.2 ± 1.6	89.9 ± 11.6	95.1 ± 11.1	0.13 ± 0.05
WISS	1.33	5.1 ± 3.2	81.3 ± 5.5	86.3 ± 3.0	0.13 ± 0.07
FLCDV	0.18	12.4 ± 1.0	64.6 ± 15.6	77.0 ± 15.5	0.31 ± 0.04
CAMT	0.30	7.9 ± 2.0	84.7 ± 9.7	92.7 ± 11.5	0.19 ± 0.02
WAOS	0.30	9.3 ± 2.2	77.1 ± 8.5	86.4 ± 9.5	0.24 ± 0.09
NYOS	0.12	5.8 ± 2.6	76.7 ± 12.5	82.6 ± 13.4	0.15 ± 0.08
COSCS	0.40	6.9 ± 2.7	70.1 ± 9.4	77.0 ± 11.8	0.18 ± 0.06
CORS	1.0	6.5 ± 2.4	71.6 ± 12.4	78.1 ± 11.1	0.17 ± 0.08
COSS	1.0	1.8 ± 1.4	85.9 ± 4.3	87.7 ± 3.7	0.05 ± 0.04
FLCPS	0.34	2.9 ± 1.2	92.9 ± 4.3	95.8 ± 4.5	0.07 ± 0.03
NYPF1	0.99	7.6 ± 2.3	80.9 ± 6.8	88.5 ± 5.0	0.19 ± 0.05
NYPF2	0.30	10.1 ± 2.9	83.1 ± 10.0	93.2 ± 8.7	0.28 ± 0.10
NYPF3	0.49	7.3 ± 2.8	85.1 ± 6.7	92.3 ± 6.7	0.20 ± 0.10
HIVS	0.73	2.0 ± 0.6	73.7 ± 5.3	75.7 ± 5.1	0.05 ± 0.01

Note. Each soil sample was administered by gavage. The results reflect cumulative excretion in urine and feces over 4 days after the dose, and are expressed as the mean ± SD for five animals. The arsenic dose is based on the arsenic concentration in the soil and the soil mass administered. Soil ID: CAMT, California mine tailings; WAOS, Washington orchard soil; NYOS, New York orchard soil; COSCS, Colorado smelter composite soil; COSS, Colorado smelter soil; FLCPS, Florida chemical plant soil; NYPF, New York pesticide facility soil; HIVS, Hawaii volcanic soil.

Table 5. For sodium arsenate–spiked soil, the average RBA was 0.94, while the RBA for arsenopyrite was 0.01 in one subject and < 0.01 in the other animals. These observations suggest that the model is capable of measuring arsenic RBA over the full range of potential values.

Arsenic mass distribution across 18 mineralogic phases was evaluated for each soil fed to the monkeys (Table 3). The results indicated significant heterogeneity in the arsenic phases reflected in the soils. Some soils were dominated by arsenic in a single phase, while for other soils, arsenic was distributed across many mineralogic phases. Stepwise linear regression was used to evaluate the apparent relationship between each of the mineralogic

TABLE 5
Arsenic Recovery and RBA from Spiked Soil Samples

Spiked sample	% dose in urine	% dose in feces	% total recovery	RBA
Sodium arsenate	38.1 ± 7.2	47.01 ± 11.7	85.1 ± 15.4	0.94 ± 0.05
Arsenopyrite	0.08 ± 0.13	101 ± 30.7	101 ± 32.8	0.002 ± 0.003

Note. Each animal received a single gavage dose of soil spiked with sodium arsenate (0.5 mg As in water per kg bw; $n = 7$) or arsenopyrite (1.0 mg As per kg bw; $n = 6$) 3 h before the dose. The results reflect cumulative excretion in urine and feces over 4 days, expressed as a percent of administered dose.

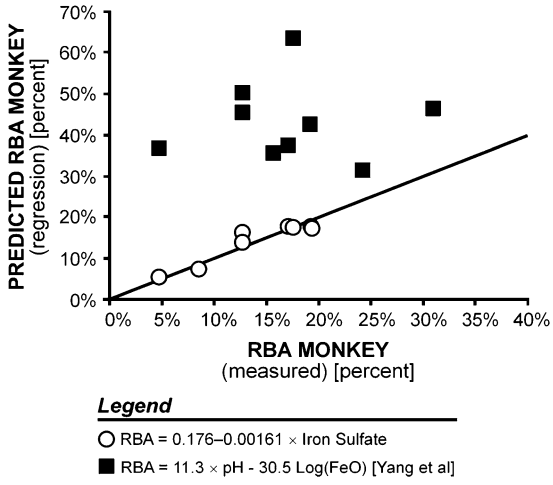

FIG. 1. Relationship between predicted and measured RBA values in cynomolgus monkeys. Open circles represent RBA values predicted based on content of arsenic in iron sulfate as described by the relationship shown. Closed square present RBA values predicted based on soil pH and iron oxide content as per Yang *et al.* (2005).

phases and RBA. In the eight samples for which arsenic was found to be present in iron sulfate, this mineral phase was the best single linear predictor of arsenic RBA ($p < 0.0005$, $R^2 = 0.883$), with RBA inversely related to arsenic present in the iron sulfate phase (Fig. 1). When all 14 samples were included in the regression analysis, the fit of the relationship was reduced ($p < 0.019$, $R^2 = 0.381$), but iron sulfate remained the best single linear predictor of RBA among the mineralogy parameters evaluated. There was no better fitting model using multiple mineralogy variables. Regression against metals, total organic carbon content, and particle size indicated no clear correlation with measured RBA.

DISCUSSION

Several species have been used as experimental models for measurement of arsenic bioavailability from soil. Among these species, the monkey is phylogenetically most similar to humans. The value of the monkey model in predicting gastrointestinal absorption in humans has been clearly demonstrated in pharmaceutical research (Ikegami *et al.*, 2003). For example, Chiou and Buehler (2002) compared the absorbed fraction of an oral dose for 43 drugs evaluated in both monkeys and humans and found excellent correlation with a slope near unity (Fig. 2). Less information is available specific to the comparative absorption of metals or metalloids in nonhuman primates, although O'Flaherty *et al.* (1996) reported that the fractional absorption of lead by cynomolgus monkeys is similar to that in fasted humans. Specifically, they found the rate of 35% absorption of lead in fasted humans (as reported in Rabinowitz *et al.*, 1980) to be comparable to the 22–44% absorption they observed in fasted monkeys.

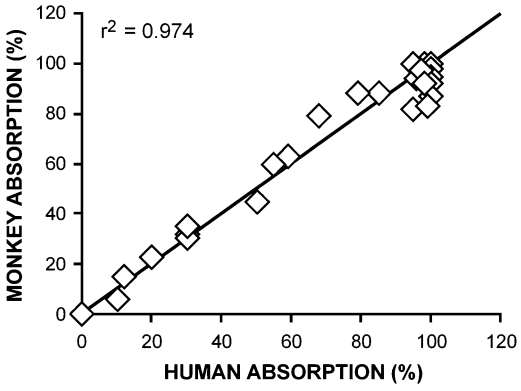

FIG. 2. Correlation of percentage oral dose absorbed between humans and monkeys for 43 drugs with a regression of equation of $F_{aM} = 0.96F_{aH} + 2.8$; $r^2 = 0.974$. Complete absorption demonstrated by 27 drugs in both species. The depicted line has a slope of unity. From Chiou and Buehler (2002).

Two previous studies have used primates to evaluate the RBA of arsenic from soil. A Battelle study measured arsenic RBA from one soil and one house dust sample collected near a Montana smelter site (Freeman *et al.*, 1995). Three female cynomolgus monkeys were used for this study. Another study (Roberts *et al.*, 2002) used five male Cebus monkeys to measure the RBA of arsenic from five soil samples collected from contaminated sites in Florida. Both previous studies measured urinary and fecal excretion of arsenic after iv and oral doses of sodium arsenate. The urinary and fecal recovery of iv administered arsenic in female cynomolgus monkeys in the Battelle study matched closely the recoveries observed in male cynomolgus monkeys reported here. In the Battelle study, 76.5 ± 2.5% (mean ± SD) of the arsenic dose was recovered in urine and 3.2 ± 1.9% was recovered in feces. Similarly, Cebus monkeys in the Florida study excreted 66.8 ± 6.5% of the iv dose in the urine and a very small percentage (0.5–0.6%) in feces.

The percent of arsenic dose recovered in urine following a gavage dose of sodium arsenate was about 40% in cynomolgus monkeys in this study, compared with about 50% in Cebus monkeys in the Florida study and almost 70% on average for cynomolgus monkeys in the Battelle study (Freeman *et al.*, 1995; Roberts *et al.*, 2002). The reason for the substantial difference in urinary excretion following oral sodium arsenate doses, particularly between studies using the same monkey species, is unclear. Total arsenic recoveries were also different, although the margin was smaller (about 80% in this study vs. 95% in the Battelle study), suggesting that at least part of the difference lies in lower gastrointestinal absorption of arsenic in water in monkeys in this study. The difference cannot be explained by dose—the Battelle study used a gavage dose (0.62 mg As/kg bw) in the middle of the range of doses in the study reported here (0.25–1.0 mg As/kg bw). It is also difficult to explain based on experimental protocol. Both studies administered the sodium arsenate dose by gavage tube without anesthesia, followed by recovery of urine and feces in metabolism cages for similar lengths of time (5 days in the Battelle study and 4 days in this study). Cage washes recovered nearly 90% of arsenic in urine (see the "Materials and Methods" section), so underrecovery of arsenic from the metabolism cages can be ruled out. The differences might be due to gender (females in the Battelle study and males in this study). Unfortunately, there are no studies of arsenic bioavailability that have included animals of both sexes to examine this possibility. It is also possible that different cynomolgus monkey strains were used in the two studies with differing gastrointestinal absorption characteristics.

Even though urinary arsenic recoveries following ingestion of sodium arsenate in water vary among studies, each serves as a valid basis for comparison within study for determination of RBA. Among the 14 soil samples tested in this study, the mean RBA values ranged from 0.05 to 0.31 (5–31%). The RBA values obtained from different subjects were variable, and the COV was near 50% for about half of the soil samples. This variability is not surprising. Gastric residence time is likely to be important in extracting arsenic from soil matrices in the low-pH intragastric environment, and gastric-emptying rates can vary substantially from one individual to another. As an example, a recent study found that the gastric half-emptying time in 10 unfed cynomolgus monkeys given a 60-ml liquid dose of acetaminophen (as a gastric-emptying marker) ranged from about 10 min to 4 h (Kondo *et al.*, 2003). Although gastric-emptying times following oral soil doses have not been reported, there is no obvious reason to expect that variability would be substantially less. Based on variability in recoveries following gavage treatment with sodium arsenate doses (Table 2), much of the variability may be intrasubject; that is, reflecting differences in absorption of arsenic on different experimental days. However, it is interesting to note that variability among subjects was small for the cattle dip vat soil (Florida cattle dip vat soil [FLCDV]), and that when previously tested in Cebus monkeys (Roberts *et al.*, 2002), this soil sample also produced relatively low intrasubject variability. This suggests that some attribute of the soil may also influence variability in RBA results among different experimental subjects.

Four soil samples tested in this study were from sites where soil arsenic RBA has been measured using different species or models. As mentioned above, the FLCDV soil sample was also evaluated in a previous study using the Cebus monkey (Roberts *et al.*, 2002) with similar results (RBA of 0.25 ± 0.03 in the Cebus vs. 0.31 ± 0.04 in the cynomolgus monkey here). Three other soil samples (namely, Montana smelter soil [MTSS], Colorado residential soil [CORS], and Western iron slag soil [WISS]) were from sites where arsenic soil bioavailability had been evaluated, but were not the same specific soil samples measured by others. MTSS (RBA 0.13 ± 0.05) was taken from a Montana smelter site where an RBA of 0.20 was measured, also using cynomolgus monkeys (Freeman *et al.*, 1995). CORS came from a site for which arsenic bioavailability had been previously measured in five soil samples using a swine model (Casteel *et al.*, 2001). The RBA values for these five samples ranged from 0.18 to 0.45 (best estimates). The RBA for arsenic in the CORS sample measured here in the monkey was at the

bottom end of this range (0.17 ± 0.08). Arsenic RBA from an iron slag site soil sample (WISS) measured in the cynomolgus monkey (0.13 ± 0.7) was lower than the value reported for another soil sample from the site measured in the swine model (0.29; Rodriguez *et al.*, 1999).

These limited comparisons suggest that the swine model might yield higher estimates of oral bioavailability than the monkey, but definitive conclusions are impossible without data from splits of the same soil sample measured in both models. The swine model uses a somewhat different protocol involving multiple doses of arsenic in soil, but there is no reason to suspect *a priori* that this would lead to higher bioavailability estimates. One important difference between the monkey and swine protocols is the volume of soil administered relative to body weight, with larger volumes administered to the monkey. To test whether this soil volume might interfere with arsenic absorption leading to underestimates of RBA, spiked soil samples were tested in the monkey model. These spiked samples showed high bioavailability from sodium arsenate (Table 5), as would be expected, suggesting that the higher soil volume in the monkey model is not an impediment to arsenic absorption.

Arsenic mineralogy data from the test soils were evaluated to determine whether they might serve as a predictor of RBA measured in the cynomolgus monkey. Arsenic is known to occur in soil as a complex mixture of mineral phases, coprecipitated and sorbed species and dissolved species, and the distribution of arsenic among these phases can control dissolution properties (Davis *et al.*, 1996; Ruby *et al.*, 1999). The distribution of arsenic among these phases may reflect the arsenic source or be altered substantially by weathering, such as association of arsenic with iron oxides within the soil (Cances *et al.*, 2005, Ruby *et al.*, 1999). Measurement of arsenic mass distribution across 18 mineralogic phases revealed significant heterogeneity among the 14 soil samples included in this study. A stepwise linear regression found arsenic present in iron sulfate was the best single linear predictor of arsenic RBA, which is consistent with proposed models of arsenic bioavailability (Ruby *et al.*, 1999). However, this result is the opposite of observations comparing soil mineralogy data with RBA measured in swine reported previously (Basta *et al.*, 2000). In that study, arsenic RBA in four samples (including two different types) of mine-waste soils increased as the percent of total arsenic in the iron sulfate fraction increased.

A number of recent studies have examined the impact of soil chemistry on the dissolution and bioavailability of arsenic. Several of these studies reported that the solubility of arsenic under physiologic conditions is inversely correlated with the soil content of other metals such as iron and aluminum (Fendorf *et al.*, 2004; Sarkar and Datta, 2004; Yang *et al.*, 2002, 2005) and/or directly related to the organic carbon content (Pouschat and Zagury, 2006; Sarkar *et al.*, 2005). With the exception of the importance of arsenic in iron sulfate, RBA measurements in the cynomolgus monkey do not support these findings. For example, Yang *et al.* (2005) have proposed a model for arsenic bioavailability from soil based on pH and extractable iron oxide content. As shown in Figure 1, this model markedly overpredicts RBA in the soils examined here, and was noted in the original report to overpredict RBA values measured previously in Cebus monkeys. Although not consistently biased in one direction, predicted arsenic bioavailability also did not correspond particularly well with RBA values measured for several soils in the swine model (Yang *et al.*, 2005).

There are several potential explanations for the apparent discrepancy between the soil chemistry studies cited above and RBA measured *in vivo*. These include the number of soils studied, soil provenance, the source of arsenic contamination, and the extraction methods used in the soil chemistry studies to approximate bioavailability. Of the six studies, four based their evaluations on two, three, or four discrete soil samples. Pouschat and Zagury evaluated 12 soils, but all were from the same source of contamination—chromated copper arsenate (CCA). Only Yang *et al.* (2002, 2005) evaluated a large diversity of soils. All but one study used soils that had been spiked with arsenic (arsenate or arsenite) and subjected to different aging or weathering regimes. Only the study of Pouschat and Zagury evaluated environmentally contaminated soils and, as noted above, this study was limited to soils affected by CCA. Finally, although some studies purported to correlate soil characteristics with "bioavailability," all the models and proposed relationships in these studies were based on data from *in vitro* extraction methods rather than actual RBA measurements *in vivo*. This suggests that information from contemporary *in vitro* "bioaccessibility" models, even those based on simulated physiological conditions, may not adequately address all the processes that affect absorption of arsenic from soils *in vivo*.

The results reported here expand considerably the number and types of soils for which arsenic bioavailability has been measured using a primate model. This study demonstrates that while the model is capable of measuring RBA values from < 10 to > 90%, results from a variety of types of contaminated sites are consistently low, i.e., about 30% or less. Recognition of the limited bioavailability of arsenic from soils is important in the evaluation of human health risks from arsenic-contaminated sites. RBA values are an important component of risk calculation and the development of risk-based cleanup goals. RBA values from *in vivo* bioavailability studies remain the "gold standard," but there is strong interest in developing more rapid, less expensive means of obtaining RBA information. Previous attempts to develop *in vitro* tools to predict arsenic RBA have met with limited success, and there are no existing *in vitro* models that predict well the RBA observations reported here for an expanded set of arsenic-contaminated soils. In order to develop a satisfactory *in vitro* model, a better understanding of factors that control gastrointestinal absorption of arsenic from soil matrices will be required.

ACKNOWLEDGMENTS

This research was supported in part by a grant from the Strategic Environmental Research and Development Program, U.S. Department of Defense

awarded to Exponent, Inc, Boulder, CO and from the Solubility/Bioavailability Research Consortium. The authors are indebted to Dr Eric Crecelius for measurement of arsenic in baseline urine samples and to Dr John Drexler, University of Colorado, Boulder, CO, for characterization of the mineralogy of soil samples.

REFERENCES

Basta, N. T., Rodgriuesz, R. R., and Casteel, S. W. (2000). *Development of Chemical Methods to Assess Bioavailability of Arsenic in Contaminated Media.* National Center for Environmental Research and Quality Assurance. Grant Progress Report.

Cances, B., Juillot, F., Morin, G., Laperche, V., Alvarez, L., Proux, O., Hazemann, J. L., Brown, G. E., and Calas, G. (2005). XAS evidence of As(V) association with iron oxyhydroxides in a contaminated soil at a former arsenical pesticide processing plant. *Environ. Sci. Technol.* **39,** 9348–9405.

Casteel, S. W., Brown, L. D., Dunsmore, M. E., Weis, C. P., Henningsen, G. M., Hoffman, E., Brattin, W. J., and Hammon, T. L. (1997). *Relative Bioavailability of Arsenic in Mining Wastes.* U.S. Environmental Protection Agency, Denver, CO.

Casteel, S. W., Evans, T., Dunsmore, M. E., Weis, C. P., Lavelle, B., Brattin, W. J., and Mannon, T. L. (2001). *Relative Bioavailability of Arsenic in Soils from the VBI70 Site. Final Report.* U.S. Environmental Protection Agency, Denver, CO.

Chiou, W. L., and Buehler, P. W. (2002). Comparison of oral absorption and bioavailability of drugs between monkey and human. *Pharm. Res.* **19,** 868–874.

Davis, A., Drexler, J. W., Ruby, M. V., and Nicholson, A. (1993). Micromineralogy of mine wastes in relation to lead bioavailability, Butte, Montana. *Environ. Sci. Technol.* **27,** 1415–1425.

Davis, A., Ruby, M. V., Bloom, M., Schoof, R., Freeman, G., and Bergstrom, P. D. (1996). Mineralogic constraints on the bioavailability of arsenic in smelter-impacted soils. *Environ. Sci. Technol.* **30,** 392–399.

Drexler, J. (2006). *Standard Operating Procedures for Metal Speciation.* http://www.colorado.edu/GeolSci/legs/EMPASOP1.html.

Fendorf, S., LaForce, M. J., and Li, G. (2004). Temporal changes in soil partitioning and bioaccessibility of arsenic, chromium and lead. *J. Environ. Qual.* **33,** 2049–2055.

Freeman, G. B., Johnson, J. D. Killinger, J. M., Liao, S. C., Davis, A. O., Ruby, M. V., Chaney, R. L., Lovre, S. C., and Bergstrom, P. D. (1993). Bioavailability of arsenic in soil impacted by smelter activities following oral administration in rabbits. *Fundam Appl. Toxicol.* **21,** 83–88.

Freeman, G. B., Schoof, R. A., Ruby, M. V., Davis, A. O., Dill, J. A., Liao, S. C., Lapin, C. A., and Bergstrom, P. D. (1995). Bioavailability of arsenic in soil and house dust impacted by smelter activities following oral administration in cynomolgus monkeys. *Fundam Appl. Toxicol.* **28,** 215–222.

Ikegami, K., Tagawa, K., Narisawa, S., and Osawa, T. (2003). Suitability of the cynomolgus monkey as an animal model for drug absorption studies of oral dosage forms from the viewpoint of gastrointestinal physiology. *Biol. Pharm. Bull.* **26,** 1442–1447.

Kelly, M. E., Brauning, S. E., Schoof, R. A., and Ruby, M. V. (2002). *Assessing Oral Bioavailability of Metals in Soil.* Battelle Press, Columbus, OH.

Kondo, H., Takahashi, Y., Watanabe, T., Yokohama, S., and Watanabe, J. (2003). Gastrointestinal transit of liquids in unfed cynomolgus monkeys. *Biopharm. Drug Dispos.* **24,** 131–140.

Lorenzana, R. M., Duncan, B., Ketterer, M., Lowry, J., Simon, J., Dawson, M., and Poppenga, R. (1996). *Bioavailability of Arsenic and Lead in Environmental Substrates. I. Results of an Oral Dosing Study of Immature Swine.* EPA 910/R-96-002. U.S. Environmental Protection Agency, Seattle, WA.

National Research Council (NRC) (2003). *Bioavailability of Contaminants in Soils and Sediments: Processes, Tools, and Applications.* National Research Council, National Academies Press, Washington, DC.

Neterm, J., Kutner, M. H., Nachtsheim, C. J., and Wasserman, W. (1989). *Applied Linear Statistical Models*, 4th ed. Richard D. Irwin, Inc, Chicago, IL.

Ng, J. C., Kratzmann, S. M., Qi, L., Crawley, H., Chiswell, B., and Moore, M. R. (1998). Speciation and absolute bioavailability: Risk assessment of arsenic-contaminated sites in a residential suburb in Canberra. *Analyst* **123,** 889–892.

O'Flaherty, E. J., Inskip, M. J., Yagminas, A. P., and Franklin, C. A. (1996). Plasma and blood lead concentrations, lead absorption, and lead excretion in nonhuman primates. *Toxicol. Appl. Pharmacol.* **138,** 121–130.

Page, E. B. (1963). Ordered hypotheses for multiple treatments: A significance test for linear ranks. *J. Am. Stat. Assn.* **58,** 216–230.

Pouschat, P., and Zagury, G. J. (2006). In vitro gastrointestinal bioavailability of arsenic in soils collected near CCA-treated utility poles. *Environ. Sci. Technol.* (in press).

Rabinowitz, M. B., Knopple, J. D., and Wetherill, G. W. (1980). Effect of food intake and fasting on gastrointestinal lead absorption in humans. *Am. J. Clin. Nutr.* **33,** 1784–1788.

Roberts, S. M., Weimer, W. R., Vinson, J. R. T., Munson, J. W., and Bergeron, R. J. (2002). Measurement of arsenic bioavailability in soil using a primate model. *Toxicol. Sci.* **67,** 303–310.

Rodriguez, R. R., Basta, N. T., Casteel, S. W., and Pace, L. W. (1999). An in vitro gastrointestinal method to estimate bioavailable arsenic in contaminated soils and solid media. *Environ. Sci. Technol.* **33,** 642–649.

Ruby, M. V., Fehling, K. A., Paustenbach, D. J., Landenberger, B. D., and Holsapple, M. P. (2002). Oral bioaccessibility of dioxins/furans at low concentrations in soil. *Environ. Sci. Technol.* **36,** 4905–4911.

Ruby, M. V., Schoof, R., Brattin, W., Goldade, M., Post, G., Harnois, M., Mosby, D. E., Casteel, S. W., Berti, W., Carpenter, M., *et al.* (1999). Advances in evaluating the oral bioavailability of inorganics in soil for use in human health risk assessment. *Environ. Sci. Technol.* **33,** 3697–3705.

Sarkar, D., and Datta, R. (2004). Human health effects from arsenic in soils: Does one model fit all? *Arch. Environ. Health* **59,** 337–341.

Sarkar, D., Datta, R., and Sharma, S. (2005). Fate and bioavailability of arsenic in organo-arsenical pesticide-applied soils. Part I. Incubation study. *Chemosphere* **60,** 188–195.

Schroder, J. L., Basta, J. T., Si, J., Casteel, S. W., Evans, T., and Payton, M. (2003). In vitro gastrointestingal method to estimate relative bioavailable cadmium in contaminated soil. *Environ. Sci. Technol.* **37,** 1365–1370.

U.S. Environmental Protection Agency (2000). *Short Sheet: TRW Recommendations for Sampling and Analysis of Soil at Lead (pb) Sites*. OSWER 9285.7–38. Washington, DC.

U.S. Environmental Protection Agency (2004). *Estimation of Relative Bioavailability of Lead in Soil and Soil-Like Materials using In Vivo and In Vitro Methods.* December 2004 Draft. Office of Solid Waste and Emergency Response. Washington, DC.

Yang, J. K., Barnett, M. O., Jardine, P. M., Basta, N. T., and Casteel, S. W. (2002). Adsorption, sequestration, and bioaccessibility of As(V) in soils. *Environ. Sci. Technol.* **36,** 4562–4569.

Yang, J.-K., Barnett, M. O., Zhuang, J., Fendorf, S. E., and Jardine, P. M. (2005). Adsorption, oxidation, and bioaccessibility of As (III) in soils. *Environ. Sci. Technol.* **39,** 7102–7110.