

## Testing Arsenic Absorption from Middleport, New York, Soils

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As part of an effort to understand the nature and behavior of arsenic in soils and sediments in the vicinity of FMC Corporation's facility in Middleport, New York, FMC has undertaken studies of the potential for arsenic uptake from soils collected from the Middleport study areas. Information obtained from these studies is intended to support work being performed as part of the FMC Middleport Facility's RCRA Facility Investigation and Corrective Measures Study. Two distinct aspects of research have been completed on Middleport soil: 1) evaluation of relative oral arsenic bioavailability, and 2) percutaneous absorption of arsenic.

The Middleport soil oral arsenic bioavailability study consisted of arsenic mineralogy testing of fourteen surficial soil samples and four subsurface soil samples; *in vitro* testing for measurement of arsenic bioaccessibility in fourteen surficial soil samples and four subsurface soil samples; and *in vivo* testing of three Middleport soil samples in cynomolgus monkeys. The scope of work and description of the protocols for the oral arsenic bioavailability study on Middleport soils are described in the "Work Plan for the Evaluation of Arsenic Bioavailability from Middleport Soil" (Exponent, February 2004), which was provided to the U.S. Environmental Protection Agency (EPA) and the New York State Department of Environmental Conservation (NYSDEC) in February 2004. The *in vivo* research effort builds on the *in vitro* extraction testing and mineralogy work that was conducted on Middleport soils and reported previously (Exponent 2005, attached).

The second study evaluated the potential for percutaneous absorption of arsenic following dermal contact for one soil sample collected from Middleport. The protocol for this study is presented in the protocol titled, "Dermal Absorption of Arsenic from Soil in Rhesus Monkeys," which was provided to EPA and NYSDEC in January 2004.

The oral bioavailability and the dermal absorption *in vivo* testing performed on the Middleport soil samples were included in a broader research effort that was funded by the Strategic Environmental Research Development Program (SERDP), which uses funding from the U.S. Department of Defense, EPA, and the U.S. Department of Energy to sponsor research that identifies, develops, and implements environmental technologies. Brief fact sheets regarding the goals of the larger research effort are attached. The *in vivo* work conducted to date to understand the potential for human exposures to arsenic from these soils represents original research that has been submitted for publication in the peer-reviewed literature.

The methods employed for both aspects of the research (oral and dermal exposures) have been used in previous evaluations, and are already incorporated into peer-reviewed publications. Summaries of the two broader research efforts, which include the results for the Middleport soil samples, are provided below. Published articles that report the new research, and a manuscript that is in press at this writing, which contain the data from the Middleport samples, are attached. These peer-reviewed articles document the methods used in this research.

## Relative Oral Bioavailability of Arsenic in Cynomolgus Monkeys

Research was conducted in the laboratory of Dr. Stephen Roberts at the University of Florida in Gainesville, Florida, and reported in the attached publication (Roberts et al. 2007). The study was conducted in an *in vivo* model using cynomolgus monkeys. This animal model was selected because of its phylogenetic similarity to humans, and because no other animal model has been specifically validated for evaluation of the relative oral bioavailability of arsenic in humans. Although this animal model also has not been specifically validated for the study of arsenic, it has been used to evaluate the relative oral bioavailability of arsenic from soils (Freeman et al. 1995), and a compilation of data across several studies indicates that the cynomolgus monkey is a reliable predictor of oral bioavailability in humans ( $r^2=0.974$  for 43 drugs for which data were available in both species, with a slope near unity) (Chiou and Buehler 2002).

For this research, monkeys (n=5 for each soil) were dosed, via gavage, with a slurry of soil in water. Urine and feces were collected and analyzed for arsenic to provide a measure of absorbed arsenic (based on urinary data), and to assess total recovery of the arsenic dose (urinary and fecal data). The study design incorporated a low-arsenic diet to minimize the potential for confounding from the normal contributions of arsenic from dietary sources. Relative oral bioavailability (RBA) was assessed by comparing the fraction of the absorbed dose of arsenic from soil to the fraction absorbed from a dose of soluble sodium arsenate, corrected for background, on an animal-specific basis.

$$RBA = \frac{(\% \text{ of soil dose in urine}) - (\text{background})}{(\% \text{ of NaAs dose in urine}) - (\text{background})}$$

The research included evaluation of fourteen discrete site soils, three of which were collected from Middleport, New York. The basis for selecting the specific soil samples from Middleport has been described previously (Exponent 2005) and included selecting soils with a range of soil arsenic concentrations (339, 549, and 1,000 mg/kg) and a range of measured *in vitro* bioaccessibility for Middleport site samples, with a bias toward samples that demonstrated *in vitro* bioaccessibility at the higher end of the measured range, thus biasing the *in vivo* results toward the samples with an expected higher bioavailability. Additionally, mineralogical characterization of soils from Middleport suggest that there may be two distinct profiles for arsenic in soil, with samples containing less than 600 mg/kg arsenic dominated by arsenic in different phases than that for the samples with higher arsenic concentrations. Therefore, the soil samples selected for further research capture the range of mineralogy reflected in soils at the site. Together, these sample selection criteria should have resulted in bioavailability data from the *in vivo* research that are either representative of the site, or constitute an upper-bound estimate of bioavailability of soils in the site vicinity. Finally, all selected samples are surface soil samples, which represent the materials most likely to be associated with direct-contact exposures.

The soils from Middleport (identified as New York Pesticide Facility, or NYPF, samples in the attached publication by Roberts et al.) were administered to monkeys at arsenic doses of 0.30, 0.49, and 0.99 mg/kg. The variation in arsenic dose is associated with the variation in arsenic

concentration in the soils, because the soil dose (in terms of mass of soil per body weight of monkey) was held constant across these three soils. Research on these soils suggested good total recovery of arsenic (88.5% to 93.2% of the administered dose) and measured RBA for the three soils of 0.28 ( $\pm 0.1$ ), 0.20 ( $\pm 0.10$ ), and 0.19 ( $\pm 0.05$ ).

The *in vivo* testing results for the soils from Middleport indicate a reduced bioavailability of arsenic from these soils, relative to absorption of soluble arsenic, with an upper limit of 0.28 (i.e., 28%). As described above, the RBA values reported for the specific Middleport soils tested likely represent the upper range of RBA for soils in the vicinity of the site. The observation of a reduced relative oral bioavailability for arsenic in Middleport soils is consistent with results for soils from other sites that have also been studied. Across the 14 soils from 12 disparate U.S. sources evaluated in the cynomolgus monkey, reported RBA values ranged from 0.05 ( $\pm 0.04$ ) to 0.31 ( $\pm 0.04$ ), indicating significantly low relative oral bioavailability of arsenic from soils, regardless of provenance.

It should be pointed out that the *in vitro* method used to assess bioaccessibility of arsenic from Middleport-area soils has been validated for estimating the RBA of lead, but has not been specifically validated with regard to arsenic. Conversely, as discussed above, data from monkey studies appear to provide a good prediction of absorption by humans (Chiou and Buehler 2002). The *in vitro* method used to assess the bioaccessibility of the broader range of surface and subsurface soils from the Middleport vicinity (Exponent 2005, attached) has now been shown to overpredict bioavailability as measured in the cynomolgus monkey (SERDP 2005). Specifically, for all of the test and control soils fed to the monkey (14 test soils and 2 control soils), the *in vitro* method underpredicts the RBA in only two. The soils for which RBA is underpredicted by the *in vitro* method are a mine tailings soil and a clay soil that absorbed arsenic spills. This indicates that the *in vitro* data provide an overestimate of relative oral bioavailability of arsenic from most soils.

For the three Middleport-area soils tested in both systems (*in vivo* in the cynomolgus monkey and *in vitro*), the results are shown below. The results for these and other soil samples indicate that the RBA of arsenic for Middleport-area soils is expected to be lower than indicated by the *in vitro* data, if each of the soils were to be studied in the monkeys.

## Relative oral bioavailability of arsenic in Middleport soils

Sample	Arsenic Concentration (mg/kg)	<i>In Vivo</i> RBA <sup>a</sup>	<i>In Vitro</i> Bioaccessibility
Sample T5E3: Surface soil sample collected from along Tributary One, south of the Erie Barge Canal	339	0.28 (±0.1)	52%
Sample T15E4: Surface soil sample collected from along Tributary One, north of the Erie Barge Canal, between Chase Road and Pearson Road	546	0.20 (±0.10)	26%
Sample A1B20: Surface soil sample collected from a residential property located adjacent to the plant (this property has subsequently been remediated)	1,000	0.19 (±0.05)	41%

<sup>a</sup> Relative oral bioavailability

## Percutaneous Absorption of Arsenic in Rhesus Monkeys

In a separate study, a soil from the vicinity of the Middleport facility was included as one of two soils evaluated for percutaneous absorption of arsenic. This research was conducted by Dr. Ronald Wester at the University of California at San Francisco, and builds on prior research conducted at this facility, using an *in vivo* Rhesus monkey research model. Research on arsenic in solution, and arsenic in solution in the presence of soil, conducted by Dr. Wester using Rhesus monkeys (Wester et al. 1993), forms the technical basis for guidance from EPA regarding dermal absorption of arsenic from soils (U.S. EPA 1992, 2004). However, our understanding of the geochemistry of arsenic suggests that, for soils that are weathered in the environment, arsenic is likely to be present in more stable alteration phases and would not be expected to behave like soluble arsenic. In order to best update the research upon which regulatory agencies rely, it was decided to use the same animal model in the same research facility, with the same principal investigator. Therefore, the research methods were updated to accept environmental samples (as opposed to radiolabeled samples). This study design has also been used to assess the percutaneous absorption of arsenic from residues present on the surface of CCA (chromated copper arsenic)-treated wood. Wester et al. (2004) established that the updated method was adequately sensitive to detect absorbed arsenic in the range detected in previous research.

In this research, Rhesus monkeys were dosed with arsenic applied to the abdominal skin, and absorption was measured based on urinary excretion of arsenic. As with the cynomolgus monkeys used for oral absorption research, the Rhesus monkeys were maintained on a low-arsenic diet, to allow for detection of arsenic absorbed from non-dietary sources. Results were expressed as the percent of the dermally applied dose that was excreted in urine, corrected for background on a monkey-specific and dosing trial-specific basis, and adjusted for the urinary arsenic excretion measured from an intravenously administered dose.

This research included an evaluation of two soils and sodium arsenate in solution. The <150- $\mu$ m particle size fraction was selected for the soil studies, because this fine fraction represents the material most likely to remain on the surface of the skin following contact with soils, and because the small particle size provides for greater surface area, thus enhancing the potential for absorption. Soils were administered at two skin hydration levels to assess whether the presence of moisture might be a controlling factor in the dermal absorption of arsenic. The test soil from the Middleport vicinity contained an arsenic concentration of 1,400 mg/kg, and was applied at a total dose of 560  $\mu$ g of arsenic in a monolayer on the skin surface. This soil was selected specifically because of the high arsenic concentration and the associated potential for demonstrating percutaneously absorbed arsenic.

The results from this research indicate that dermal application of soluble arsenic in solution is absorbed, with 4.8%  $\pm$ 5.5% percent of the applied dose excreted in urine within a few days following exposure. Conversely, following application of soils, urinary excretion of arsenic does not increase above background levels, regardless of the test soil or hydration level of the skin. These results indicate that the assumption that arsenic weathered in soil will behave like soluble arsenic or soluble arsenic in the presence of soil is unsubstantiated. Rather, they suggest that dermal absorption of arsenic from soils provides a negligible, if any, contribution to arsenic exposures.

## Summary and Conclusions

Arsenic chemistry (mineralogy), *in vitro* bioaccessibility, oral arsenic bioavailability, and dermal absorption of arsenic have been evaluated for soils collected from the Middleport study area. The information regarding arsenic mineralogy and *in vitro* bioaccessibility was used to understand the nature of arsenic in area soils, to assess the potential variability in solubility of arsenic under physiological conditions, and to provide a technical basis for selection of samples for additional study. Specifically, *in vitro* bioaccessibility data were used to identify soils for additional study that would either be representative of the site, or constitute an upper-bound estimate of bioavailability of soils in the site vicinity.

Results from animal research indicate that that the relative oral bioavailability of arsenic from site soils is lower than the absorption of soluble arsenic, and that accurate assessment of exposures to arsenic from site soils should incorporate a relative oral bioavailability adjustment factor (RAF). A summary of this research is presented above, and full details of study design, results, and uncertainty are included in the attached manuscript. The results of the oral bioavailability research indicate arsenic RAF values of 19%, 20%, and 28% for the three study soils from the Middleport study area.

Similarly, research directed at understanding the potential for dermal absorption of arsenic from soils indicates that arsenic in soil is not absorbed across the skin as well as soluble forms of arsenic. These results indicate that dermal absorption of arsenic from soil is negligible, is unlikely to contribute to human exposures, and does not warrant quantification in assessment of exposures from site soils.

## References

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## **Attachments**



## Characterization Data and *In Vitro* Testing of Surface and Subsurface Soil: Middleport, New York

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Thirteen surficial soil samples and four subsurface samples (6- to 12-inch depth) were collected from the offsite areas in the vicinity of the FMC facility in Middleport, New York. As the initial steps toward the evaluation of arsenic bioavailability from these soils, several evaluations have been conducted, including soil characterization (total organic carbon [TOC], pH, grain size, and metals concentrations), *in vitro* bioaccessibility testing for arsenic, and arsenic mineralogy. Tables containing the associated data are attached (Tables 1–4). Results of the mineralogy evaluation and *in vitro* extraction testing are discussed further below. In order to respond to the agency advice that existing *in vitro* data would be strengthened by confirmation in an animal study, recommendations for samples for oral bioavailability testing *in vivo* in monkeys are also provided.

### Arsenic Bioaccessibility from Middleport Samples

Measurement of arsenic bioaccessibility (i.e., the fraction that could become solubilized in the human gastrointestinal tract and be available for absorption) was conducted according to the Standard Operating Procedure (SOP) developed by the Solubility/Bioavailability Research Consortium, amended to incorporate an additional extraction at higher pH (presented in Appendix B of the Work Plan [Exponent 2004]). All 13 surface soil and 4 subsurface soil samples were evaluated. The arsenic concentrations observed for these soil samples ranged from <20 mg/kg (detection limit) to 2,230 mg/kg in surface soil, and from <20 mg/kg to 661 mg/kg in subsurface samples. Extraction was conducted under simulated stomach and intestinal conditions, with results reported separately for each phase of the extraction (Table 2, Figures 1 and 2). All quality control samples fell within acceptable limits (Table 3).

For the stomach-phase extractions (pH 1.5), the calculated bioaccessibility values range from 22% to 52%. For the intestinal phase (pH 7.0), calculated bioaccessibility values range from 19% to 36%, with the exception of one sample that demonstrated a lower intestinal-phase bioaccessibility of 4%. For the two samples (one surface and one subsurface) collected from location T2E1, arsenic concentrations were too low to allow for calculation of meaningful bioaccessibility values. Therefore, these samples are excluded from the following discussion. For 13 of the 15 soil samples for which bioaccessibility could be calculated, the bioaccessibility values based on the lower-pH (“stomach-phase”) extraction were higher than for the higher-pH, (“intestinal-phase”) extraction. For the two surface soil samples in which the bioaccessibility values are not higher in the stomach-phase extraction, the bioaccessibility values are very similar at both pH ranges tested (i.e., 24% vs. 26% for soil sample A1B21, and 22% vs. 28% for A1B3). Therefore, in most instances, it is reasonable to assume that the stomach-phase extraction controls solubility, and these data served as the basis for selecting samples for *in vivo* research.



For locations at which both surface soil and subsurface soil samples were collected, arsenic concentrations were similar or higher in the subsurface sample. Conversely, the bioaccessibility of arsenic from the subsurface soils was lower than that measured in the associated surface sample.

## Arsenic Mineralogy in Middleport Samples

The arsenic mineralogy in the 13 surficial offsite soil samples and 4 subsurface samples was evaluated by electron microprobe, in accordance with the SOP presented in Appendix A of the Work Plan (Exponent 2004). Results from these analyses are presented in Table 4. These results indicate that the arsenic mineralogy in samples with less than about 600 mg/kg arsenic is dominated by arsenic in iron oxide soil minerals, arsenic in manganese oxide minerals, and arsenic in iron sulfate phases. In contrast, samples with greater than 600 mg/kg arsenic appear to contain arsenic predominantly as an iron-arsenic oxide compound (Fe-As oxide).<sup>1</sup> Based on measurements of arsenic bioaccessibility in *in vitro* testing of soils, it appears that the solubility of arsenic is low from this iron-arsenic oxide compound. Most of the samples with arsenic concentrations greater than 600 mg/kg arsenic also contain limited amounts of lead arsenate (PbAsO<sub>4</sub>) and calcium arsenate (CaAsO<sub>4</sub>), whereas these arsenic forms were not found in samples with lower arsenic concentrations.

The range of arsenic concentrations reflected in the four samples from subsurface locations is smaller than that observed in the 13 surficial soil samples: <20 mg/kg to 661 mg/kg for the subsurface samples, vs. <20 mg/kg to 2,230 mg/kg for surface soils. There are three locations represented by both surface soil and subsurface soil samples. The mineralogy is similar in two samples (T2E1 and T13E4), and in the third sample (T5E3), the subsurface sample is enriched in iron-arsenic oxide phases, relative to the surface soil sample. This finding, however, is consistent with the higher arsenic concentration in the subsurface sample: all of the surface or subsurface samples with arsenic concentrations greater than 600 mg/kg are enriched in this iron-arsenic oxide phase, making the data from the subsurface samples consistent with the data from the surface soil samples. Although the mineralogy of the soils at depth is not exactly like the overlying soils from the same location, the mineralogy reflected in the subsurface soils is consistent with the mineralogy reflected in the surficial soils—both surface and subsurface soils with arsenic concentrations below 600 mg/kg are dominated by arsenic in iron oxide phases, and samples with arsenic concentrations above 600 mg/kg are dominated by an iron-arsenic oxide phases. The mineralogy of the subsurface soils indicates that the arsenic forms present in these samples are the same as those found in surface soil samples from the site, and no additional forms of arsenic are present in the subsurface horizon.

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<sup>1</sup> The literature does not indicate that an iron arsenate (or iron arsenite) pesticide was ever produced or marketed in the United States.

## Sample Selection for *In Vivo* Bioavailability Testing

The work plan for evaluation of arsenic bioavailability from Middleport soils (Exponent 2004) specifies that, following characterization and bioaccessibility testing, three soils will be selected for *in vivo* testing for arsenic bioavailability in Cynomolgus monkeys, using a modified version of the oral bioavailability model of Roberts et al. (2002). Based on the bioaccessibility extraction data, the soils that were selected for oral bioavailability testing are surface soil samples T5E3, T15E4, and A1B20. These samples were selected because they represent a range of values for soil arsenic concentrations (339, 549, and 1000 mg/kg, respectively) and a range of measured bioaccessibility. (T5E3 has the highest bioaccessibility value, and A1B20 has the next-highest value, with a soil concentration sufficiently high for *in vivo* testing, while T15E4 is at the lower end of the range of measured bioaccessibility.) These samples include two samples from the tributary area. There are other samples that demonstrate higher bioaccessibility than some of the selected samples; however, three of these contain arsenic at concentrations that are too low for *in vivo* testing (i.e., below 200 mg/kg). A fourth sample that could be selected (S11) contains an arsenic concentration similar to that selected, and so would result in a more limited range of concentrations tested. The recommended samples also provide for some variability in the associated arsenic mineralogy, and will therefore provide bioavailability data for a range of mineralogies reflected in the soils at the site that contain higher arsenic concentration.

The samples selected for oral bioavailability testing are surface soil samples, which is appropriate in this case for several reasons. Surface soils best represent the materials that might be associated with direct-contact exposure, the observed arsenic concentrations and bioaccessibility ranges are similar between the surface and subsurface soils, and there does not appear to be any unique characteristics of the arsenic in the subsurface soils that would compel including them in the samples for oral bioavailability testing. Therefore, the surface samples selected represent the most relevant study matrices for oral bioavailability testing.

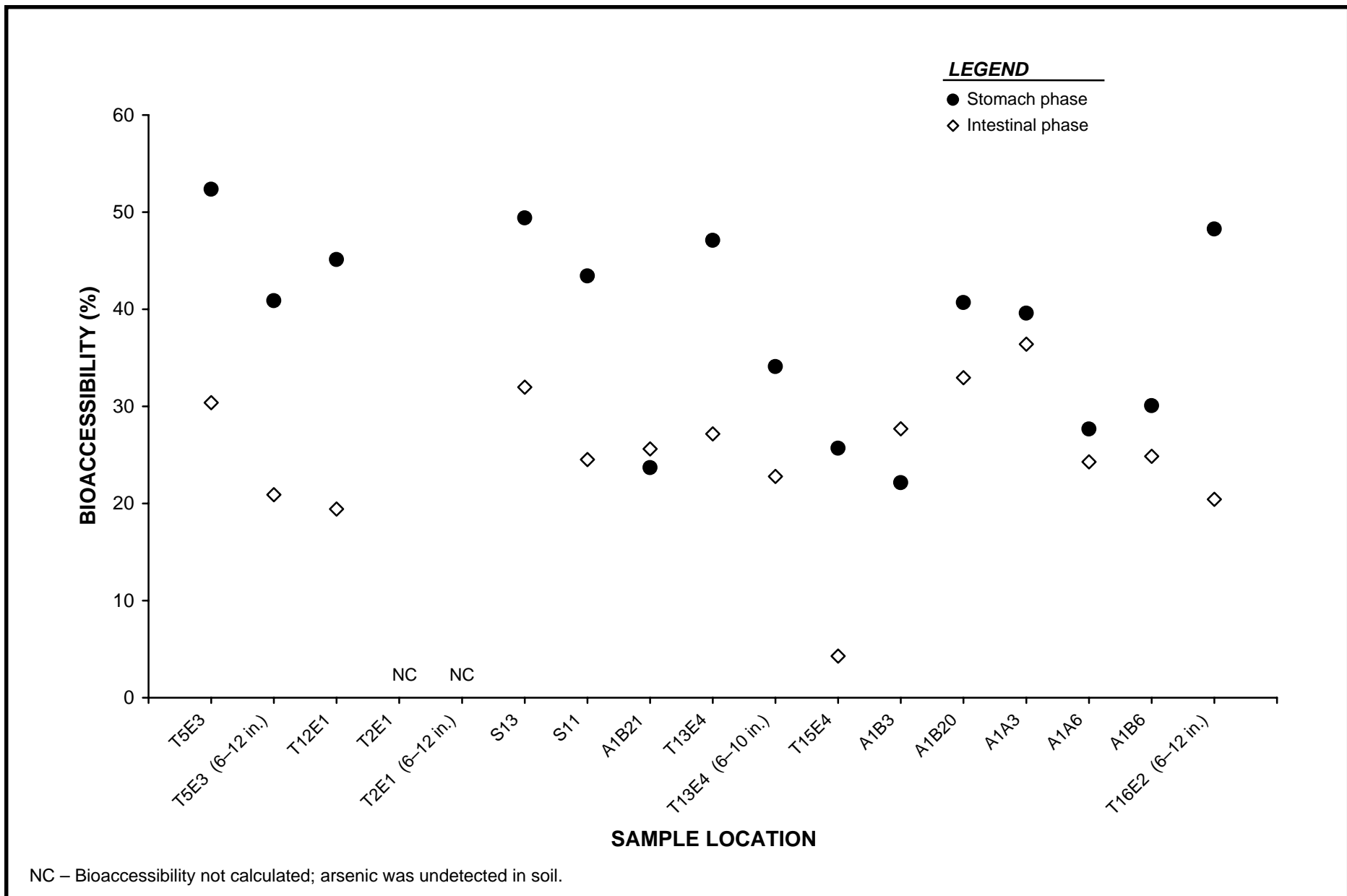


Figure 1. Arsenic *in vitro* bioaccessibility for Middleport soil samples

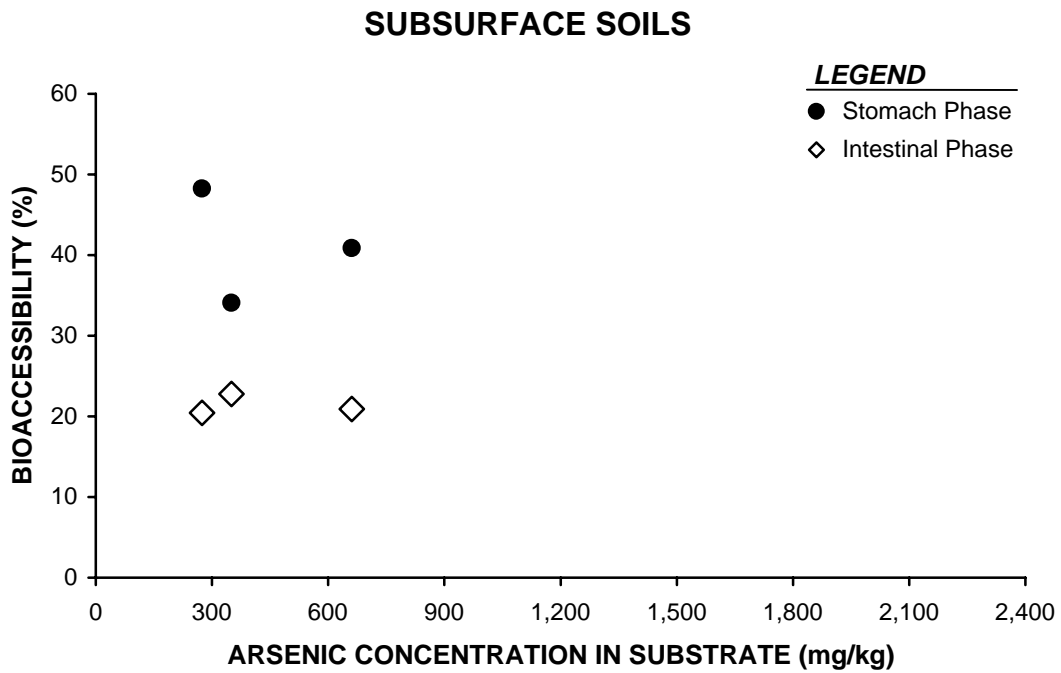
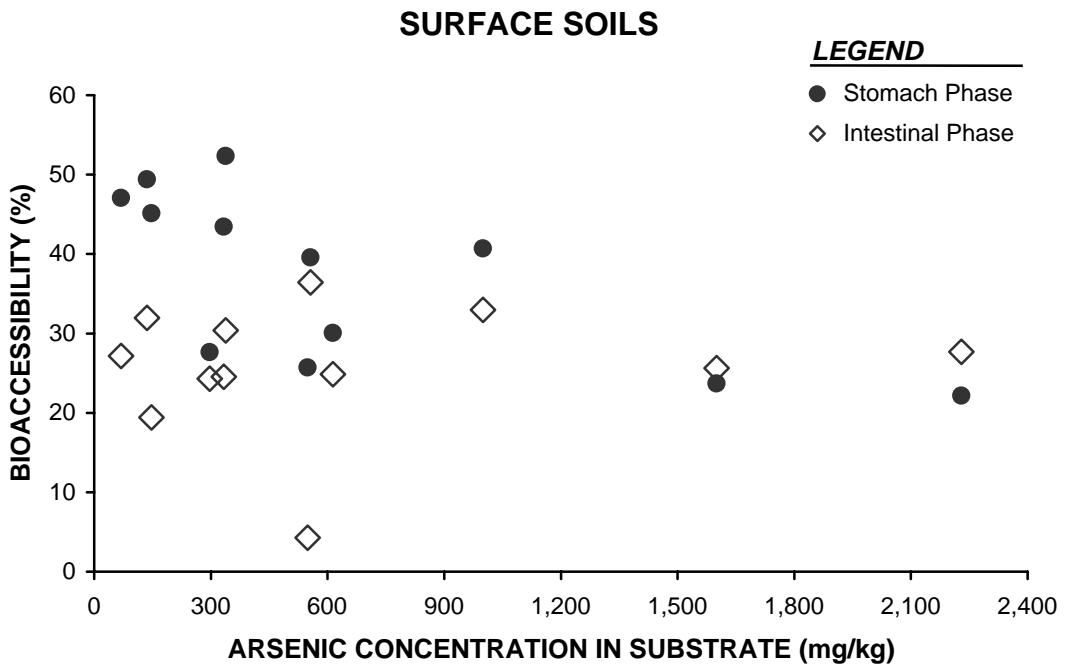


Figure 2. Substrate arsenic concentration and *in vitro* bioaccessibility of Middleport soil samples

**Table 1. Middleport soil characterization**

Analyte	Units	A1A3			A1A6			A1B3			
		10/2/03	10/6/04	10/6/04	10/2/03	10/6/04	10/6/04	10/2/03	4/1/04	10/6/04	10/6/04
		<250 µm	<2 mm	<250 µm	<250 µm	<2 mm	<250 µm	<250 µm	<150 µm	<2 mm	<250 µm
		S0001B	S0001A	S0001B	S0002B	S0002A	S0002B	S0003B	S0003C	S0003A	S0003B
<b>Conventionals</b>											
Carbon, Total Organic	%	--	--	8.68	--	--	4.56	--	--	--	3.13 <sup>a</sup>
Cation Exchange Capacity	mEq/100g	--	--	--	--	--	--	--	--	--	--
DCB Extractable Iron	mg/kg	--	--	--	--	--	--	--	8,960	--	--
pH	s.u.	--	--	6.40 <sup>a</sup>	--	--	6.00	--	--	--	5.44
<b>Grain Size</b>											
Gravel, Medium	%	--	0.01	--	--	0.0	--	--	--	0.0	--
Gravel, Fine	%	--	0.1	--	--	0.07	--	--	--	0.29	--
Sand, Very Coarse	%	--	31.8	--	--	12.5	--	--	--	11.4	--
Sand, Coarse	%	--	15.3	--	--	14	--	--	--	12.3	--
Sand, Medium	%	--	9.65	--	--	9.26	--	--	--	11.3	--
Sand, Fine	%	--	11.9	--	--	13.3	--	--	--	15.7	--
Sand, Very Fine	%	--	3.45	--	--	5.16	--	--	--	5.55	--
Clay	%	--	1.8	--	--	3.42	--	--	--	4.23	--
Silt	%	--	18.7	--	--	36.4	--	--	--	34.9	--
<b>Metals</b>											
Antimony	mg/kg	--	--	9.9 <sup>Ua</sup>	--	--	10.0 <sup>U</sup>	--	--	--	9.9 <sup>U</sup>
Arsenic	mg/kg	565	574 <sup>a</sup>	586 <sup>a</sup>	274	298	307	2,160	1,640	2,440	2,530
Beryllium	mg/kg	--	--	1.0 <sup>Ua</sup>	--	--	1.0 <sup>U</sup>	--	--	--	1.0 <sup>U</sup>
Cadmium	mg/kg	--	--	3.5 <sup>a</sup>	--	--	1.6	--	--	--	11.1
Chromium	mg/kg	--	--	17.3 <sup>a</sup>	--	--	16.0	--	--	--	13.3
Copper	mg/kg	--	--	48.3 <sup>a</sup>	--	--	35.8	--	--	--	58.8
Iron	mg/kg	--	--	--	--	--	--	--	--	--	--
Lead	mg/kg	--	--	186 <sup>a</sup>	--	--	137	--	--	--	341
Manganese	mg/kg	--	--	--	--	--	--	--	--	--	--
Mercury	mg/kg	--	--	0.48	--	--	0.54	--	--	--	1.21 <sup>a</sup>
Nickel	mg/kg	--	--	17.8 <sup>a</sup>	--	--	13.1	--	--	--	11.0
Selenium	mg/kg	--	--	1.7 <sup>a</sup>	--	--	1.0 <sup>U</sup>	--	--	--	1.5
Silver	mg/kg	--	--	2.0 <sup>Ua</sup>	--	--	2.0 <sup>U</sup>	--	--	--	2.0 <sup>U</sup>
Thallium	mg/kg	--	--	1.0 <sup>Ua</sup>	--	--	1.0 <sup>U</sup>	--	--	--	1.0 <sup>U</sup>
Zinc	mg/kg	--	--	158 <sup>a</sup>	--	--	162	--	--	--	130
<b>Triplicate Arsenic Analysis</b>											
A	mg/kg	--	--	--	--	--	--	--	--	--	--
B	mg/kg	--	--	--	--	--	--	--	--	--	--
C	mg/kg	--	--	--	--	--	--	--	--	--	--

**Table 1. (cont.)**

Analyte	Units	A1B6			A1B20				A1B21	
		10/2/03	10/6/04	10/6/04	10/2/03	4/1/04	10/6/04	10/6/04	10/2/03	10/2/03
		<250 µm S0004B	<2 mm S0004A	<250 µm S0004B	<250 µm S0005B	<150 µm S0005C	<2 mm S0005A	<250 µm S0005B	<2 mm S0006A	<250 µm S0006B
<b>Conventionals</b>										
Carbon, Total Organic	%	--	--	5.02	--	--	--	6.43	--	--
Cation Exchange Capacity	mEq/100g	--	--	--	--	--	--	--	--	--
DCB Extractable Iron	mg/kg	--	--	--	--	--	--	--	--	--
pH	s.u.	--	--	5.64	--	--	--	5.95	--	--
<b>Grain Size</b>										
Gravel, Medium	%	--	0.0	--	--	--	0.0	--	0.03	--
Gravel, Fine	%	--	0.07	--	--	--	0.03	--	0.59	--
Sand, Very Coarse	%	--	8.68	--	--	--	19.9	--	23.6	--
Sand, Coarse	%	--	15.1	--	--	--	17	--	23.9	--
Sand, Medium	%	--	11.7	--	--	--	12.3	--	15.1	--
Sand, Fine	%	--	17.7	--	--	--	15.7	--	18.1	--
Sand, Very Fine	%	--	6.78	--	--	--	5.57	--	3.95	--
Clay	%	--	2.87	--	--	--	1.66	--	1.86	--
Silt	%	--	34	--	--	--	21.5	--	14.9	--
<b>Metals</b>										
Antimony	mg/kg	--	--	10.0 <i>U</i>	--	--	--	9.9 <i>U</i>	--	--
Arsenic	mg/kg	584	646	603	991	759	1,090	846	--	1,580
Beryllium	mg/kg	--	--	1.0 <i>U</i>	--	--	--	1.0 <i>U</i>	--	--
Cadmium	mg/kg	--	--	3.5	--	--	--	7.3	--	--
Chromium	mg/kg	--	--	15.8	--	--	--	25.3	--	--
Copper	mg/kg	--	--	67.0	--	--	--	198	--	--
Iron	mg/kg	--	--	--	--	--	--	--	--	--
Lead	mg/kg	--	--	277	--	--	--	625	--	--
Manganese	mg/kg	--	--	--	--	--	--	--	--	--
Mercury	mg/kg	--	--	0.78	--	--	--	2.79	--	--
Nickel	mg/kg	--	--	13.1	--	--	--	19.3	--	--
Selenium	mg/kg	--	--	1.0 <i>U</i>	--	--	--	2.8	--	--
Silver	mg/kg	--	--	2.0 <i>U</i>	--	--	--	2.0 <i>U</i>	--	--
Thallium	mg/kg	--	--	1.0 <i>U</i>	--	--	--	1.0 <i>U</i>	--	--
Zinc	mg/kg	--	--	181	--	--	--	562	--	--
<b>Triplicate Arsenic Analysis</b>										
A	mg/kg	--	--	--	--	--	--	--	--	--
B	mg/kg	--	--	--	--	--	--	--	--	--
C	mg/kg	--	--	--	--	--	--	--	--	--

**Table 1. (cont.)**

Analyte	Units	A1B21 (cont.)				S13		T5E3	
		10/30/03		10/6/04	10/6/04	10/6/04	10/6/04	10/6/04	10/6/04
		<150 µm		<2 mm	<250 µm	<2 mm	<250 µm	<2 mm	<250 µm
		S0006C <sup>a</sup>	S0006C	S0006A	S0006B	S0007A	S0007B	S0008A	S0008B
<b>Conventionals</b>									
Carbon, Total Organic	%	4.77	4.25	--	5.08	--	6.16	--	5.72
Cation Exchange Capacity	mEq/100g	--	81.0	--	--	--	--	--	--
DCB Extractable Iron	mg/kg	--	9,720	--	--	--	--	--	--
pH	s.u.	5.34	5.61	--	5.24	--	7.31	--	7.18
<b>Grain Size</b>									
Gravel, Medium	%	--	--	0.0	--	0.0	--	0.0	--
Gravel, Fine	%	--	--	0.03	--	0.03	--	0.28	--
Sand, Very Coarse	%	--	--	22.8	--	19.2	--	8.82	--
Sand, Coarse	%	--	--	18.1	--	16.3	--	15.7	--
Sand, Medium	%	--	--	11.8	--	16.2	--	14.8	--
Sand, Fine	%	--	--	13.7	--	21.3	--	18.6	--
Sand, Very Fine	%	--	--	4.02	--	5.6	--	6.42	--
Clay	%	--	--	2.02	--	0.89	--	3.08	--
Silt	%	--	--	15.8	--	18.2	--	29.7	--
<b>Metals</b>									
Antimony	mg/kg	--	10 <i>U</i>	--	15.7	--	9.8 <i>U</i>	--	10.0 <i>U</i>
Arsenic	mg/kg	1,610	1,400	1,500	1,750	160	120	394	309
Beryllium	mg/kg	--	1 <i>U</i>	--	1.0 <i>U</i>	--	1.0 <i>U</i>	--	1.0 <i>U</i>
Cadmium	mg/kg	--	1.7	--	8.4	--	2.4	--	2.5
Chromium	mg/kg	17.3	16.2	--	19.0	--	18.3	--	16.1
Copper	mg/kg	60.1	62.3	--	61.6	--	112	--	48.8
Iron	mg/kg	16,950	15,050	--	--	--	--	--	--
Lead	mg/kg	374	399	--	416	--	657	--	145
Manganese	mg/kg	661	645	--	--	--	--	--	--
Mercury	mg/kg	--	0.44	--	2.11	--	0.21	--	0.19
Nickel	mg/kg	--	13.7	--	12.9	--	14.6	--	15.7
Selenium	mg/kg	--	1.9	--	2.2	--	1.0 <i>U</i>	--	1.0 <i>U</i>
Silver	mg/kg	--	2 <i>U</i>	--	2.0 <i>U</i>	--	2.0 <i>U</i>	--	2.0 <i>U</i>
Thallium	mg/kg	--	1 <i>U</i>	--	1.0 <i>U</i>	--	1.0 <i>U</i>	--	1.0 <i>U</i>
Zinc	mg/kg	--	244	--	254	--	453	--	251
<b>Triplicate Arsenic Analysis</b>									
A	mg/kg	--	1,350	--	--	--	--	--	--
B	mg/kg	--	1,360	--	--	--	--	--	--
C	mg/kg	--	1,360	--	--	--	--	--	--



**Table 1. (cont.)**

Analyte	Units	S11		T2E1		T12E1		T13E4		T15E4	
		10/6/04 <2 mm	10/6/04 <250 µm	10/6/04 <2 mm	10/6/04 <250 µm	10/6/04 <2 mm	10/6/04 <250 µm	10/6/04 <2 mm	10/6/04 <250 µm	10/6/04 <2 mm	10/6/04 <250 µm
		S0009A	S0009B	S0010A	S0010B	S0011A	S0011B	S0012A	S0012B	S0013A	S0013B
<b>Conventionals</b>											
Carbon, Total Organic	%	--	2.60	--	3.68	--	4.28	--	5.49	--	6.77
Cation Exchange Capacity	mEq/100g	--	--	--	--	--	--	--	--	--	--
DCB Extractable Iron	mg/kg	--	--	--	--	--	--	--	--	--	--
pH	s.u.	--	7.48	--	7.59	--	7.31	--	7.41	--	7.30
<b>Grain Size</b>											
Gravel, Medium	%	0.0	--	0.0	--	0.0	--	0.0	--	0.0	--
Gravel, Fine	%	0.05	--	0.05	--	0.25	--	0.11	--	1.76	--
Sand, Very Coarse	%	11.5	--	11.8	--	6.99	--	4.63	--	43.5	--
Sand, Coarse	%	15.1	--	7.34	--	14.7	--	14.9	--	15.5	--
Sand, Medium	%	19.6	--	7.79	--	19.4	--	15.8	--	8.9	--
Sand, Fine	%	21.9	--	12.7	--	28.1	--	26.3	--	9.14	--
Sand, Very Fine	%	5.12	--	5.24	--	7.63	--	8.91	--	3.22	--
Clay	%	3.2	--	5.22	--	0.95	--	1.44	--	1.28	--
Silt	%	21.9	--	49.1	--	19.3	--	24.7	--	14.3	--
<b>Metals</b>											
Antimony	mg/kg	--	10.0 <i>U</i>	--	10.0 <i>U</i>	--	10.0 <i>U</i>	--	9.9 <i>U</i>	--	9.8 <i>U</i>
Arsenic	mg/kg	364	305	20 <i>U</i>	14.5	200	123	73.2	58.8	503	489
Beryllium	mg/kg	--	1.0 <i>U</i>	--	1.0 <i>U</i>	--	1.0 <i>U</i>	--	1.0 <i>U</i>	--	1.0 <i>U</i>
Cadmium	mg/kg	--	1.7	--	1.0 <i>U</i>	--	1.2	--	2.2	--	3.8
Chromium	mg/kg	--	13.8	--	22.0	--	14.3	--	15.4	--	21.1
Copper	mg/kg	--	58.5	--	36.8	--	33.7	--	36.0	--	47.4
Iron	mg/kg	--	--	--	--	--	--	--	--	--	--
Lead	mg/kg	--	135	--	175	--	89.1	--	94.9	--	121
Manganese	mg/kg	--	--	--	--	--	--	--	--	--	--
Mercury	mg/kg	--	0.16	--	0.28	--	0.15	--	0.12	--	0.26
Nickel	mg/kg	--	13.7	--	27.5	--	12.8	--	13.8	--	19.1
Selenium	mg/kg	--	1.0 <i>U</i>	--	1.0 <i>U</i>	--	1.0 <i>U</i>	--	1.0 <i>U</i>	--	1.0 <i>U</i>
Silver	mg/kg	--	2.0 <i>U</i>	--	2.0 <i>U</i>	--	2.0 <i>U</i>	--	2.0 <i>U</i>	--	2.0 <i>U</i>
Thallium	mg/kg	--	1.0 <i>U</i>	--	1.0 <i>U</i>	--	1.0 <i>U</i>	--	1.0 <i>U</i>	--	1.0 <i>U</i>
Zinc	mg/kg	--	196	--	165	--	222	--	264	--	390
<b>Triplicate Arsenic Analysis</b>											
A	mg/kg	--	--	--	--	--	--	--	--	--	--
B	mg/kg	--	--	--	--	--	--	--	--	--	--
C	mg/kg	--	--	--	--	--	--	--	--	--	--

**Table 1. (cont.)**

Analyte	Units	T13E4 (6–10 in.)			T2E1 (6–12 in.)		T5E3 (6–12 in.)		T16E2 (6–12 in.)	
		10/15/04	10/15/04	10/15/04	10/15/04	10/15/04	10/15/04	10/15/04	11/23/04	11/23/04
		<2 mm	<250 µm	<250 µm	<2 mm	<250 µm	<2 mm	<250 µm	<2 mm	<250 µm
		S0014A	S0018	S0014B	S0015A	S0015B	S0016A	S0016B	S0017A	S0017B
<b>Conventionals</b>										
Carbon, Total Organic	%	--	--	2.72	--	3.25	--	4.49	--	3.94
Cation Exchange Capacity	mEq/100g	--	--	--	--	--	--	--	--	--
DCB Extractable Iron	mg/kg	--	--	--	--	--	--	--	--	--
pH	s.u.	--	--	7.33	--	7.00	--	7.32	--	7.20
<b>Grain Size</b>										
Gravel, Medium	%	0.0	--	--	0.0	--	0.0	--	0.0	--
Gravel, Fine	%	0.0	--	--	0.10	--	0.0	--	0.0	--
Sand, Very Coarse	%	6.05	--	--	5.80	--	8.89	--	3.48	--
Sand, Coarse	%	12.0	--	--	11.0	--	13.9	--	9.93	--
Sand, Medium	%	15.0	--	--	10.0	--	15.8	--	10.6	--
Sand, Fine	%	26.1	--	--	17.4	--	24.6	--	22.8	--
Sand, Very Fine	%	8.02	--	--	7.65	--	6.90	--	8.79	--
Clay	%	3.97	--	--	2.71	--	2.92	--	5.24	--
Silt	%	28.0	--	--	43.6	--	24.4	--	35.9	--
<b>Metals</b>										
Antimony	mg/kg	--	--	10 U	--	10 U	--	10 U	--	10 U
Arsenic	mg/kg	364	357	342	22.7	7.1	732	661	271	274
Beryllium	mg/kg	--	--	1.0 U	--	1.0 U	--	1.0 U	--	1.0 U
Cadmium	mg/kg	--	--	3.3	--	1.0 U	--	4.1	--	2.5
Chromium	mg/kg	--	--	22.4	--	15.5	--	18.4	--	31.0
Copper	mg/kg	--	--	51.9	--	24.2	--	70.2	--	70.2
Iron	mg/kg	--	--	--	--	--	--	--	--	--
Lead	mg/kg	--	--	151	--	58.9	--	188	--	147
Manganese	mg/kg	--	--	--	--	--	--	--	--	--
Mercury	mg/kg	--	--	0.34	--	0.07	--	0.23	--	0.36
Nickel	mg/kg	--	--	20.7	--	13.3	--	17.2	--	23.2
Selenium	mg/kg	--	--	1.0 U	--	1.0 U	--	1.0 U	--	1.0 U
Silver	mg/kg	--	--	2.0 U	--	2.0 U	--	2.0 U	--	2.0 U
Thallium	mg/kg	--	--	2.0 U	--	1.0 U	--	1.0 U	--	1.0 U
Zinc	mg/kg	--	--	290	--	76.2	--	307	--	527
<b>Triplicate Arsenic Analysis</b>										
A	mg/kg	--	--	--	--	--	--	--	--	--
B	mg/kg	--	--	--	--	--	--	--	--	--
C	mg/kg	--	--	--	--	--	--	--	--	--

(notes on following page)

**Table 1. (cont.)**

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**Note:** -- -- Not available or not applicable  
*U* – Undetected; value represents reporting limit

<sup>a</sup> Average of duplicate results.

**Table 2. Results from *in vitro* bioaccessibility testing of arsenic in Middleport soil samples (<250 µm fraction)**

Soil Sample ID	Arsenic Conc. in Substrate (mg/kg)	Mass of Soil Tested (g)	Mass of Arsenic in Soil Extracted (mg)	Extraction Date	Phase	Final pH (s.u.)	Arsenic Conc. in Extract (mg/L)	Volume of Extract (L)	Mass of Arsenic in Extract (mg)	Arsenic Bioaccessibility (%)
<b>Surface Soils</b>										
T5E3	339 <sup>a</sup>	1.0005 <sup>a</sup>	0.3387	9/16/04	stomach intestinal	1.79 <sup>a</sup> 7.01 <sup>a</sup>	1.77 <sup>a</sup> 1.08 <sup>a</sup>	0.100 0.095	0.177 0.103	52 30
T12E1	147	1.0009	0.1471	9/16/04	stomach intestinal	1.77 7.07	0.66 0.30	0.100 0.095	0.066 0.029	45 19
T2E1	20 <sup>U</sup>	1.0001	NC	9/16/04	stomach intestinal	1.77 7.15	0.06 0.04	0.100 0.095	0.006 0.004	NC NC
S13	136	1.0029	0.1364	9/16/04	stomach intestinal	1.78 7.01	0.67 0.46	0.100 0.095	0.067 0.044	49 32
S11	334 <sup>b</sup>	1.0009	0.3338	9/16/04	stomach intestinal	1.76 7.15	1.45 0.86	0.100 0.095	0.145 0.082	43 25
A1B21	1,600 <sup>a</sup>	1.0037 <sup>a</sup>	1.6060	9/17/04	stomach intestinal	1.53 <sup>a</sup> 6.83 <sup>a</sup>	3.80 <sup>a</sup> 4.33 <sup>a</sup>	0.100 0.095	0.380 0.411	24 26
T13E4	68.9	1.0049	0.0692	9/17/04	stomach intestinal	1.76 7.06	0.33 0.20	0.100 0.095	0.033 0.019	47 27
T15E4	549	1.0068	0.5527	9/17/04	stomach intestinal	1.79 6.84	1.42 0.25	0.100 0.095	0.142 0.024	26 4
A1B3	2,230	1.0045	2.2400	9/17/04	stomach intestinal	1.53 6.64	4.96 6.53	0.100 0.095	0.496 0.620	22 28
A1B20	1,000	1.0002	1.0002	9/17/04	stomach intestinal	1.57 6.87	4.07 3.47	0.100 0.095	0.407 0.330	41 33
A1A3	556 <sup>b</sup>	0.9996	0.5558	9/17/04	stomach intestinal	1.55 6.84	2.20 2.13 <sup>a</sup>	0.100 0.095	0.220 0.202	40 36
A1A6	297	1.0050	0.2985	9/17/04	stomach intestinal	1.54 6.87	0.83 <sup>a</sup> 0.76	0.100 0.095	0.083 0.072	28 24
A1B6	614 <sup>b</sup>	1.0022	0.6154	9/17/04	stomach intestinal	1.53 6.98	1.85 1.61	0.100 0.095	0.185 0.153	30 25

**Table 2. (cont.)**

Soil Sample ID	Arsenic Conc. in Substrate (mg/kg)	Mass of Soil Tested (g)	Mass of Arsenic in Soil Extracted (mg)	Extraction Date	Phase	Final pH (s.u.)	Arsenic Conc. in Extract (mg/L)	Volume of Extract (L)	Mass of Arsenic in Extract (mg)	Arsenic Bioaccessibility (%)
<b>Subsurface Soils</b>										
T13E4 (6–10 in.)	350 <sup>b</sup>	1.0514	0.3680	12/20/04	stomach intestinal	1.54 6.83	1.26 <sup>b</sup> 0.88 <sup>b</sup>	0.100 0.095	0.126 0.084	34 23
T2E1 (6–12 in.)	20 <i>U</i>	1.0328	NC	12/20/04	stomach intestinal	1.47 6.99	0.04 0.06	0.100 0.095	0.004 0.005	NC NC
T5E3 (6–12 in.)	661	1.0175	0.6726	12/20/04	stomach intestinal	1.50 7.05	2.75 1.48	0.100 0.095	0.275 0.141	41 21
T16E2 (6–12 in.)	274	1.0133	0.2776	12/20/04	stomach intestinal	1.48 6.84	1.34 0.60	0.100 0.095	0.134 0.057	48 20

**Note:** *U* - not detected; value represents detection limit

NC - Not calculated due to the non-detect soil concentration

<sup>a</sup> Average of triplicate results.

<sup>b</sup> Average of duplicate results.

**Table 3. QA sample results for *in vitro* bioaccessibility testing of arsenic in Middleport soils (<250 µm fraction)**

Sample ID	Final pH		Arsenic Conc. in Substrate (mg/kg)	Relative Percent Deviation (%)	Relative Standard Deviation (%)	Arsenic Spike Conc. (mg/L)	Arsenic Concentration in Extract		Percent Recovery		Relative Standard Deviation		Control Limits
	Stomach Phase (s.u.)	Intestinal Phase (s.u.)					Stomach Phase (mg/L)	Intestinal Phase (mg/L)	Stomach Phase (%)	Intestinal Phase (%)	Stomach Phase (%)	Intestinal Phase (%)	
<b>Soil Duplicates</b>													
S11	--	--	338	--	--	--	--	--	--	--	--	--	
S11 (A)	--	--	329	2.7	--	--	--	--	--	--	--	--	0–20%
A1A3	--	--	557	--	--	--	--	--	--	--	--	--	
A1A3 (A)	--	--	555	0.4	--	--	--	--	--	--	--	--	0–20%
A1B6	--	--	623	--	--	--	--	--	--	--	--	--	
A1B6 (A)	--	--	605	2.9	--	--	--	--	--	--	--	--	0–20%
T13E4 (6–10 in.)	--	--	342 <sup>a</sup>	--	--	--	--	--	--	--	--	--	
T13E4 (6–10 in.) (A)	--	--	357	4.3	--	--	--	--	--	--	--	--	0–20%
<b>Soil Triplicates</b>													
T5E3	--	--	338 <sup>a</sup>	--	--	--	--	--	--	--	--	--	
T5E3 (A)	--	--	339	--	--	--	--	--	--	--	--	--	
T5E3 (B)	--	--	339	--	0.3	--	--	--	--	--	--	--	0–20%
A1B21	--	--	1,600	--	--	--	--	--	--	--	--	--	
A1B21 (A)	--	--	1,590	--	--	--	--	--	--	--	--	--	
A1B21 (B)	--	--	1,610	--	0.6	--	--	--	--	--	--	--	0–20%
<b>Duplicate Extractions</b>													
T13E4 (6–10 in.)	1.56	6.85	--	--	--	--	1.26 <sup>a</sup>	0.935 <sup>a</sup>	--	--	--	--	
T13E4 (6–10 in.) (A)	1.52	6.80	--	--	--	--	1.25	0.831	--	--	0.6	8.3	0–20%
<b>Triplicate Extractions</b>													
T5E3	1.83	7.10	--	--	--	--	1.76 <sup>a</sup>	1.16 <sup>a</sup>	--	--	--	--	
T5E3 (A)	1.79	7.02	--	--	--	--	1.74	1.05	--	--	--	--	
T5E3 (B)	1.76	6.92	--	--	--	--	1.82	1.04	--	--	2.3	6.1	0–20%
A1B21	1.51	6.95	--	--	--	--	3.91 <sup>a</sup>	4.09	--	--	--	--	
A1B21 (A)	1.55	6.78	--	--	--	--	3.74	4.77	--	--	--	--	
A1B21 (B)	1.52	6.75	--	--	--	--	3.76	4.13	--	--	2.4	8.8	0–20%
<b>QC Samples (Sept. 16–17, 2004)</b>													
Reagent Blank	--	--	--	--	--	--	0.01 U	0.01 U	--	--	--	--	<0.005 mg/L
Method Blank	--	--	--	--	--	--	0.01 U	0.01 U	--	--	--	--	<0.01 mg/L
Matrix Spike	--	--	--	--	--	1.00	0.955	0.895	96	90	--	--	85–115% <sup>b</sup>
SRM NIST 2711	--	--	--	--	--	--	0.529	0.399	--	--	--	--	0.5–0.68 mg/L <sup>b</sup>

**Table 3. (cont.)**

Sample ID	Final pH		Arsenic Conc. in Substrate (mg/kg)	Relative Percent Deviation (%)	Relative Standard Deviation (%)	Arsenic Spike Conc. (mg/L)	Arsenic Concentration in Extract		Percent Recovery		Relative Standard Deviation		Control Limits	
	Stomach Phase (s.u.)	Intestinal Phase (s.u.)					Stomach Phase (mg/L)	Intestinal Phase (mg/L)	Stomach Phase (%)	Intestinal Phase (%)	Stomach Phase (%)	Intestinal Phase (%)		
	<b>QC Samples (December 20, 2005)</b>													
Reagent Blank	--	--	--	--	--	--	0.005 <i>U</i>	--	--	--	--	--	--	<0.005 mg/L
Method Blank	--	--	--	--	--	--	0.005 <i>U</i>	0.005 <i>U</i>	--	--	--	--	--	<0.01 mg/L
Matrix Spike	--	--	--	--	--	1.00	1.020	0.886	102	89	--	--	--	85–115% <sup>b</sup>
SRM NIST 2711	--	--	--	--	--	--	0.525	0.413	--	--	--	--	--	0.5–0.68 mg/L <sup>b</sup>

**Notes:** -- - not available/not applicable

*U* - not detected; value represents detection limit

<sup>a</sup> Average of analytical laboratory replicate results.

<sup>b</sup> Control limit relevant for recovery in the stomach phase only.



**Table 4. Arsenic mineralogy results for Middleport offsite surface and subsurface soils**

Soil Sample ID: Sample No.:	Surface Soil Samples								
	T2E1 S010B			T13E4 S012B			S13 S007B		
	Frequency (%)	Arsenic Mass Distribution (%)	Average Particle Size <sup>a</sup> (µm)	Frequency (%)	Arsenic Mass Distribution (%)	Average Particle Size <sup>a</sup> (µm)	Frequency (%)	Arsenic Mass Distribution (%)	Average Particle Size <sup>a</sup> (µm)
Arsenic Form									
Iron-arsenic oxide (Fe-As oxide)	--	--	--	--	--	--	--	--	--
Lead arsenate (PbAsO <sub>4</sub> )	--	--	--	--	--	--	--	--	--
Calcium arsenate (CaAsO <sub>4</sub> )	--	--	--	--	--	--	--	--	--
Iron oxides (FeOOH)	85.3	84.3	16.8	100.0	100.0	43.8	79.9	99.0	28.0
Manganese oxides (MnOOH)	--	--	--	--	--	--	1.0	0.1	12.0
Phosphates	--	--	--	--	--	--	8.4	0.9	11.7
Iron sulfate (FeSO <sub>4</sub> )	14.7	15.7	26.0	--	--	--	--	--	--
Calcium-iron silicate (CaFeSiO <sub>2</sub> )	--	--	--	--	--	--	--	--	--
Lead metal oxide (PbMO)	--	--	--	--	--	--	--	--	--
No. particles counted			10			10			112
Arsenic concentration (mg/kg)			20 <i>U</i>			68.9			136

**Table 4. (cont.)**

Soil Sample ID: Sample No.:	Surface Soil Samples								
	T12E1 S011B			A1A6 S002B			S11 S009B		
	Frequency (%)	Arsenic Mass Distribution (%)	Average Particle Size <sup>a</sup> (µm)	Frequency (%)	Arsenic Mass Distribution (%)	Average Particle Size <sup>a</sup> (µm)	Frequency (%)	Arsenic Mass Distribution (%)	Average Particle Size <sup>a</sup> (µm)
Arsenic Form									
Iron-arsenic oxide (Fe-As oxide)	--	--	--	1.5	18.5	5.0	0.7	9.9	5.0
Lead arsenate (PbAsO <sub>4</sub> )	--	--	--	--	--	--	--	--	--
Calcium arsenate (CaAsO <sub>4</sub> )	--	--	--	--	--	--	--	--	--
Iron oxides (FeOOH)	100.0	100.0	14.8	42.1	51.2	22.7	95.4	88.6	15.5
Manganese oxides (MnOOH)	--	--	--	55.2	28.7	21.7	2.9	1.0	11.0
Phosphates	--	--	--	--	--	--	--	--	--
Iron sulfate (FeSO <sub>4</sub> )	--	--	--	1.2	1.6	5.0	1.0	0.6	7.5
Calcium-iron silicate (CaFeSiO <sub>2</sub> )	--	--	--	--	--	--	--	--	--
Lead metal oxide (PbMO)	--	--	--	--	--	--	--	--	--
No. particles counted			100			107			101
Arsenic concentration (mg/kg)			147			297			334 <sup>b</sup>

**Table 4. (cont.)**

Soil Sample ID: Sample No.:	Surface Soil Samples								
	T5E3 S008B			T15E4 S013B			A1A3 S001B		
	Frequency (%)	Arsenic Mass Distribution (%)	Average Particle Size <sup>a</sup> (µm)	Frequency (%)	Arsenic Mass Distribution (%)	Average Particle Size <sup>a</sup> (µm)	Frequency (%)	Arsenic Mass Distribution (%)	Average Particle Size <sup>a</sup> (µm)
Arsenic Form									
Iron-arsenic oxide (Fe-As oxide)	--	--	--	--	--	--	4.2	34.5	12.8
Lead arsenate (PbAsO <sub>4</sub> )	--	--	--	--	--	--	--	--	--
Calcium arsenate (CaAsO <sub>4</sub> )	--	--	--	--	--	--	--	--	--
Iron oxides (FeOOH)	100.0	100.0	19.9	99.8	99.9	37.5	94.2	65.2	17.8
Manganese oxides (MnOOH)	--	--	--	0.2	0.1	8.0	1.2	0.4	22.0
Phosphates	--	--	--	--	--	--	--	--	--
Iron sulfate (FeSO <sub>4</sub> )	--	--	--	--	--	--	0.4	0.0	7.0
Calcium-iron silicate (CaFeSiO <sub>2</sub> )	--	--	--	--	--	--	--	--	--
Lead metal oxide (PbMO)	--	--	--	--	--	--	--	--	--
No. particles counted			88			104			105
Arsenic concentration (mg/kg)			339 <sup>c</sup>			549			556 <sup>b</sup>

**Table 4. (cont.)**

Soil Sample ID: Sample No.:	Surface Soil Samples											
	A1B6 S004B			A1B20 S005B			A1B21 S006B			A1B3 S003B		
	Frequency	Arsenic Mass Distribution (%)	Average Particle Size <sup>a</sup> (µm)	Frequency	Arsenic Mass Distribution (%)	Average Particle Size <sup>a</sup> (µm)	Frequency	Arsenic Mass Distribution (%)	Average Particle Size <sup>a</sup> (µm)	Frequency	Arsenic Mass Distribution (%)	Average Particle Size <sup>a</sup> (µm)
Arsenic Form	(%)	(%)	(µm)	(%)	(%)	(µm)	(%)	(%)	(µm)	(%)	(%)	(µm)
Iron-arsenic oxide (Fe-As oxide)	17.0	76.7	3.4	7.7	54.5	7.5	46.4	94.8	3.8	67.8	97.6	4.8
Lead arsenate (PbAsO <sub>4</sub> )	0.2	1.1	2.0	0.9	8.1	3.7	--	--	--	--	--	--
Calcium arsenate (CaAsO <sub>4</sub> )	--	--	--	0.4	1.7	12.0	--	--	--	--	--	--
Iron oxides (FeOOH)	68.1	20.9	22.8	69.7	32.1	33.4	38.3	4.4	19.6	16.2	1.8	36.8
Manganese oxides (MnOOH)	14.8	1.4	30.5	17.3	3.0	61.0	11.0	0.5	10.1	16.0	0.6	29.2
Phosphates	--	--	--	2.3	0.1	11.0	--	--	--	--	--	--
Iron sulfate (FeSO <sub>4</sub> )	--	--	--	0.9	0.5	3.7	2.2	0.2	6.7	--	--	--
Calcium-iron silicate (CaFeSiO <sub>2</sub> )	--	--	--	--	--	--	--	--	--	--	--	--
Lead metal oxide (PbMO)	--	--	--	--	--	--	--	--	--	--	--	--
No. particles counted			106			118			146			137
Arsenic concentration (mg/kg)			614 <sup>b</sup>			1,000			1,600 <sup>c</sup>			2,230

**Table 4. (cont.)**

Soil Sample ID: Sample No.:	Subsurface Soil Samples											
	T13E4 (6–10 in.) S0014B			T2E1 (6–12 in.) S0015B			T5E3 (6–12 in.) S0016B			T16E2 S0017B		
	Frequency	Arsenic Mass Distribution	Average Particle Size <sup>a</sup>	Frequency	Arsenic Mass Distribution	Average Particle Size <sup>a</sup>	Frequency	Arsenic Mass Distribution	Average Particle Size <sup>a</sup>	Frequency	Arsenic Mass Distribution	Average Particle Size <sup>a</sup>
Arsenic Form												
Iron-arsenic oxide (Fe-As oxide)	--	--	--	--	--	--	5.7	58.6	9.5	--	--	--
Lead arsenate (PbAsO <sub>4</sub> )	--	--	--	--	--	--	--	--	--	--	--	--
Calcium arsenate (CaAsO <sub>4</sub> )	--	--	--	--	--	--	--	--	--	--	--	--
Iron oxides (FeOOH)	89.5	90.0	22.8	100	100	13.8	72.3	23.5	22.9	99.5	99.6	49.5
Manganese oxides (MnOOH)	10.1	9.8	20.3	--	--	--	8.2	2.6	32.0	0.4	0.4	18.0
Phosphates	--	--	--	--	--	--	--	--	--	0.09	0.04	4.0
Iron sulfate (FeSO <sub>4</sub> )	0.3	0.2	4.0	--	--	--	--	--	--	--	--	--
Calcium-iron silicate (CaFeSiO <sub>2</sub> )	--	--	--	--	--	--	8.6	14.6	50.3	--	--	--
Lead metal oxide (PbMO)	--	--	--	--	--	--	3.9	0.5	45.5	--	--	--
No. particles counted			108			8			101			90
Arsenic concentration (mg/kg)			350 <sup>b</sup>			20 <sup>U</sup>			661			274

**Note:** -- -- not present or not relevant

*U* – not detected; value represents detection limit

Forms contributing less than 0.5% of arsenic mass in any sample are not shown.

<sup>a</sup> Based on long-axis dimensions.

<sup>b</sup> Average of duplicate results.

<sup>c</sup> Average of triplicate results.



## Extraction Tests for Determining the Bioavailability of Metals in Soil

Cleanup  
CU-1165

### Contact Information:

Yvette W. Lowney  
Exponent  
4875 Pearl East Circle, Suite 201  
Boulder, Colorado 80301  
Phone: (303) 544-2000  
E-mail: [lowneyy@exponent.com](mailto:lowneyy@exponent.com)

### Background:

Environmentally acceptable endpoints (EAEs) for soil most commonly are defined as concentrations of chemicals that are judged acceptable by a regulatory agency, and are derived from standard guidelines. Standard practice in determining EAEs is to assume that the absorption of chemicals from soil is the same as that from a soluble solution of a given test chemical. Considerable research conducted in recent years indicates that site-specific factors can affect chemical solubility from soil, and thereby affect absorption by a biological system (i.e., human or ecological receptors).

### Objective:

The research conducted under this project was designed to yield a database that establishes whether site- or soil-specific factors affect the bioavailability of target metals from soils. Where the database (from *in vivo* research) identifies that these types of factors are operating, an additional goal of the research has been to develop simple extraction tests that are inexpensive to perform and that are predictive of metals bioavailability from soil. These tools can then be available to U.S. Department of Defense (DoD) personnel for site-specific evaluation of metals bioavailability from soil at field sites and will result in more accurate exposure and risk estimates that are still protective of human health and the environment.

### Summary of Findings:

#### Metals that Drive Remedial Decisions at DoD Sites

Based on this research, lead was the most frequent soil contaminant associated with DoD sites that exceeded screening criteria, for both human health and ecological scenarios. Other metals that have been determined to be of concern for human health include arsenic, chromium, cadmium, and antimony. The most frequent metals of concern based on the ecological screening criteria were lead, zinc, mercury, chromium, and selenium for birds, and arsenic for mammals.

### Relative Oral Bioavailability of Arsenic — Human Receptors

A research model using cynomolgus monkeys was used to assess the relative bioavailability (RBA) of arsenic from soils. The mean RBA values for the 10 soil samples studied varied from 5% to 31%, indicating that absorption of arsenic from soil is controlled by soil- or site-specific factors. The presence of arsenic in insoluble mineralogic forms is likely a factor in controlling the RBA.

Table 1 provides a summary of the RBA estimates for the soils studied, together with information regarding the sample mineralogy.

**Table 1. Relative oral bioavailability of arsenic from 10 soil samples**

Soil Sample	RBA <sup>a</sup>
MTSS	0.13 ± 0.05 (36)
WISS	0.13 ± 0.07 (52)
FLCDV	0.31 ± 0.04 (14)
CAMT	0.19 ± 0.02 (11)
WAOS	0.24 ± 0.09 (36)
NYOS	0.16 ± 0.08 (49)
COSCS	0.23 ± 0.13 (54)
CORS	0.17 ± 0.08 (49)
COSS	0.05 ± 0.04 (81)
FLCPS	0.08 ± 0.04 (50)

<sup>a</sup> Results expressed as mean ± SD (N=5); COV in parentheses. The RBA was calculated by dividing the percent of dose excreted in urine by the average percent of dose excreted in urine after administration of sodium arsenate in water by gavage for each animal.

### Relative Oral Bioavailability of Cadmium — Human Receptors

A juvenile swine model was used to assess the relative oral bioavailability of cadmium in soil from four sites with varying soil characteristics. Results indicate that soil-specific factors control the relative bioavailability of cadmium, and that the solubility of the predominant cadmium phases may be a more significant factor in controlling relative bioavailability than is particle size.

### Percutaneous Absorption of Arsenic from Soils — Human Receptors

Female Rhesus monkeys were selected for the research on percutaneous absorption of arsenic because of their ability to duplicate the biodynamics of percutaneous absorption in humans, and because previous studies of percutaneous arsenic absorption have used this same model. For the soluble dose, calculated absorption rates averaged 2.9% for the group. These results are consistent with earlier research that utilized a radioactive marker, and indicated that the research model was effective at detecting dermally absorbed arsenic without radiolabel. Converse to the results for soluble arsenic, data from dermal application of arsenic in soils indicate virtually no absorption, regardless of hydration level. Figure 1 demonstrates the results of the testing of percutaneous absorption of arsenic from soil in comparison to absorption of arsenic when administered as a soluble solution of sodium arsenate.

### Relative Oral Bioavailability of Metals — Ecological Receptors

The research conducted under the SERDP project involved the development of a novel animal model for assessing the relative bioavailability of metals from soil using the least shrew. Results indicate that the relative bioavailability of arsenic, cadmium, and lead ranged from 7% to 49%, 13% to 81%, and 21% to 60%, respectively. Cr(III) was not absorbed from soil, even at very high doses, and Cr(VI) was absorbed to a slight extent from a soil that was spiked with a high concentration of Cr(VI). Based on the study results, it is clear that arsenic, cadmium, and lead



are absorbed to varying extents from different soils in this shrew model, and that site-specific (or soil-specific) factors affect the relative absorption of the metals.

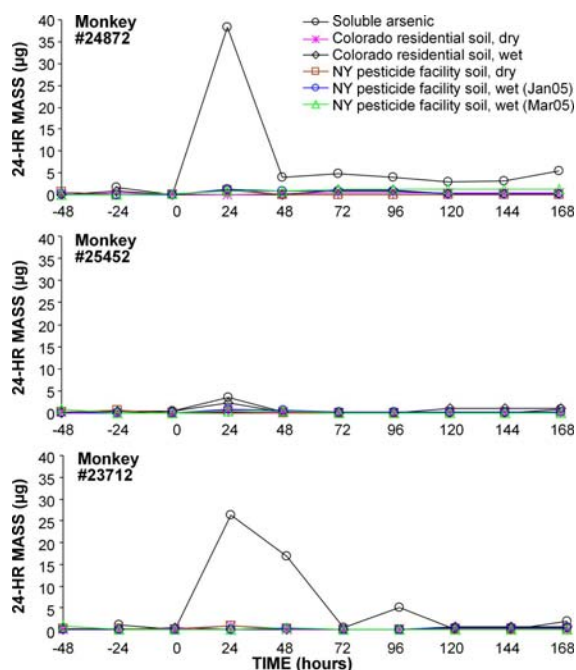


Figure 1. Dermal absorption of arsenic from solution or soils using the Rhesus monkey research model

### ***In Vitro* Research**

In order to evaluate the potential for development of an *in vitro* method for each receptor/pathway combination investigated in the *in vivo* research component of this project, soils were tested *in vitro* under a variety of conditions, and the results were evaluated for correlation to the *in vivo* results. The *in vivo* database for oral absorption of arsenic provided a robust database for assessing an *in vitro* approach for evaluating relative bioavailability of arsenic from soil, and indicated that existing *in vitro* methods likely overestimate RBA for many soil types, as predicted by the cynomolgus monkey. A better correlation was achieved by changing the extraction system, and the revised system can be used to assess soils on a site-specific basis. For dermal exposure to arsenic, the *in vivo* research indicates poor absorption of arsenic from soils, thus negating the need for an *in vitro* method for this route of exposure. For cadmium, *in vitro* methods were developed that were highly predictive of relative oral bioavailability measured in the swine. Similarly, highly specific extraction methods are required for assessing the RBA of metals in soils to ecological receptors.

### **Benefit:**

This research has established that there are soil- and site-specific factors that affect the bioavailability of metals from soils, for both human and ecological receptors. Addressing these issues in the site evaluation or risk assessment process will allow DoD personnel to produce more accurate exposure and risk estimates, which may diverge from what would be calculated using default assumptions, yet are still protective of human health and the environment. The *in vitro* methods developed as part of this research provide a useful tool for assessing possible RBA on a site-specific basis, in a manner that is not cost prohibitive.

### **Transition:**

The methods developed during this research are available for application. Research will continue, with a primary goal of gaining approval of these methods from regulatory agencies. Progress will be reported in the open literature.

Cleanup  
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*In vitro* extraction apparatus used to conduct bioaccessibility testing of soils

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

Stephen M. Roberts,\*<sup>1</sup> 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

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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

<sup>1</sup> 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 pelleted 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  $\mu\text{m}$ . 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- $\mu\text{m}$  sieved soil was stored in sealed containers at room temperature until utilized. The total arsenic concentration in an aliquot of the 250- $\mu\text{m}$  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  $\times$  1/4" 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  $\mu\text{g/l}$ .

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  $87.2 \pm 2.3\%$  (mean  $\pm$  SD,  $n = 3$ ) of arsenic added to the cage. Feces samples were collected in tared 7  $\times$  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  $\mu\text{g/l}$ . 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  $\mu\text{g/l}$  and 0.5  $\mu\text{g/g}$ , 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  $\mu\text{g/day}$ ) 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 ( $U_{\text{As}}$ ). The percent of arsenic dose recovered in urine ( $U_{\text{As,arsenate}}/\text{Dose}_{\text{As,arsenate}}$ ) 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 (Neter *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 <sup>a</sup>	0.3 <sup>a</sup>	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.

<sup>a</sup>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)			Mean ± SD
	0.25 mg As/kg bw	0.50 mg As/kg bw	1.0 mg As/kg bw	
% 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 <sub>2</sub> O <sub>3</sub> )	—	—	—	—	—	—	—	87.3	—	—	—	—	—	—
As (metals) oxide	6.4	—	—	—	—	—	30.0	0.2	—	—	—	—	—	—
As (metals) sulfate	—	7.5	—	—	—	—	—	—	—	—	—	—	—	—
Calcium arsenate (CaAsO <sub>4</sub> )	—	—	—	—	—	—	—	—	—	—	—	—	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 <sub>4</sub> )	23.1	9.3	—	2.3	—	—	1.4	0.1	76.7	64.8	—	—	0.5	—
Lead arsenate (PbAsO <sub>4</sub> )	—	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  $\mu$ m. 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 ( $\pm$  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.

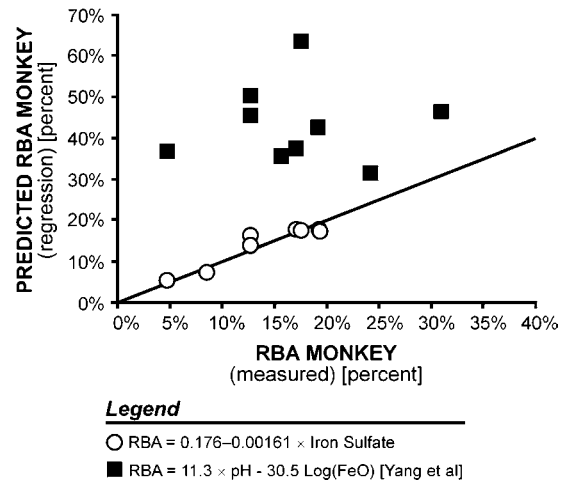
Table 5. For sodium arsenate–spiked soil, the average RBA was 0.94, while the RBA for arsenopyrite was 0.01 in one subject and < 0.01 in the other animals. These observations suggest that the model is capable of measuring arsenic RBA over the full range of potential values.

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.



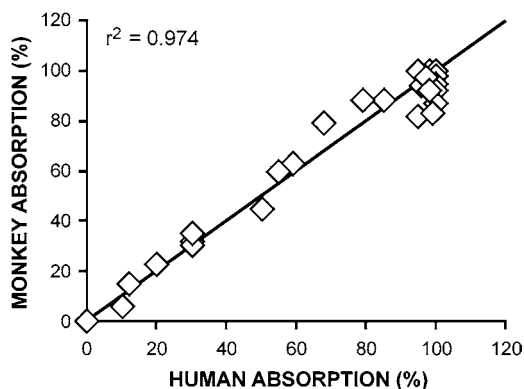


FIG. 2. Correlation of percentage oral dose absorbed between humans and monkeys for 43 drugs with a regression of equation of  $F_{aM} = 0.96F_{aH} + 2.8$ ;  $r^2 = 0.974$ . Complete absorption demonstrated by 27 drugs in both species. The depicted line has a slope of unity. From Chiou and Buehler (2002).

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,  $76.5 \pm 2.5\%$  (mean  $\pm$  SD) of the arsenic dose was recovered in urine and  $3.2 \pm 1.9\%$  was recovered in feces. Similarly, Cebus monkeys in the Florida study excreted  $66.8 \pm 6.5\%$  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 intra-subject; 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  $0.25 \pm 0.03$  in the Cebus vs.  $0.31 \pm 0.04$  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  $0.13 \pm 0.05$ ) 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 ( $0.17 \pm 0.08$ ). Arsenic RBA from an iron slag site soil sample (WISS) measured in the cynomolgus monkey ( $0.13 \pm 0.7$ ) 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.

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## *In Vivo* Percutaneous Absorption of Arsenic from Water and CCA-Treated Wood Residue

Ronald C. Wester,<sup>\*†1</sup> Xiaoying Hui,<sup>†</sup> Sherry Barbadillo,<sup>†</sup> Howard I. Maibach,<sup>†</sup> Yvette W. Lowney,<sup>‡</sup> Rosalind A. Schoof,<sup>§</sup> Stewart E. Holm,<sup>¶</sup> and Michael V. Ruby<sup>‡</sup>

<sup>\*</sup>University of California, San Francisco, Dermatology Department, PO Box 0989, San Francisco, California 94143-0989; <sup>†</sup>University of California, San Francisco, California 94143-0989; <sup>‡</sup>Exponent, Boulder, Colorado 80301; <sup>§</sup>Integral Consulting, Inc., Mercer Island, Washington; <sup>¶</sup>Georgia-Pacific Corporation, Atlanta, Georgia 30303-1847

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This study was conducted to evaluate the dermal absorption of arsenic from residues present on the surface of wood preserved with chromated copper arsenate (CCA). The research reported herein used methods parallel to those of earlier research on the dermal absorption of radiolabeled arsenic (R. C. Wester *et al.*, 1993, *Fund. Appl. Toxicol.* 20, 336–340), with modifications to allow use of environmental matrices that are not radiolabeled. These modifications include the surface area of application and dietary intake of arsenic, thus maximizing the potential for detection of dermally absorbed arsenic in exposed animals above diet-associated background levels of exposure. Two forms of arsenic were administered in this work. The first, arsenic in solution, was applied to the skin of monkeys to calibrate the model against prior absorption research and to serve as the basis of comparison for absorption of arsenic from CCA-treated wood residues. The second substrate was residue that resides on the surface of CCA-treated wood. Results from this research indicate that this study methodology can be used to evaluate dermally absorbed arsenic without the use of a radiolabel. Urinary excretion of arsenic above background levels can be measured following application of soluble arsenic, and absorption rates (0.6–4.4% absorption) are consistent with prior research using the more sensitive, radiolabeled technique. Additionally, the results show that arsenic is poorly absorbed from CCA-treated wood residues (i.e., does not result in urinary arsenic excretion above background levels).

**Key Words:** dermal arsenic absorption; CCA; arsenic exposure; environmental arsenic.

Prior research on the dermal absorption of soluble arsenic administered in water, and soluble arsenic mixed with soil, in Rhesus monkeys (Wester *et al.*, 1993) produced mean dermal absorption rates for soluble arsenic in the range of 2.0–6.4% of the applied dose. Percent absorption did not vary across five orders of magnitude in the applied dose. Also, in Wester *et al.*

(1993), the absorption rates for arsenic from the test soil fell within the range of the rates for percutaneous absorption of the arsenic administered in water. The research method was based on dermal application and subsequent urinary excretion of radiolabeled arsenic (As<sup>73</sup>), thereby permitting detection of very small amounts of absorbed arsenic in the urine. Subsequent to this research, questions arose as to whether the data on dermal absorption of soluble arsenic mixed with soil immediately prior to dermal application are representative of arsenic absorption from environmental media (U.S. EPA, 2001a). Specifically, this issue affects the ongoing discussion of dermal absorption of arsenic from wood treated with chromated copper arsenate (CCA). Currently, the U.S. EPA is evaluating whether children who repeatedly contact playground equipment or decks made from CCA-treated wood may face increased risks from the associated arsenic exposures (U.S. EPA 2001a, 2003). The U.S. EPA assessment currently relies on dermal arsenic absorption data generated for soluble arsenic and soluble arsenic mixed with soil, and may not be representative of exposures associated with contact with CCA-treated wood. This paper used a methodology similar to that used by Wester *et al.* (1993) to assess dermal arsenic absorption from the residues that would adhere to an individual's skin after contacting the surface of CCA-treated wood.

Among several challenges associated with studying exposure to arsenic from environmental media is the large degree of exposure to background levels of arsenic from the diet (Schoof, 1999a,b; Yost *et al.*, 2004). Typical daily urinary arsenic excretion for Rhesus monkeys consuming the standard diet of Purina monkey chow is 5–15  $\mu\text{g}$  As/day. In the Wester *et al.* (1993) research, the use of a radiolabeled arsenic source circumvented the confounding effects of concomitant dietary exposures and associated difficulties in data interpretation. For study of environmental samples (e.g., contaminated soils or treated wood), it is not practicable to use a radiolabeled source. Therefore, a new research protocol was designed, incorporating a low-arsenic diet. Urine samples were analyzed using inductively coupled plasma/mass spectrometry, which pro-

<sup>1</sup> To whom correspondence should be addressed. Fax: (415) 753-5304. E-mail: rcwgx@itsa.ucsf.edu.

vided an adequately low detection limit for total arsenic in urine. This alteration in the study design allows for a sensitive evaluation of dermal arsenic absorption from natural environmental media.

The research reported herein describes the use of the Rhesus monkey model to measure the dermal absorption of arsenic from water and from residues collected from the surface of CCA-treated wood. The Rhesus monkey is a relevant animal model for *in vivo* human percutaneous absorption (Wester and Maibach, 1975, 1989).

## MATERIALS AND METHODS

**Formulations and dosing rates.** An open crossover design was used, in which each animal is dosed in each of the trials (soluble arsenic in solution applied to the skin, CCA residue applied to the skin, and iv injection), with a washout period of at least 14 days between each dose. This design allows for each animal to serve as its own internal control.

The iv dose (1060  $\mu\text{g}$  arsenic/monkey) was administered as a solution of sodium arsenate heptahydrate in deionized (DI) water (2120 mg/l arsenic). For the iv dose, each monkey received 0.5 ml of the dosing solution injected into the saphenous vein. The iv dose was given while the monkeys were in their metabolic cages, so the monkeys did not spend any time in the metabolic restraint chairs, as they did with the topical doses.

For the soluble arsenic dose, arsenic was administered in water onto the monkey's skin at an application rate of 5  $\mu\text{l}/\text{cm}^2$  evenly applied across 100  $\text{cm}^2$  of skin, to achieve a total dermal dose of 1430  $\mu\text{g}$  arsenic (Table 1). The solution was prepared from sodium arsenate heptahydrate in DI water, which was acidified with 1% nitric acid (trace-metal grade). The soluble arsenic dose was designed to match the arsenic dose applied in the CCA-treated wood residue.

The CCA residue used in this study is the easily dislodgeable material present on the surface of CCA-treated wood, and was collected from the surface of CCA-treated wood that had been weathered in the environment. This represents the material that a human might contact during play or use of a CCA-treated wooden structure. Consideration was given to using an actual piece of CCA-treated wood in this research, but we elected to use the "collected residue" for the following reasons:

- If actual wood were used, it would be impossible to accurately characterize the dose of arsenic applied to the skin.

- There was concern that the environment of the skin (e.g., transdermal water loss, irritation) may be modified if a solid structure such as wood was applied directly.
- We could not ensure adequate wood-to-skin contact for a solid wood material. If the wood was not held in good contact with the skin, then the results would be biased low.
- Prior to using the "collected residue," we evaluated the chemical structure of arsenic in the residue and on the surface of CCA-treated wood (new and aged). These results indicate that the nature of the arsenic in the residue is identical to the arsenic on the surface of the wood (Nico *et al.*, 2003).
- Because the form of arsenic in the residue was the same as in the treated wood samples, and because use of the residue circumvented the issues associated with items 1–3 above, we determined that use of the residue provided the best study matrix.

The residue, in the form of a fine particulate, was supplied by the American Chemistry Council (ACC, 2003), and represents the material present on the surface of CCA-treated wood, which an individual might contact during use of, or play on, structures made of treated wood. In collecting the "residue" from the surface of the wood, efforts were made to collect the material on the surface of the wood that might be dislodged during direct human contact with the wood. Specifically, CCA-treated boards consisting of either Southern Yellow Pine or Ponderosa Pine were removed from in-service residential decks in Michigan and Georgia. Deck structures ranged from one to four years of age and had no coatings applied. Aged structures were selected, because they were believed to best represent the material that an individual might contact over time. As described below, recent chemical characterization work indicates that the chemical structure of the arsenic in the residue collected from the surface of decks is indistinguishable from the form of arsenic in newly treated or aged CCA-treated wood structures. A total of 1456 board sections (each 2 ft. long) were collected and shipped to Michigan State University, where the residue was collected as a single composite from multiple boards. The residue was collected by wiping the boards with a soft-bristle test-tube brush while rinsing with DI water. The rinsate and residue collected in this manner were filtered through glass wool, concentrated by rotary evaporation under vacuum at 46°C, and then air dried in a fume hood at 22°C and 65% humidity. The dried residue was irradiated using Cobalt-60 irradiation for 3 h, to eliminate possible microbial contamination of the sample.

Duplicate aliquots of the residue material used in the dermal dosing studies were analyzed for arsenic, chromium, copper, iron, and manganese concentrations, which involved digestion in refluxing nitric acid and analysis by inductively coupled plasma mass spectroscopy (ICP-MS; EPA Method 6010B; U.S. EPA, 1997). This analytical method was used to ensure adequate sensitivity for all metals of interest. As a means of comparing the composition of the

TABLE 1  
Arsenic Doses Given during This Study and Earlier Dermal Absorption Studies

Study	Concentration <sup>a</sup>	Volume <sup>b</sup>	Arsenic mass	
			Dosed ( $\mu\text{g}$ )	Per unit area ( $\mu\text{g}/\text{cm}^2$ )
Soluble dose	2860 mg/l <sup>c</sup>	0.5 ml	1430	14.3
CCA residue	3555 mg/kg <sup>c</sup>	400 mg	1422	14.2
Intravenous dose	2120 mg/l	0.5 ml	1060	—
Soluble dose (Wester <i>et al.</i> , 1993)				
High dose	—	0.06 ml	76	2.1
Low dose	—	0.06 ml	0.00086	0.000024

Note. —, not available or not applicable.

<sup>a</sup>Arsenic concentration in dosing material.

<sup>b</sup>Volume of dosing material administered.

<sup>c</sup>Average of duplicate analyses.



CCA residue with the composition of treated wood, samples (a 1-cm<sup>2</sup> wood chip from the top 0.2 cm of wood surface) of newly treated wood and a sample of weathered wood from a five-year-old CCA-treated residential deck were subjected to identical digestion and analyses.

For very fine soil (i.e., silty clay), a loading of 5.4 mg/cm<sup>2</sup> of skin results in a monolayer (U.S. EPA, 2001b). Because the residue appears similar in particle size distribution to silty clay, and a loading rate of 4 mg/cm<sup>2</sup> of the residue provides complete coverage on a flat surface, a loading rate of 4 mg/cm<sup>2</sup> was selected for this study. Application of 4 mg/cm<sup>2</sup> on 100 cm<sup>2</sup> of skin area resulted in a total dose of 1422 µg arsenic (Table 1). The residue was applied as a dry powder, and spread in an even layer across the exposure area.

**In Vivo Model.** Female Rhesus monkeys were selected for this research because of their ability to duplicate the biodynamics of percutaneous absorption in humans, and because previous studies of percutaneous arsenic absorption have used this same model. Prior research indicates that percutaneous absorption in the Rhesus monkey is similar to absorption in humans across a variety of chemicals and range of dermal penetration characteristics (Wester and Maibach, 1975). This research indicates that measurements from the monkey are just slightly higher than their counterparts in the human. Results from other species (pig, rat, rabbit) are not nearly as close to the values measured in humans, and indicate that, of the species tested, absorption in the monkey is closest to that in the human.

The monkeys were approximately 20 years old, which is the same approximate age as the monkeys used in the previous dermal arsenic absorption research (Wester *et al.*, 1993). The animals reside within the monkey colony maintained by the University of California, San Francisco, and have not been used for active research for 18 months. Prior to the beginning of the current series of studies, no topical doses had been applied to the skin of these animals for more than four years.

Each topical dose was applied to a pre-measured 100-cm area of abdominal skin of three monkeys. The dosing area was demarked by "masking" the boundaries with a single layer of Tegaderm (a water-vapor-permeable adhesive membrane available from 3M Health Care, St. Paul, MN) and then was dosed by spreading the fluid (5 µl/cm<sup>2</sup>) or residue (4 mg/cm<sup>2</sup>) evenly across the 100-cm<sup>2</sup> dosing area. The dosing area was then covered with a layer of Tegaderm to ensure that the material remained in contact with the skin. The Tegaderm patch over the dosing area extended well beyond the boundaries of the exposure area. In addition to the Tegaderm patch, the abdomen of each monkey was wrapped with Spandage Instant Stretch Bandage (MEDI-TECH International Corp., Brooklyn, NY) to ensure that the applied dose was kept in direct contact with the skin throughout the dosing period. This bandage is of a web construction; most of the Tegaderm was exposed to the open air for moisture and air exchange. Following application of the topical doses, the monkeys were placed in metabolic restraint chairs for the duration of the eight-h dosing period. The eight-h dosing period was selected to represent an upper bound of time that an individual might remain in contact with residues, and is also the upper limit of time that the monkey can remain in the metabolic restraint chair. During this time, the monkeys had free access to water, but were restricted from touching their abdominal area. Researchers remained in the room and interacted with the monkeys, and the monkeys were hand fed bananas and liquid diet during this stage.

Urine was collected during the 8-h dosing period in a pan under the metabolic chair. After 8 h, the monkeys were removed from the chairs, the Spandage bandage and Tegaderm patch were removed, and the applied doses were removed using a soap and water wash (50/50 v/v, soap and water, followed by water, soap, and two final water washes). The monkeys were then transferred to metabolic cages for continued urine collection over the following seven days.

As with humans, significant exposure to arsenic occurs from the normal diet (Schoof, 1999a,b; Yost, 2004). Urinary excretion of total arsenic for Rhesus monkeys on the standard diet of Purina Monkey Chow falls in the range of 5 to 15 µg/day—levels that would obscure accurate detection of the arsenic that might be absorbed following topical application of arsenic. Therefore, the monkeys were provided a low-arsenic diet (Primate Liquidiet from BioServe,

Inc.) for seven days prior to each dose. The powdered Liquidiet formulation also was prepared into meal bars, which were provided *ad libitum* to the monkeys during the research period (seven days prior to dosing through seven days after dosing). The diet was supplemented with pieces of banana and apple, which are both known to be low in total arsenic (Schoof *et al.*, 1999a). DI water was provided *ad libitum*. The liquid diet was provided as both liquid and solid forms. Preference was for the solid form. The monkeys maintained their body weight during the study.

The monkey urine samples were preserved with nitric acid (2%) at the time of collection, and shipped to Battelle Pacific Northwest Laboratories in Sequim, Washington, for analysis. At Battelle, the urine samples were acidified with an additional 2% (by volume) of concentrated nitric acid and analyzed for total arsenic by ICP/MS (Method 1638, U.S. EPA, 2002). This method provides a method detection limit (MDL) of approximately 0.1 µg/l arsenic in monkey urine. Quality assurance and quality control (QA/QC) samples included a method blank, duplicates, matrix spikes, and a laboratory control sample at a 5% frequency of analysis.

## RESULTS

Total metals concentrations of arsenic, chromium, and copper in the residue are presented in Table 2, along with corresponding data for a sample of newly treated wood (recently purchased from a local retailer), and a sample of weathered wood from a five-year-old residential deck. The relative concentrations of these three metals are similar for all three samples, indicating that the residue contains a proportion of the CCA metals that is similar to both freshly treated and aged wood. As expected, concentrations of all three metals are somewhat lower in the wood-chip samples than in residue. Although the residue is largely composed of decayed wood from the wood surface, larger wood fragments were removed from the sample during preparation of the residue, when the residue is filtered through glass wool. In contrast, the wood-chip samples contained a larger proportion of wood matter. More instructive is the ratio of the different metals from these analyses, which are similar across the samples.

Data for the mass of urinary arsenic excreted by the monkeys following dermal dosing are presented in Table 3 (soluble arsenic), Table 4 (CCA residue), and Table 5 (iv dose). Data on the background arsenic excretion for each monkey for the days prior to the dosing period are included. The value reported for the 0- to 24-h period is the combined arsenic mass from the urine collected during the 8-h dosing period, a wash of the

TABLE 2  
Metal Concentrations in CCA Residue and Wood

Sample	Arsenic (mg/kg)	Chromium (mg/kg)	Copper (mg/kg)
CCA residue			
Sample 1	3600	4120	2260
Sample 2	3510	4070	2220
Weathered CCA-treated wood	1760	2700	942
Freshly-treated CCA wood	2730 <sup>a</sup>	3080 <sup>a</sup>	1545 <sup>a</sup>

<sup>a</sup>Average of lab duplicates.

**TABLE 3**  
Urinary Arsenic Data following Dermal Application of  
Arsenic in Soluble Dose

	24-h Mass excreted	
	( $\mu\text{g}$ )	Corrected <sup>a</sup> ( $\mu\text{g}$ )
Animal 1		
Background		
24–48 h	5.07	0.00
0–24 h	1.56	0.00
0–24 h	41.58 <sup>b</sup>	35.50
24–48 h	7.22	1.13
48–72 h	8.08	1.99
72–96 h	7.21	1.12
Total arsenic mass excreted (0–96 h)		39.74
Total arsenic mass excreted with urinary arsenic excretion fraction correction (0–96 h)		48.41 <sup>c</sup>
Percent absorption (0–96 h)		3.4% <sup>d</sup>
Animal 2		
Background		
24–48 h	6.30	0.82
0–24 h	7.08	1.61
0–24 h	10.22 <sup>b</sup>	4.75
24–48 h	6.96	1.48
48–72 h	5.32	0.00
72–96 h	6.53	1.05
Total arsenic mass excreted (0–96 h)		7.28
Total arsenic mass excreted with urinary arsenic excretion fraction correction (0–96 h)		8.87 <sup>c</sup>
Percent absorption (0–96 h)		0.62% <sup>d</sup>
Animal 3		
Background		
24–48 h	5.20	1.79
0–24 h	3.07	0.00
0–24 h	30.35 <sup>b</sup>	26.94
24–48 h	20.98	17.56
48–72 h	4.52	1.10
72–96 h	9.16	5.75
Total arsenic mass excreted (0–96 h)		51.35
Total arsenic mass excreted with urinary arsenic excretion fraction correction (0–96 h)		62.55 <sup>c</sup>
Percent absorption (0–96 h)		4.4% <sup>d</sup>

<sup>a</sup>Corrected mass calculated by subtracting median of the eight background arsenic masses for each monkey. If corrected mass is calculated less than zero, corrected mass is set to zero.

<sup>b</sup>Sum of (0–8 h), pan wash, and (8–24 h). Pan wash concentration is calculated using pan wash concentration minus average of wash water concentrations.

<sup>c</sup>Calculated by correcting excreted mass for fractional excretion of arsenic from iv dose (i.e., 0.821 or 82.1%).

<sup>d</sup>Percent absorption calculated using soluble applied dose mass of 1430  $\mu\text{g}$ .

urine collection pan, and the urine collected from 8 h to 24 h after the monkeys were returned to their cages. The right-most column in each of these tables presents the mass of arsenic excreted for each 24-h period, corrected for background levels

**TABLE 4**  
Urinary Arsenic Data following Dermal Application of  
Arsenic in CCA Residue

	24-h Mass excreted	
	( $\mu\text{g}$ )	Corrected <sup>a</sup> ( $\mu\text{g}$ )
Animal 1		
Background		
96–120 h	7.88	1.79
48–72 h	6.44	0.35
0–24 h	5.73	0.00
0–24 h	4.84 <sup>b</sup>	0.00
24–48 h	4.90	0.00
48–72 h	4.86	0.00
72–96 h	5.89 <sup>c</sup>	0.00
Total arsenic mass excreted (0–96 h)		0.00
Total arsenic mass excreted with urinary arsenic excretion fraction correction (0–96 h)		0.00 <sup>d</sup>
Percent absorption (0–96 h)		0.00% <sup>e,f</sup>
Animal 2		
Background		
96–120 h	5.79	0.32
48–72 h	1.92	0.00
0–24 h	4.59	0.00
0–24 h	4.17 <sup>b</sup>	0.00
24–48 h	2.93	0.00
48–72 h	3.77	0.00
72–96 h	3.78 <sup>c</sup>	0.00
Total arsenic mass excreted (0–96 h)		0.00
Total arsenic mass excreted with urinary arsenic excretion fraction correction (0–96 h)		0.00 <sup>d</sup>
Percent absorption (0–96 h)		0.00% <sup>e,f</sup>
Animal 3		
Background		
96–120 h	4.40	0.99
48–72 h	4.88	1.47
0–24 h	3.44	0.03
0–24 h	4.24 <sup>b</sup>	0.83
24–48 h	3.26	0.00
48–72 h	3.94	0.53
72–96 h	3.39 <sup>c</sup>	0.00
Total arsenic mass excreted (0–96 h)		1.37
Total arsenic mass excreted with urinary arsenic excretion fraction correction (0–96 h)		1.66 <sup>d</sup>
Percent absorption (0–96 h)		0.12% <sup>e,f</sup>

<sup>a</sup>Corrected mass calculated by subtracting median of the eight background arsenic masses for each monkey. If corrected mass is calculated less than zero, corrected mass is set to zero.

<sup>b</sup>Sum of (0–8 h), pan wash, and (8–24 h). Pan wash concentration is calculated using pan wash concentration minus average of wash water concentrations.

<sup>c</sup>24-h mass excreted is estimated as 1/4 of 72–168 h sample mass.

<sup>d</sup>Calculated by correcting excreted mass for fractional excretion of arsenic from iv dose (i.e., 0.821 or 82.1%).

<sup>e</sup>Percent absorption calculated using CCA residue applied dose mass of 1422  $\mu\text{g}$ .

<sup>f</sup>Not statistically different from background.

TABLE 5  
Urinary Arsenic Data following Intravenous Arsenic Dose

	24-h Mass excreted	
	$\mu\text{g}$	Corrected <sup>a</sup> ( $\mu\text{g}$ )
Animal 1		
Background		
96–120 h	5.14	0.00
48–72 h	8.64	2.55
0–24 h	7.10	1.01
0–24 h	767.28 <sup>b</sup>	761.19
24–48 h	65.88	59.79
48–72 h	19.54 <sup>c</sup>	13.45
72–96 h	19.54 <sup>c</sup>	13.45
Total arsenic mass excreted (0–96 h)		847.88
Percent absorption (0–96 h)		80.0% <sup>d</sup>
Animal 2		
Background		
96–120 h	5.16	0.00
48–72 h	7.26	1.79
0–24 h	4.54	0.00
0–24 h	761.84 <sup>b</sup>	756.36
24–48 h	80.45	74.97
48–72 h	24.60 <sup>c</sup>	19.13
72–96 h	24.60 <sup>c</sup>	19.13
Total arsenic mass excreted (0–96 h)		869.59
Percent absorption (0–96 h)		82.0% <sup>d</sup>
Animal 3		
Background		
96–120 h	2.25	0.00
48–72 h	2.91	0.00
0–24 h	3.38	0.00
0–24 h	706.09 <sup>b</sup>	702.68
24–48 h	123.50	120.09
48–72 h	38.68 <sup>c</sup>	35.26
72–96 h	38.68 <sup>c</sup>	35.26
Total arsenic mass excreted (0–96 h)		893.29
Percent absorption (0–96 h)		84.3% <sup>d</sup>

<sup>a</sup>Corrected mass calculated by subtracting median of the eight background arsenic masses for each monkey. If corrected mass is calculated less than zero, corrected mass is set to zero.

<sup>b</sup>Sum of (0–8 h), cage wash, and (8–24 h). Cage wash concentration is calculated using cage wash concentration minus average of wash water concentrations. [iv-dosed monkeys did not use the metabolic chair, and the cage wash was collected from below the cages after collection of the (0–8 h) sample.]

<sup>c</sup>24-h mass excreted is estimated as  $\frac{1}{2}$  of 48–96 hr sample mass.

<sup>d</sup>Percent absorption calculated using intravenous dose of 1060  $\mu\text{g}$ .

of arsenic in urine by subtracting out the median of the eight background data points for each monkey, on a monkey-specific basis. (In other words, the eight background values for each monkey were compiled, and the median was calculated for each monkey. The median values of 6.09, 5.48, and 3.41  $\mu\text{g}$  arsenic/24-h period for monkeys 1, 2, and 3, respectively, were subtracted out of the 24-h urine value to yield “background-corrected” values.) The median value was selected because it is

the best representation of the central tendency of background urinary arsenic excretion over time, and is less sensitive to potential outlier effects (Fig. 1). This correction was applied to the data to reduce the influence of dietary arsenic on the excreted arsenic mass. The mass of arsenic excreted that is associated with the dermally applied dose is calculated by adding the mass excreted from the time of dosing through 96 h after dosing. After 96 h, the arsenic excretion has returned to background levels.

Prior research indicates that for female Rhesus monkeys, urinary excretion of an iv dose of arsenic was  $80 \pm 6.7\%$  of the administered dose (Wester *et al.*, 1993). The iv dose given during this study resulted in  $82.1 \pm 2.2\%$  of the administered arsenic dose excreted in urine (Table 5). The average urinary arsenic excretion value from this study (82.1%) was used to adjust the assumed total mass of arsenic excreted over the 96-h collection period, by dividing the calculated mass excreted by 0.821. This correction is intended to account for the fraction of arsenic that might be retained within the body or excreted by other routes (e.g., feces). This calculated mass excreted was then divided by the applied dose to calculate the percent of the applied dose that was absorbed for each animal and each dosing substrate. The percent absorption of arsenic was calculated in the following manner:

$$\text{Percent absorption} = \frac{(\text{Corrected mass excreted}_{0-96 \text{ hours}} \div \text{Urinary Excretion Fraction})}{\text{Applied dose}} \times 100 \quad (1)$$

For the soluble dose, absorption rates were 3.4, 0.62, and 4.4% for the three monkeys in the study (Table 3). Dosing levels used in our earlier research on the dermal absorption of arsenic are compared to those used in this study in Table 1. Despite the nearly seven-fold difference in the dermal loading rate between the two studies, the average absorption rate for the group dosed with soluble arsenic (2.8%) is consistent with results from Wester *et al.* (1993) (Table 6). These results are consistent with the previous study, wherein absorption rates were relatively consistent (range of 2–6.4%) despite a five-orders-of-magnitude change in the dose levels (i.e., an applied dose range of 0.000024 to 2.1  $\mu\text{g}/\text{cm}^2$ ). These data strongly support the suggestion that the difference in the measured absorption rates in the Wester *et al.* (1993) research reflects experimental variability rather than dose-related differences in absorption (U.S. EPA, 2001b). This is consistent with our understanding of individual variability in percutaneous absorption in humans and animals (Wester and Maibach, 1991, 1997).

Converse to the results for soluble arsenic, data from dermal application of CCA residue indicate virtually no absorption. Absorption rates following dermal application of residue are presented in Table 4. These data show that urinary excretion of arsenic following dermal application of the CCA residue does



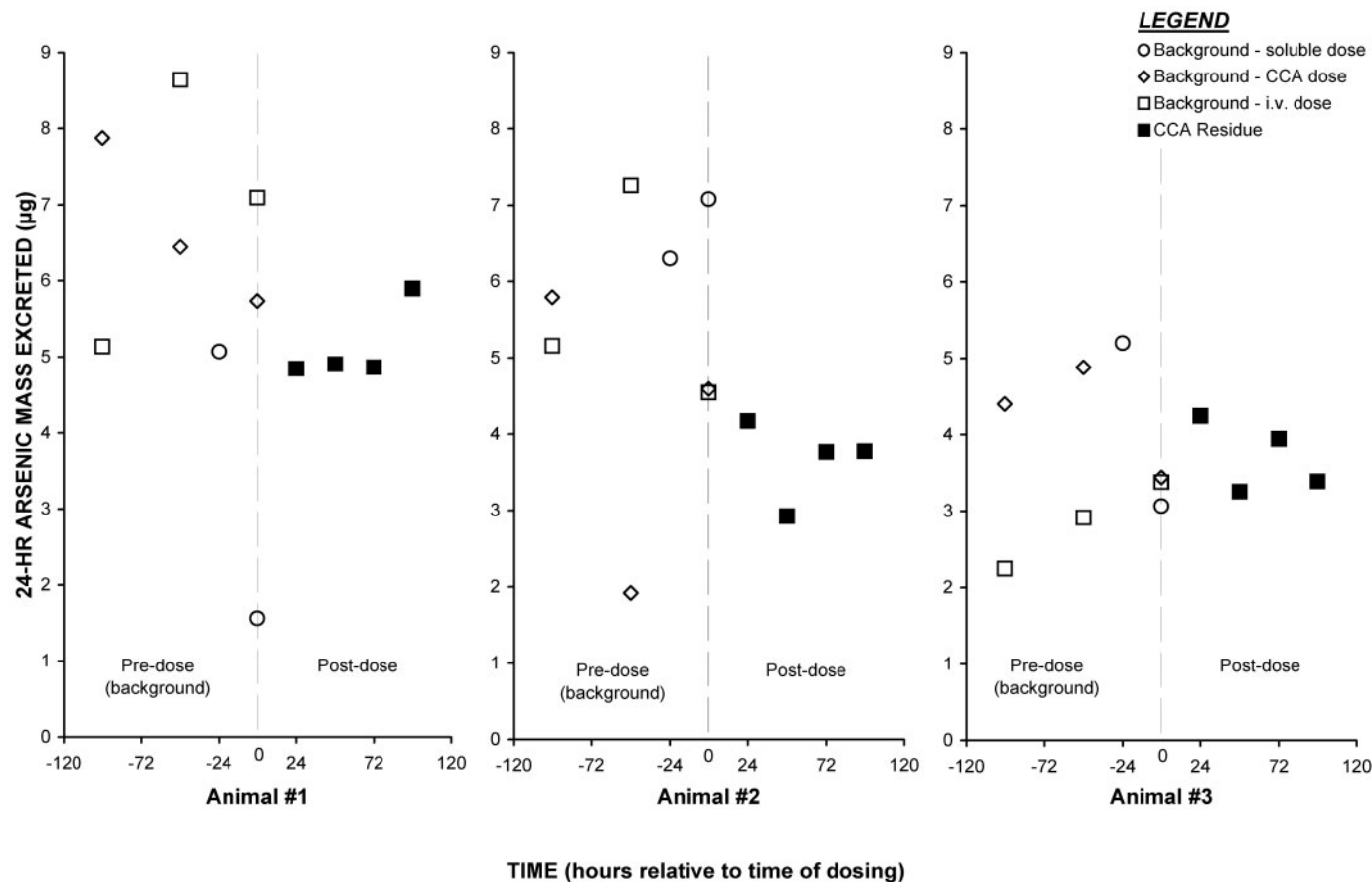


FIG. 1. Background urinary arsenic mass excretion in comparison to excretion following dosing with CCA residue.

not cause a detectable increase in urinary arsenic excretion, despite the fact that equivalent doses of arsenic were applied for both soluble arsenic and residue.

TABLE 6

Summary of Dermal Arsenic Absorption Values from Various Dosing Substrates

Substrate	Percent absorption	
	Average $\pm$ SD	(Range)
Soluble dose	2.8 $\pm$ 1.9	(0.62–4.4)
CCA residue	0.04 $\pm$ 0.07 <sup>a</sup>	(0.00–0.12)
Wester <i>et al.</i> (1993)		
Soluble		
Low dose	6.4 $\pm$ 3.9	—
High dose	2.0 $\pm$ 1.2	—
Soluble mixed with soil		
Low dose	4.5 $\pm$ 3.2	—
High dose	3.2 $\pm$ 1.9	—

Note. —, not available or not applicable.

<sup>a</sup>Not statistically different from background for any monkey.

The time profiles for urinary arsenic excretion by each monkey are provided in Figure 2. These charts show a consistent time course for the three monkeys; peak excretion of arsenic occurs within 24 h of the dermal application of the soluble dose, with a rapid return to near-background levels of excretion within 48 to 72 h. Peak 24-h urinary arsenic excretion following the soluble dose ranged up to a maximum value of 41.6  $\mu\text{g}$ . The time profile for arsenic excretion following dermal application of the CCA residue is also consistent across all three monkeys. Figure 2 depicts that, following application of the CCA residue, there is no increase in urinary arsenic excretion, followed out through time.

Because the number of animals that can be used in primate research is constrained, the crossover study design—wherein each individual animal is dosed in each dose group, and data from each individual monkey can be used as its own “comparison control”—was specifically selected for use in this research. This study design optimizes the potential to observe statistically significant results despite the small sample size. It does necessitate, however, use of specific statistical approaches that are consistent with the study design. To determine whether the difference in the

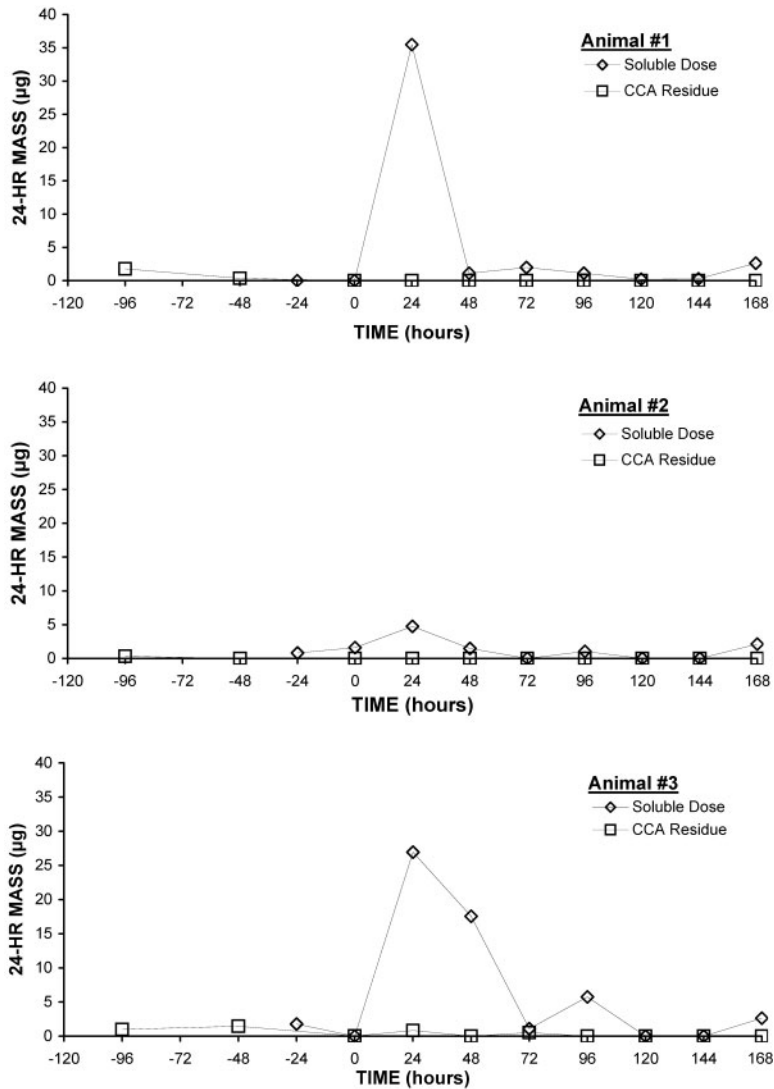


FIG. 2. Urinary arsenic mass excretion in 24-h increments.

results for the two dermal exposure groups was statistically different from background or from each other, an ANOVA analysis followed by a Tukey's multiple comparison test was conducted. In a study with a small number of animals, the variability between animals could be greater than the differences in absorption for different treatment groups; thus, statistical differences should be assessed after accounting for overall differences between monkeys. Because of the sequential nature of the data generated (i.e., at specified time points after dosing), analyses must also account for any time-dependent patterns present over the sampling period evaluated (e.g., comparing data within a given timepoint). The ANOVA model used to evaluate these data included factors for monkey, time, and treatment group. The factor for monkey controls for inter-monkey differences in mass excreted, allowing each monkey to serve as its own control. Monkey number was included as a random factor, because the monkeys tested were not specifically of interest but

rather a random selection of monkeys. In order to incorporate the sampling order, time period was included in the ANOVA model as an ordered factor. After accounting for monkey and time period differences, the treatment factor (i.e., soluble or residue dose group) was assessed for significance and followed by Tukey's multiple comparison test to identify which treatments are different from one another, using an overall significance level of 0.05 or 95% confidence. Results indicate that the urinary arsenic excretion levels in the animals exposed to the CCA residue are not statistically greater than background. This is also depicted in Figure 1, which shows a scatter plot of the daily urinary excretion values for each monkey, including background urinary excretion for each animal (i.e., prior to dosing trials), in comparison to the daily urinary excretion following exposure to the CCA residue. This figure demonstrates that the range of daily urinary excretion following exposure to CCA residue falls well within the range of background

urinary arsenic excretion. Conversely, the urinary arsenic excretion in the animals exposed to soluble arsenic in solution is significantly greater than background, and significantly greater than the residue exposure group.

## DISCUSSION

The results from this research indicate that the methodology described above can be used to evaluate dermally absorbed arsenic from environmental samples. The development of this method was challenging because of the high degree of background arsenic exposure from the diet, and the potential for that background exposure to obscure any signal from a dermally applied dose. Use of the low-arsenic diet resulted in an approximately four-fold decrease in urinary arsenic excretion relative to the standard primate diet, and allowed for detection above background of a dermally applied dose of arsenic.

Although the results indicate that the urinary arsenic levels following topical administration of arsenic in CCA residues are not distinguishable from background, the non-zero values for background urinary arsenic excretion, and the variability of the measured background values, impose some limits regarding the sensitivity of the model to detect an absorbed dose. A statistical evaluation using a comparison of means (*t*-test) for our data indicates that the absorbed dose would need to be in the range of 0.10 to 0.16% of the applied dose, at the dosing levels used in this study, for daily arsenic excretion levels to be detectable above background. Thus, while these data suggest that there may not be any dermal absorption of arsenic from CCA residue (no monkey demonstrated urinary arsenic excretion that was statistically different from background), the uncertainty associated with this research model tells us that dermal absorption of arsenic from CCA residues is at least an order of magnitude lower than absorption of soluble arsenic from solution.

Extensive chemical analyses indicate that the arsenic present in the CCA residue used in this study is structurally and chemically identical to the arsenic present on the surface of newly treated or aged CCA-treated wood (Nico *et al.*, 2003), thus making it an appropriate study substrate for understanding the potential dermal absorption of arsenic following contact with CCA-treated wood. The negligible absorption of arsenic from the CCA residues derives from the fact that this arsenic is chemically bound with other metals (particularly chromium) and ultimately to the wood structure (Bull, 2001; Nico *et al.*, 2003). The physico-chemical conditions on the surface of the skin do not result in the liberation of arsenic from the residue, thus precluding absorption. These results indicate that percutaneous absorption of arsenic from environmental media can be significantly different from soluble arsenic or even soluble arsenic mixed with environmental media (Table 6). Therefore, it is not appropriate to apply generic assumptions regarding dermal absorption to these unique matrices, and medium-

specific analysis may be required to understand the dermal absorption from them (and potential associated risks). This appears to be true for arsenic, and may be true for other metals that form similarly stable complexes in the environment. The latter point should not be overgeneralized until additional metals have been thoroughly studied.

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## Dermal Absorption of Arsenic from Soils, as Measured in the Rhesus Monkey

Yvette W. Lowney, Exponent, Boulder, Colorado (corresponding author)  
Ronald C. Wester, Department of Dermatology, University of California, San Francisco  
Rosalind A. Schoof, Integral Consulting, Mercer Island, WA  
Colleen A. Cushing, Exponent, Chicago, IL  
Melanie Edwards, Exponent, Bellevue, WA  
and Michael V. Ruby, formerly with Exponent, Boulder, CO

### Abstract

Regulatory agencies have relied on dermal absorption data for soluble forms of arsenic as the technical basis for specific absorption values that are used to calculate exposure to arsenic in weathered soil. These evaluations indicate that percutaneous absorption of arsenic from soil ranges from 3.2 to 4.5% of the dermally applied dose, based on studies of arsenic freshly mixed with soil. When this value is incorporated into risk assessments and combined with other assumptions about dermal exposures to soil, the conclusion is often that dermal exposure to arsenic from soil may contribute significantly to overall exposure to arsenic in soil.

Prior characterization research has indicated that the solubility of arsenic in soil varies, depending on the provenance of the soil, the source of the arsenic, and the chemical interaction of arsenic with other minerals present within the soil matrix. Weathering produces forms of arsenic that are more tightly bound within the soil and less available for absorption. Our research expands on prior *in vivo* studies to provide insights into the potential for dermal absorption of arsenic from the more environmentally relevant substrate of soil. Specifically, two soils with very high concentrations of arsenic were evaluated under two levels of skin hydration. One soil, containing 1,400 mg/kg arsenic, was collected adjacent to a pesticide production facility in New York. The other soil, containing 1,230 mg/kg arsenic, was collected from a residential area with a history of application of arsenical pesticides. Although the results of this research are constrained by the small study size dictated by the selection of an animal research model using monkeys, the statistical power was optimized by using a “crossover” study design, wherein each animal could serve as its own comparison control. No other models (animal or *in vitro*) were deemed adequate for studying the dermal absorption of soil arsenic.

Our results show dermal absorption of soluble arsenic in solution to be  $4.8\% \pm 5.5\%$ , which is similar to results reported earlier for arsenic in solution (and used by regulatory agencies in recommendations regarding dermal absorption of arsenic). Conversely, absorption following application of arsenic in the soil matrices resulted in mean estimated arsenic absorption of 0.5% or less for all soils, and all individual estimates were less than 1%. More specifically, following application of arsenic-bearing soils to the abdomens of monkeys, urinary arsenic excretion could not be readily distinguished from background. This was true across all five soil dosing trials, including application of the two soils dry, and three trials with wet soil. These findings are consistent with our understanding of the environmental chemistry of arsenic, wherein arsenic can be present in soils in complexed mineral forms. This research addresses an important component involved in estimating the true contribution of percutaneous exposures to arsenic in soil relative to exposures via ingestion. Our findings suggest that dermal absorption of arsenic

from soil is truly negligible, and that EPA's current default assumption of 3% dermal absorption of arsenic from soils results in significant overestimates of exposure.

## **Background**

Regulatory agencies have relied on dermal absorption data (developed by Wester et al. 1993) for soluble forms of arsenic as the technical basis for specific absorption values that are used to calculate exposure to arsenic in weathered soil (U.S. EPA 2004). These evaluations indicate that percutaneous absorption may contribute significantly to overall exposure to arsenic in soil (U.S. EPA 2001). However, data from biomonitoring studies of human populations exposed to arsenic from environmental sources suggest that percutaneous absorption of arsenic does not contribute significantly relative to other pathways of exposure (i.e., ingestion or inhalation) (Walker and Griffin, 1998). This apparent discrepancy suggests the need for new research using more relevant substrates.

To that end, recent research indicates that the percutaneous absorption of arsenic from soil and other solid environmental media can be different from the absorption of arsenic from solution, or arsenic freshly mixed with soil. Data from research with Rhesus monkeys indicated *in vivo* percutaneous absorption of arsenic from water, or from arsenic in water freshly mixed with soil, ranges from 2.0% to 6.4%, with no statistical difference in absorption across the five-order-of-magnitude concentration range that was tested. Parallel research using human cadaver skin indicated a lower fraction absorbed of 0.76% (Wester et al., 1993). Research conducted on the residue collected from the surface of preserved wood indicated negligible percutaneous absorption of arsenic from this matrix (Wester et al., 2004). Subsequent evaluations indicated that the arsenic present at the surface of treated wood exists in a complexed form with a stable molecular structure (Nico et al., 2004).

The element arsenic is considered to be a metalloid, and occurs naturally in primary minerals that are found in soils, such as the metal-arsenate minerals (Sposito, 1989). It may also occur as arsenopyrite (FeAsS), which is associated with the mining of sulfide ore deposits (Prinz et al., 1978). Also, salts of methanearsenic acid (MA) or cacodylic acid (CA) can occur as a result of past herbicide use (EPRI, 1984), but these salts typically weather in the soil matrix, becoming incorporated into more stable secondary mineral phases (Cances et al., 2005; Fendorf et al., 1997; Kneebone et al., 2002; Ruby et al., 1999).

The solubility of arsenic in soil can vary, depending on the provenance of the soil, the source of the arsenic, and the chemical interaction of arsenic with both primary and secondary minerals present within the soil matrix. The main chemical factor that controls the availability of arsenic in soil is that it can co-precipitate with metal oxides through the process of adsorption, by forming a homogeneous mixed-solid phase with a metal oxide at the host-mineral/soil-solution interface (Sposito, 1989). This process produces a more tightly bound, less available arsenic form. Mineralogical analyses of soils (Drexler, 2005) have shown arsenic to be present with these secondary metal oxide minerals.

Arsenic in solution occurs as an arsenite or arsenate oxyanion in the +3 or +5 oxidation state (Lytle et al. 2004) and it remains in solution until the solubility product for the arsenate species

is exceeded. Of the various forms of arsenic that can exist, dissolved arsenic is expected to have the greatest potential for absorption through skin, whereas mineral forms of arsenic in soil may have much lower absorption potential. A soluble arsenic species freshly mixed with soil may exhibit variable behavior, depending on several soil-specific factors, including arsenic concentration, water content, mixing procedures, organic carbon content, the amount of metal oxides that are available in the soil, the pre-test incubation time, and the binding kinetics (Ruby et al., 1999; Sarkar and Datta, 2004; Yang et al., 2002, 2005; Fendorf et al., 2004; Pouschat and Zagury (2006); Zhang and Selim, 2005).

Despite the understanding that mineral forms of arsenic in soil behave much differently from arsenic in solution or freshly mixed with soil, current guidance from regulatory agencies recommends the use of an absorption fraction of 0.03 for dermal absorption of arsenic from soil (U.S. EPA, 2004), a value based on the early research on soluble arsenic freshly mixed with soil.

The research reported herein expands on prior *in vivo* studies using the Rhesus monkey research model (Wester et al., 1993, 2004), to provide insights into the potential for dermal absorption of arsenic from the more environmentally relevant substrate of soil. Specifically, two soils have been evaluated under two levels of skin hydration. Results from application of the soils are presented, along with findings regarding dermal absorption of arsenic following application in solution.

## **Methods and Materials**

***In vivo* research model:** The *in vivo* model used in this research is discussed in detail in Wester et al. (2004), including dosing procedures and the relevance of the Rhesus monkey model to understanding dermal absorption by humans; therefore, the model is described only in summary fashion herein. Female Rhesus monkeys, approximately 20 years old, were selected for this research. The animals reside within the monkey colony maintained by the University of California, San Francisco, and had not been used in active research for 18 months prior to this effort. No topical doses had been applied to the skin of these animals for more than 4 years. All research protocols were approved by the U.S. Department of Health and Human Services, Office of Laboratory Animal Welfare, and work conducted under the review of the Institutional Animal Care and Use Committee of the University of California, San Francisco. The *in vivo* model used an open-crossover design, in which the abdomens of female Rhesus monkeys are exposed to soil with elevated arsenic concentrations or to arsenic in solution. The crossover design, wherein each individual animal is dosed in each dose group, allow data from each individual monkey can be used as its own “comparison control.” This design provides greater power to observe statistically significant results despite the small sample size dictated by primate research. Before and during the dose applications, the monkeys were maintained on a low-arsenic diet to allow adequate detection of percutaneously absorbed arsenic, which would otherwise be obscured due to normal dietary sources of inorganic arsenic. The low-arsenic diet (Primate Liquidiet from BioServe, Inc.) was provided *ad libitum* for 7 days prior to each dose, continuing through 7 days after dosing. Between dosing trials, the monkeys were maintained on the standard diet of Purina Monkey Chow.



Each topical dose was applied to a pre-measured 100-cm<sup>2</sup> area of abdominal skin. The dosing area was demarked by “masking” the boundaries with a single layer of Tegaderm™ and then was dosed by spreading the fluid or soil evenly across the dosing area. (Tegaderm™ is a transparent and breathable medical bandage manufactured by 3M Corporation. It is available in sheets that are large enough to cover the entire dosing area, retaining the soil dose in place without an occlusive barrier.) The dosing area was then covered with Tegaderm™, and the abdomen of each monkey wrapped with Spandage Instant Stretch bandage to ensure that the material remained in contact with the skin. To prevent contact with and possible removal of the dosed material, the monkeys were maintained in metabolic restraint chairs for the duration of the 8-hour dosing period. Following the dosing period, the applied doses were removed using a soap and water wash (50:50 v/v soap and water, followed by water, soap and water, and two final water washes), and the animals were returned to metabolism cages. Urine was collected from three days prior to the time of dosing (for characterization of pre-dosing urinary arsenic), during dosing, and through day 7 following dosing.

Monkey urine samples were preserved with nitric acid at the time of collection and shipped to Battelle Pacific Northwest Laboratories in Sequim, Washington for analysis. At Battelle, the urine samples were analyzed for total arsenic by ICP/MS (Method 1638, U.S. EPA, 2002a) with a method detection limit of approximately 0.1 µg/L.

Arsenic absorption is calculated based on urinary excretion of total arsenic, corrected for urinary arsenic excretion fraction determined from intravenous dosing of sodium arsenate heptahydrate. The iv dose (1060 µg arsenic/monkey) was administered as 0.5 mls of a solution of sodium arsenate heptahydrate in dionized water (2120 mg/l arsenic). Implicit in this approach is the assumption that the urinary excretion fraction of the iv dose is applicable to a dermally-absorbed dose. Although this remains an area of uncertainty, research in this laboratory, using radiolabeled arsenic (Wester et al., 1993), suggests that this is a reasonable assumption for assessing the urinary excretion fraction of any absorbed dose of arsenic. For assessing the absorption of arsenic from solution, sodium arsenate was applied at 5 µL/cm<sup>2</sup> for a total dermal dose of 1,305 or 1,430 µg arsenic for the first and second dosing trials, respectively. The analyses reported herein utilize the pre-existing data (Wester et al., 2004), as well as an additional dermal dosing trial that was conducted with arsenic in solution.

**Study substrates:** The soils evaluated in this research were surficial soil samples collected from areas known through previous sampling to contain substantial arsenic concentrations (i.e., >1,000 mg As/kg soil). One sample, containing arsenic concentrations of 1,400 mg/kg, was collected adjacent to a pesticide production facility in New York that had historically produced inorganic arsenical pesticides. The other sample, containing arsenic concentrations of 1,230 mg/kg, was collected from a residential area in Denver, Colorado, with a history of application of the herbicide PAX (composed of 25.11% arsenic trioxide and 8.25% lead arsenate), among other potential arsenic sources. The New York pesticide facility sample was collected from the top 6 inches of soil, and the Colorado residential sample was a composite of 40 discrete samples collected from 0–2 inches. Studies of the relative oral bioavailability of arsenic from 14 soils (Roberts et al., 2007), demonstrated these soils to be in the middle or high end of observed relative oral arsenic bioavailability values. Although the factors affecting the release of arsenic from soil would be less aggressive at the skin surface than in the acidic



environment of the gastrointestinal tract, it might be assumed that the soils included in this study can provide representative, or higher end estimates of dermal absorption of arsenic from soil.

The soils were air-dried at  $<30^{\circ}\text{C}$ , sieved through a 2-mm screen, and then thoroughly mixed. An aliquot of each soil was retained for future reference, and the remainder was sieved to less than  $150\ \mu\text{m}$  (U.S. Standard Sieve Mesh No. 100) using a Meinzer Sieve Shaker, Norcross GA (Fisher Scientific). The  $150\text{-}\mu\text{m}$  soil fraction was stored in sealed containers at room temperature. Duplicate aliquots of the sieved soils used in the dermal dosing studies were analyzed for arsenic and other metals with digestion in refluxing nitric acid and analysis by inductively coupled plasma mass spectroscopy (ICP-MS; EPA Method 6010B, U.S. EPA, 1997). Additionally, soils were evaluated for arsenic mineralogy by electron microprobe analysis (EMPA) using standard methods (Brattin et al., 2004; Drexler and Brattin, 2007) at the Department of Geological Sciences, University of Colorado, Boulder. The very fine particle size fraction ( $<150\ \mu\text{m}$ ) was selected for study, because the fines are the soil fraction that would be expected to adhere to the surface of the skin, and because the smaller particle size has a larger surface area from which absorption may occur.

The relation between arsenic concentrations in different particle size fractions can be very site specific (SERDP, 2005; U.S. EPA, 1997). For the soils included in this research, arsenic was enriched in the smaller particle size fraction of one soil, but not the other. Specifically, for the New York soil, arsenic concentrations in the  $<2\text{-mm}$ ,  $<250\text{-}\mu\text{m}$ , and  $<150\text{-}\mu\text{m}$  particle size fractions were 1,500 mg/kg, 1,665 mg/kg, and 1,400 mg/kg, respectively, indicating no arsenic enrichment in the smaller fraction. For the Colorado residential soil, arsenic concentrations in the  $<250\text{-}\mu\text{m}$  and  $<150\text{-}\mu\text{m}$  particle size fractions were 869 mg/kg and 1,230 mg/kg, respectively, suggesting that for this site, the smaller particle size fraction is enriched with regard to arsenic concentrations.

For very fine soil (i.e., silty clay), a loading of  $5.4\ \text{mg}/\text{cm}^2$  on skin results in a monolayer of soil at the skin surface (U.S. EPA, 2001). In order not to exceed a monolayer of application, a dermal soil loading of  $4\ \text{mg}/\text{cm}^2$  was selected for the study. This soil application load resulted in total doses of  $560\ \mu\text{g}$  arsenic and  $492\ \mu\text{g}$  arsenic for the New York and Colorado soils, respectively (Table 1). In order to investigate whether arsenic dissolution from soil and/or dermal absorption may be controlled by the hydration level of the skin, each soil was evaluated both wet and dry. In all cases, the soil was applied dry, and spread in an even layer across the exposure area prior to being covered. To study the soils wet, once the soil was spread on the skin, a fine spray mist was used to wet the soil in place. Misting was conducted to add 20% to 30% moisture to the soil, resulting in moist soil but no free water that might run off of the dosing area.

**Methodological Changes from Prior Work:** The research that forms the basis of current regulatory guidance regarding percutaneous absorption of arsenic (Wester, 1993) was carried out in our laboratories, utilizing the same animal model (i.e., Rhesus monkey), and a similar sample size (i.e.  $n=3$  or  $4$ ). Sensitivity to absorbed and excreted arsenic was ensured in the 1993 research by use of a radiolabeled arsenic source. A significant change in these more recent

studies, in comparison to Wester 1993, is that the new study design was specifically tailored to evaluate environmental substrates, rather than constructed substrates of soil mixed with radiolabeled arsenic. In order to accomplish this, background exposures to arsenic from the diet were minimized, to allow detection of an absorbed dose. Additional changes implemented in this more recent effort were to use a larger skin surface area, and to use Tegaderm and a stretch bandage as a superior method of retaining the soil in place at the skin surface. Although the nature of primate research constrained the number of animals that could be dosed in these study trials, the research reported herein reflects a study design with fewer methodological limitations than the earlier research, and incorporates more relevant study matrices (i.e., environmental soils rather than soluble arsenic or soluble arsenic freshly mixed with soil).

The values reported herein for percutaneous absorption of arsenic from soluble arsenic during the first dosing trial and the i.v. dosing vary slightly from the values reported in Wester et al. (2004). This difference arises from a slight difference in how the background (i.e., pre-dosing) data were incorporated into the analysis. Specifically, the research reported herein provided data for a sufficient number of specific dosing trials to determine that comparison to background could be conducted on a dose-specific basis, with correction for background levels of arsenic in urine by subtracting out the average of the three background time points on a dosing trial- and monkey-specific basis. For the prior research, such specificity was not substantiated by the more limited data set on urinary arsenic excretion during the pre-dosing time frame, and background data were therefore aggregated across dosing trials. For this study, background excreted arsenic levels were evaluated to determine the best correction for treatment measurement by monkey.

In general, three background measurements were made on each monkey prior to treatment applications, for a total of 23 measurements per monkey. In the first two treatments (intravenous and first trial of soluble arsenic), the monkeys were not consistently fed a low-arsenic diet, as evidenced by the elevated measurements. Further, comparison of the background excretion levels by treatment group using an ANOVA followed by multiple comparison test showed a significant elevation relative to later treatment-group background measurements.

Overall, each monkey's background measurements were not significantly different prior to a specific treatment, but there were significant differences between background measurements for different treatment groups. Due to these differences, measurements of excretion during treatment applications were corrected using the average of the background measurements that preceded each specific treatment.

## **Results**

Details regarding the dosing trials (concentrations in substrates, volume dosed, arsenic mass dose) are provided in Table 1. These soils served as part of a more extensive soil characterization effort (SERDP, 2005), for which other parameters were evaluated. These soil characterization data are presented in Table 2, and arsenic mineralogy is presented in Figure 1. Arsenic mineralogy of the two soils is quite different. The mineralogy analyses indicate that the arsenic in the Colorado residential soil is dominated by arsenic in arsenic oxide phases (87% of the arsenic mass in the sample) and lead arsenate phases (10%), with small amounts present in

iron oxides (1.7%) or manganese oxides (0.3%). For the New York pesticide facility soil, arsenic is present primarily complexed with iron, in the iron-arsenic oxide phase (95% of the arsenic mass in the sample), with some arsenic in iron oxides (4.4%) and manganese oxides (0.5%) phases.<sup>1</sup>

Data for the mass of urinary arsenic excreted by the monkeys following intravenous and dermal dosings of soluble arsenic are presented in Tables 3 and 4, respectively. The intravenous dose resulted in  $82 \pm 2.4\%$  of the administered arsenic dose being excreted in urine. This finding is consistent with prior research that used more sensitive methods; i.e., evaluating excretion of a radiolabeled dose of arsenic. The average urinary arsenic excretion value of 82% was used to adjust the data from the other dosing trials to account for the fraction of arsenic that might be retained within the body or excreted by other routes.

For the soluble dose, absorption ranged from 0.32% to 4.3% (average of  $2.9\% \pm 2.3\%$ ) in the first dosing trial, and from 1.9 to 16% (average of  $6.7\% \pm 7.8\%$ ) for the second dosing trial for soluble arsenic. Combining all of these measurements provides an estimated absorption for soluble arsenic of  $4.8\% \pm 5.5\%$ . These data are generally consistent with the earlier report by Wester et al. (1993), which indicated dermal absorption of radiolabeled arsenic of  $4.5\% \pm 3.2\%$  for a low dose of soluble arsenic in solution, or  $3.2\% \pm 1.9\%$  for a higher dose of arsenic in solution. Of note for the data reported herein, five of the six doses of soluble arsenic that were applied resulted in calculated absorption fractions of 4.3% or less. One monkey demonstrated absorption of 16% of the applied dose. An earlier dosing of this monkey resulted in 4.1% absorption of the applied dose. Thus, the 16% absorption value for this dosing appears to be an outlier of unknown origin.

In contrast, mean estimates of arsenic absorption from soil were 0.5% or less for all soils, and all individual estimates were less than 1% (Table 5). Following application of arsenic-bearing soils to the abdomens of monkeys, urinary arsenic excretion could not be readily distinguished from background. This was true across all five soil dosing trials, including application of the two soils dry, and three trials with wet soil. Figure 2 provides a graphical depiction of the uncorrected 24-hour urinary arsenic excretion for all five soil applications and for the application of soluble arsenic. Included are data for urinary arsenic excretion prior to dosing, on a trial-specific basis. Figure 3 depicts a time course of urinary arsenic excretion for each dosing trial, corrected for background on a trial- and monkey-specific basis. Urinary arsenic excretion peaks within the first 24 hours following application of the soluble arsenic. The mass of arsenic excreted in urine then quickly decreases, returning to near-background levels within 48 to 72 hours, with minimal subsequent excretion. In contrast to the findings for urinary arsenic excretion following application of a soluble dose, none of the soils produced readily visible elevated urinary arsenic excretion at any time point after application. Although application of soluble arsenic resulted in some variability across the six applications (two trials with three

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<sup>1</sup> The “iron-arsenic oxide” phase is defined as an iron oxide that contains over 5 wt% arsenic, with the arsenic incorporated into the mineral structure, while “arsenic in iron oxide” is an iron oxide that contains less than 5 wt% arsenic and likely represents arsenic sorbed to soil iron oxide.

monkeys each), none of the soils resulted in urinary arsenic excretion above background across the five dosing trials (total of 15 applications) of soil.

## **Discussion**

Few good animal models exist for understanding the dermal absorption of chemicals by humans. Dermal absorption in primates has been shown to be similar to or somewhat higher than absorption in humans (Wester and Maibach, 1975). The costs and handling considerations associated with primate research constrain the number of animals that can be used. Therefore, the crossover study design, wherein each monkey can serve as its own comparison control, was selected, because it optimizes the potential to observe statistically significant results despite the small sample size. It does, however, necessitate the use of specific statistical approaches that are consistent with the study design. The reader is referred to prior research (Wester et al. 2004) for information on the specific statistical approach employed in the evaluation of these data. Previous research (Wester et al. 2004) has demonstrated that the methodology used in this study provides adequate sensitivity to evaluate dermally absorbed arsenic without using a radiolabeled marker. Maintaining the research animals on a low-arsenic diet prior to dosing and throughout the study period is a critical element of this approach due to the presence of significant background levels of inorganic arsenic in the diet (Schoof 1999; Yost 2004).

Consistent with model limitations discussed in earlier research (Wester et al., 2004), although the results reported herein indicate that the urinary arsenic levels following topical administration of arsenic in weathered soils are not distinguishable from background, the non-zero values for background urinary arsenic excretion, and the variability of the measured background values, impose some limits regarding the sensitivity of the model to detect an absorbed dose. A statistical evaluation using a comparison of means (t-test) for our data indicates that the absorbed dose of arsenic from soils would need to be in the range of 0.02 to 0.22% of the applied dose from soils, at the dosing levels used in this study, for daily arsenic excretion levels to be detectable above background. Thus, while these data suggest that there may not be any dermal absorption of arsenic from weathered soils (i.e., urinary arsenic excretion was not statistically different from background following application of soils), the uncertainty associated with this research model tells us that dermal absorption of arsenic from weathered soils is well below absorption of soluble arsenic. Additionally, estimates of the dermally-absorbed dose of arsenic from soil were six- to ten-fold lower than default estimates recommended by EPA for use in risk assessments (USEPA 2004). These findings are consistent for the two soils studied and are independent of skin hydration levels. These results are also consistent with previous research that showed that absorption of dermally applied arsenic-containing wood was negligible (Wester et al. 2004).

These findings are also consistent with our understanding of the environmental chemistry of arsenic. As described above, arsenic can be present in soils in complexed mineral forms. Even if the arsenic is introduced to the environment in soluble forms, secondary minerals that form during the soil weathering process can alter the form and behavior of arsenic within the soil matrix, causing it to become more stable over time. Secondary mineral formation can vary, depending on the redox conditions, pH, water content, and primary minerals that are present as the soil weathers, and arsenic can co-precipitate with other minerals, resulting in a more tightly bound, less available arsenic form (Sposito, 1989). Mineralogical analyses of soils (Drexler

2005) have shown arsenic to be present with these secondary minerals, as well as in other complexed forms.

This research addresses an important component involved in estimating the true contribution of percutaneous exposures to arsenic in soil relative to exposures via ingestion. Using current default parameters for children's soil ingestion and dermal contact, from EPA's guidance on soil screening levels (U.S. EPA, 2002b), calculations show that dermal exposures to arsenic would contribute a fairly small fraction of exposure to arsenic in soil, and that a majority of exposure would occur via ingestion. Specifically, using the standard default exposure assumptions of a soil ingestion rate of 200 mg/day, relative oral bioavailability of 100%, surface area of 2,800 cm<sup>2</sup>, soil-to-skin adherence factor of 0.2, and assuming 3% dermal absorption for arsenic, exposures via dermal contact for a child would contribute approximately 8% of total exposures to arsenic.

However, recent studies on parameters associated with the soil ingestion pathway suggest that the default parameters may significantly overestimate exposures via ingestion. As the estimates for ingestion become lower, it becomes even more vital that we refine the estimates for dermal contact, or risk assessors could significantly overestimate the contribution of dermal contact to total exposures from arsenic in soil. For example, recent studies on children's soil ingestion rates (Stanek and Calabrese, 2000; Stanek et al., 2001) suggest that central-tendency values are likely to be less than 50 mg/day, and perhaps as low as 24 mg/day (Stanek and Calabrese, 2001), while the 95<sup>th</sup> percentile is likely in the range of 100 to 125 mg/day. Thus, even an upper bound soil ingestion rate is almost 2-fold lower than the current default of 200 mg/day.

In addition, studies indicate that the relative oral bioavailability of arsenic in weathered soil is typically less than 50% (Freeman et al. 1995, Roberts et al., 2002, 2007, USEPA 2005). Using more refined soil ingestion rates and relative bioavailability assumptions with the current default dermal contact parameters would inappropriately suggest that dermal exposures might contribute roughly one-third of total exposures to arsenic in soil. Using these improved estimates of oral bioavailability, combined with more refined estimates of soil ingestion rates alongside default parameters for dermal contact, could lead to the erroneous conclusion that exposures to arsenic in soil via dermal contact actually exceed exposures via ingestion. In contrast, our findings suggest that dermal absorption of arsenic from soil is truly negligible, and that EPA's current default assumption of 3% dermal absorption of arsenic from soils results in significant overestimates of exposure.

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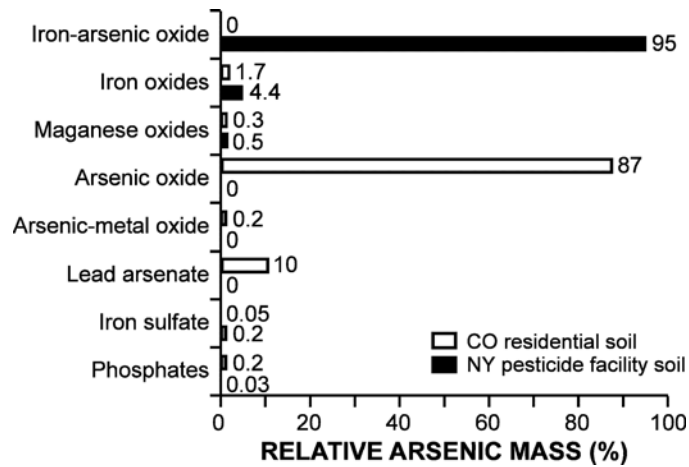


Figure 1. Arsenic mineralogy of soils used in dermal arsenic absorption study

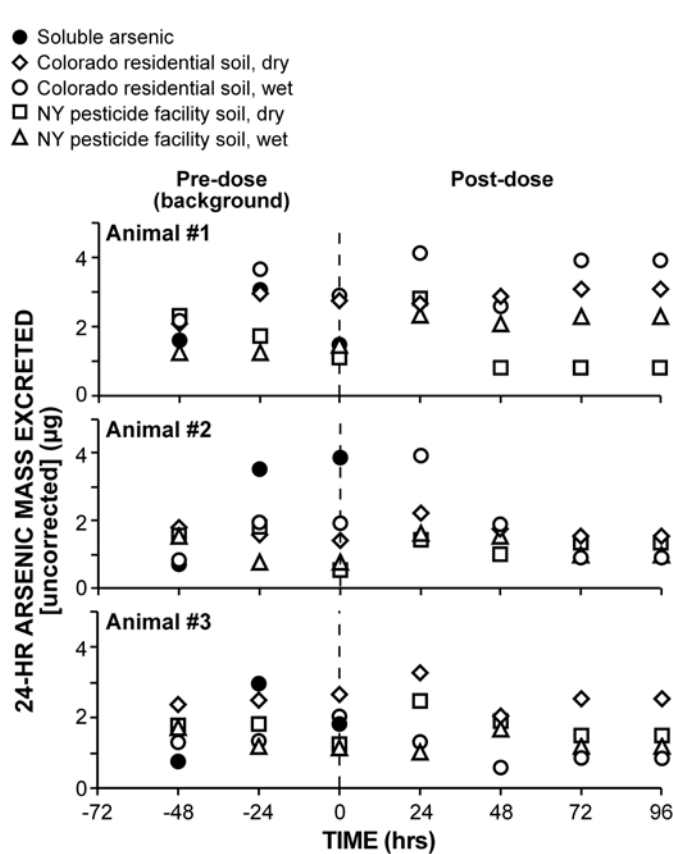


Figure 2. Urinary arsenic mass excreted (uncorrected) in dermal absorption studies. For soluble arsenic, only pre-dose/background values are plotted because the post-dose data for soluble arsenic extends beyond the range of values included on the figures.

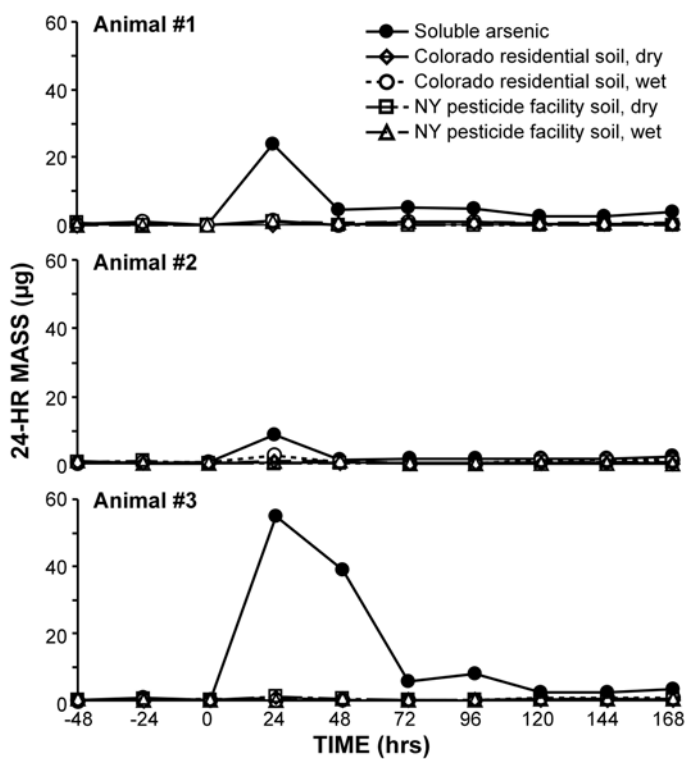


Figure 3. Urinary arsenic mass excretion (corrected) in 24-hour increments

**Table 1. Summary of applied arsenic doses for dermal absorption studies**

Study		Arsenic Concentration in Dosing Material	Volume of Dosing Material Administered	Arsenic Mass Dosed ( $\mu\text{g}$ )	Arsenic Mass per Unit Area ( $\mu\text{g}/\text{cm}^2$ )
Intravenous <sup>a</sup>	--	2,120 mg/L	0.5 mL	1,060	--
Soluble dose <sup>a</sup>	Trial 1	2,860 mg/L	0.5 mL	1,430	14.3
Soluble dose	Trial 2	2,610 mg/L		1,305	13.1
Soluble dose	average	2,735 mg/L		--	13.7
Colorado residential soil	dry	1,230 $\mu\text{g}/\text{g}$	400 mg	492	4.9
	wet				
New York pesticide facility soil	dry	1,400 $\mu\text{g}/\text{g}$	400 mg	560	5.6
	wet - trial 1				
	wet - trial 2				
	wet - average				

**Note:** Soils sieved to the <150  $\mu\text{m}$  size fraction.

-- - not available or not applicable

<sup>a</sup> Data have been reported previously (Wester et al., 2004). The monkeys for these dose groups in this previous study were not consistently fed a low-arsenic diet (see text for more detail).

**Table 2. Soil characteristics for dermal absorption study**

Chemical	Units	Colorado Residential Soil <sup>a</sup>	New York Pesticide Facility Soil
<b>Conventionals</b>			
pH	s.u.	5.33 <sup>b</sup>	5.48 <sup>c</sup>
Total organic carbon	%	2.76 <sup>b</sup>	4.51 <sup>c</sup>
Arsenic	mg/kg	1,230	1,400 <sup>c</sup>
<b>Other Metals</b>			
Antimony	mg/kg	10.0 <sup>b</sup>	10 <sup>U</sup>
Beryllium	mg/kg	1.0 <sup>U<sup>b</sup></sup>	1.0 <sup>U</sup>
Cadmium	mg/kg	5.0 <sup>b</sup>	1.7
Chromium	mg/kg	51.8	16.7 <sup>c</sup>
Copper	mg/kg	30.4 <sup>b</sup>	61.2 <sup>c</sup>
Iron	mg/kg	13,650	16,000 <sup>c</sup>
Lead	mg/kg	469 <sup>b</sup>	387 <sup>c</sup>
Manganese	mg/kg	--	653 <sup>c</sup>
Mercury	mg/kg	0.80 <sup>b</sup>	0.44
Nickel	mg/kg	11.2 <sup>b</sup>	13.7
Selenium	mg/kg	2.5 <sup>U<sup>b</sup></sup>	1.9
Silver	mg/kg	2.0 <sup>U<sup>b</sup></sup>	2.0 <sup>U</sup>
Thallium	mg/kg	10.0 <sup>U<sup>b</sup></sup>	1.0 <sup>U</sup>
Zinc	mg/kg	314 <sup>b</sup>	244

**Note:** -- - not analyzed  
 U - undetected; value represents detection or reporting limit

<sup>a</sup> Soil obtained from Syracuse Research Corp.

<sup>b</sup> Results for this parameter for the Colorado Residential Soil were obtained from a sample sieved to <250  $\mu\text{m}$ .

<sup>c</sup> Average of replicates.

**Table 3. Urinary arsenic excretion following application of intravenous arsenic dose**

	24-Hr Mass Excreted	
	( $\mu\text{g}$ )	Corrected <sup>a</sup> ( $\mu\text{g}$ )
<b>Animal #1</b>		
Background		
96–120 hr	5.14	0
48–72 hr	8.64	1.68
0–24 hr	7.10	0.14
0–24 hr	767 <sup>b</sup>	760
24–48 hr	65.9	58.9
48–72 hr	19.5 <sup>d</sup>	12.6
72–96 hr	19.5 <sup>d</sup>	12.6
Total arsenic mass excreted (0–96 hrs):		844
<b>Percent excretion (0–96 hrs):</b>		<b>80%</b> <sup>d</sup>
<b>Animal #2</b>		
Background		
96–120 hr	5.16	0
48–72 hr	7.26	1.61
0–24 hr	4.54	0
0–24 hr	762 <sup>b</sup>	756
24–48 hr	80.4	74.8
48–72 hr	24.6 <sup>c</sup>	18.9
72–96 hr	24.6 <sup>c</sup>	18.9
Total arsenic mass excreted (0–96 hrs):		869
<b>Percent excretion (0–96 hrs):</b>		<b>82%</b> <sup>d</sup>
<b>Animal #3</b>		
Background		
96–120 hr	2.25	0
48–72 hr	2.91	0.066
0–24 hr	3.38	0.53
0–24 hr	706 <sup>b</sup>	703
24–48 hr	124	121
48–72 hr	38.7 <sup>c</sup>	35.8
72–96 hr	38.7 <sup>c</sup>	35.8
Total arsenic mass excreted (0–96 hrs):		895
<b>Percent excretion (0–96 hrs):</b>		<b>84%</b> <sup>d</sup>

**Note:** Data have been reported previously (Wester et al., 2004).

<sup>a</sup> Corrected mass calculated by subtracting average of the background arsenic masses by monkey and by dose. If corrected mass is calculated less than zero, corrected mass is set to zero.

<sup>b</sup> Sum of (0–8 hr), cage wash, and (8–24 hr). Cage wash concentration is calculated using cage wash concentrations minus wash water concentration [iv-dosed monkeys did not use the metabolic chair, and the cage wash was collected from below the cages after collection of the (0–8 hr) sample.]

<sup>c</sup> 24-hour mass excreted is estimated as one-half of 48–96 hr sample mass.

<sup>d</sup> Percent excretion calculated using intravenous dose of 1,060  $\mu\text{g}$ .

**Table 4. Urinary arsenic excretion following application of arsenic in soluble dose**

	Trial 1		Trial 2	
	24-hr Mass Excreted		24-hr Mass Excreted	
	( $\mu\text{g}$ )	Corrected <sup>a</sup> ( $\mu\text{g}$ )	( $\mu\text{g}$ )	Corrected <sup>a</sup> ( $\mu\text{g}$ )
<b>Animal #1</b>				
Background				
48–72 hr	--	--	1.57	0.24
24–48 hr	5.07	1.75	1.01	0
0–24 hr	1.56	0	1.40	0.073
0–24 hr	41.6 <sup>b</sup>	38.3	11.0 <sup>b</sup>	9.72
24–48 hr	7.22	3.90	6.81	5.48
48–72 hr	8.08	4.76	7.13 <sup>c</sup>	5.80
72–96 hr	7.21	3.90	7.13 <sup>c</sup>	5.80
Total arsenic mass excreted (0–96 hrs):		50.8		26.8
Total arsenic mass excreted with urinary arsenic excretion fraction correction (0–96 hrs):		62.0 <sup>d</sup>		32.7 <sup>d</sup>
<b>Percent absorption (0–96 hrs):</b>		<b>4.3%</b> <sup>e</sup>		<b>2.5%</b> <sup>f</sup>
<b>Animal #2</b>				
Background				
48–72 hr	--	--	0.688	0.044
24–48 hr	6.30	0	0.675	0.032
0–24 hr	7.08	0.39	0.567	0
0–24 hr	10.2 <sup>b</sup>	3.53	13.9 <sup>b</sup>	13.2
24–48 hr	6.96	0.27	2.25	1.61
48–72 hr	5.32	0	3.22 <sup>c</sup>	2.58
72–96 hr	6.53	0	3.22 <sup>c</sup>	2.58
Total arsenic mass excreted (0–96 hrs):		3.80		20.0
Total arsenic mass excreted with urinary arsenic excretion fraction correction (0–96 hrs):		4.63 <sup>d</sup>		24.4 <sup>d</sup>
<b>Percent absorption (0–96 hrs):</b>		<b>0.32%</b> <sup>e</sup>		<b>1.9%</b> <sup>f</sup>
<b>Animal #3</b>				
Background				
48–72 hr	5.20	1.07	0.726	0.062
24–48 hr	3.07	0	0.703	0.039
0–24 hr	30.3 <sup>b</sup>	26.2	0.563	0
0–24 hr	21.0	16.8	84.5 <sup>b</sup>	83.9
24–48 hr	4.52	0.38	61.9 <sup>c</sup>	61.2
48–72 hr	9.16	5.03	11.7 <sup>c</sup>	11.0
Total arsenic mass excreted (0–96 hrs):		48.5		167
Total arsenic mass excreted with urinary arsenic excretion fraction correction (0–96 hrs):		59.1 <sup>d</sup>		204 <sup>d</sup>
<b>Percent absorption (0–96 hrs):</b>		<b>4.1%</b> <sup>e</sup>		<b>16%</b> <sup>f</sup>

**Note:** -- - not analyzed

Trial 1 data presented in this table have been reported previously (Wester et al., 2004).

<sup>a</sup> Corrected mass calculated by subtracting average of the background arsenic masses by monkey and by dose. If corrected mass is calculated less than zero, corrected mass is set to zero.

<sup>b</sup> Sum of (0–8 hr), pan wash, and (8–24 hr). Pan wash concentration is calculated using pan wash concentration minus "water for pan wash" or "blank sample" concentration for this experiment.

<sup>c</sup> 24-hour mass excreted is estimated as one-half of 48–96 hr sample mass.

<sup>d</sup> Calculated by correcting excreted mass for fractional excretion of arsenic from IV dose (i.e., 0.82 or 82%).

<sup>e</sup> Percent absorption calculated using soluble applied dose mass of 1,430  $\mu\text{g}$ .

<sup>f</sup> Percent absorption calculated using soluble applied dose mass of 1,305  $\mu\text{g}$ .

**Table 5. Urinary arsenic excretion following application of arsenic-containing soil: Colorado residential soil**

	Dry		Wet	
	24-hr Mass Excreted		24-hr Mass Excreted	
	( $\mu\text{g}$ )	Corrected <sup>a</sup> ( $\mu\text{g}$ )	( $\mu\text{g}$ )	Corrected <sup>a</sup> ( $\mu\text{g}$ )
<b>Animal #1</b>				
Background				
48–72 hr	2.10	0	2.10	0
24–48 hr	2.96	0.36	3.66	0.81
0–24 hr	2.76	0.15	2.77	0
0–24 hr	2.68 <sup>b</sup>	0.075	4.11 <sup>b</sup>	1.27
24–48 hr	2.89	0.28	2.62	0
48–72 hr	3.10 <sup>c</sup>	0.49	3.92 <sup>c</sup>	1.08
72–96 hr	3.10 <sup>c</sup>	0.49	3.92 <sup>c</sup>	1.08
Total arsenic mass excreted (0–96 hrs):		1.34		3.42
Total arsenic mass excreted with urinary arsenic excretion fraction correction (0–96 hrs):		1.63 <sup>d</sup>		4.17 <sup>d</sup>
<b>Percent absorption (0–96 hrs):</b>		<b>0.33%</b> <sup>e</sup>		<b>0.85%</b> <sup>e</sup>
<b>Animal #2</b>				
Background				
48–72 hr	1.81	0.22	0.79	0
24–48 hr	1.56	0	1.93	0.39
0–24 hr	1.41	0	1.90	0.37
0–24 hr	2.21 <sup>b</sup>	0.61	3.91 <sup>b</sup>	2.37
24–48 hr	1.73	0.14	1.78	0.24
48–72 hr	1.54 <sup>c</sup>	0	0.90 <sup>c</sup>	0
72–96 hr	1.54 <sup>c</sup>	0	0.90 <sup>c</sup>	0
Total arsenic mass excreted (0–96 hrs):		0.75		2.62
Total arsenic mass excreted with urinary arsenic excretion fraction correction (0–96 hrs):		0.92 <sup>d</sup>		3.19 <sup>d</sup>
<b>Percent absorption (0–96 hrs):</b>		<b>0.19%</b> <sup>e</sup>		<b>0.65%</b> <sup>e</sup>
<b>Animal #3</b>				
Background				
48–72 hr	2.37	0	1.29	0
24–48 hr	2.48	0	1.35	0
0–24 hr	2.66	0.16	2.01	0.46
0–24 hr	3.28 <sup>b</sup>	0.78	1.32 <sup>b</sup>	0
24–48 hr	2.06	0	0.59	0
48–72 hr	2.53 <sup>c</sup>	0.029	0.84 <sup>c</sup>	0
72–96 hr	2.53 <sup>c</sup>	0.029	0.84 <sup>c</sup>	0
Total arsenic mass excreted (0–96 hrs):		0.84		0
Total arsenic mass excreted with urinary arsenic excretion fraction correction (0–96 hrs):		1.02 <sup>d</sup>		0 <sup>d</sup>
<b>Percent absorption (0–96 hrs):</b>		<b>0.21%</b> <sup>e</sup>		<b>0%</b> <sup>e</sup>

**Note:** -- - not available or not applicable

<sup>a</sup> Corrected mass calculated by subtracting average of the background arsenic masses by monkey and by dose. If corrected mass is calculated less than zero, corrected mass is set to zero.

<sup>b</sup> Sum of (0–8 hr), pan wash, and (8–24 hr).

<sup>c</sup> 24-hour mass excreted is estimated as one-half of 48–96 hr sample mass.

<sup>d</sup> Calculated by correcting excreted mass for fractional excretion of arsenic from IV dose (i.e., by dividing by 0.82 or 82%).

<sup>e</sup> Percent absorption calculated using applied dose mass of 492  $\mu\text{g}$ .

**Table 6. Urinary arsenic excretion following application of arsenic-containing soil: New York pesticide facility**

	Dry		Wet, Trial 1		Wet, Trial 2	
	24-hr Mass Excreted		24-hr Mass Excreted		24-hr Mass Excreted	
	( $\mu\text{g}$ )	Corrected <sup>a</sup> ( $\mu\text{g}$ )	( $\mu\text{g}$ )	Corrected <sup>a</sup> ( $\mu\text{g}$ )	( $\mu\text{g}$ )	Corrected <sup>a</sup> ( $\mu\text{g}$ )
<b>Animal #1</b>						
Background						
48–72 hr	2.29	0.60	1.31	0.20	1.20	0
24–48 hr	1.70	0.012	0.91	0	1.54	0.034
0–24 hr	1.07	0	1.10	0	1.78	0.27
0–24 hr	2.80 <sup>b</sup>	1.12	2.28 <sup>c</sup>	1.17	2.36 <sup>c</sup>	0.85
24–48 hr	0.80	0	1.84	0.73	2.31	0.80
48–72 hr	0.78 <sup>d</sup>	0	1.85 <sup>d</sup>	0.74	2.75 <sup>d</sup>	1.24
72–96 hr	0.78 <sup>d</sup>	0	1.85 <sup>d</sup>	0.74	2.75 <sup>d</sup>	1.24
Total arsenic mass excreted (0–96 hrs):		1.12		3.39		4.13
Total arsenic mass excreted with urinary arsenic excretion fraction correction (0–96 hrs):		1.36 <sup>e</sup>		4.13 <sup>e</sup>		5.03 <sup>e</sup>
<b>Percent absorption (0–96 hrs):</b>		<b>0.24%</b> <sup>f</sup>		<b>0.74%</b> <sup>f</sup>		<b>0.90%</b> <sup>f</sup>
Background						
48–72 hr	1.53	0.25	0.778	0.073	2.27	0.93
24–48 hr	1.81	0.53	0.577	0	0.963	0
0–24 hr	0.505	0	0.759	0.054	0.785	0
0–24 hr	1.42 <sup>b</sup>	0.14	1.45 <sup>c</sup>	0.75	1.83 <sup>c</sup>	0.49
24–48 hr	0.97	0	1.31	0.61	1.73	0.39
48–72 hr	1.30 <sup>d</sup>	0.023	0.874 <sup>d</sup>	0.17	1.10 <sup>d</sup>	0
72–96 hr	1.30 <sup>d</sup>	0.023	0.874 <sup>d</sup>	0.17	1.10 <sup>d</sup>	0
Total arsenic mass excreted (0–96 hrs):		0.19		1.70		0.88
Total arsenic mass excreted with urinary arsenic excretion fraction correction (0–96 hrs):		0.23 <sup>e</sup>		2.07 <sup>e</sup>		1.07 <sup>e</sup>
<b>Percent absorption (0–96 hrs):</b>		<b>0.04%</b> <sup>f</sup>		<b>0.37%</b> <sup>f</sup>		<b>0.19%</b> <sup>f</sup>
<b>Animal #3</b>						
Background						
48–72 hr	1.75	0.16	0.985	0	2.47	0.82
24–48 hr	1.80	0.20	1.11	0.048	1.25	0
0–24 hr	1.23	0	1.09	0.027	1.24	0
0–24 hr	2.46 <sup>b</sup>	0.86	1.09 <sup>c</sup>	0.034	0.94 <sup>c</sup>	0
24–48 hr	1.88	0.29	1.28	0.22	2.08	0.43
48–72 hr	1.46 <sup>d</sup>	0	0.924 <sup>d</sup>	0	1.46 <sup>d</sup>	0
72–96 hr	1.46 <sup>d</sup>	0	0.924 <sup>d</sup>	0	1.46 <sup>d</sup>	0
Total arsenic mass excreted (0–96 hrs):		1.15		0.25		0.43
Total arsenic mass excreted with urinary arsenic excretion fraction correction (0–96 hrs):		1.41 <sup>e</sup>		0.30 <sup>e</sup>		0.53 <sup>e</sup>
<b>Percent absorption (0–96 hrs):</b>		<b>0.25%</b> <sup>f</sup>		<b>0.05%</b> <sup>f</sup>		<b>0.09%</b> <sup>f</sup>

**Note:** -- - not available or not applicable

<sup>a</sup> Corrected mass calculated by subtracting average of the background arsenic masses by monkey and by dose. If corrected mass is calculated less than zero, corrected mass is set to zero.

<sup>b</sup> Sum of (0–8 hr), pan wash, (8–24 hr), and cage wash (8–24 hr).

<sup>c</sup> Sum of (0–8 hr), pan wash, and (8–24 hr).

<sup>d</sup> 24-hour mass excreted is estimated as one-half of 48–96 hr sample mass.

<sup>e</sup> Calculated by correcting excreted mass for fractional excretion of arsenic from IV dose (i.e., by dividing by 0.82 or 82%).

<sup>f</sup> Percent absorption calculated using applied dose mass of 560  $\mu\text{g}$ .



**Table 7. Summary of percent absorption of arsenic**

Study		Percent Absorption (0–96 hrs)	
		Average ± S.D.	
Intravenous	--	82%	± 2.4%
Soluble dose	Trial 1	2.9%	± 2.3%
Soluble dose	Trial 2	6.7%	± 7.8%
Soluble dose	average	4.8%	± 5.5%
Colorado residential soil	dry	0.24%	± 0.08%
	wet	0.50%	± 0.44%
New York pesticide facility soil	dry	0.18%	± 0.12%
	wet - trial 1	0.39%	± 0.34%
	wet - trial 2	0.39%	± 0.44%
	wet - average	0.39%	± 0.35%

**Note:** Soils sieved to the <150  $\mu\text{m}$  size fraction.