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

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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 1400 mg/kg arsenic, was collected adjacent to a pesticide production facility in New York. The other soil, containing 1230 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 ± 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 dry soils 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 mixed with soil 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

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(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 occur as arsenopyrite, which is associated with the mining of sulfide ore deposits. Also, salts of methanearsenic acid or cacodylic acid can occur as a result of past herbicide use, but these salts typically weather in the soil matrix, becoming incorporated into more stable secondary mineral phases (Cances *et al*., 2005; Fendorf *et al*., 2004; 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 coprecipitate 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 pretest incubation time, and the binding kinetics (Fendorf *et al*., 2004; Pouschat and Zagury, 2006; Ruby *et al*., 1999; Sarkar and Datta, 2004; Yang *et al*., 2002, 2005; 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, allows 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, Frenchtown N.J.) 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 premeasured 100-cm^2 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 was 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-h dosing period. Following the dosing period, the applied doses were removed using a soap and water wash (50:50 vol/vol 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 3 days prior to the time of dosing (for characterization of predosing 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 inductively coupled plasma/mass spectroscopy (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 iv dosing of sodium arsenate heptahydrate. The iv dose (1060 μg arsenic per monkey) was administered as 0.5 ml 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^2 for a total dermal dose of 1305 or 1430 μg arsenic for the first and second dosing trials, respectively. The analyses reported herein utilize the preexisting 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., > 1000 mg As/kg soil). One sample, containing arsenic concentrations of 1400 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 1230 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 in. of soil, and the Colorado residential sample was collected as a surface grab sample (lower depth not reported but likely 0–1 inch or 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°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 μm (U.S. Standard Sieve Mesh No. 100) using a Meinzer Sieve Shaker (Fisher Scientific, Norcross, GA). The 150-μm 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 using standard methods (Brattin *et al*., 2004; Drexler and Brattin, 2007 manuscript) at the Department of Geological Sciences, University of Colorado, Boulder. The very fine particle size fraction (< 150 μm) 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-mm, < 250-μm, and < 150-μm particle size fractions were 1500, 1665, and 1400 mg/kg, respectively, indicating no arsenic enrichment in the smaller fraction. For the Colorado residential soil, arsenic concentrations in the < 250- and < 150-μm particle size fractions were 869 and 1230 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 mg/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 mg/cm^2 was selected for the study. This soil application load resulted in total doses of 560 and 492 μg 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 20–30% 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 iv dosing vary slightly from the values reported in Wester *et al*. (2004). This difference arises from a slight

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 (μg)	Arsenic mass per unit area (μg/cm^2)
IV[a]	na	2120 mg/l	0.5 ml	1060	na
Soluble dose[a]	Trial 1	2860 mg/l	0.5 ml	1430	14.3
Soluble dose	Trial 2	2610 mg/l	0.5 ml	1305	13.1
Soluble dose	Average	2735 mg/l	0.5 ml	na	13.7
Colorado residential soil	Dry	1230 μg/g	400 mg	492	4.9
Colorado residential soil	Wet	1230 μg/g	400 mg	492	4.9
New York pesticide facility soil	Dry	1400 μg/g	400 mg	560	5.6
New York pesticide facility soil	Wet—trial 1	1400 μg/g	400 mg	560	5.6
New York pesticide facility soil	Wet—trial 2	1400 μg/g	400 mg	560	5.6
New York pesticide facility soil	Wet—average	1400 μg/g	400 mg	560	5.6

Note. Soils sieved to the < 150-μm size fraction;.

na, not available or not applicable.

[a]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).

difference in how the background (i.e., predosing) 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 predosing 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 (iv 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

TABLE 2
Soil Characteristics for Dermal Absorption Study

Chemical	Units	Colorado residential soil[a]	New York pesticide facility soil
Conventionals			
pH	SU	5.33[b]	5.48[c]
Total organic carbon	%	2.76[b]	4.51[c]
Arsenic	mg/kg	1230	1400[c]
Other metals			
Antimony	mg/kg	10.0[b]	10 U
Beryllium	mg/kg	1.0 U[b]	1.0 U
Cadmium	mg/kg	5.0[b]	1.7
Chromium	mg/kg	51.8	16.7[c]
Copper	mg/kg	30.4[b]	61.2[c]
Iron	mg/kg	13,650	16,000[c]
Lead	mg/kg	469[b]	387[c]
Manganese	mg/kg	—	653[c]
Mercury	mg/kg	0.80[b]	0.44
Nickel	mg/kg	11.2[b]	13.7
Selenium	mg/kg	2.5 U[b]	1.9
Silver	mg/kg	2.0 U[b]	2.0 U
Thallium	mg/kg	10.0 U[b]	1.0 U
Zinc	mg/kg	314[b]	244

Note. —, not analyzed; U, undetected.
Value represents detection or reporting limit.
[a]Soil obtained from Syracuse Research Corp.
[b]Results for this parameter for the Colorado Residential Soil were obtained from a sample sieved to < 250 μm.
[c]Average of replicates.

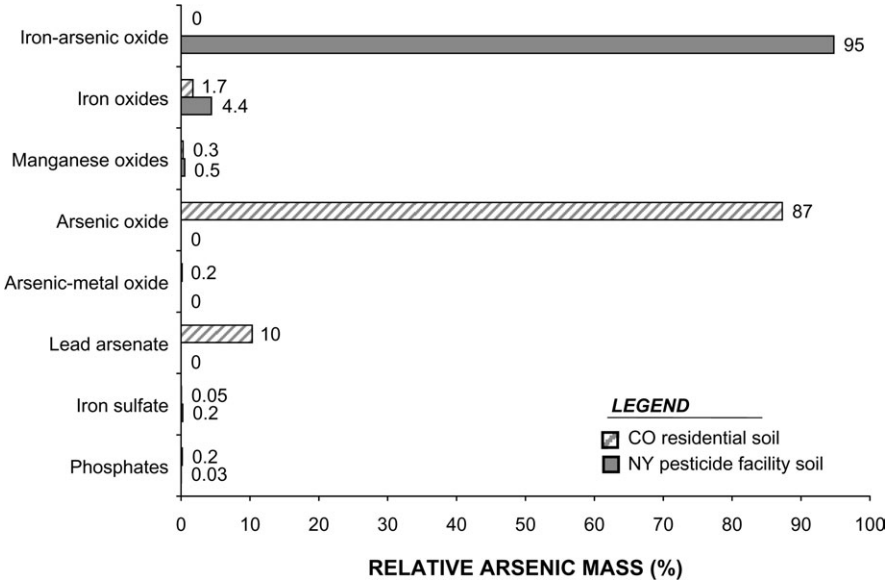


FIG. 1. Arsenic mineralogy of soils used in dermal arsenic absorption study.

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 oxide (0.5%) phases. (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.)

Data for the mass of urinary arsenic excreted by the monkeys following iv and dermal dosings of soluble arsenic are presented in Tables 3 and 4, respectively. The iv dose resulted in 82 ± 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 ± 2.3%) in the first dosing trial, and from 1.9 to 16% (average of 6.7 ± 7.8%) for the second dosing trial for soluble arsenic. Combining all these measurements provides an estimated absorption for soluble arsenic of 4.8 ± 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 ± 3.2% for a low dose of soluble arsenic in solution, or 3.2 ± 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, 6 & 7). 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 dry soils, and three trials with wet soil. Figure 2 provides a graphical depiction of the uncorrected 24-h 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 h following application of the soluble arsenic. The mass of arsenic excreted in urine then quickly decreases, returning to near-background levels within 48–72 h, 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 monkeys each), none of the soils resulted in urinary arsenic excretion above background across the five dosing trials (total of 15 applications) of soil.

TABLE 3
Urinary Arsenic Excretion following Application of IV Arsenic Dose

	24-h mass excreted (μg)	
		Corrected[a]
Animal no. 1		
Background (h)		
96–120	5.14	0
48–72	8.64	1.68
0–24	7.10	0.14
0–24	767[b]	760
24–48	65.9	58.9
48–72	19.5[c]	12.6
72–96	19.5[c]	12.6
Total arsenic mass excreted (0–96 h)		844
Percent excretion (0–96 h)		**80%**
Animal no. 2		
Background (h)		
96–120	5.16	0
48–72	7.26	1.61
0–24	4.54	0
0–24	762[b]	756
24–48	80.4	74.8
48–72	24.6[d]	18.9
72–96	24.6[d]	18.9
Total arsenic mass excreted (0–96 h)		869
Percent excretion (0–96 h)		**82%**
Animal no. 3		
Background (h)		
96–120	2.25	0
48–72	2.91	0.066
0–24	3.38	0.53
0–24	706[b]	703
24–48	124	121
48–72	38.7[d]	35.8
72–96	38.7[d]	35.8
Total arsenic mass excreted (0–96 h)		895
Percent excretion (0–96 h)		**84%**

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

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

[b]Sum of 0–8 h, cage wash, and 8–24 h. 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- to 8-h sample).

[c]Percent excretion calculated using iv dose of 1060 μg.

[d]24-h mass excreted is estimated as one-half of 48- to 96-h sample mass.

TABLE 4
Urinary Arsenic Excretion following Application of Arsenic in Soluble Dose

	Trial 1		Trial 2	
	24-h mass excreted (μg)		24-h mass excreted (μg)	
		Corrected[a]		Corrected[a]
Animal no. 1				
Background (h)				
48–72	—	—	1.57	0.24
24–48	5.07	1.75	1.01	0
0–24	1.56	0	1.40	0.073
0–24	41.6[b]	38.3	11.0[b]	9.72
24–48	7.22	3.90	6.81	5.48
48–72	8.08	4.76	7.13[c]	5.80
72–96	7.21	3.90	7.13[c]	5.80
Total arsenic mass excreted (0–96 h)		50.8		26.8
Total arsenic mass excreted with urinary arsenic excretion fraction correction (0–96 h)		62.0[d]		32.7[d]
Percent absorption (0–96 h)		**4.3[e]%**		**2.5%[f]**
Animal no. 2				
Background (h)				
48–72	—	—	0.688	0.044
24–48	6.30	0	0.675	0.032
0–24	7.08	0.39	0.567	0
0–24	10.2[b]	3.53	13.9[b]	13.2
24–48	6.96	0.27	2.25	1.61
48–72	5.32	0	3.22[c]	2.58
72–96	6.53	0	3.22[c]	2.58
Total arsenic mass excreted (0–96 h)		3.80		20.0
Total arsenic mass excreted with urinary arsenic excretion fraction correction (0–96 h)		4.63[d]		24.4[d]
Percent absorption (0–96 h)		**0.32[e]%**		**1.9%[f]**
Animal no. 3				
Background (h)	—	—	0.726	0.062
48–72	5.20	1.07	0.703	0.039
24–48	3.07	0	0.563	0
0–24	30.3[b]	26.2	84.5[b]	83.9
0–24	21.0	16.8	61.9	61.2
24–48	4.52	0.38	11.7[c]	11.0
48–72	9.16	5.03	11.7[c]	11.0
Total arsenic mass excreted (0–96 h)		48.5		167
Total arsenic mass excreted with urinary arsenic excretion fraction correction (0–96 h)		59.1[d]		204[d]
Percent absorption (0–96 h)		**4.1[e]%**		**16%[f]**

Note. —, not analyzed. Trial 1 data presented in this table have been reported previously (Wester *et al*., 2004).

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

[b]Sum of 0–8 h, pan wash, and 8–24 h. Pan wash concentration is calculated using pan wash concentration minus "water for pan wash" or "blank sample" concentration for this experiment.

[c]24-h mass excreted is estimated as one-half of 48- to 96-h sample mass.

[d]Calculated by correcting excreted mass for fractional excretion of arsenic from iv dose (i.e., 0.82 or 82%).

[e]Percent absorption calculated using soluble applied dose mass of 1430 μg.

[f]Percent absorption calculated using soluble applied dose mass of 1305 μg.

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,

TABLE 5
Urinary Arsenic Excretion following Application of Arsenic-Containing Soil: Colorado Residential Soil

	Dry		Wet	
	24-h mass excreted (μg)		24-h mass excreted (μg)	
		Corrected[a]		Corrected[a]
Animal no. 1				
Background (h)				
48–72	2.10	0	2.10	0
24–48	2.96	0.36	3.66	0.81
0–24	2.76	0.15	2.77	0
0–24	2.68[b]	0.075	4.11[b]	1.27
24–48	2.89	0.28	2.62	0
48–72	3.10[c]	0.49	3.92[c]	1.08
72–96	3.10[c]	0.49	3.92[c]	1.08
Total arsenic mass excreted (0–96 h)		1.34		3.42
Total arsenic mass excreted with urinary arsenic excretion fraction correction (0–96 h)		1.63[d]		4.17[d]
Percent absorption (0–96 h)		**0.33[e]%**		**0.85**[e]%
Animal no. 2				
Background (h)				
48–72	1.81	0.22	0.79	0
24–48	1.56	0	1.93	0.39
0–24	1.41	0	1.90	0.37
0–24	2.21[b]	0.61	3.91[b]	2.37
24–48	1.73	0.14	1.78	0.24
48–72	1.54[c]	0	0.90[c]	0
72–96	1.54[c]	0	0.90[c]	0
Total arsenic mass excreted (0–96 h)		0.75		2.62
Total arsenic mass excreted with urinary arsenic excretion fraction correction (0–96 h)		0.92[d]		3.19[d]
Percent absorption (0–96 h)		**0.19[e]%**		**0.65**[e]%
Animal no. 3				
Background (h)				
48–72	2.37	0	1.29	0
24–48	2.48	0	1.35	0
0–24	2.66	0.16	2.01	0.46
0–24	3.28[b]	0.78	1.32[b]	0
24–48	2.06	0	0.59	0
48–72	2.53[c]	0.029	0.84[c]	0
72–96	2.53[c]	0.029	0.84[c]	0
Total arsenic mass excreted (0–96 h)		0.84		0
Total arsenic mass excreted with urinary arsenic excretion fraction correction (0–96 h)		1.02[d]		0[d]
Percent absorption (0–96 h)		**0.21[e]%**		**0**[e]%

Note. —, not available or not applicable.

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

[b]Sum of 0–8 h, pan wash, and 8–24 h.

[c]24-h mass excreted is estimated as one-half of 48- to 96-h sample mass.

[d]Calculated by correcting excreted mass for fractional excretion of arsenic from iv dose (i.e., by dividing by 0.82 or 82%).

[e]Percent absorption calculated using applied dose mass of 492 μg.

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 *et al*., 1999; Yost *et al*., 2004).

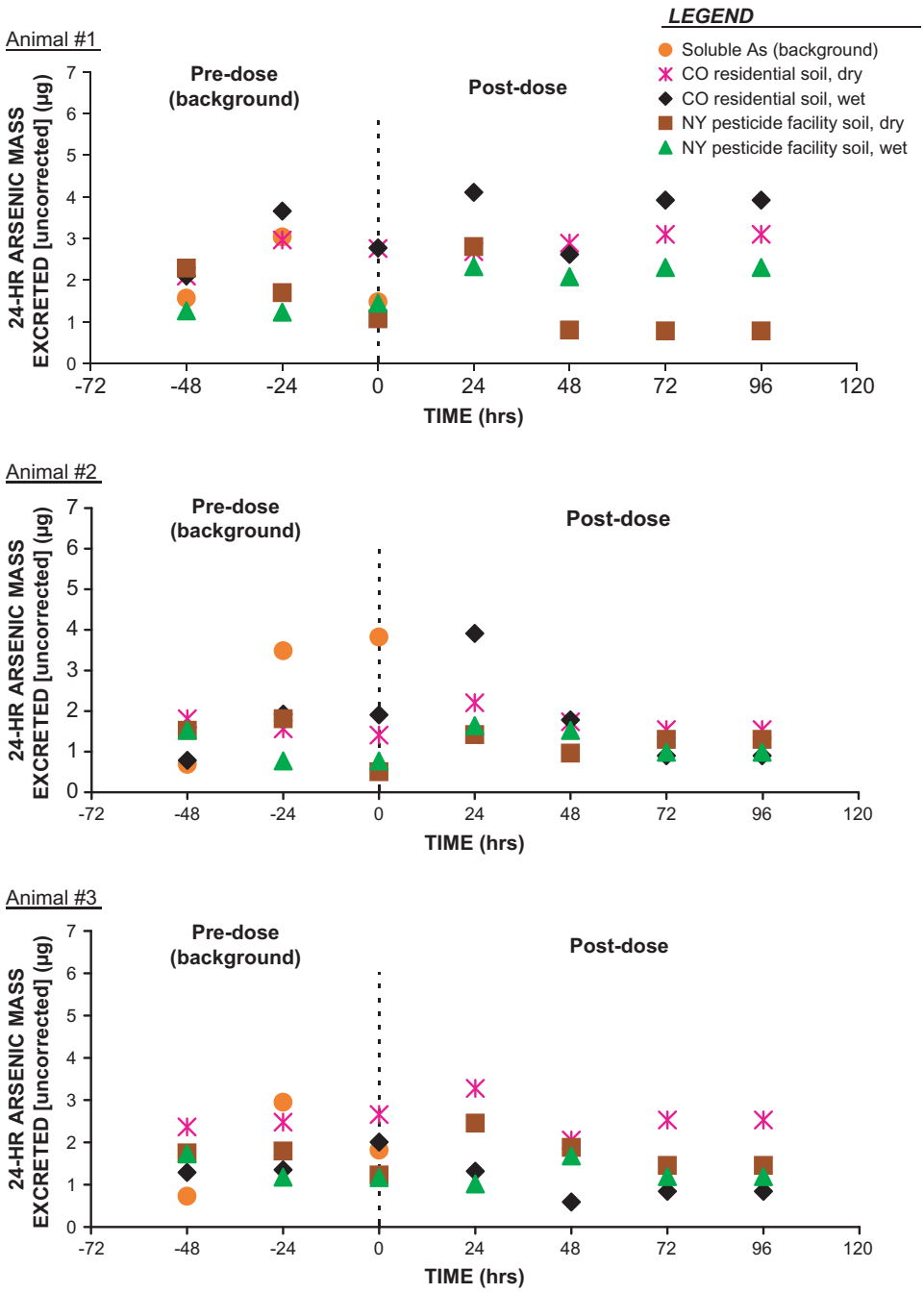
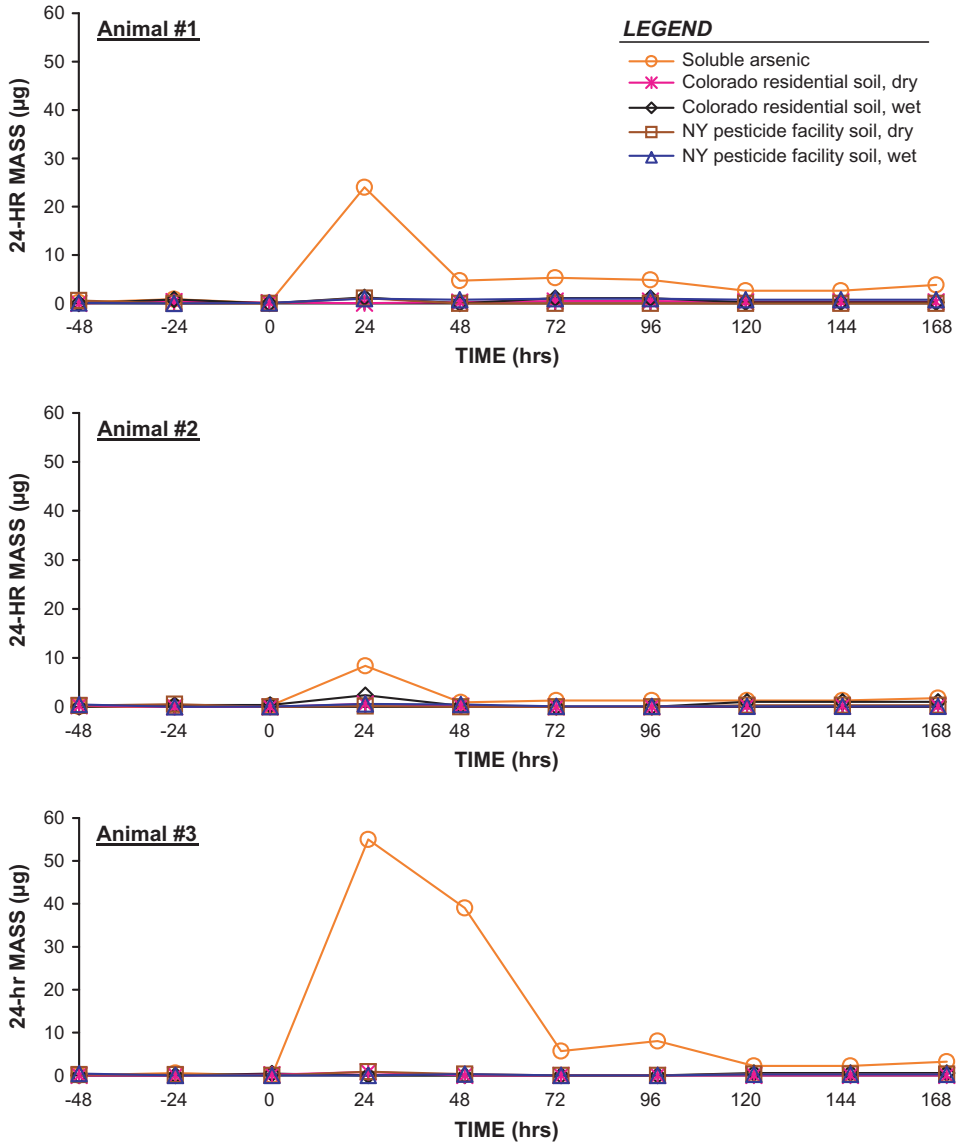


FIG. 2. Urinary arsenic mass excreted (uncorrected) in dermal absorption studies. For soluble arsenic, only predose/background values are plotted because the post-dose data for soluble arsenic extend beyond the range of values included on the figures.

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 nonzero 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 0.02–0.22% 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



Note: 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.

FIG. 3. Urinary arsenic mass excretion (corrected) in 24-h increments.

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 6- to 10-fold lower than default estimates recommended by EPA for use in risk assessments (U.S. EPA, 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 coprecipitate with other minerals, resulting in a more tightly bound, less available arsenic form (Sposito, 1989). Mineralogical analyses of soils

TABLE 6
Urinary Arsenic Excretion following Application of Arsenic-Containing Soil: New York Pesticide Facility

	Dry		Wet, trial 1		Wet, trial 2	
	24-h mass excreted (μg)		24-h mass excreted (μg)		24-h mass excreted (μg)	
		Corrected[a]		Corrected[a]		Corrected[a]
Animal no. 1						
Background (h)						
48–72	2.29	0.60	1.31	0.20	1.20	0
24–48	1.70	0.012	0.91	0	1.54	0.034
0–24	1.07	0	1.10	0	1.78	0.27
0–24	2.80[b]	1.12	2.28[c]	1.17	2.36[c]	0.85
24–48	0.80	0	1.84	0.73	2.31	0.80
48–72	0.78[d]	0	1.85[d]	0.74	2.75[d]	1.24
72–96	0.78[d]	0	1.85[d]	0.74	2.75[d]	1.24
Total arsenic mass excreted (0–96 h)		1.12		3.39		4.13
Total arsenic mass excreted with urinary arsenic excretion fraction correction (0–96 h)		1.36[e]		4.13[e]		5.03[e]
Percent absorption (0–96 h)		0.24[f]%		0.74[f]%		**0.90**[f]%
Animal no. 2						
Background (h)						
48–72	1.53	0.25	0.778	0.073	2.27	0.93
24–48	1.81	0.53	0.577	0	0.963	0
0–24	0.505	0	0.759	0.054	0.785	0
0–24	1.42[b]	0.14	1.45[c]	0.75	1.83[c]	0.49
24–48	0.97	0	1.31	0.61	1.73	0.39
48–72	1.30[d]	0.023	0.874[d]	0.17	1.10[d]	0
72–96	1.30[d]	0.023	0.874[d]	0.17	1.10[d]	0
Total arsenic mass excreted (0–96 h)		0.19		1.70		0.88
Total arsenic mass excreted with urinary arsenic excretion fraction correction (0–96 h)		0.23[e]		2.07[e]		1.07[e]
Percent absorption (0–96 h)		0.04[f]%		0.37[f]%		**0.19**[f]%
Animal no. 3						
Background (h)						
48–72	1.75	0.16	0.985	0	2.47	0.82
24–48	1.80	0.20	1.11	0.048	1.25	0
0–24	1.23	0	1.09	0.027	1.24	0
0–24	2.46[b]	0.86	1.09[c]	0.034	0.94[c]	0
24–48	1.88	0.29	1.28	0.22	2.08	0.43
48–72	1.46[d]	0	0.924[d]	0	1.46[d]	0
72–96	1.46[d]	0	0.924[d]	0	1.46[d]	0
Total arsenic mass excreted (0–96 h)		1.15		0.25		0.43
Total arsenic mass excreted with urinary arsenic excretion fraction correction (0–96 h)		1.41[e]		0.30[e]		0.53[e]
Percent absorption (0–96 h)		0.25[f]%		0.05[f]%		**0.09**[f]%

Note. —, not available or not applicable.

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

[b]Sum of 0–8 h, pan wash, 8–24 h, and cage wash (8–24 h).

[c]Sum of 0–8 h, pan wash, and 8–24 h.

[d]24-h mass excreted is estimated as one-half of 48- to 96-h sample mass.

[e]Calculated by correcting excreted mass for fractional excretion of arsenic from iv dose (i.e., by dividing by 0.82 or 82%).

[f]Percent absorption calculated using applied dose mass of 560 μg.

(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

TABLE 7
Summary of Percent Absorption of Arsenic

Study		Percent absorption (0–96 h) Average ± SD (%)
IV	—	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-μm size fraction.

bioavailability of 100%, surface area of 2800 cm^2, 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 95th percentile is likely in the range of 100–125 mg/day. Thus, even an upper bound soil ingestion rate is almost twofold 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; U.S. EPA, 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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