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Compilation and Review of Data on Relative Bioavailability of Arsenic in Soil

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACRONYMS AND ABBREVIATIONS

ABA	Absolute bioavailability
AF_o	Oral absorption fraction
AM	Arithmetic mean
As	Arsenic
AUC	Area-under-the-curve
bw	Body weight
CI	Confidence interval
CTE	Central tendency estimate
D	Dose
FeAs	Iron arsenide
ICP-AES	Inductively coupled plasma-atomic emission spectrometry
ICP-MS	Inductively coupled plasma-mass spectrometry
INAA	Instrumental neutron activation analysis
IRIS	Integrated Risk Information System
IVBA	*In vitro* bioaccessibility
kg	Kilogram
LCL	Lower confidence limit
mg	Milligram
n	Number of data points
NIST	National Institute of Standards and Technology
ppm	Parts per million
QA	Quality assurance
RAGS	Risk Assessment Guidance for Superfund
RBA	Relative bioavailability
RM	Reference material
SD	Standard deviation
SE	Standard error
SRM	Standard reference material
TM	Test material
UCL	Upper confidence limit
UEF	Urinary excretion fraction
µg	Microgram
µm	Micrometer
U.S. EPA	United States Environmental Protection Agency

1.0 INTRODUCTION

1.1 Background

The Risk Assessment Guidance for Superfund (RAGS) Part A (U.S. EPA, 1989), Framework for Metals Risk Assessment (U.S. EPA, 2007b), and Guidance for Evaluating the Bioavailability of Metals in Soils for Use in Human Health Risk Assessment (U.S. EPA, 2007c) discuss using site-specific bioavailability data to make adjustments to exposure estimates or toxicity values in Superfund site-specific risk assessments when the medium of exposure in the exposure assessment differs from the medium of exposure associated with the toxicity value (e.g., cancer slope factor, reference dose value, etc.). In the absence of reliable site-specific data, the default assumption is that the bioavailability of the contaminant is the same in the exposure medium at the site (e.g., soil, water, etc.) as in the exposure medium used to derive the toxicity value. For arsenic, the toxicity values in EPA's Integrated Risk Information System (IRIS) are based upon exposure to arsenic in water (U.S. EPA, 2012). The default assumption for assessing risk from arsenic in soil is that the bioavailability of arsenic in soil is the same as the bioavailability of arsenic dissolved in water. In other words, the relative bioavailability (RBA) of arsenic (all forms) in soil compared to water-soluble arsenic is assumed to be 1. This assumption will result in an overestimate of the true risk if the bioavailability of arsenic in soil is less than that of arsenic in water. The EPA is evaluating the general applicability and potential uncertainties associated with the assumption that the bioavailability of arsenic in soil is the same as that of water-soluble arsenic, and is also evaluating and developing laboratory methods for estimating RBA of soil arsenic. In support of these assessments, EPA is compiling information on bioassays that have been used to measure RBA of arsenic in soil along with estimates of RBA that have been derived from these bioassays. This report summarizes RBA estimates compiled as of September 2011. EPA expects that future data collection efforts will add to this data set and that the analyses in this report would be periodically updated.

1.2 Bioavailability – Definitions

In this report, the term *bioavailability* refers to the fraction or percentage of an ingested dose of arsenic that is absorbed into the systemic circulation. Bioavailability of arsenic in soil can be expressed either in absolute terms (absolute bioavailability) or in relative terms (relative bioavailability):

1. Absolute bioavailability (ABA) is defined as the ratio of the amount of arsenic absorbed to the amount ingested. This ratio is also referred to as the oral absorption fraction (AF_o).

2. Relative bioavailability (RBA) is defined as the ratio of the ABA or AF_o of arsenic present in the soil (test material, TM) to the absolute bioavailability of arsenic in some appropriate reference material (RM, Equation 1):

$$RBA = \frac{ABA_{TM}}{ABA_{RM}} \qquad \text{Eq. (1)}$$

3. Bioaccessibility refers to the physiological solubility of arsenic in the gastrointestinal tract (NRC, 2003). Ingested arsenic must become bioaccessible in the gastrointestinal tract in order to be absorbed. This process may include physical transformation of arsenic-bearing particles (e.g., break down of the particle to expose arsenic to gastrointestinal tract fluids), dissolution of arsenic, and chemical transformation of dissolved arsenic.

For human health risk assessment purposes, relative bioavailability is important because we are most often interested in knowing the extent to which the absolute bioavailability of a chemical increases or decreases in different exposure matrices (e.g., food vs. water vs. soil) or with the physical or chemical form(s) of the chemical to which humans are exposed.

For example, if 100 micrograms (µg) of arsenic dissolved in drinking water were ingested and a total of 50 µg were absorbed, the ABA (or AF_o) would be 50/100 or 0.50 (50%). Likewise, if 100 µg of arsenic contained in soil were ingested and 30 µg were absorbed into the body, the ABA (or AF_o) for arsenic in soil would be 30/100 or 0.30 (30%). The RBA for arsenic in soil, relative to arsenic in water, would be 0.30/0.50 or 0.60 (60%).

The form of arsenic typically used as the reference material in a RBA bioassay is an arsenic compound dissolved in water or a readily soluble form (e.g., sodium arsenate) that is expected to completely dissolve when ingested (i.e., 100% bioaccessible).

2.0 KEY AND RELEVANT STUDIES

A search of the literature was conducted to identify studies in which soil arsenic RBA was estimated from data collected in controlled human clinical studies or from animal bioassays. Studies that reported only bioaccessibility measurements (e.g., *in vitro* extraction of soils) or that attempted to predict arsenic RBA from bioaccessibility measurements were not included in this data compilation for several reasons. Although there is good evidence to suggest that bioaccessibility influences and may be an important determinant of RBA, there is no current consensus on whether or not *in vitro* bioaccessibility measurements can be used to accurately predict soil arsenic RBA. EPA has not identified a validated *in vitro* assay for predicting RBA. Other on-going efforts by EPA are evaluating methods for predicting arsenic RBA from bioaccessibility measurements.

Pertinent studies from the published literature were identified by searching bibliographic databases (i.e., PUBMED, TOXLINE) and other secondary source documents including the cited references of the retrieved literature. The search period for TOXLINE covered 1980 through August 2011 and for PUBMED was comprehensive through August 2011. Reference lists from selected literature were also searched. For additional information or clarification of published data, study authors were contacted as necessary.

Studies were classified as “key” or “relevant” based on considerations of experimental design, the number of different test materials analyzed in each animal species, and the source of test materials. RBA estimates were taken from studies that included a wide variety of bioassay

protocols that reflect methods currently being used to assess arsenic RBA. Requirements for inclusion in the analyses were that:

(1) the study was conducted by or for EPA in which EPA developed the RBA estimates from the raw data using established standard protocols and/or the raw data were available for Quality Assurance (QA) review by the U.S. EPA Bioavailability Committee of the Technical Review Workgroup (e.g., EPA swine and mouse studies); or

(2) the study was conducted by other research groups and results had been subjected to peer review as a requirement for publication. No attempt was made to reanalyze the primary data on which each RBA was based (e.g., to verify the RBA value or to apply the same data reduction methods to the raw data derived from different study protocols).

Evaluation of multiple test materials in each animal species was considered important for characterization of uncertainty and variability in RBA estimates. Studies described in this report assessed RBA of soils that were contaminated *in situ*. Studies of soils that were spiked with arsenic in the laboratory (Juhasz et al., 2008; Konstantinos et al., 2008; Nagar et al., 2009) were not considered based on evidence that RBA of soils spiked with highly bioaccessible sodium arsenate can change as the soil ages (Juhasz et al., 2008). Studies that assessed absolute bioavailability and did not report RBA or provide data for calculation of RBA (i.e., Ellickson et al., 2001) were not considered. As described in Section 2.2 (Key Studies), all "key" studies were conducted in swine, monkey, or mouse; multiple test materials were analyzed using these animal models to estimate arsenic RBA. In "key" studies, a total of 103 RBA estimates for 88 unique test materials were obtained in swine (64 RBA estimates), monkeys (24 RBA estimates), and mice (15 RBA estimates). Among these "key" studies, direct comparisons of swine, monkey, and mouse RBA estimates are available for only 4 test materials and direct comparisons of swine and mice RBA estimates are available for 11 test materials. Data obtained from "key" studies were analyzed to develop summary statistics describing the distribution of RBA values and to explore sources of variability in the RBA values (i.e., using regression analysis). As described in Section 2.3 (Relevant Studies), "relevant" studies analyzed a single test material using a unique animal model (i.e., rabbit). "Relevant" studies provided supportive data, but were not included in the statistical summary.

A single human experimental study of bioavailability of arsenic soil was reported (Stanek et al., 2010). This study was not selected for inclusion in this report as a key or relevant study because of several methodological limitations and uncertainties, which are summarized in Appendix A.

2.1 Methodologies Used in Key and Relevant Studies

A variety of different *in vivo* methods have been utilized for estimating soil arsenic RBA. All of these methods share a common general approach in which biomarkers of arsenic absorption (blood arsenic concentration or urinary arsenic excretion) were measured following a single dose or during a period of repeated dosing with arsenic in soil (the test material) and

following dosing with sodium arsenate (the reference material). The study protocols differ with respect to dose (e.g., mg/kg), dosing frequency, the absorption biomarker measured (blood or urine arsenic), and the computational methods applied to the data for calculating RBA.

In studies that measured urinary arsenic excretion, the absorption dose metric was the urinary excretion fraction (UEF) which is the amount or rate of arsenic excreted in urine (U_{As}) divided by the arsenic dose (D_{As}, Equation 2).

$$UEF = \frac{U_{As}}{D_{As}} \quad \text{Eq. (2)}$$

The RBA was estimated as the ratio of the UEF for arsenic when administered in soil (test material, TM) to that of the reference material (RM; i.e., sodium arsenate, Equation 3).

$$RBA = \frac{UEF_{TM}}{UEF_{RM}} \quad \text{Eq. (3)}$$

In studies in which animals were dosed one time, the UEF was the cumulative amount of arsenic excreted during a defined post-dose observation period (e.g., 4 days) divided by the administered dose. In studies in which doses of arsenic were administered repeatedly to achieve a quasi-steady state, the UEF was the rate of excretion of arsenic (e.g., µg As/day) divided by the dosing rate (e.g., µg As/day). In studies in which arsenic was administered at more than one dose (e.g., 25, 50, or 100 µg As/kg bw/day), the UEF was estimated as the regression slope of the relationship between urinary arsenic excretion and dose.

In studies that relied on blood arsenic concentration for estimating RBA, the absorption dose metric was the time-integrated arsenic blood concentration. This was typically measured as the time-integrated blood concentration of arsenic, referred to in this report and in most of the literature as the area under the curve (AUC) of the arsenic blood concentration-time profile (e.g., estimated using a geometric approximation such as the trapezoid rule). The AUC estimate was divided by the administered dosage, and the RBA was estimated as the ratio of AUC/dose for the test and reference materials (Equation 4).

$$RBA = \frac{AUC_{TM}}{D_{TM}} \div \frac{AUC_{RM}}{D_{RM}} \quad \text{Eq. (4)}$$

If arsenic was administered at more than one dose (mg/kg), the AUC/dose ratio was estimated as the regression slope of the relationship between the blood AUC and dose.

Each of these methods is described in greater detail in the sections that follow.

2.1.1 Single Dose Urinary Excretion Fraction Method

In studies conducted using this method, a one-time oral dose of test material or reference material (sodium arsenate) was administered. Following administration of the arsenic dose, urine was collected for up to 7 days. Relative bioavailability in test materials was calculated as the ratio of the UEFs for the test and reference materials, where the UEF was the cumulative urinary excretion of arsenic divided by the arsenic dose.

2.1.2 Repeated Dose Steady-State Urinary Excretion Fraction Method

In studies conducted using this method, groups of animals typically were dosed with the test material or reference material (sodium arsenate) repeatedly for 10–15 days. At various times during the dosing period, urine samples were collected from each animal and analyzed for arsenic. The RBA of a test material was calculated as the ratio of the UEFs for the test and reference materials. In studies in which a single dose level was administered, UEF was estimated as the cumulative urinary arsenic excretion (e.g., µg As) divided by the dose. In studies in which arsenic was administered at more than one dose level (e.g., 25, 50, or 100 µg As/kg bw/day), UEF was calculated by fitting a regression model to the data on dose and urinary excretion and estimating UEF as the regression slope.

2.1.3 Single Dose Blood-Time Concentration Curve Method

In studies conducted using this method, groups of animals were administered a one-time oral dose of test material or reference material (sodium arsenate) or an intravenous dose of the reference material. Test and reference materials were administered at multiple dose levels. Blood samples were collected at various time points up to 6 days after dosing. For the calculation of RBA, the time-integrated blood arsenic concentration (AUC) and arsenic dose for both the test material and reference material were subjected to regression analysis. RBA was estimated as the ratio of the regression slopes.

2.2 Key Studies

Methods and protocols of key studies are summarized below. Many of these studies estimated RBA for multiple test materials. Sources of uncertainties that were considered in assessing confidence in RBA estimates and making statistical inference regarding arsenic RBA in soils are summarized in Table 1. The identity of the individual test materials, dosing schedules, and dose levels used to assess RBA for each test material are provided in Table 2.

2.2.1 U.S. EPA, 2010

The RBA of arsenic was estimated for several test materials using the steady-state urinary excretion fraction method described in U.S. EPA (2010). These studies were sponsored by U.S. EPA Region 8. Test materials were obtained from various locations throughout the U.S. and included residential and non-residential soils and mining slag. The concentration of arsenic in these test materials ranged from 72 to 1050 ppm. All studies were performed using young, intact male swine (genetically defined Line 26 strain), typically 5 to 7 weeks old, weighing 7 to 12 kg. Groups of animals (usually 4–5 per dose group) were exposed to 1 to 3 dose levels of test material or reference material (sodium arsenate) daily for 12–15 days. Test materials were placed in the center of moistened feed (dough ball) and administered to the animals by hand. Sodium arsenate (reference material) was administered by gavage or intravenous injection. Samples of urine were collected from each animal on several different days during the study (the exact days varied from study to study, with collection periods ranging from 24–48 hours). Urine samples were prepared for analysis using one of two alternative methods referred to as Phase II

(acid digestion) and Phase III (acid digestion and ashing). Arsenic in digested urine samples was measured by hydride generation using atomic absorption spectrometry (limit of detection ~1–2 µg/L). Detailed descriptions of the acid digestion and ashing methodologies are provided in U.S. EPA (2010). The Phase II method yielded a poor recovery of organic metabolites of arsenic, which could result in underestimates of urinary arsenic. However, comparative studies using the same test materials showed that the Phase II and Phase III methods yielded essentially the same RBA estimates. Therefore, RBA estimates using Phase II methods are considered reliable. For the RBA calculation, regression was used to estimate the slope of the relationship between urinary arsenic excretion (e.g., µg/day) and arsenic dose (e.g., µg/day) for both the test and reference materials. The RBA of the test material was calculated as the ratio of the slopes. A total of 24 test materials were evaluated with RBA estimates ranging from 8 to 61%.

2.2.2 Casteel and SRC, 2005

The RBA of arsenic was estimated for one test material using the steady-state urinary excretion fraction method described in U.S. EPA (2010). This study was sponsored by U.S. EPA Region 6. The test material was a soil sample containing 47 ppm arsenic, obtained from a U.S. Superfund site in Palestine, Texas. The study was conducted using Phase III methodology as described in U.S. EPA (2010), with groups of 5 intact male swine (genetically defined Line 26 strain) administered 3 dose levels of test material or reference material (sodium arsenate) daily for 15 days. The estimated RBA of the test material was 15%.

2.2.3 Casteel and SRC, 2009a

The RBA of arsenic was estimated for four test materials using the steady-state urinary excretion fraction method described in U.S. EPA (2010). This study was sponsored by U.S. EPA Office of Superfund Remediation and Technology Innovation. The test materials were soil samples containing 290 to 388 ppm arsenic obtained from a former commercial apple orchard, the Barber Orchard site located near Waynesville, Haywood County, North Carolina. The study was conducted using Phase III methodology as described in U.S. EPA (2010), with groups of 4 intact male swine (genetically defined Line 26 strain) administered 2 to 3 dose levels of test material or reference material (sodium arsenate) daily for 14 days. The RBA of the test materials ranged from 31 to 53%. Arsenic RBA estimates for these four Barber Orchard test materials were also obtained in monkeys (U.S. EPA, 2009; see Section 3.2.8).

2.2.4 Casteel and SRC, 2009b

The RBA of arsenic was estimated for one test material using the steady-state urinary excretion fraction method described in U.S. EPA (2010). This study was sponsored by U.S. EPA Office of Superfund Remediation and Technology Innovation. The test material was a sample of National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 2710. This soil sample, collected in Montana from an area contaminated by mine tailings deposits, contained 626 ppm arsenic. The study was conducted using Phase III methodology as described in U.S. EPA (2010), with groups of 4 intact male swine (genetically defined Line 26 strain) administered 3 dose levels of test material or reference material (sodium arsenate) daily for 14 days. The RBA of the test material was 44%.

2.2.5 Casteel and SRC, 2009c

The RBA of arsenic was estimated for one test material using the steady-state urinary excretion fraction method described in U.S. EPA (2010). This study was sponsored by U.S. EPA Office of Superfund Remediation and Technology Innovation. The test material was a sample of soil from the Mohr Orchard site located in Region 3, Lehigh County, Pennsylvania. The arsenic concentration of the Mohr Orchard soil sample was 340±4.5 mg/kg (mean±SD). The study was conducted using Phase III methodology as described in U.S. EPA (2010), with groups of 4 intact male swine (genetically defined Line 26 strain) administered 3 dose levels of test material or reference material (sodium arsenate) daily for 14 days. The RBA of the test material was 53%.

2.2.6 Casteel and SRC, 2010a

The RBA of arsenic was estimated for two test materials using the steady-state urinary excretion fraction method described in U.S. EPA (2010). This study was sponsored by U.S. EPA Office of Superfund Remediation and Technology Innovation. The test materials were samples of soil from the Iron King Mine – Humboldt Smelter Superfund Site. The soil samples (HSJ583 and IKJ583) were collected from the Chaparral Gulch near a residential area (HSJ583) and a tailings pile (IKJ583). The mean arsenic concentrations of the soil samples were 200 ppm (HSJ583, TM1) and 3957 ppm (IKJ583, TM2). The study was conducted using Phase III methodology as described in U.S. EPA (2010), with groups of 4 intact male swine (genetically defined Line 26 strain) administered 3 dose levels of test material or reference material (sodium arsenate) daily for 14 days. The RBA of the test materials were 60% (TM1) and 19% (TM2).

2.2.7 Casteel and SRC, 2010b

The RBA of arsenic was estimated for two test materials (ASARCO and Hawaii) using the steady-state urinary excretion fraction method described in U.S. EPA (2010). This study was sponsored by U.S. EPA Office of Superfund Remediation and Technology Innovation. The ASARCO material was collected from a former smelter site near Tacoma, Washington. Multiple samples were collected from a stockpile of soil that was removed from residential properties and composited prior to analysis. The Hawaii material was collected from a garden plot used by Kea'au Middle School, located in the town of Kea'au on the island of Hawaii. The garden has high arsenic concentrations attributable to herbicide use between 1920 and 1950 in former sugar mill plantation lands in the area. The soil samples contained 182 ppm (ASARCO) and 769 ppm (Hawaii) arsenic. The study was conducted using Phase III methodology as described in U.S. EPA (2010), with groups of 4 intact male swine (genetically defined Line 26 strain) administered 3 dose levels of test material or reference material (sodium arsenate) daily for 14 days. The RBA of the test materials were 49% (ASARCO) and 33% (Hawaii).

2.2.8 Casteel and SRC, 2010c

The RBA of arsenic was estimated for one test material using the steady-state urinary excretion fraction method described in U.S. EPA (2010). This study was sponsored by U.S. EPA Office of Superfund Remediation and Technology Innovation. The test material was a sample of

NIST SRM 2710a. This soil sample, obtained in Montana from an area contaminated by mine tailings deposits, contained 1540 ppm arsenic. The study was conducted using Phase III methodology as described in U.S. EPA (2010), with groups of 4 intact male swine (genetically defined Line 26 strain) administered 3 dose levels of test material or reference material (sodium arsenate) daily for 14 days. The RBA of the test material was 42%.

2.2.9 Basta et al., 2007; Rodriguez et al., 1999

Rodriguez et al. (1999) estimated the RBA of arsenic in several test materials in juvenile swine using the same steady-state urinary excretion fraction method described in U.S. EPA (2010). Test materials (soils and slags), with arsenic concentrations ranging from 233 to 17,500 ppm, were collected from mining/smelter sites in the western U.S. Studies were performed in young, intact male swine (Line 26 strain), weighing 10–12 kg. Test groups of animals (2–5 per dose group) were administered a single dose level of test material (in a dough ball) and a control group was administered a reference material (sodium arsenate). The animals were dosed daily for 15 days, and urine was collected for five 24-hour periods. For the calculation of RBA, the UEF of arsenic (cumulative urinary excretion/dose) administered in test material and in reference material (sodium arsenate) was calculated, and the RBA was calculated as the ratio of the UEF values. The Rodriguez et al. (1999) report did not include standard deviations (SD), standard errors (SE), or confidence limits (CI) for mean RBA values. Due to concerns regarding recovery of organoarsenical compounds in urine, Basta et al. (2007) re-analyzed urine samples from nine test materials reported in Rodriguez et al. (1999) using the Phase III analytical method (U.S. EPA, 2010). Revised RBA estimates for these nine samples were reported graphically in Basta et al. (2007); numeric values (mean RBA estimates and standard deviations) were provided for this report through a personal communication with Dr. Basta. A total of 14 test materials were evaluated in the Basta et al. (2007) and Rodriguez et al. (1999) studies, with RBA estimates ranging from 4 to 43%.

2.2.10 U.S. EPA, 1996

In a study sponsored by U.S. EPA Region 10, the RBA of arsenic was estimated for two test materials (mining soil and slag collected from the Ruston/North Tacoma Superfund site) using the single dose blood-time concentration curve method. Arsenic concentrations in the test materials were 1600 ppm for the mining soil and 10,100 ppm for the slag. The study was conducted in young, female swine (bred from Hampshire sires and Landrace/Large White/Duroc dams), 6–7 weeks of age, weighing approximately 15 kg. Groups of three animals were administered a single oral dose of test material as an aqueous suspension or single oral or intravenous dose of reference material (sodium arsenate); multiple dose levels of test and reference materials were evaluated. Following administration, blood samples were obtained at various time points from 15 minutes to 144 hours after dosing. Following acid digestion and heat treatment, arsenic was measured by hydride generation using atomic absorption spectrometry (limit of detection = 1 μg/L). Regression models were fit to the data on time-integrated blood arsenic concentration (AUC) and dose, and RBA was calculated as the ratio of slopes for test and reference materials. The study report did not include standard deviations or standard errors, but reported 95% confidence limits. RBA estimates ranged from 42% (slag) to 78% (soil).

2.2.11 Juhasz et al., 2007

Juhasz et al. (2007) estimated the RBA of arsenic in several Australian test materials, with arsenic concentrations ranging from 42 to 1114 ppm, using the single dose blood-time concentration curve method. Test materials were collected from railway corridors, cattle tick dip sites, mining sites, and gossans (areas containing naturally elevated concentrations of arsenic). Groups of 3 female swine (strain: large white; body weight: 20 to 25 kg) were administered single doses of test materials as soil slurries or sodium arsenate by gavage. Blood samples were collected at various times up to 26 hours following dosing. Samples were digested by nitric acid or ammonium hydroxide; arsenic was measured by inductively coupled plasma-mass spectrometry (ICP-MS; limit of detection not reported). Relative bioavailability of arsenic in test materials was determined using the ratio of the time-integrated blood arsenic concentration (AUC) divided by the dose, for the test and reference material. Although Juhasz et al. (2007) did not report RBA estimates for individual test materials, study authors provided means and standard deviations for individual test materials in a personal communication (dated June 18, 2008). A total of 12 test materials were evaluated in this study, with RBA estimates ranging from 7 to 75%.

2.2.12 Roberts et al., 2007

The RBA of arsenic was estimated for several soils (arsenic concentration range: 125 to 1492 ppm) collected from various locations throughout the U.S. (California, Colorado, Florida, Hawaii. Montana, New York, Washington, and Wisconsin) using the single dose urinary excretion fraction method. The study was conducted in young adult male cynomolgus monkeys, weighing 4 to 5 kg. Five animals were administered single doses of test materials (as soil slurry) or reference material (sodium arsenate) by gavage. Each monkey received the test and reference material, with dosing of each material separated by at least 3 weeks. Urine and feces were collected for 4 days after dosing. Urine samples were treated with nitric acid, heat, and hydrogen peroxide; urin arsenic was measured using inductively coupled plasma-atomic emission spectrometry (ICP-AES) (limit of detection = 2.3 µg/L). The relative bioavailability in test materials was determined using the ratio of the UEF for test and reference materials, where UEF was the cumulative urinary arsenic (µg) excretion divided by the arsenic dose (µg). A total of 14 test materials were evaluated in this study, with RBA estimates ranging from 5 to 31%.

2.2.13 U.S. EPA, 2009

The RBA of arsenic was estimated for 4 soils collected from the Barber Orchard site near Waynesville, Haywood County, North Carolina (a former commercial apple orchard, soil arsenic concentration range: 290 to 388 ppm) using the single dose urinary excretion fraction method. Single doses of test materials (as soil slurry) or reference material (sodium arsenate) were administered by gavage to 5 young adult male cynomolgus monkeys, weighing 4 to 5 kg. Each monkey received the test and reference material, with dosing of each material separated by at least 3 weeks. Urine and feces were collected for 4 days after dosing. Urine samples were treated with nitric acid, heat, and hydrogen peroxide; urinary arsenic was measured using ICP-AES (limit of detection = 0.3 µg/L). Relative bioavailability in test materials was determined

using the ratio of the UEF for test and reference materials, where UEF was the cumulative urinary arsenic (µg) excretion divided by the arsenic dose (µg). RBA estimates for the Barber Orchard test materials assayed in this study ranged from 25 to 38%. RBA estimates for these 4 Barber Orchard test materials were also obtained in swine (Casteel and SRC, 2009a; see Section 3.2.3).

2.2.14 Roberts et al., 2002

The RBA of arsenic was estimated for contaminated Florida surface soils (arsenic concentration range: 101 to 743 ppm) using the single dose urinary excretion fraction method. The study was conducted using adult male *Cebus apella* monkeys, weighing 2.5 to 3 kg. Single doses of test materials (as soil slurry) or reference material (sodium arsenate) were administered by gavage to 5 animals. Urine and feces were collected for 4 days after dosing. Urine samples were treated with nitric acid, heat, and hydrogen peroxide; urinary arsenic was measured using ICP-AES (limit of detection = 2.5 µg/L). Relative bioavailability in test materials was determined using the ratio of the UEF for test and reference materials, where UEF was the cumulative urinary arsenic (µg) excretion divided by the arsenic dose (µg). A total of 5 test materials were evaluated in this study, with RBA estimates ranging from 11 to 25%.

2.2.15 Freeman et al., 1995

Freeman et al. (1995) estimated the RBA of arsenic in a single test material (residential soil, arsenic concentration: 410 ppm) using both the single dose urinary excretion fraction and single dose blood-time concentration curve methods in female cynomolgus monkeys (weighing 2 to 3 kg). Three female monkeys were administered single doses of the test material in a capsule by gavage or reference material (sodium arsenate in solution) by gavage or intravenous injection. Each monkey received the test and reference material. Urine was collected for 7 days after dosing, and blood samples were collected at several time points from 15 minutes to 120 hours after dosing. In this study, the ABA of arsenic was calculated for the test and reference materials. For this report, RBA was calculated as the ratio of the reported ABA for the test and reference material.

Freeman et al. (1995) estimated arsenic ABA from both measurements of UEF and time-integrated arsenic blood concentration (AUC). For each, the ABA was calculated as the ratio of the biomarker measured following the oral dose to that measured following an intravenous dose (i.e., 100% absorption, Equations 5 and 6):

$$ABA = \frac{UEF_{TM,oral}}{UEF_{RM,iv}} \quad \text{Eq. (5)}$$

$$ABA = \frac{AUC_{TM,oral}}{D_{TM,oral}} \div \frac{AUC_{RM,iv}}{D_{RM,iv}} \quad \text{Eq. (6)}$$

The arsenic RBA calculated based on the UEF data for the individual animals (n=3) was 20.1% (SD=6.9%), compared to 11.0% (SD=7.7%) based on the blood AUC data. These estimates are not significantly different (paired t-test, p=0.37).

2.2.16 Bradham et al., 2011, 2012

The RBA of arsenic was estimated for contaminated surface soils (arsenic concentration range: 182 to 4495 ppm) using the repeated dose steady state urinary excretion fraction method (Bradham et al., 2011, 2012). Test materials were obtained from various locations throughout the U.S. and included agricultural soils and soils impacted by mining and smelting. Four to six week-old female C57BL/6 mice were fed diets containing the test soil or sodium arsenate. The test soil and sodium arsenate groups typically consisted of 12 mice that were housed in metabolic cages containing 3 mice per cage. The test soil was mixed into the powdered AIN-93G purified rodent diet to achieve a 1% (w/w) soil:diet ratio. Mice received the diets for a period of 10 days during which urine and feces were collected daily. Arsenic concentrations in diet, soil, urine, and feces were determined by Instrumental Neutron Activation Analysis (INAA). Daily arsenic dosages were estimated from measurements of daily diet consumption. Doses ranged from 0.32 to 6.10 mg As/kg bw/day, and soil dose ranged from 1.15 to 1.65 g soil/kg bw/day (over a 10-day period). Arsenic RBA was estimated as the ratio of UEFs for soil arsenic and sodium arsenate treatment groups, where the UEF was the cumulative urinary arsenic (µg) excretion divided by the cumulative arsenic dose (µg). A total of 15 test materials were evaluated in these studies, with RBA estimates ranging from 11 to 52%.

2.3 Relevant Studies

Studies that evaluated soil arsenic RBA bioavailability using a unique animal model (i.e., rabbit) were considered to be "relevant" studies in that they provided supportive data but were not included in the data analysis.

2.3.1 Freeman et al., 1993

Freeman et al. (1993) estimated the RBA of arsenic in a single test material using the single dose urinary excretion fraction method in New Zealand white rabbits. The arsenic concentration of the test material (soil contaminated through smelter activities) was 3900 ppm. Groups of 5 male and 5 female rabbits (9 to 12 weeks old, body weight 2 kg) were administered single oral doses of test material (formulated in a gelatin capsule) or reference material (sodium arsenate solution). Urine was collected for 120 hours after dosing. Urine samples were digested with nitric acid and hydrogen peroxide, and urine arsenic was measured using ICP-MS (limit of detection = 30 µg/L). The RBA of the test material was estimated by calculating the ratio of the UEF values for test and reference materials normalized for dose. This study did not report standard deviations, standard errors, or confidence limits for the mean RBA values of 48%.

3.0 LIMITATIONS OF DATA

The data used to estimate RBA for arsenic in soil materials have the following limitations and uncertainties for making generic prediction of soil arsenic RBA in humans.

Extrapolation of results to humans: The swine and monkey models have been utilized to predict human RBA of arsenic for site risk assessment because the gastric physiology of both animal species is similar to that of humans (U.S. EPA, 2007a) and because of a prior history of

using these models for assessing RBA of other inorganic contaminants (e.g., lead; U.S. EPA, 2007a) and gastrointestinal absorption of drugs (Chiou and Buehler, 2002; Roberts et al., 2007). Although estimates of RBA of arsenic in soil materials in animal models have not been quantitatively compared to estimates made in humans for the same material, this report shows that RBA estimates obtained from swine, monkey, and mouse for the same test materials are sufficiently similar to suggest that large differences in RBA across mammalian species are unlikely. This increases confidence in extrapolating RBA estimates obtained from these assays to humans.

Comparability of estimates from swine, monkey, and mouse assays: When applied to the same test materials, the swine, monkey, and mouse assays yielded remarkably similar RBA estimates for some materials and widely different estimates for other materials (see Section 4.2.1). However, collectively, the differences in the RBA estimates were relatively small. The absolute difference in the RBA estimates (e.g., RBA_{swine} - RBA_{mouse}, RBA_{swine} - RBA_{monkey}) ranged from <1 to 28%, and the average difference was 12%. This magnitude of difference is relatively small in the context of risk assessment, where uncertainties in other parameters in risk calculations can exceed several orders of magnitude. Therefore, from the perspective of use of the assays to support risk assessment, the swine, monkey, and mouse assays appear to yield essentially equivalent information about arsenic RBA.

The reason why the same test materials give different outcomes in the three animal models are discussed in Section 4.4.1.

Single dose vs. steady-state models: Animal models that estimate RBA with steady state dosing have some useful advantages over single dose assays.

(1) Steady state models more closely mimic the status of the human receptor who receives continuous daily exposure to soil.

(2) At steady state, urinary excretion of arsenic will be relatively constant over time, and as a result, urinary arsenic excretion rate and UEF can be estimated by averaging multiple estimates obtained from several urine samples collected over time. By contrast, in a single dose study, UEF must be estimated as the cumulative urinary arsenic excretion. This requires absolute accuracy in sampling urine at each interval of the post-dosing observation period.

(3) Random errors in urine sampling (e.g., completeness of collection) would be expected to have a larger impact on estimates of the cumulative arsenic excretion than on average steady state arsenic excretion.

Single vs. multiple dose level models: Assays that estimate RBA at multiple arsenic dose levels have some useful advantages over single dose level assays.

(1) Potential dependence of UEF on arsenic dose level can be detected and accounted for in the data reduction and estimate of RBA. Thus far, dose dependence of arsenic

UEF has not been demonstrated in swine or monkeys, at least not with the range of arsenic doses examined in reported studies (Roberts et al., 2007; U.S. EPA, 2010).

(2) In multiple dose level studies, UEF can be estimated from regression models of the relationship between excretion and dose (i.e. change in urinary arsenic excretion/change in dose level) This provides a statistical alternative to discrete estimates of UEF based on results obtained at a single dose level.

Test material dose levels: Ideally, animal bioassays should administer test material doses (i.e., mg soil/kg bw/day) that are similar to those expected in the human receptor population. This would reduce uncertainty related to possible dependences of arsenic RBA on test material dose. However, the design of animal RBA assays, particularly detection limits for blood and urinary arsenic and the wide variation in the arsenic concentrations of test materials, has placed constraints on experimental control of both the arsenic dose and test material dose used in each assay. The doses (single doses were administered) of test material in key studies ranged from approximately 0.4 to 3528 mg soil/kg bw in swine, 490 to 2970 mg soil/kg bw in monkeys and 1150 to 1650 mg soil/kg bw in mice. These ranges include values that are substantially higher than typical daily soil ingestion rates in children or adults (U.S. EPA, 2008). The implication of these high test material doses in extrapolating RBA estimates from animals to humans (e.g., effect of the test material dose on RBA) has not been thoroughly investigated.

4.0 SUMMARY OF ARSENIC RBA ESTIMATES

4.1 Summary of Arsenic RBA Estimates

Relative bioavailability estimates for individual test materials evaluated in "key" and "relevant" studies are summarized in Table 2. Summary statistics for RBA estimates from "key" studies are provided in Table 3. "Key" studies consist of 64 RBA estimates based on bioassays in juvenile swine (Basta et al., 2007; Casteel and SRC, 2005, 2009a,b,c, 2010a,b,c; Juhasz et al., 2007; Rodriguez et al., 1999; U.S. EPA, 1996, 2010), 24 RBA estimates based on bioassays in monkeys (Freeman et al., 1995; Roberts et al., 2002, 2007; U.S. EPA, 2009), and 15 RBA estimates based on bioassays in mice (Bradham et al., 2011, 2012). Eleven test materials were evaluated in both swine and mice, and 4 test materials (Barber Orchard soils) were evaluated in swine, monkeys, and mice. Test materials assessed in "key" studies come from sites impacted by various arsenic sources: mining/smelting (n=57); agriculture, including orchards and livestock dipping sites (n=12); other chemical manufacturing/processes, mainly pesticide manufacture (n=9); railway corridors (n=6); and miscellaneous or uncharacterized sites such as volcanic soils (n=1). In developing summary statistics shown in Table 3, two approaches were used:

(1) RBA estimates for materials tested in more than one assay were treated either as independent estimates (where RBA is represented in sample statistics), or

(2) as repeated measurements of the same sample (where the average value for all assays of the same test material is represented in the sample statistics).

The two approaches yield essentially the same values for the summary statistics (n=103 or n=88, see Table 3). For the entire data set (n=103), RBA estimates ranged from 4.1 to 78%, with an arithmetic mean of 31% (±16, SD, 5th–95th percentile range: 7–57%).

Summary statistics shown in Table 3 give equal weight to each of the RBA estimates in the key study data set. However, each RBA estimate represents a mean value for a group of animals, and each mean has an associated uncertainty given by the standard error and confidence limits. If each RBA estimate were to be weighted according to its associated confidence, the resulting distribution of RBA estimates would be a more accurate reflection of the confidence in each RBA estimate. Monte Carlo simulation was used to derive an uncertainty-weighted estimate of the mean and selected percentiles and to derive confidence limits for these empirical parameters. Monte Carlo analysis was conducted as follows.

(1) For each test material, a mean RBA and standard error (SE) were identified.

(2) A distribution for the mean RBA for each test material was defined as

TRUNCATED NORMAL (mean, SE, 0, 100)

where 0 and 100 were the truncation limits and represent the minimum and maximum values possible for RBA, respectively, and SE is the standard error. If the standard deviation (SD) was reported but not a SE, the SE was estimated as SD/n$^{0.5}$, where *n* was the number of animals represented in the mean. If confidence limits were available but not standard errors, the standard error was estimated assuming the standard normal distribution of error and the appropriate value for Z value for the standard normal distribution (i.e., 1.96 for 95% confidence limits). For 95% upper and lower confidence limits (UCL, LCL), the corresponding SE was calculated as follows (Equation 7).

$$SE = \frac{95\%UCL - 95\%LCL}{2 \cdot 1.96} \qquad \text{Eq. (7)}$$

(3) Each iteration of the Monte Carlo simulation consisted of a random selection from the distribution of means from each and every test material (i.e., sampling without replacement). Iteration yielded 10,000 sets of RBA estimates (one per test material).

(4) The mean and 5th, 50th and 95th percentile RBA values were calculated for each iteration of the Monte Carlo, yielding 10,000 realizations of each parameter.

(5) The 2.5th percentile and 97.5th percentile values were calculated from the 10,000 values for each parameter. These were used to represent the 95% confidence intervals on the mean 5th, 50th, and 95th percentile RBA values.

Results of the Monte Carlo analysis are shown in Table 4. The uncertainty-weighted estimates from the Monte Carlo simulation are very similar to the unweighted estimates (see Table 3). For example, the weighted estimate of the 50th percentile (n=103) is 28.5% (unweighted = 29.1%), and the confidence interval is 26–31%. The weighted estimate of the 95th percentile RBA is 58.1% (compared to 56.8% for the unweighted estimate), and the confidence

interval is 53–64%. Truncation of the distributions used in the Monte Carlo analysis had a negligible effect on the weighted parameter estimates and confidence limits. Only one RBA estimate, the Tacoma, WA sample (U.S. EPA, 1996), which had an RBA of 78% (±14 SE) in swine, would have been affected by truncation. A random draw from this distribution would be expected to yield values 2 SE above the mean (106%) at a frequency of approximately 2.5%. However, this had a minimal effect on the weighted estimates and confidence limits for the full data set.

4.2 Factors Influencing RBA Estimates

RBA estimates showed a wide range (i.e., 4.1 to 78%). Variability in RBA estimates may be due to several factors, including differences between animal species, experimental methods and methods of data reduction, arsenic source, arsenic soil concentration and dose, soil characteristics, and arsenic mineralogy. Not all of these factors could be assessed with the available data.

4.2.1 Species Differences

Comparisons of RBA estimates assayed in swine, monkeys, and mice show that arsenic RBA estimates for materials assayed in swine and mice tended to be higher than estimates for test materials assayed in monkeys (see Table 3, Figure 1). The mean RBA estimates for test materials assayed in swine and mice are 34.5% (95% CI: 30.2–38.8, n=64) and 33.5% (95% CI: 27.1–39.8, n=15), respectively, compared to 19.2% (95% CI: 15.8–22.6, n=24) in monkeys. Data from two different species of monkey, cynomolgus (Freeman et al., 1995; Roberts et al., 2007) and *C. apella* (Roberts et al., 2002), are represented in the data set. These data were combined in the summary statistics reported above because comparison of RBA estimates from cynomolgus and *C. apella* bioassays did not show significant differences. The mean RBA values were 19.9% (±9.2 SD, n=19) for cynomolgus and 16.7 (±5.1 SD, n=5) for *C. apella*. However, these estimates correspond to different test materials assayed in the two species. Available data do not allow comparisons of RBA estimates for the same test materials assayed in different monkey species to determine if different species actually yield different RBA values. Given the lack of information on which to distinguish RBA estimates from cynomolgus and *C. apella*, RBA estimates from both monkeys species were combined for comparison of RBA estimates from swine, monkey, and mouse assays (described below).

Differences between RBA estimates from swine, monkey, and mouse assays may also be attributable to:

(1) species difference in RBA;

(2) differences in assay protocols;

(3) differences in data reduction methods used to calculate RBA;

(4) differences in methods used to measure arsenic concentration in soils and biological samples, and

(5) differences in the test materials assayed.

Theoretically, direct comparison of results from different bioassays when applied to the same test materials would provide a test of whether or not differences can be attributed to the test materials, rather than to the bioassay protocols and/or species. Thus far, such direct comparisons between swine, monkey, and mouse assays are available for only 4 test materials, all of which were obtained from the same site (Barber Orchard, Region 4). These data are shown in Table 5 and Figure 2. The sample size (n=4) is too small to make meaningful statistical comparisons. However, based on the 95% confidence limits, the uncertainty bounds on estimates obtained from the three assays show substantial overlap. Furthermore, the 95% confidence limits on the group mean RBA (n=4) also overlap substantially (see Figure 2). Therefore, if these four soil samples were used in a risk assessment to represent the RBA for the Barber Orchard site (it is not unusual to base site-wide RBA estimates on a few samples of *in vivo* RBA estimates), the site-wide RBA estimates from the swine, monkey, and mouse assays would be statistically indistinguishable.

A larger set of comparisons are available for swine and mouse RBA estimates. The data set includes 2 standard reference materials (NIST 2710 and 2710a), the 4 Barber Orchard samples, and 5 soil samples from 4 other sites (see Table 6). Collectively, these comparisons show that the assays yielded similar results for 5 of the materials (95% confidence limits overlap) and dissimilar estimates for 6 of the materials (see Figure 3). In all of the latter cases, the RBA from the mouse bioassay was less than the RBA from the swine assay. Figure 4 shows a scatter plot of RBA estimates in swine and mice for these 11 test materials. The data tend to cluster around the line of identity; however, the linear regression model showed a relatively weak association between the RBA estimates obtained in swine and mice (R^2=0.35, p=0.053). Although different RBA values were obtained from the swine and mouse assays for some test materials, the differences were relatively small. The absolute difference in the RBA estimates (RBA_{swine} - RBA_{mouse}) ranged from ≤1% (NIST 2710 and 2710a) to 28% (Barber Orchard MS-5), and the average difference was 12%. For the 4 Barber Orchard soils, the absolute difference between swine and monkey RBA values (RBA_{swine} - RBA_{monkey}) ranged from 2% (Barber Orchard MS-1) to 28% (Barber Orchard MS-8), and the average difference was 8%; and the absolute difference between monkey and mouse (RBA_{mouse} - RBA_{monkey}) ranged from 7% (Barber Orchard MS-1 and MS 4) to 17% (Barber Orchard MS-5), and the average difference was 10%.

4.2.2 Urinary Excretion Fraction (UEF) Method vs. Blood AUC Method

In theory, we expect RBA estimates based on blood AUC measurements to be equivalent to RBA estimates based on urinary excretion measurements. The underlying assumption for both methods is that arsenic absorbed from the test and reference materials have the same toxicokinetics; and therefore, for both test and reference material, the same fraction of the absorbed dose is expected to appear in blood or urine.

The only direct comparison of the two methods is from Freeman et al. (1995). This study used blood AUC and UEF to estimate arsenic ABA for an oral dose of sodium arsenate and arsenic in soil, using the same three monkeys. These data allow calculation of the RBA for each monkey, for each method, and for the same test material (see Table 7). The RBA estimates based on the two methods were not significantly different based on paired t-test ($p=0.37$) or unpaired t-test ($p=0.20$). As there is no evidence to suggest that the blood AUC method and UEF method would yield different estimates of RBA, and there is no theoretical argument for a difference, RBA estimates obtained from the UEF method and blood AUC method are combined in summary statistics of RBA estimates for the entire data set (see Table 3).

4.2.3 Test Material Arsenic Dose and Concentration

Doses of arsenic varied with test material and study. In general, arsenic doses administered to monkeys were higher than those administered to swine, although the range of doses evaluated in each species overlapped. The range of arsenic doses evaluated in swine was approximately 1.5 to 1540 µg As/kg bw/day, in monkeys approximately 120 to 1330 µg As/kg bw (single dose), and in mice approximately 320–6100 µg As/kg bw/day. It is not possible to evaluate potential effects of arsenic dose on RBA because of the different dosing protocols used in the various studies. In some protocols, repeated doses of arsenic were administered at multiple dose levels, and RBA was derived from the composite data (e.g., Casteel and SRC, 2009a,b,c, 2010a,b,c), whereas other protocols administered repeated doses of arsenic at the same dose level (e.g., Basta et al., 2007; Bradham et al., 2011, 2012; Casteel and SRC, 2009a,b,c, 2010a,b,c; Rodriguez et al., 1999) or administered a single dose of arsenic (e.g., Freeman et al., 1995; Juhasz et al., 2007; Roberts et al., 2002, 2007; U.S. EPA, 1996, 2009). Doses used in these different protocols are not directly comparable. In studies conducted in swine, arsenic urinary excretion rate (µg As/day) was a linear function of arsenic dose for both sodium arsenate (dose range ≤310 µg As/kg bw/day) and test material arsenic (dose range ≤1540 µg As/kg bw/day). This observation suggests that arsenic absorption (based on UEF) was not strongly dependent on arsenic dose (Casteel and SRC, 2009a,b,c, 2010a,b,c; U.S. EPA, 2010). In studies conducted in cynomolgus monkeys, the arsenic UEF was shown to be independent of dose (administered as a single gavage dose) over the dose range 250–1000 µg/kg (Roberts et al., 2007). In mice, arsenic UEF was shown to be independent of dose over a dose range of 580–2600 µg As/kg bw/day (Bradham et al., 2011, 2012).

Arsenic levels in the test materials assayed in swine ranged from 42 to 17,500 mg/kg, in monkeys from 101 to 1492 mg/kg, and in mice from 182 to 4495 mg/kg. The wide range of arsenic concentrations resulted in a similarly wide range of soil doses given to the animals (e.g., lower soil arsenic concentrations required larger doses of soil to be administered to achieve the same arsenic dose). The soil doses ranged from approximately 0.4 to 3528 mg soil/kg bw/day in swine, 490 to 2970 mg soil/kg (single dose) in monkeys, and 1150 to 1650 mg soil/kg bw/day in mice. A direct evaluation of the influence of soil dose on arsenic RBA cannot be made from these data because of the differences in dosing regimens used in the various bioassays. However, a strong dependence of RBA on soil dose would be expected to also result in a dependence on soil arsenic concentration since these two variables would be strongly negatively correlated if soil dose was adjusted to achieve a fixed range of soil arsenic doses. Simple regression analysis of these data indicated a relatively small influence (≤14%) of arsenic level on

RBA, with values for R^2 of 0.10 (p=0.01, n=64) for test materials assayed in swine, 0.14 (p=0.07, n=24) for test materials assayed in monkeys, 0.03 (p=0.51, n=15) for test materials assayed in mice, and 0.06 (p=0.01, n=1036) for swine, monkey, and mice combined.

4.2.4 Explanatory Variables Influencing RBA Estimates in Key Studies

Multivariate regression analyses were conducted using factors found to be significant variables in simple regression analyses (species, iron arsenide [FeAs] sulfate content of arsenic-bearing particles, and arsenic levels in test materials) as explanatory variables. These analyses were restricted to data from swine and monkey studies for which data on arsenic mineralogy were available. Content of FeAs sulfate was examined because it has been shown to be an influential variable on RBA in monkeys (Roberts et al., 2007). The R^2 for the model that included all three variables was 0.38 (p=0.006, n=29); however, only species (i.e., monkey or swine) was significant (p=0.02). When the analysis was restricted to monkeys, the dominant influential variable was relative mass of the FeAs sulfate phase of arsenic-bearing particles (R^2= 0.70, p=0.015, n=10), as reported in Roberts et al. (2007). When the analysis was restricted to swine none of the variables (i.e., arsenic level, FeAs sulfate) were found to be significant predictors of RBA (R^2= 0.05, p=0.68, n=19).

Based on these analyses, the dominant influential variable on RBA in this data set appears to be species (i.e., whether the test material was assayed in monkeys or swine) and for test materials assayed in monkeys, the relative mass of the FeAs sulfate phase of arsenic-bearing particles. As previously noted, an explanation for the difference between RBA estimates from monkey and swine assays is not apparent from these analyses.

Other factors, not explored in this analysis, may contribute to the unexplained variability in the arsenic RBA estimates. Approximately 62% of the RBA estimates are based on an R^2 value of 0.38 for the model that included species, FeAs sulfate content of arsenic-bearing particles, and arsenic levels in test materials. Likely candidates are arsenic mineralogy (chemical composition and morphology of the arsenic-bearing particles) and soil characteristics, which together may determine arsenic bioaccessibility and/or absorption of bioaccessible arsenic.

4.3 Uncertainties in Use of Compiled RBA Estimates for Prediction of Arsenic RBA

Table 1 summarizes sources of uncertainties to be considered in assessing confidence in RBA estimates and making statistical inference regarding arsenic RBA in soils. These include the following.

- **Adequacy of Approach:**
    - Confidence in predictions of arsenic RBA in humans based on animal bioassays has not been assessed. This would require measuring RBA of the same soils in both humans and animal models.
    - When applied to the same test materials (see results for Barber Orchard soil samples in Table 5), the swine, monkey, and mouse assays yielded remarkably similar RBA

estimates for some materials and widely different estimates for other materials. However, collectively, the differences in the RBA estimates were relatively small. The average absolute difference in the RBA estimates for assays conducted on the same test materials ranged from <1 to 28%, and the average differences were 8, 12, and 10% for RBA_{swine} - RBA_{monkey}, RBA_{swine} - RBA_{mouse}, and RBA_{mouse} - RBA_{monkey}, respectively. When the three assays were applied to multiple samples from the same site (i.e., 4 samples from the Barber Orchard site), 95% confidence limits on the site-wide mean RBA values overlapped substantially, suggesting that for these samples, assays in the 3 species provided site-wide estimates of RBA that were statistically indistinguishable. The reason why the same test materials give different RBA outcomes for some of the Barber Orchard samples tested in the three animal models is not apparent from available data and could be related to one or more factors (as described in Section 4.7.1):

(1) animal species differences in arsenic absorption;

(2) differences in assay protocols;

(3) differences in data reduction methods used to calculate RBA; and

(4) differences in methods used to measure arsenic concentration in soils and biological samples.

- Experimental protocols of RBA bioassays differ (e.g., multiple dose levels vs. single dose level, repeated dosing vs. single dose), and each protocol may have different sources and magnitudes of measurement error.

- The arsenic dose range for test materials administered in the bioassays includes values that are substantially higher than typical daily soil ingestion rates in children or adults. The implication of these high test material doses in extrapolating RBA estimates from animal bioassays to humans (e.g., the effect of test material dose on RBA) has not been thoroughly investigated; however, based on measurements of urinary arsenic, the absorption fraction does not appear to be strongly dependent on dose.

- **Representativeness:** The RBA estimates considered in this analysis are derived from an opportunistic sample of soils and do not represent a statistical sample of soils in any geographic region (e.g., U.S.) or source of arsenic contamination. The samples were collected because of regulatory interest in specific sites. Although the data set includes samples from sites impacted by various sources of arsenic contamination (e.g., mining/smelting, agricultural, chemical/pesticide manufacturing facilities, and railway corridors), the dominant arsenic sources in the data set are mining and smelting (54 of 88 test materials). The absence of a statistical sampling design limits confidence in statistical inference based on the data set. For example, sample statistics such as the mean and standard deviation, even for specific categories of arsenic contamination, mineralogy, or soil characteristics, cannot be assumed to represent these categories in

general. Nevertheless, the data set does describe the distribution of RBA values that have been encountered in soils from various sites of regulatory interest. The empirical distribution of RBA values in this data set suggests that values for arsenic RBA exceeding 60% are relatively uncommon (i.e., less than 5% of the estimates exceed 60% RBA). Based on this experience, it is reasonable to expect that future RBA estimates exceeding 60% would also be uncommon if samples were to be drawn from a collection of similar types of sites and soils. This prediction could be further evaluated with additional data collection efforts and may be of value for informing assumptions about soil arsenic RBA at sites where RBA estimates have not yet been made (e.g., screening level assessments).

- **Variability of Test Material RBA Estimates:** Multivariate regression models used to explore the contribution of bioassay and soil variables to variability in RBA estimates yielded R^2 values ≤38%. Therefore, these models could explain no more than 38% of the variability observed in the RBA estimates, most of which was attributed to bioassay species. The relatively low explanatory power of the models explored in this analysis precludes their use in making predictions about RBA of arsenic in soil. It is likely that more informative regression models (or other variance models) could be developed that account for test material variables not considered in this analysis (e.g., arsenic mineralogy and soil characteristics). These variables are currently being explored as part of on-going EPA research. In addition to variables related to the soil test materials, other variables are likely to have contributed to the unexplained variability in the RBA estimates. These include the bioassay methods (e.g., dosing regimens), biomarkers used to estimate absorption (e.g., urine and blood), methods used to measure arsenic in soil and in biological samples, measurement error (e.g., doses administered, urinary arsenic excretion, and blood arsenic concentrations), and differences in data reduction methods. It is expected that differences in experimental design and protocol, data reduction methods, and measurement error contribute to variability in the RBA estimates. The above variables may explain differences in RBA estimates for some test materials that have been assayed in swine, monkey, and mouse. This complicates analyses of the impacts of other variables (e.g., arsenic mineralogy and soil characteristics) on RBA.

- **Interindividual Variability in RBA:** The RBA estimates for each test material represent mean values derived from experiments made on groups of animals. Estimates of interindividual variability in RBA were not possible for all studies and study designs. Interindividual variability in UEF for the test and reference material groups were accounted for in the calculation of group mean RBA estimates in the swine and mouse studies; however, the statistical design of the studies does not yield an estimate of interindividual variability in RBA, although it does provide an estimate of uncertainty in the RBA represented by the confidence limits. The monkey studies used a repeated measures design in which each animal received the soil and reference materials. This design allowed estimation of a group mean and standard deviation for RBA for each study, representing the interindividual variability in the RBA for each test material. Coefficients of variation (SD/mean) for the 20 RBA estimates derived from monkey bioassays ranged from 0.11 to 0.80 (mean 0.38 ± 0.17 SD). This outcome suggests that interindividual variability in RBA in monkeys that received the same test material varies

across test materials and/or studies. Numerous other factors may contribute to interindividual variability in arsenic RBA, including diet, nutrition, and age. Since these variables were controlled in the animal bioassays, interindividual variability observed in the animal bioassays is presumably dominated by contributions from the test material and physiological variables that affect bioaccessibility and absorption of arsenic. However, in human populations, interindividual variability in diet/nutrition, disease states, and other factors may also contribute to variability in RBA.

- **Intraindividual Variability in RBA:** This analysis did not attempt to estimate intraindividual variability in RBA. The RBA studies compiled in this review did not provide data on intraindividual variability, which would have required repeated measurements of RBA in the same animals. As noted above, the controlled conditions of the bioassays would have eliminated variables that may contribute to intraindividual variability in RBA estimates in humans. Variables that may contribute to intraindividual variability in arsenic RBA include age, diet/nutrition, disease states, etc.

- **Relevance of Soil Arsenic Concentrations Tested:** Arsenic RBA was not significantly correlated with arsenic concentration (<100 to 17,500 mg kg^{-1}). Nevertheless, RBA estimates at sites that have arsenic concentrations well below or above the risk-based decision level may not influence cleanup decisions.

- **Data Collection Period and Relevance of Soil Aging to Arsenic RBA:** RBA estimates in this report cannot represent temporal changes in soil characteristics (e.g., changes in soil composition or arsenic speciation) at the sites that might alter RBA. Bioavailability of arsenic in soil may change over time. Although direct evidence for this for *in situ* contaminated soils is not available, studies of laboratory-contaminated soils suggest that changes over time in certain soils can be substantial. Juhasz et al. (2008) found that RBA decreased from 100 to 25% in 3 months and then remained constant for the next 9 months following addition of sodium arsenate to a soil containing a high iron content (99,671 mg Fe/kg soil). Arsenic RBA remained approximately 100% in a similarly spiked soil that contained lower iron content (7980 mg/kg). The predominant arsenic phase in the high iron content soil was associated with iron oxides. Although this study was limited to soils spiked in the laboratory with sodium arsenate, it suggests the possibility that arsenic RBA may change over time and that the magnitude of the change may depend on soil characteristics. Studies in which arsenic RBA is measured repeatedly over time, in a variety of soils, would be needed to determine the relevance of this observation to arsenic-contaminated sites. On-going EPA research is attempting to evaluate the long-term stability of arsenic bioaccessibility of soils contaminated *in situ*.

- **Extrapolation to Humans:** Studies comparing arsenic RBA in humans and animals for the same soils are not available and are not likely to be undertaken. This limitation introduces uncertainty into predictions of arsenic RBA in humans based on results from animal bioassay studies; however, it should not preclude making extrapolations of animal bioassay data to humans. EPA currently recommends use of a swine RBA assay (or an *in vitro* bioaccessibility (IVBA) assay that was validated with a swine assay) for predicting site-specific lead RBA in human health risk assessments (U.S. EPA,

2007a,b,c). As noted previously, when applied to the same test materials, RBA estimates based on the swine, monkey, and mouse assays yielded remarkably similar RBA estimates for some materials and collectively, the differences in the RBA estimates were relatively small. The similarity of RBA estimates based on assays in three mammalian species increases confidence in extrapolation of these results to humans.

- **Quality Assurance:** For some studies, information on quality assurance/quality control was limited or absent.

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Table 1. Confidence in Arsenic RBA Estimates		
General Assessment Factors	**Rationale**	**Rating**
Soundness		
Adequacy of Approach	Methodologies included several limitations: (1) Estimates of RBA of arsenic in soil materials in humans have not been reported. The monkey and swine models have been utilized for predicting RBA of arsenic in humans because the gastric physiology of both animal species share many similarities to that of humans and because of a prior history of use of the models for assessing RBA of other inorganic contaminants (e.g., lead) and gastrointestinal absorption of drugs. Estimates of RBA of arsenic in soil materials in animal models cannot be quantitatively compared to estimates made in humans, as estimates in humans are not available for these test materials. (2) Reported estimates of RBA for arsenic in soil materials obtained from monkey assays are significantly lower than reported estimates obtained from swine or mouse assays. The mechanism for the different outcomes from the two assays is not apparent and could be related to several factors (e.g., species differences, protocol differences, test material differences). (3) Experimental protocols utilizing a steady-state design with multiple dose levels may introduce less error than experimental protocols using a steady-state design with a single dose level or a single dose (i.e., non steady-state) design. (4) Variations in the design of animal RBA assays, in particular, different detection limits for blood and urinary arsenic and wide variations in arsenic concentrations of test materials, has placed constraints on experimental control of both the arsenic dose and test material dose used in each assay. Therefore, the dose range for test materials administered in the animal bioassays includes values that are substantially higher than typical daily soil ingestion rates in children or adults. The implication of these high test material doses in extrapolating RBA estimates from monkey and swine assays to humans has not been thoroughly investigated (e.g., effect of test material dose on RBA).	Medium
Bias	Numerous sources of measurement error exist. Studies utilizing multiple dose levels and dosing regimens to achieve steady-state are more likely to have less measurement error in the critical parameter (i.e., UEF). The upper bound estimate may be biased by sample selection bias (samples dominated by mining/smelter sources).	

Table 1. Confidence in Arsenic RBA Estimates		
General Assessment Factors	**Rationale**	**Rating**
Applicability and Utility		
Default Value of Interest	All "key" and "relevant" studies focus on the relative bioavailability of arsenic.	Medium
Representativeness	The RBA estimates considered in this analysis do not represent a statistical sample of soils in any geographic region (e.g., U.S.). Although not a statistical sample of soils, nearly all samples were collected at hazardous waste sites. These included test materials collected from mining and/or smelter operations, pesticides (orchards), and manufacturing/electrical waste. Therefore, the samples may provide adequate representation of soils at sites of the highest regulatory interest or concern.	
Currency	Test materials assayed reflect recent conditions (samples collected over ≤10–15 years).	
Data Collection Period	Test materials assayed represent a cross-sectional sample of soils. However, RBA estimates of those test materials cannot assess temporal change in soil characteristics (e.g., changes in soil composition or arsenic speciation) at the sites and potential related changes in RBA estimates of those materials.	
Clarity and Completeness		
Accessibility	Observations for individual data on which RBA estimates were based are available in the published literature or online.	Low
Reproducibility	Reproducibility has not been evaluated across methodologies.	
Quality Assurance	For some studies, information on quality assurance/quality control was limited or absent.	
Variability and Uncertainty		
Variability in Estimates	The sample of test materials is not a statistical sample of soils. Therefore, variability in arsenic RBA for soils in general or for any subset of characteristics of the test materials (e.g., arsenic mineralogy, soil characteristics) cannot be inferred from the variability represented in the data set.	Low
Minimal Uncertainty	Estimates of the mean and percentiles for RBAs of test material sample are reasonably certain; however, the representativeness of the sample for making statistical inference about arsenic RBA estimates for soils in general, or about soils at specific sites is uncertain.	
Evaluation and Review		
Peer Review	The animal bioassays used in all studies either appeared in peer reviewed journals or the study was conducted by or for EPA in which EPA developed the RBA estimates from the raw data using established standard protocols and/or the raw data were available for QA review by the U.S. EPA Bioavailability Committee of the Technical Review Workgroup (e.g., EPA swine studies); or, the study was conducted by other research groups and results had been subjected to peer review as a requirement for publication.	Medium

Table 1. Confidence in Arsenic RBA Estimates		
General Assessment Factors	**Rationale**	**Rating**
Number and Agreement of Studies	Application of similar assay methodologies produced highly variable estimates of arsenic RBA. However, these differences may reflect differences in test material characteristics, differences in assay protocols, or differences in species (monkeys, swine, mouse). Direct comparisons of swine, monkey, and mouse RBA estimates are available for only 4 test materials and direct comparisons of swine and mouse RBA estimates are available for 11 test materials. Based on this limited comparison, the magnitude of difference between RBA estimates derived from swine, monkey, and mouse assays is relatively small in the context of risk assessment, where uncertainties in other parameters in risk calculations can exceed several orders of magnitude. Therefore, from the perspective of use of the assays to support risk assessment, the swine, monkey, and mouse assays appear to yield essentially equivalent information about arsenic RBA.	Medium
Overall Rating		Medium

Table 2. Key and Relevant Study Results				
Test Material	**Species**	**Method/Dose**	**RBA (%)**	**Reference**
Key Studies				
Source: Bingham Creek Channel soil (sieved to <250 µm) Type: Mining/smelting As concentration: 149 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 5, 20, or 50 µg As/kg bw/day; 5 animals/group Test material dose: 15.8 µg As/kg bw/day (106.0 mg soil/kg bw/day); 5 animals/group	39±8 Mean±SE	U.S. EPA, 2010
Source: Murray smelter slag (sieved to <250 µm) Type: Mining/smelting As concentration: 695 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 5, 20, or 50 µg As/kg bw/day; 5 males/group Test material dose: 13.4 µg As/kg bw/day (19.2 mg soil/kg bw/day); 5 animals/group	55±10 Mean±SE	U.S. EPA, 2010
Source: Butte soil, composite soil waste rock dumps (sieved to <250 µm) Type: Mining/smelting As concentration: 234 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 5, 20, or 50 µg As/kg bw/day; 5 animals/group Test material dose: 6.3 µg As/kg bw/day (26.2 mg soil/kg bw/day); 5 animals/group	9±3 Mean±SE	U.S. EPA, 2010
Source: Midvale slag, composite sample Midvale smelter slag pile (sieved to <250 µm) Type: Mining/smelting As concentration: 591 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 5, 20, or 50 µg As/kg bw/day; 5 animals/group Test material dose: 16.8 µg As/kg bw/day (28.5 mg soil/kg bw/day); 5 animals/group	23±4 Mean±SE	U.S. EPA, 2010
Source: California Gulch Phase I residential soil, composite residential soil, Leadville, CO (sieved to <250 µm) Type: Mining/smelting As concentration: 203 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 5, 20, or 50 µg As/kg bw/day; 5 animals/group Test material dose: 6.1 µg As/kg bw/day (30.0 mg soil/kg bw/day); 5 animals/group	8±3 Mean±SE	U.S. EPA, 2010
Source: California Gulch Fe/Mn PbO, composite soil, Leadville, CO (sieved to <250 µm) Type: Mining/smelting As concentration: 110 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 5, 20, or 50 µg As/kg bw/day; 5 animals/group Test material dose: 5.7 µg As/kg bw/day (52.1 mg soil/kg bw/day); 5 animals/group	57±12 Mean±SE	U.S. EPA, 2010
Source: Palmerton Location 2, composite soil, Palmerton, PA (sieved to <250 µm) Type: Mining/smelting As concentration: 110 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 5, 20, or 50 µg As/kg bw/day; 5 animals/group Test material dose: 7.7 µg As/kg bw/day (70.0 mg soil/kg bw/day); 5 animals/group	49±10 Mean±SE	U.S. EPA, 2010

Table 2. Key and Relevant Study Results				
Test Material	**Species**	**Method/Dose**	**RBA (%)**	**Reference**
Source: Palmerton Location 4, composite soil, Palmerton, PA (sieved to <250 µm) Type: Mining/smelting As concentration: 134 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 5, 20, or 50 µg As/kg bw/day; 5 animals/group Test material dose: 14.0 µg As/kg bw/day (104.7 mg soil/kg bw/day); 5 animals/group	61±11 Mean±SE	U.S. EPA, 2010
Source: California Gulch AV slag, Leadville, CO (sieved to <250 µm) Type: Mining/smelting As concentration: 1050 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 5, 20, or 50 µg As/kg bw/day; 2 animals/group Test material dose: 22.3 µg As/kg bw/day (21.2 mg soil/kg bw/day); 2 animals/group	18±2 Mean±SE	U.S. EPA, 2010
Source: Murray Smelter Soil, composite (sieved to <250 µm) Type: Mining/smelting As concentration: 310 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 5, 20, or 50 µg As/kg bw/day; 5 animals/group Test material dose: 65.4 µg As/kg bw/day (211.0 mg soil/kg bw/day); 5 animals/group	33±5 Mean±SE	U.S. EPA, 2010
Source: Clark Fork Tailings, MT (sieved to <250 µm) Type: Mining/smelting As concentration: 181 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 20 or 50 µg As/kg bw/day; 4 animals/group Test material dose: 10.0 or 25 µg As/kg bw/day (55.2 or 138.1 mg soil/kg bw/day); 4 animals/group	51±6 Mean±SE	U.S. EPA, 2010
Source: Sample TM1 Vasquez Boulevard and I-70, composite residential, Denver CO (sieved to <250 µm) Type: Mining/smelting As concentration: 312 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 50 or 125 µg As/kg bw/day; 4 animals/group Test material dose: 37.0 or 92.5 µg As/kg bw/day (59.2 or 148.1 mg soil/kg bw/day); 4 animals/group	40±4 Mean±SE	U.S. EPA, 2010
Source: Sample TM2 Vasquez Boulevard and I-70, composite residential, Denver CO (sieved to <250 µm) Type: Mining/smelting As concentration: 983 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 50 or 125 µg As/kg bw/day; 4 animals/group Test material dose: 33.9 or 84.7 µg As/kg bw/day (17.2 or 43.1 mg soil/kg bw/day); 4 animals/group	42±4 Mean±SE	U.S. EPA, 2010

Table 2. Key and Relevant Study Results				
Test Material	**Species**	**Method/Dose**	**RBA (%)**	**Reference**
Source: Sample TM3 Vasquez Boulevard and I-70, composite residential, Denver CO (sieved to <250 µm) Type: Mining/smelting As concentration: 390 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 50 or 125 µg As/kg bw/day; 4 animals/group Test material dose: 27.5 or 68.7 µg As/kg bw/day (35.2 or 88.0 mg soil/kg bw/day); 4 animals/group	37±3 Mean±SE	U.S. EPA, 2010
Source: Sample TM4 Vasquez Boulevard and I-70, composite residential, Denver, CO (sieved to <250 µm) Type: Mining/smelting As concentration: 813 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 50 or 125 µg As/kg bw/day; 4 animals/group Test material dose: 37.4 or 93.5 µg As/kg bw/day (22.9 or 57.5 mg soil/kg bw/day); 4 animals/group	24±2 Mean±SE	U.S. EPA, 2010
Source: Sample TM5 Vasquez Boulevard and I-70, composite residential, Denver, CO (sieved to <250 µm) Type: Mining/smelting As concentration: 368 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 50 or 125 µg As/kg bw/day; 4 animals/group Test material dose: 41.1 or 102.7 µg As/kg bw/day (55.8 or 139.5 mg soil/kg bw/day); 4 animals/group	21±2 Mean±SE	U.S. EPA, 2010
Source: Sample TM6 Vasquez Boulevard and I-70, composite residential, Denver, CO (sieved to <250 µm) Type: Mining/smelting As concentration: 516 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 50 or 125 µg As/kg bw/day; 4 animals/group Test material dose: 32.4 or 81.0 µg As/kg bw/day (31.4 or 78.5 mg soil/kg bw/day); 4 animals/group	24±3 Mean±SE	U.S. EPA, 2010
Source: Butte TM1, composite waste rock dumps (U.S. EPA Sample #8-37926) (sieved to <250 µm) Type: Mining/smelting As concentration: 234 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 34, 59, or 94 µg As/kg bw/day; 4 animals/group Test material dose: 30.4, 60.5, or 92.0 µg As/kg bw/day (130.0, 258.5, or 393.2 mg soil/kg bw/day); 4 animals/group	18±3 Mean±SE	U.S. EPA, 2010
Source: Butte TM2, composite (U.S. EPA Sample #BPSOU-0501-ASBIO) (sieved to <250 µm) Type: Mining/smelting As concentration: 367 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 34, 59, or 94 µg As/kg bw/day; 4 animals/dose Test material dose: 25.7, 62.5, or 92.6 µg As/kg bw/day (70.0, 170.3, or 252.3 mg soil/kg bw/day); 4 animals/dose	24±2 Mean±SE	U.S. EPA, 2010

Table 2. Key and Relevant Study Results				
Test Material	**Species**	**Method/Dose**	**RBA (%)**	**Reference**
Source: Aberjona River sediment composite TM1 (fine sieved, but no information was reported on size) Type: Mining/smelting As concentration: 676 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 30, 60, or 90 µg As/kg bw/day; 4 animals/dose Test material dose: 18.3, 40.2, or 46.9 µg As/kg bw/day (27.1, 59.5, or 73.3 mg soil/kg bw/day); 4 animals/dose	38±2 Mean±SE	U.S. EPA, 2010
Source: Aberjona River sediment composite TM2 (fine sieved, but no information was reported on size) Type: Mining/smelting As concentration: 313 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 30, 60, or 90 µg As/kg bw/day; 4 animals/group Test material dose: 18.8, 35.9, or 61.9 µg As/kg bw/day (60.1, 114.7, or 197.8 mg soil/kg bw/day); 4 animals/group	52±2 Mean±SE	U.S. EPA, 2010
Source: Soil sample (TM1) American Canal, El Paso County, TX (sieved to <250 µm) Type: Mining/smelting As concentration: 74 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 25 or 50 µg As/kg bw/day; 5 animals/group Test material dose: 40, 80, or 160 µg As/kg bw/day (540.5, 1081.1, or 2162.2 mg soil/kg bw/day); 5 animals/group	44±3 Mean±SE	U.S. EPA, 2010
Source: Soil sample (TM2) American Canal, El Paso County, TX (sieved to <250 µm) Type: Mining/smelting As concentration: 73 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 25 or 50 µg As/kg bw/day; 5 animals/group Test material dose: 40, 80, or 160 µg As/kg bw/day (547.9, 1095.9, or 2191.8 mg soil/kg bw/day); 5 animals/group	37±3 Mean±SE	U.S. EPA, 2010
Source: Utility pole soil, Conley, GA (sieved to <250 µm) Type: Pesticide application As concentration: 320 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 30 or 60 µg As/kg bw/day; 5 animals/group Test material dose: 46.5 or 91.0 µg As/kg bw/day (145.3 or 284.4 mg soil/kg bw/day); 5 animals/group	47±3 Mean±SE	U.S. EPA, 2010
Source: Soil, Superfund site, Palestine, TX (sieved to <250 µm) Type: Mining/smelting As concentration: 47 mg/kg soil	Swine (Line 26, male, immature, 5–6 weeks old, 7–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 30, 60, or 121 µg As/kg bw/day; 5 animals/group Test material dose: 42.6, 84.8, or 165.8 µg As/kg bw/day (906.4, 1804.3, or 3527.7 mg soil/kg bw/day); 5 animals/group	15±1.1 Mean±SE	Casteel and SRC, 2005

Table 2. Key and Relevant Study Results				
Test Material	**Species**	**Method/Dose**	**RBA (%)**	**Reference**
Source: Barber Orchard NC, sample MS-1 (sieved to <250 µm) Type: Agriculture As concentration: 290 mg/kg soil	Swine (Line 26, male, immature, 6–7 weeks old)	**Steady-state urinary excretion fraction method** Reference material dose: 32.0, 55.7, or 125.2 µg As/kg bw/day; 4 animals/group Test material dose: 72.9 or 145.7 µg As/kg bw/day (251.0 or 502.4 mg soil/kg bw/day); 4 animals/group	31±4.0 Mean±SE	Casteel and SRC, 2009a
Source: Barber Orchard NC, sample MS-4 (sieved to <250 µm) Type: Agriculture As concentration: 388 mg.kg soil	Swine (Line 26, male, immature, 6–7 weeks old)	**Steady-state urinary excretion fraction method** Reference material dose: 25.4, 53.6, or 104.6 µg As/kg bw/day; 4 animals/group Test material dose: 52.6, 77.3, or 144.4 µg As/kg bw/day (135.6, 199.2, or 372.2 mg soil/kg bw/day); 4 animals/group	41±1.8 Mean±SE	Casteel and SRC, 2009a
Source: Barber Orchard NC, sample MS-5 (sieved to <250 µm) Type: Agriculture As concentration: 382 mg/kg soil	Swine (Line 26, male, immature, 6–7 weeks old)	**Steady-state urinary excretion fraction method** Reference material dose: 29.7 or 57.3 µg As/kg bw/day; 4 animals/group Test material dose: 46.0, 71.0, or 138.9 µg As/kg bw/day (120.4, 185.8, or 363.6 mg soil/kg bw/day); 4 animals/group	49±4.7 Mean±SE	Casteel and SRC, 2009a
Source: Barber Orchard NC, sample MS-8 (sieved to <250 µm) Type: Agriculture As concentration: 364 mg/kg soil	Swine (Line 26, male, immature, 6–7 weeks old)	**Steady-state urinary excretion fraction method** Reference material dose: 25.4, 53.6, or 104.6 µg As/kg bw/day; 4 animals/group Test material dose: 44.6, 72.0, or 155.0 µg As/kg bw/day (122.5, 197.8, or 425.8 mg soil/kg bw/day); 4 animals/group	53±2.3 Mean±SE	Casteel and SRC, 2009a
Source: NIST SRM 2710 (sieved to 74 µm) Type: Mining/smelting As concentration: 626±38 mg/kg soil	Swine (Line 26, male, immature, 6–7 weeks old, ~9–10 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 24.1, 47.5, or 95.9 µg As/kg bw/day; 4 animals/group Test material dose: 58.2 or 114.5 µg As/kg bw/day (93.0 or 182.9 mg soil/kg bw/day); 4 animals/group	44±2.3 Mean±SE	Casteel and SRC, 2009b
Source: Mohr Orchard PA sample (sieved to <250 µm) Type: Agriculture As concentration: 340 mg/kg soil	Swine (Line 26, male, immature, 6–7 weeks old, ~9–10 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 29, 62, or 130 µg As/kg bw/day; 4 animals/group Test material dose: 52, 72, or 153 µg As/kg bw/day (153, 212, or 450 mg soil/kg bw/day); 4 animals/group	53 (51–57; 90% CI)	Casteel and SRC, 2009c

Table 2. Key and Relevant Study Results				
Test Material	**Species**	**Method/Dose**	**RBA (%)**	**Reference**
Source: Iron King, AZ soil sample TM1 (sieved to <250 µm) Type: Mining/smelting As concentration: 200±5.3 mg/kg soil	Swine (Line 26, male, immature, 6–7 weeks old, ~9–10 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 25, 50, or 100 µg As/kg bw/day; 4 animals/group Test material dose: 40, 60, or 120 µg As/kg bw/day (200, 300, or 600 mg soil/kg bw/day); 4 animals/group	60±2.7 Mean±SE	Casteel and SRC, 2010a
Source: Iron King, AZ soil sample TM2 (sieved to <250 µm) Type: Mining/smelting As concentration: 3957±332.7 mg/kg soil	Swine (Line 26, male, immature, 6–7 weeks old, ~9–10 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 25, 50, or 100 µg As/kg bw/day; 4 animals/group Test material dose: 116, 175, or 349 µg As/kg bw/day (29, 44, or 88 mg soil/kg bw/day); 4 animals/group	19±1.0 Mean±SE	Casteel and SRC, 2010a
Source: ASARCO soil sample (sieved to <250 µm) Type: Mining/smelting As concentration: 181.9±6.3 mg/kg soil	Swine (Line 26, male, immature, 6–7 weeks old, ~9–10 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 25, 50, or 100 µg As/kg bw/day; 4 animals/group Test material dose: 40, 60, or 120 µg As/kg bw/day (220, 330, or 660 mg soil/kg bw/day); 4 animals/group	49±2.5 Mean±SE	Casteel and SRC, 2010b
Source: Hawaiian soil sample (sieved to <250 µm) Type: Agriculture As concentration: 768.85±32.3 mg/kg soil	Swine (Line 26, male, immature, 6–7 weeks old, ~9–10 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 25, 50, or 100 µg As/kg bw/day; 4 animals/group Test material dose: 40, 60, 120 µg As/kg bw/day (80, 120, or 240 mg soil/kg bw/day); 4 animals/group	33±1.7 Mean±SE	Casteel and SRC, 2010b
Source: NIST SRM 2710a (sieved to <74 µm) Type: Mining/smelting As concentration: 1540±100 mg/kg soil	Swine (Line 26, male, immature, 6–7 weeks old, ~9–10 kg)	**Steady-state urinary excretion fraction method** Reference material dose: 26, 52, or 105 µg As/kg bw/day; 4 animals/group Test material dose: 41, 62, or 121 µg As/kg bw/day (27, 40, or 79 mg soil/kg bw/day); 4 animals/group	42±1.4 Mean±SE	Casteel and SRC, 2010c
Source: Mining smelter soil (sample #1) (sieved to <250 µm) Type: Mining/smelting As concentration: 11,300 mg/kg soil	Swine (Line 26, male, 10–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: not reported; 5 animals/group Test material dose: 70.6 µg As/kg/day (6.25 mg soil/kg/day); 5 animals/group	8.6±6.9 Mean±SD	Basta et al., 2007

Table 2. Key and Relevant Study Results				
Test Material	**Species**	**Method/Dose**	**RBA (%)**	**Reference**
Source: Mining smelter soil (sample #2) (sieved to <250 µm) Type: Mining/smelting As concentration: 17,500 mg/kg soil	Swine (Line 26, male, 10–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: not reported; 5 animals/group Test material dose: 109 µg As/kg bw/day (6.25 mg soil/kg bw/day); 5 animals/group	4.1±2.1 Mean±SD	Basta et al., 2007
Source: Mining smelter soil (sample #3) (sieved to <250 µm) Type: Mining/smelting As concentration: 13,500 mg/kg soil	Swine (Line 26, male, 10–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: not reported; 4 animals/group Test material dose: 84.4 µg As/kg bw/day (6.25 mg soil/kg bw/day); 4 animals/group	7.9±4.3 Mean±SD	Basta et al., 2007
Source: Mining smelter soil (sample #4) (sieved to <250 µm) Type: Mining/smelting As concentration: 11,500 mg/kg soil	Swine (Line 26, male, 10–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: not reported; 5 animals/group Test material dose: 71.9 µg As/kg bw/day (6.25 mg soil/kg bw/day); 5 animals/group	22.8±4.6 Mean±SD	Basta et al., 2007
Source: Mining smelter soil (sample #6) (sieved to <250 µm) Type: Mining/smelting As concentration: 405 mg/kg soil	Swine (Line 26, male, 10–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: not reported; 2 animals/group Test material dose: 2.5 µg As/kg bw/day (6.25 mg soil/kg bw/day); 2 animals/group	38.7±15.3 Mean±SD	Basta et al., 2007
Source: Mining smelter soil (sample #7) (sieved to <250 µm) Type: Mining/smelting As concentration: 450 mg/kg soil	Swine (Line 26, male, 10–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: not reported; 4 animals/group Test material dose: 2.8 µg As/kg bw/day (6.25 mg soil/kg bw/day); 4 animals/group	43.0±23.8 Mean±SD	Basta et al., 2007
Source: Mining smelter soil (sample #8) (sieved to <250 µm) Type: Mining/smelting As concentration: 1180 mg/kg soil	Swine (Line 26, male, 10–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: not reported; 4 animals/group Test material dose: 7.4 µg As/kg bw/day (6.25 mg soil/kg bw/day); 4 animals/group	39.1±15.5 Mean±SD	Basta et al., 2007

Table 2. Key and Relevant Study Results				
Test Material	**Species**	**Method/Dose**	**RBA (%)**	**Reference**
Source: Mining smelter soil (sample #9) (sieved to <250 µm) Type: Mining/smelting As concentration: 5020 mg/kg soil	Swine (Line 26, male, 10–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: not reported; 5 animals/group Test material dose: 31.4 µg As/kg bw/day (6.25 mg soil/kg/day); 5 animals/group	32.9±7.4 Mean±SD	Basta et al., 2007
Source: Mining smelter soil (sample #10) (sieved to <250 µm) Type: Mining/smelting As concentration: 4650 mg/kg soil	Swine (Line 26, male, 10–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: not reported; 4 animals/group Test material dose: 29.1 µg As/kg bw/day (6.25 mg soil/kg bw/day); 4 animals/group	21.9±5.6 Mean±SD	Basta et al., 2007
Source: Mining smelter soil (sample #11) (sieved to <250 µm) Type: Mining/smelting As concentration: 331 mg/kg soil	Swine (Line 26, male, 10–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: not reported; 5 animals/group Test material dose: 2.2 µg As/kg bw/day (6.25 mg soil/kg bw/day); 5 animals/group	6.2 Mean (SE or SD not reported)	Rodriguez et al., 1999
Source: Mining smelter soil (sample #12) (sieved to <250 µm) Type: Mining/smelting As concentration: 233 mg/kg soil	Swine (Line 26, male, 10–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: not reported; 5 animals/group Test material dose: 1.5 µg As/kg bw/day (6.25 mg soil/kg bw/day); 5 animals/group	42.8 Mean (SE or SD not reported)	Rodriguez et al., 1999
Source: Mining smelter soil (sample #13) (sieved to <250 µm) Type: Mining/smelting As concentration: 799 mg/kg soil	Swine (Line 26, male, 10–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: not reported; 5 animals/group Test material dose: 5.0 µg As/kg bw/day (6.25 mg soil/kg bw/day); 5 animals/group	29.1 Mean (SE or SD not reported)	Rodriguez et al., 1999
Source: Mining smelter soil (sample #14) (sieved to <250 µm) Type: Mining/smelting As concentration: 1460 mg/kg soil	Swine (Line 26, male, 10–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: not reported; 5 animals/group Test material dose: 9.1 µg As/kg bw/day (6.25 mg soil/kg bw/day); 5 animals/group	18.7 Mean (SE or SD not reported)	Rodriguez et al., 1999

Table 2. Key and Relevant Study Results				
Test Material	**Species**	**Method/Dose**	**RBA (%)**	**Reference**
Source: Mining smelter soil (sample #15) (sieved to <250 µm) Type: Mining/smelting As concentration: 401 mg/kg soil	Swine (Line 26, male, 10–12 kg)	**Steady-state urinary excretion fraction method** Reference material dose: not reported; 5 animals/group Test material dose: 2.5 µg As/kg bw/day (6.25 mg soil/kg bw/day); 5 animals/group	36.5 Mean (SE or SD not reported)	Rodriguez et al., 1999
Source: Smelter composite soil Ruston/North Tacoma Superfund site (no information available on particle size of test material) Type: Mining/smelting As concentration: 1600 mg/kg soil	Swine (sires: Hampshire hybrid; dams: crossbred Landrace/Large White/Duroc, immature, ~6–7 weeks old, ~15 kg)	**Single dose blood-time concentration curve method** Reference material dose: 10, 110, or 310 µg As/kg bw; 3 animals/group Test material dose: 40, 100, 160, or 240 µg As/kg bw (25, 62.5, 100, or 150 mg soil/kg bw); 3 animals/group	78 Mean (SE or SD not reported)	U.S. EPA, 1996
Source: Smelter composite slag Ruston/North Tacoma Superfund site (no information available on particle size of test material) Type: Mining/smelting As concentration: 10,100 mg/kg soil	Swine (sires: Hampshire hybrid; dams: crossbred Landrace/Large White/Duroc, immature, ~6–7 weeks old, ~15 kg)	**Single dose blood-time concentration curve method** Reference material dose: 10, 110, or 310 µg As/kg bw; 3 animals/group Test material dose: 610, 1010, or 1540 µg As/kg bw (60.4, 100, or 152.5 mg soil/kg bw); 3 animals/group	42 Mean (SE or SD not reported)	U.S. EPA, 1996
Source: Australian railway corridor soil (sample #2) (sieved to <250 µm) Type: Railway corridor As concentration: 267 mg/kg soil	Swine (large white, female, 20–25 kg)	**Single dose blood-time concentration curve method** Reference material dose: 100 µg As/kg bw; 3 animals/group Test material dose: 119 to 297 µg As/kg bw (0.4 to 1.1 mg soil/kg bw); 3 animals/group	67.4±32.2 Mean±SD	Juhasz et al., 2007
Source: Australian railway corridor soil (sample #4) (sieved to <250 µm) Type: Railway corridor As concentration: 42 mg/kg soil	Swine (large white, female, 20–25 kg)	**Single dose blood-time concentration curve method** Reference material dose: 100 µg As/kg bw; 3 animals/group Test material dose: 19 to 47 µg As/kg bw (0.4 to 1.1 mg soil/kg bw); 3 animals/group	41.6±11.5 Mean±SD	Juhasz et al., 2007
Source: Australian railway corridor soil (sample #5) (sieved to <250 µm) Type: Railway corridor As concentration: 1114 mg/kg soil	Swine (large white, female, 20–25 kg)	**Single dose blood-time concentration curve method** Reference material dose: 100 µg As/kg bw; 3 animals/group Test material dose: 495 to 1238 µg As/kg bw (0.4 to 1.1 mg soil/kg bw); 3 animals/group	20.0±16.5 Mean±SD	Juhasz et al., 2007

Table 2. Key and Relevant Study Results				
Test Material	**Species**	**Method/Dose**	**RBA (%)**	**Reference**
Source: Australian railway corridor soil (sample #10) (sieved to <250 µm) Type: Railway corridor As concentration: 257 mg/kg soil	Swine (large white, female, 20–25 kg)	**Single dose blood-time concentration curve method** Reference material dose: 100 µg As/kg bw; 3 animals/group Test material dose: 114 to 285 µg As/kg bw (0.4 to 1.1 mg soil/kg bw); 3 animals/group	11.2±4.7 Mean±SD	Juhasz et al., 2007
Source: Australian railway corridor soil (sample #16) (sieved to <250 µm) Type: Railway corridor As concentration: 751 mg/kg soil	Swine (large white, female, 20–25 kg)	**Single dose blood-time concentration curve method** Reference material dose: 100 µg As/kg bw; 3 animals/group Test material dose: 334 to 834 µg As/kg bw (0.4 to 1.1 mg soil/kg bw); 3 animals/group	22.5±3.8 Mean±SD	Juhasz et al., 2007
Source: Australian railway corridor soil (sample #18) (sieved to <250 µm) Type: Railway corridor As concentration: 91 mg/kg soil	Swine (large white, female, 20–25 kg)	**Single dose blood-time concentration curve method** Reference material dose: 100 µg As/kg bw; 3 animals/group Test material dose: 40 to 101 µg As/kg bw (0.4 to 1.1 mg soil/kg bw); 3 animals/group	74.7±11.2 Mean±SD	Juhasz et al., 2007
Source: Australian cattle tick dip soil (sample #24) (sieved to <250 µm) Type: Agriculture As concentration: 713 mg/kg soil	Swine (large white, female, 20–25 kg)	**Single dose blood-time concentration curve method** Reference material dose: 100 µg As/kg bw; 3 animals/group Test material dose: 317 to 792 µg As/kg bw (0.4 to 1.1 mg soil/kg bw); 3 animals/group	33.0±17.0 Mean±SD	Juhasz et al., 2007
Source: Australian cattle tick dip soil (sample #27) (sieved to <250 µm) Type: Agriculture As concentration: 228 mg/kg soil	Swine (large white, female, 20–25 kg)	**Single dose blood-time concentration curve method** Reference material dose: 100 µg As/kg bw; 3 animals/group Test material dose: 100 to 250 µg As/kg bw (0.4 to 1.1 mg soil/kg bw); 3 animals/group	49.9±11.0 Mean±SD	Juhasz et al., 2007
Source: Australian mine site (sample #33) Type: Mining/smelting (sieved to <250 µm) As concentration: 807 mg/kg soil	Swine (large white, female, 20–25 kg)	**Single dose blood-time concentration curve method** Reference material dose: 100 µg As/kg bw; 3 animals/group Test material dose: 359 to 897 µg As/kg bw (0.4 to 1.1 mg soil/kg bw); 3 animals/group	40.8±7.4 Mean±SD	Juhasz et al., 2007
Source: Australian mine site (sample #34) (sieved to <250 µm) Type: Mining/smelting As concentration: 577 mg/kg soil	Swine (large white, female, 20–25 kg)	**Single dose blood-time concentration curve method** Reference material dose: 100 µg As/kg bw; 3 animals/group Test material dose: 248 to 619 µg As/kg bw (0.4 to 1.1 mg soil/kg bw); 3 animals/group	6.9±5.0 Mean±SD	Juhasz et al., 2007

Table 2. Key and Relevant Study Results				
Test Material	**Species**	**Method/Dose**	**RBA (%)**	**Reference**
Source: Australian gossan soil (sample #44) (sieved to <250 µm) Type: Mining/smelting As concentration: 190 mg/kg soil	Swine (large white, female, 20–25 kg)	**Single dose blood-time concentration curve method** Reference material dose: 100 µg As/kg bw; 3 animals/group Test material dose: 84 to 211 µg As/kg bw (0.4 to 1.1 mg soil/kg bw); 3 animals/group	16.4±9.1 Mean±SD	Juhasz et al., 2007
Source: Australian gossan soil (sample #45) (sieved to <250 µm) Type: Mining/smelting As concentration: 88 mg/kg soil	Swine (large white, female, 20–25 kg)	**Single dose blood-time concentration curve method** Reference material dose: 100 µg As/kg bw; 3 animals/group Test material dose: 39 to 98 µg As/kg bw (0.4 to 1.1 mg soil/kg bw); 3 animals/group	12.1±8.5 Mean±SD	Juhasz et al., 2007
Source: Montana smelter soil (sieved to <250 µm) Type: Mining/smelting As concentration: 650 mg/kg soil	Cynomolgus monkeys (male, 4–5 kg)	**Single dose urinary excretion fraction method** Reference material dose: 250, 500, or 1000 µg As/kg bw; 5 animals/group Test material dose: 650 µg As/kg bw (1000 mg soil/kg bw); 5 animals/group	13±5 Mean±SD	Roberts et al., 2007
Source: Wisconsin smelter soil (sieved to <250 µm) Type: Mining/smelting As concentration: 1412 mg/kg soil	Cynomolgus monkeys (male, 4–5 kg)	**Single dose urinary excretion fraction method** Reference material dose: 250, 500, or 1000 µg As/kg bw; 5 animals/group Test material dose: 1330 µg As/kg bw (942 mg soil/kg bw); 5 animals/group	13±7 Mean±SD	Roberts et al., 2007
Source: Florida cattle dip site (sieved to <250 µm) Type: Agriculture As concentration: 189 mg/kg soil	Cynomolgus monkeys (male, 4–5 kg)	**Single dose urinary excretion fraction method** Reference material dose: 250, 500, or 1000 µg As/kg bw; 5 animals/group Test material dose: 180 µg As/kg bw (952 mg soil/kg bw); 5 animals/group	31±4 Mean±SD	Roberts et al., 2007
Source: California mine tailings (sieved to <250 µm) Type: Mining/smelting As concentration: 300 mg/kg soil	Cynomolgus monkeys (male, 4–5 kg)	**Single dose urinary excretion fraction method** Reference material dose: 250, 500, or 1000 µg As/kg bw; 5 animals/group Test material dose: 300 µg As/kg bw (1000 mg soil/kg bw); 5 animals/group	19±2 Mean±SD	Roberts et al., 2007
Source: Washington orchard soil (sieved to <250 µm) Type: Agriculture As concentration: 301 mg/kg soil	Cynomolgus monkeys (male, 4–5 kg)	**Single dose urinary excretion fraction method** Reference material dose: 250, 500, or 1000 µg As/kg bw; 5 animals/group Test material dose: 300 µg As/kg bw (997 mg soil/kg bw); 5 animals/group	24±9 Mean±SD	Roberts et al., 2007

Table 2. Key and Relevant Study Results				
Test Material	**Species**	**Method/Dose**	**RBA (%)**	**Reference**
Source: New York orchard soil (sieved to <250 µm) Type: Agriculture As concentration: 125 mg/kg soil	Cynomolgus monkeys (male, 4–5 kg)	**Single dose urinary excretion fraction method** Reference material dose: 250, 500, or 1000 µg As/kg bw; 5 animals/group Test material dose: 120 µg As/kg bw (960 mg soil/kg bw); 5 animals/group	15±8 Mean±SD	Roberts et al., 2007
Source: Colorado smelter soil (sieved to <250 µm) Type: Mining/smelting As concentration: 394 mg/kg soil	Cynomolgus monkeys (male, 4–5 kg)	**Single dose urinary excretion fraction method** Reference material dose: 250, 500, or 1000 µg As/kg bw; 5 animals/group Test material dose: 400 µg As/kg bw (1015 mg soil/kg bw); 5 animals/group	18±6 Mean±SD	Roberts et al., 2007
Source: Colorado smelter (sieved to <250 µm) Type: Mining/smelting As concentration: 1230 mg/kg soil	Cynomolgus monkeys (male, 4–5 kg)	**Single dose urinary excretion fraction method** Reference material dose: 250, 500, or 1000 µg As/kg bw; 5 animals/group Test material dose: 1000 µg As/kg bw (813 mg soil/kg bw); 5 animals/group	17±8 Mean±SD	Roberts et al., 2007
Source: Colorado smelter soil (sieved to <250 µm) Type: Mining/smelting As concentration: 1492 mg/kg soil	Cynomolgus monkeys (male, 4–5 kg)	**Single dose urinary excretion fraction method** Reference material dose: 250, 500, or 1000 µg As/kg bw; 5 animals/group Test material dose: 1000 µg As/kg bw (670 mg soil/kg bw); 5 animals/group	5±4 Mean±SD	Roberts et al., 2007
Source: Florida chemical plant soil (sieved to <250 µm) Type: Chemical manufacturing As concentration: 268 mg/kg soil	Cynomolgus monkeys (male, 4–5 kg)	**Single dose urinary excretion fraction method** Reference material dose: 250, 500, or 1000 µg As/kg bw; 5 animals/group Test material dose: 340 µg As/kg bw (1269 mg soil/kg bw); 5 animals/group	7±3 Mean±SD	Roberts et al., 2007
Source: New York pesticide facility soil #1 (sieved to <250 µm) Type: Chemical manufacturing As concentration: 1000 mg/kg soil[a]	Cynomolgus monkeys (male, 4–5 kg)	**Single dose urinary excretion fraction method** Reference material dose: 250, 500, or 1000 µg As/kg bw; 5 animals/group Test material dose: 990 µg As/kg bw (2920 mg soil/kg bw); 5 animals/group	19±5 Mean±SD	Roberts et al., 2007
Source: New York pesticide facility soil #2 (sieved to <250 µm) Type: Chemical manufacturing As concentration: 339 mg/kg soil[a]	Cynomolgus monkeys (male, 4–5 kg)	**Single dose urinary excretion fraction method** Reference material dose: 250, 500, or 1000 µg As/kg bw; 5 animals/group Test material dose: 300 µg As/kg bw (549 mg soil/kg bw); 5 animals/group	28±10 Mean±SD	Roberts et al., 2007

Table 2. Key and Relevant Study Results				
Test Material	**Species**	**Method/Dose**	**RBA (%)**	**Reference**
Source: New York pesticide facility soil #3 (sieved to <250 µm) Type: Chemical manufacturing As concentration: 546 mg/kg soil[a]	Cynomolgus monkeys (male, 4–5 kg)	**Single dose urinary excretion fraction method** Reference material dose: 250, 500, or 1000 µg As/kg bw; 5 animals/group Test material dose: 490 µg As/kg bw (490 mg soil/kg bw); 5 animals/group	20±10 Mean±SD	Roberts et al., 2007
Source: Hawaiian volcanic soil (sieved to <250 µm) Type: Volcanic As concentration: 724 mg/kg soil	Cynomolgus monkeys (male, 4–5 kg)	**Single dose urinary excretion fraction method** Reference material dose: 250, 500, or 1000 µg As/kg bw; 5 animals/group Test material dose: 730 µg As/kg bw (1008 mg soil/kg bw); 5 animals/group	5±1 Mean±SD	Roberts et al., 2007
Source: Barber Orchard NC, sample MS-1 (sieved to <250 µm) Type: Agriculture As concentration: 290 mg/kg soil	Cynomolgus monkeys (adult male, 4–5 kg)	**Single dose urinary excretion fraction method** Reference material dose: 300 and 500 µg As/kg bw; 5 animals/group Test material dose: 290 µg As/kg bw (1000 mg soil/kg bw); 5 animals/group	33±5 Mean±SE	U.S. EPA, 2009
Source: Barber Orchard NC, sample MS-4 (sieved to <250 µm) Type: Agriculture As concentration: 388 mg/kg soil	Cynomolgus monkeys (adult male, 4–5 kg)	**Single dose urinary excretion fraction method** Reference material dose: 300 and 500 µg As/kg bw; 5 animals/group Test material dose: 388 µg As/kg bw (1000 mg soil/kg bw); 5 animals/group	28±3 Mean±SE	U.S. EPA, 2009
Source: Barber Orchard NC, sample MS-5 (sieved to <250 µm) Type: Agriculture As concentration: 382 mg/kg soil	Cynomolgus monkeys (adult male, 4–5 kg)	**Single dose urinary excretion fraction method** Reference material dose: 300 and 500 µg As/kg bw; 5 animals/group Test material dose: 382 µg As/kg bw (1000 mg soil/kg bw); 5 animals/group	38±7 Mean±SE	U.S. EPA, 2009
Source: Barber Orchard NC, sample MS-8 (sieved to <250 µm) Type: Agriculture As concentration: 364 mg/kg soil	Cynomolgus monkeys (adult male, 4–5 kg)	**Single dose urinary excretion fraction method** Reference material dose: 300 and 500 µg As/kg bw; 5 animals/group Test material dose: 364 µg As/kg bw (1000 mg soil/kg bw); 5 animals/group	25±5 Mean±SE	U.S. EPA, 2009
Source: Florida electrical substation soil (sieved to <250 µm) Type: Other manufacturing As concentration: 312 mg/kg soil	*Cebus apella* monkeys (adult male, 2.5–3.0 kg)	**Single dose urinary excretion fraction method** Reference material dose: 1000 µg As/kg bw; 5 animals/group Test material dose: 500 µg As/kg bw (1602 mg soil/kg bw); 5 animals/group	14.6±5.1 Mean±SD	Roberts et al., 2002

Table 2. Key and Relevant Study Results				
Test Material	**Species**	**Method/Dose**	**RBA (%)**	**Reference**
Source: Cattle dip site soil (sieved to <250 µm) Type: Agriculture As concentration: 189 mg/kg soil	*Cebus apella* monkeys (adult male, 2.5–3.0 kg)	**Single dose urinary excretion fraction method** Reference material dose: 1000 µg As/kg bw; 5 animals/group Test material dose: 500 µg As/kg bw (2646 mg soil/kg bw); 5 animals/group	24.7±3.2 Mean±SD	Roberts et al., 2002
Source: Florida pesticide site #1 soil (sieved to <250 µm) Type: Chemical manufacturing As concentration: 743 mg/kg soil	*Cebus apella* monkeys (adult male, 2.5–3.0 kg)	**Single dose urinary excretion fraction method** Reference material dose: 1000 µg As/kg bw; 5 animals/group Test material dose: 1000 µg As/kg bw (1346 mg soil/kg bw); 5 animals/group	10.7±4.9 Mean±SD	Roberts et al., 2002
Source: Wood preservative site #2 soil (sieved to <250 µm) Type: Chemical manufacturing As concentration: 101 mg/kg soil	*Cebus apella* monkeys (adult male, 2.5–3.0 kg)	**Single dose urinary excretion fraction method** Reference material dose: 1000 µg As/kg bw; 5 animals/group Test material dose: 300 µg As/kg bw (2970 mg soil/kg bw); 5 animals/group	16.3±6.5 Mean±SD	Roberts et al., 2002
Source: Pesticide site soil (sieved to <250 µm) Type: Chemical manufacturing As concentration: 329 mg/kg soil	*Cebus apella* monkeys (adult male, 2.5–3.0 kg)	**Single dose urinary excretion fraction method** Reference material dose: 1000 µg As/kg bw; 5 animals/group Test material dose: 500 µg As/kg bw (1520 mg soil/kg bw); 5 animals/group	17.0±10.0 Mean±SD	Roberts et al., 2002
Source: Composite residential soil, Anaconada, MT (sieved to <250 µm) Type: Mining/smelting As concentration: 410 mg/kg soil	Cynomolgus monkeys (adult female, 2–3 kg)	**Single dose urinary excretion fraction method** Reference material dose: 620 µg As/kg bw; 3 animals/group Test material dose: 620 µg As/kg bw (1500 mg soil/kg bw); 3 animals/group	20.1 Mean (SE or SD not reported)	Freeman et al., 1995
Source: NIST SRM 2710 (sieved to 74 µm) Type: Mining/smelting As concentration: 601 mg/kg soil (INAA)	C57BL/6 mice (female, 6 weeks, 15–20 g)	**Steady-state urinary excretion fraction method** Reference material dose: 820–1160 µg As/kg bw/day Test material dose: 650–1020 µg As/kg bw/day (1150–1420 mg soil/kg bw/day)	42.9 (40.5–45.4) Mean (95% CI)	Bradham et al., 2011, 2012
Source: NIST SRM 2710a (sieved to <74 µm) Type: Mining/smelting As concentration: 1513 mg/kg soil (INAA)	C57BL/6 mice (female, 6 weeks, 15–20 g)	**Steady-state urinary excretion fraction method** Reference material dose: 820–1160 µg As/kg bw/day Test material dose: 580–2360 µg As/kg bw/day (1460–1490 mg soil/kg bw/day)	42.1 (39.8–44.4) Mean (95% CI)	Bradham et al., 2011, 2012

Table 2. Key and Relevant Study Results				
Test Material	**Species**	**Method/Dose**	**RBA (%)**	**Reference**
Source: Iron King, AZ soil sample TM1 (sieved to <250 µm) Type: Mining/smelting As concentration: 280 mg/kg soil (INAA)	C57BL/6 mice (female, 6 weeks, 15–20 g)	**Steady-state urinary excretion fraction method** Reference material dose: 820–1160 µg As/kg bw/day Test material dose: 390 µg As/kg bw/day (1490 mg soil/kg bw/day)	39.9 (36.2–43.8) Mean (95% CI)	Bradham et al., 2011, 2012
Source: Iron King, AZ soil sample TM2 (sieved to <250 µm) Type: Mining/smelting As concentration: 4495 mg/kg soil (INAA)	C57BL/6 mice (female, 6 weeks, 15–20 g)	**Steady-state urinary excretion fraction method** Reference material dose: 820–1160 µg As/kg bw/day Test material dose: 6100 µg As/kg bw/day (1430 mg soil/kg bw/day)	14.5 (11.2–17.8) Mean (95% CI)	Bradham et al., 2011, 2012
Source: ASARCO soil sample (sieved to <250 µm) Type: Mining/smelting As concentration: 182 mg/kg soil (INAA)	C57BL/6 mice (female, 6 weeks, 15–20 g)	**Steady-state urinary excretion fraction method** Reference material dose: 820–1160 µg As/kg bw/day Test material dose: 320 µg As/kg bw/day (1460 mg soil/kg bw/day)	26.7 (22.8–30.7) Mean (95% CI)	Bradham et al., 2011, 2012
Source: Sample TM2 Vasquez Boulevard and I-70, composite residential, Denver CO (sieved to <250 µm) Type: Mining/smelting As concentration: 990 mg/kg soil (INAA)	C57BL/6 mice (female, 6 weeks, 15–20 g)	**Steady-state urinary excretion fraction method** Reference material dose: 820–1160 µg As/kg bw/day Test material dose: 1580 µg As/kg bw/day (1450 mg soil/kg bw/day)	48.7 (43.4–54.2) Mean (95% CI)	Bradham et al., 2011, 2012
Source: Sample TM4 Vasquez Boulevard and I-70, composite residential, Denver, CO (sieved to <250 µm) Type: Mining/smelting As concentration: 829 mg/kg soil (INAA)	C57BL/6 mice (female, 6 weeks, 15–20 g)	**Steady-state urinary excretion fraction method** Reference material dose: 820–1160 µg As/kg bw/day Test material dose: 1190 µg As/kg bw/day (1400 mg soil/kg bw/day)	49.7 (45.0–54.5) Mean (95% CI)	Bradham et al., 2011, 2012
Source: Sample TM5 Vasquez Boulevard and I-70, composite residential, Denver, CO (sieved to <250 µm) Type: Mining/smelting As concentration: 379 mg/kg soil (INAA)	C57BL/6 mice (female, 6 weeks, 15–20 g)	**Steady-state urinary excretion fraction method** Reference material dose: 820–1160 µg As/kg bw/day Test material dose: 520 µg As/kg bw/day (1580 mg soil/kg bw/day)	51.6 (47.0–56.3) Mean (95% CI)	Bradham et al., 2011, 2012

Table 2. Key and Relevant Study Results				
Test Material	**Species**	**Method/Dose**	**RBA (%)**	**Reference**
Source: Midvale slag, composite sample Midvale smelter slag pile (sieved to <250 µm) Type: Mining/smelting As concentration: 837 mg/kg soil (INAA)	C57BL/6 mice (female, 6 weeks, 15–20 g)	**Steady-state urinary excretion fraction method** Reference material dose: 820–1160 µg As/kg bw/day Test material dose: 1040 µg As/kg bw/day (1650 mg soil/kg bw/day)	11.2 (10.6–11.8) Mean (95% CI)	Bradham et al., 2011, 2012
Source: Hawaiian soil sample (sieved to <250 µm) Type: Agriculture As concentration: 769 mg/kg soil (INAA)	C57BL/6 mice (female, 6 weeks, 15–20 g)	**Steady-state urinary excretion fraction method** Reference material dose: 820–1160 µg As/kg bw/day Test material dose: 1100 µg As/kg bw/day (1500 mg soil/kg bw/day)	24.0 (20.9–27.2) Mean (95% CI)	Bradham et al., 2011, 2012
Source: Barber Orchard NC, sample MS-1 (sieved to <250 µm) Type: Agriculture As concentration: 322 mg/kg soil (INAA)	C57BL/6 mice (female, 6 weeks, 15–20 g)	**Steady-state urinary excretion fraction method** Reference material dose: 820–1160 µg As/kg bw/day Test material dose: 470 µg As/kg bw/day (1470 mg soil/kg bw/day)	26.3 (23.4–29.4) Mean (95% CI)	Bradham et al., 2011, 2012
Source: Barber Orchard NC, sample MS-4 (sieved to <250 µm) Type: Agriculture As concentration: 387 mg/kg soil (INAA)	C57BL/6 mice (female, 6 weeks, 15–20 g)	**Steady-state urinary excretion fraction method** Reference material dose: 820–1160 µg As/kg bw/day Test material dose: 600 µg As/kg bw/day (1480 mg soil/kg bw/day)	35.2 (30.9–39.6) Mean (95% CI)	Bradham et al., 2011, 2012
Source: Barber Orchard NC, sample MS-5 (sieved to <250 µm) Type: Agriculture As concentration: 467 mg/kg soil (INAA)	C57BL/6 mice (female, 6 weeks, 15–20 g)	**Steady-state urinary excretion fraction method** Reference material dose: 820–1160 µg As/kg bw/day Test material dose: 630 µg As/kg bw/day (1370 mg soil/kg bw/day)	20.9 (15.9–26.0) Mean (95% CI)	Bradham et al., 2011, 2012
Source: Barber Orchard NC, sample MS-8 (sieved to <250 µm) Type: Agriculture As concentration: 396 mg/kg soil (INAA)	C57BL/6 mice (female, 6 weeks, 15–20 g)	**Steady-state urinary excretion fraction method** Reference material dose: 820–1160 µg As/kg bw/day Test material dose: 640 µg As/kg bw/day (1510 mg soil/kg bw/day)	35.0 (31.2–38.9) Mean (95% CI)	Bradham et al., 2011, 2012

Table 2. Key and Relevant Study Results				
Test Material	**Species**	**Method/Dose**	**RBA (%)**	**Reference**
Source: Mohr Orchard PA sample (sieved to <250 µm) Type: Agriculture As concentration: 340 mg/kg soil (INAA)	C57BL/6 mice (female, 6 weeks, 15–20 g)	**Steady-state urinary excretion fraction method** Reference material dose: 820–1160 µg As/kg bw/day Test material dose: 500 µg As/kg bw/day (1440 mg soil/kg bw/day)	33.2 (27.7–38.7) Mean (95% CI)	Bradham et al., 2011, 2012
Relevant Studies				
Source: Residential soil, Anaconda, MT (test material particle size 19 µm) Type: Mining/smelting As concentration: 3900 mg/kg soil	Rabbit (New Zealand white rabbits male and female; 9–12 weeks old, ~2 kg)	**Single dose urinary excretion fraction method** Reference material dose: 1950 µg As/kg bw; 5 animals/sex/group Test material dose: 780, 1970, or 3900 µg As/kg bw (200, 500, or 1000 mg soil/kg bw); 5 animals/sex/group	48.2 Mean (SE or SD not reported)	Freeman et al., 1993

[a] Arsenic concentrations based on personal communication from the co-authors S. Roberts and Y. Lowney (09/24/2010) which corrects an error in column headings in Table 3 of Roberts et al. (2007); reported values: NYPF1=339 ppm, NYPF2=546 ppm, and NYPF3=1000 ppm)

Table 3. Summary Statistics for RBA (%) Estimates Based on Key Studies					
Parameter	**Swine**	**Monkeys**	**Mice**	**All Species[a]**	**All Species[b]**
N[c]	64	24	15	103	88
AM	34.5	19.2	33.5	30.8	29.9
SD	17.5	8.6	12.6	16.4	16.8
SE	2.2	1.7	3.3	1.6	1.8
95LCL[d]	30.2	15.8	27.1	27.6	26.4
95UCL[d]	38.8	22.6	39.8	34.0	33.4
MIN	4.1	5.0	11.2	4.1	4.1
5th %	7.9	5.3	13.5	7.1	6.9
10th %	9.7	8.1	17.0	10.8	8.9
25th %	20.8	14.2	25.2	18.0	16.9
50th %	37.0	18.5	35.0	29.1	28.3
75th %	44.8	24.8	42.5	42.0	42.0
90th %	54.4	30.1	49.3	51.5	50.3
95th %	60.9	32.7	50.2	56.8	56.3
MAX	78.0	38.0	51.6	78.0	78.0
SKEW	0.21	0.29	-0.24	0.47	0.55
KURT	-0.42	-0.21	-0.92	-0.23	-0.14

[a] Each RBA estimate for materials evaluated in more than one assay is given equal weight.
[b] RBA estimates for materials evaluated in more than on assay are represented by the average of values from all assays. These include the following test materials: Barber Orchard MS-1, -4, -5, and -8 (swine, monkey, and mouse); and Iron King TM1 and TM2, Ruston/ASARCO, Hawaii, Mohr Orchard, NIST 2710 and NIST 2710A (swine and mouse).
[c] Number of RBA estimates.
[c] Number of RBA estimates.
[d] Assumes central limit and Z=1.96 for standard normal

AM, arithmetic mean; KURT, kurtosis; LCL, lower confidence limit on the mean; MAX, maximum; MIN, minimum; SD, standard deviation; SE, standard error; UCL, upper confidence limit on the mean; 5th %, 5th percentile

Table 4. Weighted RBA Summary Statistics and Confidence Limits[a]			
Parameter	**CTE**	**95% LCL**	**95% UCL**
AM	30.8	29.8	31.7
5th %	6.6	5.1	8.3
50th %	28.5	26.2	31.0
95th %	58.1	53.3	64.0

[a] Weighted for uncertainty (SE of mean, based on Monte Carlo analysis of all RBA estimates from swine, monkey, and mouse studies [n=103]).

AM, arithmetic mean; CTE, central tendency estimate; LCL, lower confidence limit; UCL, upper confidence limit

Table 5. RBA Estimates for Barber Orchard Soils Administered to Mice, Monkeys, and Swine				
	RBA % (95% Confidence Limits)			
Species	**MS-1 (290 ppm)[a]**	**MS-4 (388 ppm)[a]**	**MS-5 (382 ppm)[a]**	**MS-8 (364 ppm)[a]**
Mice	26 (23–29)	35 (31–40)	21 (16–26)	35 (31–39)
Monkey	33 (23–43)[b]	28 (22–34)[b]	38 (24–52)[b]	25 (15–35)[b]
Swine	31 (24–40)	41 (37–44)	49 (40–59)	53 (48–57)

[a] Test material number (As concentration): arsenic concentration measured on sieved (250 µm) fractions.
[b] Estimated as SE x 1.96 (Z=1.96 for standard normal), where SE values were reported in U.S. EPA, 2009.

Table 6. Comparison Between RBA Estimates Based on Mice and Swine Bioassays		
Test Materials	**RBA % (95% Confidence Limits)**	
	Mice	**Swine**
Iron King HSJ-583	40 (36–44)	60 (55–66)[a]
Iron King IKJ-583	14 (11–18)	19 (17–20)
Ruston ASARCO	27 (23–31)	49 (44–54)[a]
Hawaii	24 (21–27)	33 (30–36)[a]
Barber Orchard MS-1	26 (23–29)	31 (24–40)
Barber Orchard MS-4	35 (31–40)	41 (37–44)
Barber Orchard MS-5	21 (16–26)	49 (40–59)[a]
Barber Orchard MS-8	35 (31–39)	53 (48–57)[a]
Mohr Orchard	33 (28–39)	53 (50–57)[a]
NIST 2710	43 (40–45)	44 (40–49)
NIST 2710A	42 (40–44)	42 (39–45)

[a] Confidence limits do not overlap.

Table 7. Comparison Between RBA Estimates Based on UEF and Blood AUC in Monkeys		
Monkey Number	**RBA based on UEF**	**RBA based on Blood AUC**
30–544	27.7	6.1
20–784	18.6	6.9
30–537	14.1	19.9
Mean	20.1	11.0
SD	6.9	7.7

Based on Freeman et al. (1995). RBA estimates based on the two methods are not significantly different based on paired t-test ($p=0.37$) or unpaired t-test ($p=0.20$).
AUC, area under the blood concentration – time curve; UEF, urinary excretion fraction

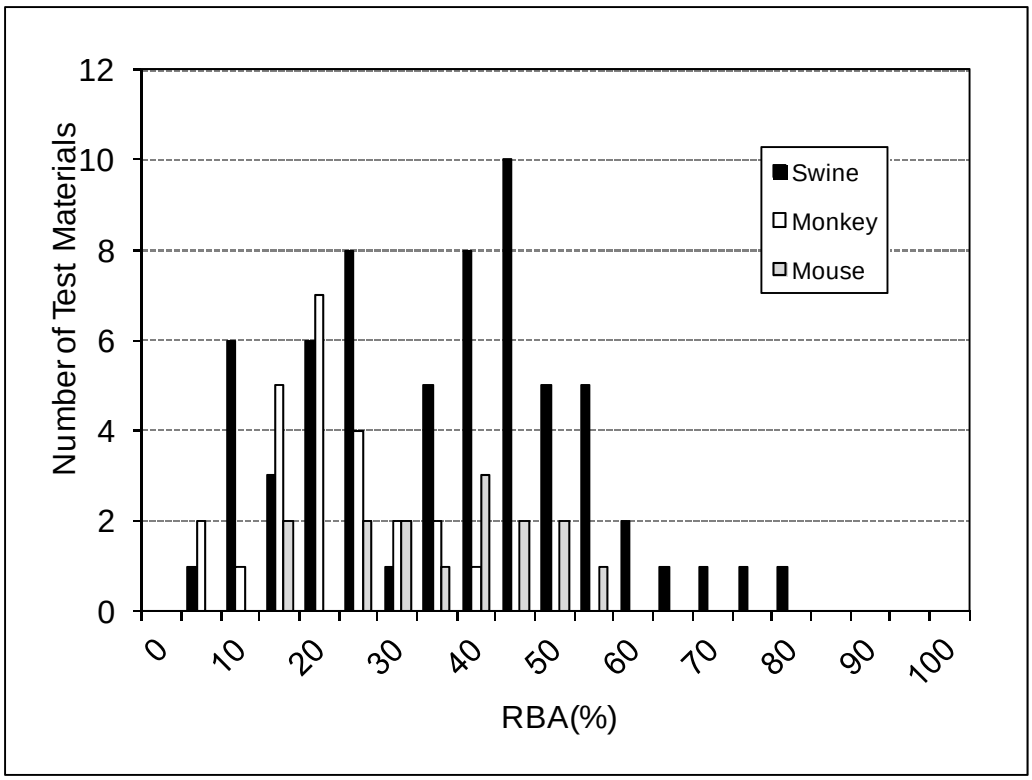

Figure 1. Distribution of RBA Values for Materials Assayed in Swine, Monkey, and Mouse.

The mean RBA value for test materials assayed in monkeys is 19.2% (95% CI: 15.8–22.6, n=24); the mean for test materials assayed in swine is 34.5% (95% CI: 30.2–38.8, n=64); the mean for test materials assayed in mice is 33.5% (95% CI: 27.1–39.8, n=15).

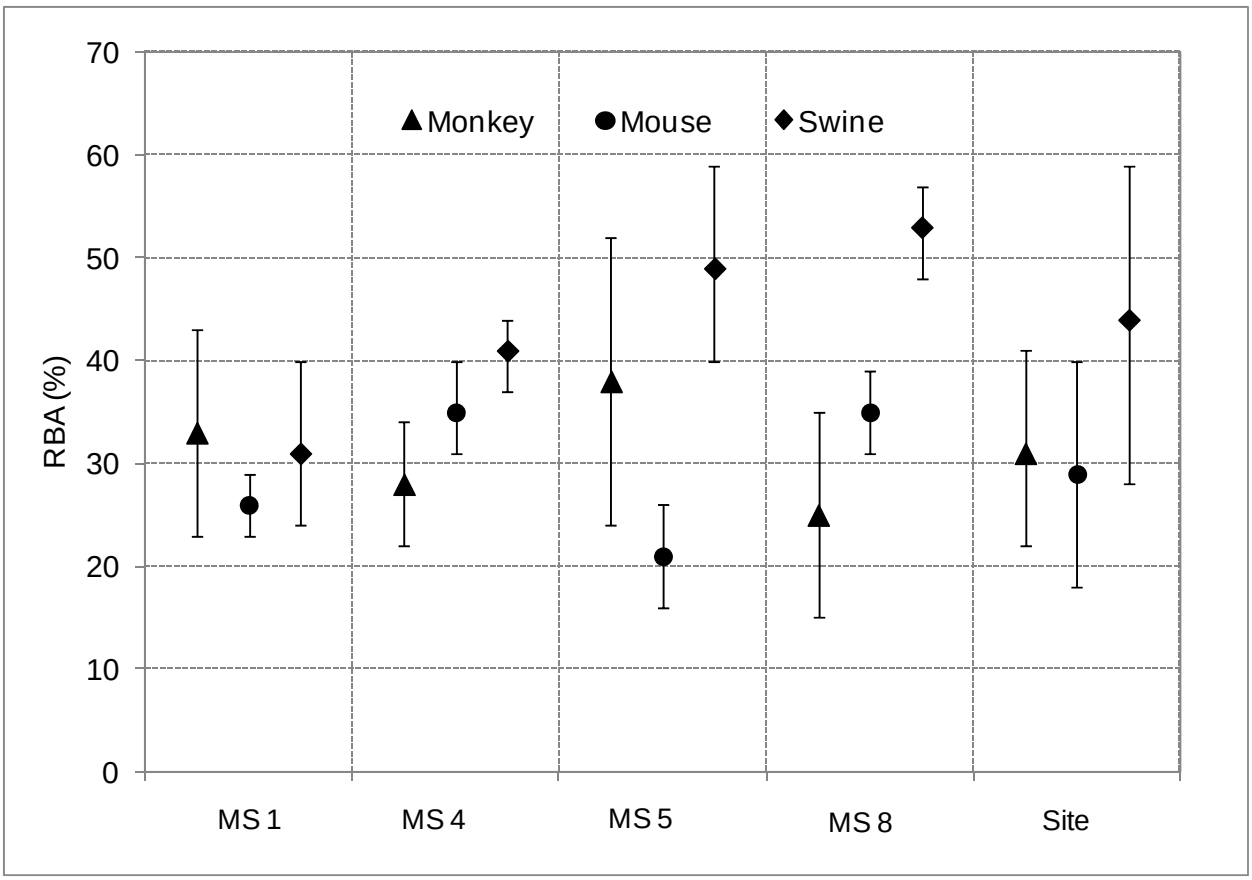

Figure 2. Comparison Between Arsenic RBA Estimates from Swine, Monkey, and Mouse Bioassays of Four Soil Samples from the Barber Orchard Site.

Shown are mean and 95% confidence limits. The values shown for "site" are the means for all four soil samples.

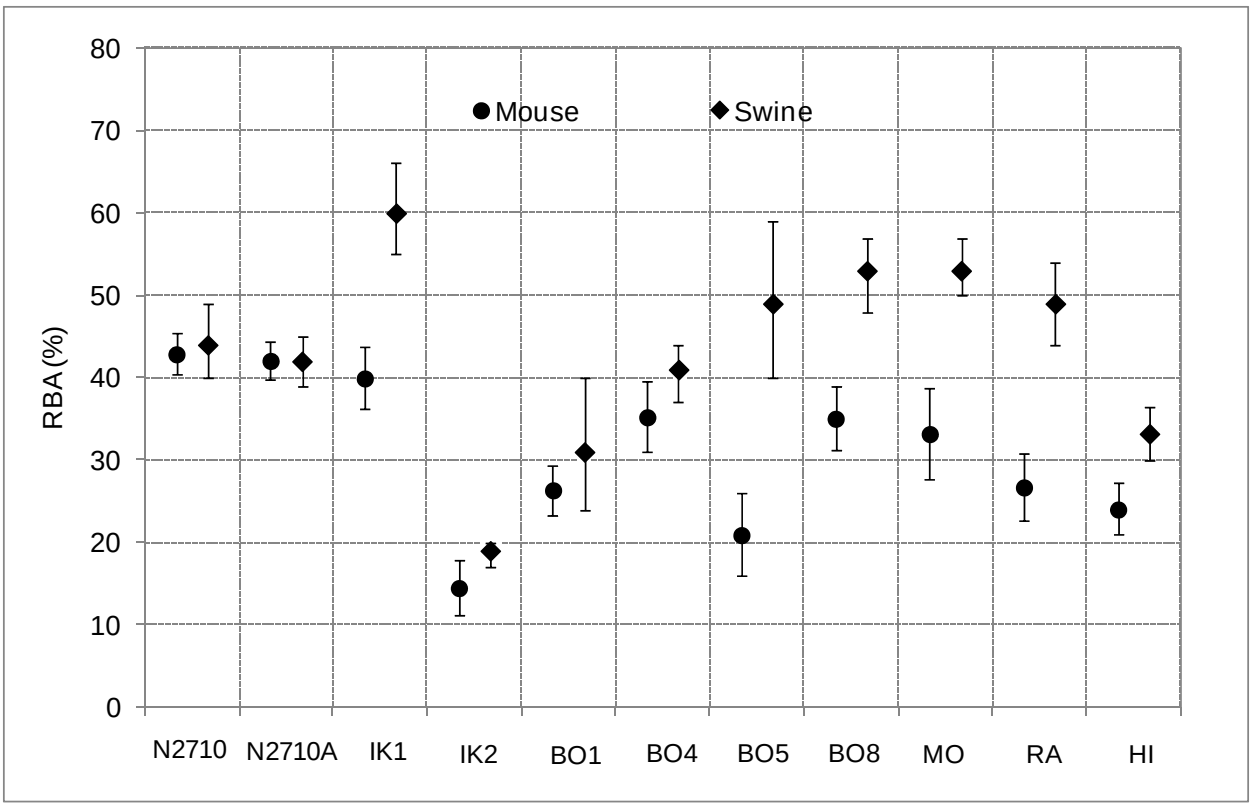

Figure 3. Comparison Between Arsenic RBA Estimates from Swine or Mouse Bioassays of 11 Test Materials.

Shown are mean and 95% confidence limits. The values shown for "site" are the means for all four soil samples.

BO, Barber Orchard; HI, Hawaii; IK, Iron King; MO, Mohr Orchard; N, NIST; RA, Ruston-ASARCO

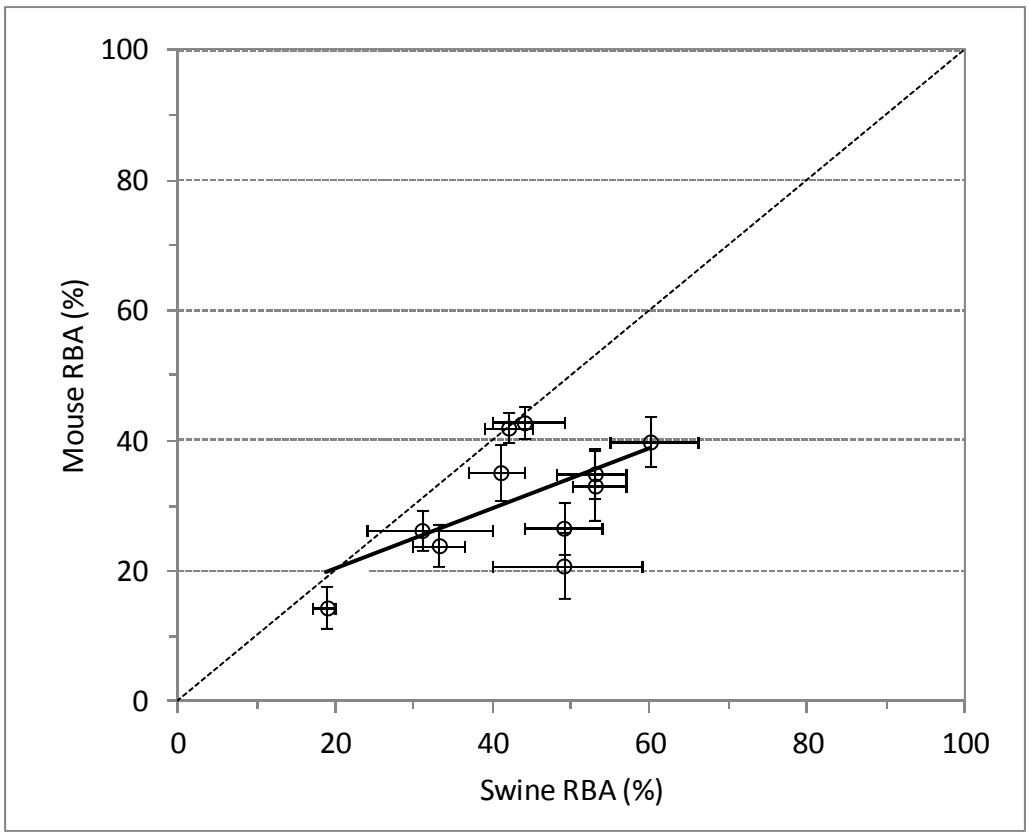

Figure 4. Relationship Between Arsenic RBA Estimates Based on Mouse and Swine Bioassays Applied to 11 Test Materials.

Error bars for mice are 95% confidence limits. Solid line is the linear regression model (R^2=0.35, p=0.053).The mouse and swine RBA estimates are not significantly correlated (Pearson r=0.60, p=0.053; Spearman r=0.42, p=0.19).

APPENDIX A: Summary Description of Human Arsenic Bioavailability Study (Stanek et al., 2010)

A single human experimental study of bioavailability of arsenic in soil was reported (Stanek et al., 2010). This study was not used selected for inclusion in this report as a key or relevant study because of several methodological limitations and uncertainties, which are briefly summarized below. Stanek et al. (2010) utilized a mass balance approach to estimate absolute bioavailability of arsenic in food and soil in a small group of human subjects (n=13 subjects including 7 females and 6 males, age 26–53 years). The study consisted of two phases conducted approximately 2–3 years apart, with partial overlap of subjects in both phases. Phase 1 of the study estimated absolute bioavailability of arsenic in food and included 11 subjects (6 females and 5 males, age 26–53 years). Daily complete urine and fecal samples, and duplicate diet samples were collected from each subject for a period of 7 consecutive days. For each subject, for each day, absolute bioavailability of ingested arsenic was calculated as follows (Equation A-1):

$$ABA_{food} = \frac{As_{food} - As_{fecal}}{As_{food}} \qquad \text{Eq. (A-1)}$$

where ABA is absolute bioavailability and As_{food} and As_{fecal} are the rate of intake of arsenic in food and rate of excretion of arsenic in feces (µg/day), respectively.

Phase 2 estimated the absolute bioavailability of arsenic in soil and included 11 subjects, 9 of whom participated in Phase 1. Subjects were asked to avoid eating seafood, rice, mushrooms, spinach, or grape juice (foods typically having high levels of arsenic) for 4 days preceding the 7-day observation period. On day 2 of the observation period, each subject ingested a gelatin capsule containing 111.7 µg As in 0.636 g of soil. The soil was obtained from a cattle dip site (see Roberts et al., 2007). Absolute bioavailability of arsenic in soil was calculated as follows (Equation A-2):

$$ABA_{soil} = \frac{As_{fecal} - As_{food} \cdot (1 - ABA_{food})}{As_{soil}} \qquad \text{Eq. (A-2)}$$

The above calculation utilizes the estimate of the absolute bioavailability of arsenic in food to calculate the amount of fecal arsenic attributable to food in Phase 2. The difference between total fecal arsenic and fecal arsenic attributed to food was attributed to the soil dose. Bioavailable arsenic from the soil dose was calculated as the difference between the soil arsenic dose and fecal arsenic attributed to the soil dose.

Stanek et al. (2010) reported estimates of 87.5% (95% CI: 81.2, 93.8) and 89.7% (95% CI: 83.4, 96.0) for absolute arsenic bioavailability in food, based on Phase 1 and Phase 2 respectively. The estimate for absolute bioavailability of arsenic in soil was 48.7% (95% CI: 36.2, 61.3). The estimate for bioavailability of arsenic from soil relative to food was 54.5% (48.7%/89.7%).

Several important uncertainties attend these above estimates of bioavailability, which precluded the using the estimates in the calculation of soil RBA for the upper bound estimate for soil RBA:

- Stanek et al. (2010) does not provide an estimate of the RBA for arsenic in soil relative to that of a completely bioaccessible form of arsenic (e.g., to sodium arsenate). The ratio of the absolute bioavailability of arsenic in soil to that of arsenic in food, reported in Stanek et al. (2010), is not directly comparable to RBAs based on key studies described in this report (e.g., soil RBA relative to sodium arsenate).

- The two study phases were separated by ~2.5 years and, although there was substantial overlap among subjects in both phases, individual subjects could not serve as their own measures for absolute bioavailability of dietary arsenic in the calculation of absolute bioavailability of soil arsenic.

- Sample collection (duplicate diets, feces, and urine) appears to have been unsupervised and was performed by individual subjects outside of a clinical research center where adherence to sampling protocols could have been assured.

- No attempt was made to control dietary arsenic intake, other than the 4-day voluntary "arsenic suppression" diet that preceded Phase 2. As a result, intra- and inter-subject variability in dietary intakes was substantial (e.g., maximum/minimum arsenic intake ratio in Phase 1 ranged from 6 to 84). This magnitude of variability in dietary arsenic intakes during the study is likely to have contributed substantial dietary noise to the estimation the fraction of fecal arsenic attributed to the soil dose in Phase 2.

- The recovery of arsenic from a duplicate diet spiked with a known amount of soil arsenic was reported to have been 78.9% and no explanation is given for the low recovery. The resulting uncertainty in the dietary and soil arsenic doses contributes to uncertainty in the corresponding bioavailability estimates for food and soil. The magnitude of the error in the bioavailability estimates attributable to error in the arsenic dose estimates depends on whether or not the low arsenic recovery represents arsenic in soil, and/or arsenic in food, and/or arsenic in soil added to food. Therefore, without an understanding of the recovery problem, or of the reproducibility of recovery, the magnitude of the error cannot be reliably determined. Based on data reported in the Appendix to Stanek et al. (2010), the estimates of soil RBA may have ranged from 40 to 60%, depending on the assignment of the recovery error to food, soil, or both media.