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REPORT

INGESTION BIOAVAILABILITY OF ARSENIC, LEAD AND CADMIUM IN HUMAN HEALTH RISK ASSESSMENTS: CRITICAL REVIEW, AND RECOMMENDATIONS

HEALTH CANADA
Environmental Health Assessment Services
Safe Environments Program
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Safe Environments Program
Ottawa, On
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ON **INGESTION BIOAVAILABILITY OF ARSENIC, LEAD AND CADMIUM IN HUMAN HEALTH RISK ASSESSMENTS: DATABASE, CRITICAL REVIEW, AND RECOMMENDATIONS**

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1.0 INTRODUCTION

Since 2003, the Environmental Health Assessment Services Division of Health Canada has been developing guidance documents for use in completing federal contaminated site risk assessments in Canada. To date three documents have been completed and released for use:

Part I: Guidance on Human Health Screening Preliminary Quantitative Risk Assessment (PQRA), September 2004.(Health Canada, 2004a)

Part II: Health Canada Toxicological Reference Values (TRVs), September, 2004 (Health Canada, 2004b)

Part III: Guidance on Peer Review of Human Health Risk Assessments, September 2004 (Health Canada, 2004c)

These documents have been prepared in support of the Federal Contaminated Sites Accelerated Action Plan (FCSAAP). The objective of this program is to provide improved and continuing federal environmental stewardship as it relates to contaminated sites located on federally owned or operated properties. A cornerstone of FCSAAP is to assess the potential risk posed to human health from exposure to contaminants of concern (CoCs) on these sites. The results of the human health risk assessments conducted on each individual site serve two purposes:

To aid in ranking the priority of contaminated sites in support of FCSAAP funding

Provide guidance on contaminants of concern that warrant either risk management or remediation in order to ensure the protection of human health.

Human Health Risk Assessment (HHRA) can be complex in its undertaking and is by no means an exact science. In Canada, the manner in which HHRA is regulated, guided, and conducted varies by provincial jurisdiction (see Dillon, 2004) and, until only recently, had very little guidance at the federal level. In addition, there is a wide range of variability in how risk assessment practitioners carry out studies, even when preparing assessments in the same jurisdiction (CMHC, 1997). When preparing federal guidance documents for conducting HHRA, Health Canada is attempting to remove the variability (as much as is possible and reasonable) in its conduct, while still allowing for professional judgement to be exercised.

In keeping with the need to standardize the manner in which risk assessment is conducted, Health Canada retained Jacques Whitford Limited (Jacques Whitford) to conduct a critical review of the most current data on the oral bioavailability of arsenic, lead and cadmium from soil and to provide recommendations on how it can be incorporated into site specific risk assessment (SSRA). In addition to this document an accompanying database was compiled that summarizes the most recent findings of bioavailability and bioaccessibility of arsenic, lead and cadmium from soil.

2.0 BACKGROUND

Regulations and guidelines governing contaminated site assessment and remediation are based on the total concentration of the target substance in a particular substrate; soil, sediment, or water (CCME, 1997). Chemicals and inorganic elements dissolved in water may be readily available for uptake by various organisms, such as plants, animals, and humans, but this is not the case for contaminants in solid substrates such as soils. This is particularly true for inorganic elements that may be tightly bound within the soil matrix.

In recent years there has been a considerable amount of research conducted world-wide into the bioavailability of contaminants from soil, and its incorporation into risk assessment, to develop scientifically defensible suitable endpoints for site remediation. The primary focus of this review is on the current literature involving the bioavailablity of arsenic, lead and cadmium from soil substrates. However, much of this line of thought is directly relevant to other inorganic elements. It should be noted that very little research has been conducted on the field of organic contaminant bioavailability, thus organic contaminants are outside the scope of this work.

2.1 Canadian Regulatory Guidance on Oral Bioavailability in Risk Assessment

Incidental ingestion of soil (and contaminants contained within it) is often the driving exposure pathway in human health risk assessment. Health Canada (2004a) has adopted the following equation for deriving the dose from soil ingestion:

Table 2-1. Health Canada Recommended Inadvertent Ingestion of Contaminated Soil Equation to be Used to Estimate Doses (Health Canada, 2003a)

INADVERTENT INGESTION OF CONTAMINATED SOIL		
The predicted intake of each contaminant via soil ingestion is calculated as:		
$$Dose\ (mg/kg\ /\ day) = \frac{C_S \times IR_S \times AF_{GIT} \times D_1 \times D_2 \times D_3}{BW \times LE}$$		
Where:		
C_S	=	concentration of contaminant in soil (mg/kg)
IR_S	=	receptor soil ingestion rate (kg/d)
AF_{GIT}	=	absorption factor from the gastrointestinal tract (unitless)
D_1	=	days per week exposed/7 days
D_2	=	weeks per year exposed/52 weeks
D_3	=	total years exposed to site (to be employed for assessment of carcinogens only)
BW	=	body weight (kg)
LE	=	life expectancy (yr) (to be employed for assessment of carcinogens only)

The variable of importance from this equation is the AF_{GIT} or the absorption factor from the gastrointestinal tract, which is expressed as a proportion and is, therefore, unitless.

The Health Canada PQRA guidance document (Health Canada, 2004a) states that for the purposes of a screening level risk assessment:

> *"Oral exposure should always be assumed to have a relative absorption of 100% (RAF=1)."*

This is the only time oral bioavailability or absorption factors are mentioned in the PQRA document. Therefore, PQRAs conducted for federal contaminated sites do not normally adjust the oral bioavailability of contaminants in soil, and use a default of 100%. This guidance was provided for two reasons, both of which are reasonable for conducting PQRAs:

1. *There has been only limited information published on the oral bioavailability of contaminants in soil and thus the use of 100% bioavailability by all risk assessment practitioners will reduce variability in PQRAs.*

2. *The purpose of a screening level risk assessment is to provide a conservative quantification of potential human health risk posed by the presence of contaminants at a site. The use of 100% bioavailability for the oral ingestion pathway is the most conservative approach.*

In general, PQRAs are the conservative first step in determining whether or not a potential risk to human health exists from exposure to the maximum concentrations of contaminants at a site, using 100% oral bioavailability (Health Canada, 2004a). In the case of sites where there is a large degree of variability in concentrations of contaminants, size of the site and land-uses then site specific risk assessments SSRAs are employed to provide a more accurate quantification of potential risk. SSRAs are a critical tool in preparing complex remedial and risk management objectives to reduce risk to acceptable levels for receptors using the site.

SSRAs involve a much greater level of detail and complexity, in that statistical approaches to data analysis and interpretation are employed, a more thorough understanding of the nature of the site is sought, and scientifically defensible modifications to equations and input parameters are made. It is at this stage that one may consider oral bioavailabilities different from 100%.

In the Health Canada Part III document (Health Canada, 2004c), the peer review checklist contains the following statement on oral bioavailability/bioaccessibility:

> *Absorption factors for ingestion are usually 100% in screening level risk assessments.*
>
> *Oral bioavailability is commonly measured in vitro as bioaccessibility (% solubility in simulated gastric fluid), which depends upon the properties of the soil and the site specific characteristics of the contaminant. In complex risk assessments, direct assays of contaminant bioaccessibility may be conducted to directly measure potential bioavailability. Therefore, if a value for oral bioavailability of less than 100% is used, ideally it is based on site specific measurements of bioaccessibility.*

This statement suggests that the risk assessment practitioner has the option of incorporating site specific bioaccessibility values into a federal site-specific risk assessment. However, there is little to no guidance as to how bioavailability should be

incorporated into the assessment. It is assumed that further guidance will be provided by Health Canada their forthcoming:

> *Part V: Guidance on Complex Human Health Site-Specific Risk Assessment (HHSSRA); (in preparation)*

An examination of Provincial jurisdiction guidelines for use at contaminated sites revealed that only the Ontario Ministry of the Environment (OMOE, 1996) contains any reference or guidance for use of bioavailability in site specific risk assessment. In the 1996, *Guidance on Site Specific Risk Assessment for Use at Contaminated Sites in Ontario*, the MOE states in Appendix D:

> *Conversions for bioavailability should only be undertaken on the basis of strong observational data from human and/or animal studies, and not on model prediction or assumptions.*

Although the OMOE has previously dictated that oral bioavailability must rely on human and/or animal studies, a recent human health risk assessment of residents of Port Colborne exposed to metal impacted soil incorporated the use of *in vitro* bioaccessibility studies into the exposure equations (OMOE, 2002). Therefore, it is assumed that the Standards Development Branch of the OMOE now accept risk assessments that incorporate oral bioaccessibility into the exposure equations, at least for metals in soil.

2.2 Background on Bioavailability/Bioaccessibility

In the early 1990s risk assessment practitioners and regulatory authorities began to realize that the oral bioavailability of contaminants in soil is a key factor in the conduct of human health risk assessment. Knowledge of the oral bioavailable fraction of inorganic elements from soils is critical for understanding the actual risk posed to human receptors through this pathway at contaminated sites. Although SSRAs and PQRAs have traditionally employed a conservative bioavailabiltity of 100%, this may lead to an over-estimation of potential risk. Such an over-estimation in a site specific risk assessment may result in remediation or risk management plans being adopted that are over protective of an area where there may be no actual risk to human receptors. This results in the expenditure of capital on remediating sites, but no real or actual reduction in risk to human health.

In 1999, a critical review article on oral bioavailability/bioaccessibility of inorganic elements in soil for use in human health risk assessment defined the various aspects of bioavailability (Ruby *et. al.*, 1999). These definitions have been widely adopted by the scientific community and have become common jargon used in this field of investigation:

> ***Bioavailability:*** *Oral bioavailability is defined as the fraction of an administered dose that reaches the central blood compartment from the gastrointestinal tract. Bioavailability defined in this manner is commonly referred to as "absolute bioavailability", and is equal to the oral absorption fraction.*
>
> ***Relative Bioavailability:*** *Relative bioavailability refers to comparative bioavailabilities of different forms of a substance or for different exposure media containing the substance (e.g., bioavailability of*

a metal from soil relative to its bioavailability from water), expressed in this document as a relative absorption factor (RAF).

Relative Absorption Factor (RAF): *The RAF describes the ratio of the absorbed fraction of a substance from a particular exposure medium relative to the fraction absorbed from the dosing vehicle used in the toxicity study for that substance. (The term relative bioavailability adjustment [RBA] is a synonym for RAF).*

Bioaccessibility: *The oral bioaccessibility of a substance is the fraction that is soluble in a gastrointestinal environment and is available for absorption (into the central blood compartment). The bioaccessible fraction is not necessarily equal to the RAF but depends on the relation between results from a particular in vitro test system and an appropriate in vivo model.*

2.2.1 Absolute versus Relative Oral Bioavailability of Contaminants in Soil

The oral bioavailability (relative or absolute) of an inorganic element in soil is best derived through *in vivo* models. The *in vivo* experiments are often conducted in a controlled laboratory setting and employ the oral administration of contaminated soil (typically through gavage) to animals; such as rats, rabbits, swine, or monkeys, and in some limited instances humans.

The bioavailability of inorganic elements from soil in *in vivo* tests may be expressed in either absolute or relative terms. Absorption of the soluble fraction of inorganic elements across the GI tract into the body varies by element and the chemical form that it is present in the gastric fluid. Nearly all soluble forms of arsenic in the GI tract are absorbed extensively (~100%) into the body in both humans and laboratory animals. However, in the case of most metals, a smaller fraction of soluble metal is actually transported across the intestinal wall.

Experimentally, absolute bioavailability (ABA) is expressed as a percentage of the original dose or concentration of inorganic element measured in biological tissues (such as blood, organs, urine, etc.), or

$$ABA_{soil} = \text{absorbed dose / administered (ingested) dose}$$

Reporting of the ABA_{soil} does not necessarily include an understanding of the concentration of the inorganic element that was soluble in the GI tract or the bioaccessible concentration. In addition, with the exception of arsenic, many of the bioavailability papers published provide only values for the relative bioavailability of contaminants from soil.

The relative bioavailability (RBA) of an orally administered dose is the ratio of

$$RBA_{soil} = ABA_{soil} / ABA_{reference\ material}$$

Where:

RBA_{soil}	relative bioavailability of contaminant in relation to reference material
ABA_{soil}	absolute bioavailability of contaminant in soil
$ABA_{lead\ acetate}$	absolute bioavailability of reference material

The reference material ABA that is most often used in deriving the RBA is that used in the derivation of the toxicity reference dose used in human health risk assessment. For example, the arsenic cancer slope factor (CSF), used by both Health Canada (Health Canada, 2004b) and the US EPA (1988), was derived from the oral ingestion of arsenic contaminated drinking water by a human population. Given that the ABA of arsenic in water is assumed to be 100% (1.0) then the RBA_{soil} for arsenic will be the same as the absolute bioavailability determined during *in vivo* laboratory experiments.

However, this is not the case for lead where approximately 50% of soluble lead (as lead acetate, or dietary lead) was shown to be absorbed into the bloodstream (Ziegler et. al., 1978; Alexander et. al., 1973; WHO 1986). The WHO provides a summary that suggests that lead absorption from lead acetate and dietary exposure ranges from 25% to 50% in children, while adults normally only absorb typically 10% of dietary lead. Baseline risk assessments in the United States for lead contaminated sites assume a default relative bioavailability (RBA) of Pb in soil of 60%, based on a default $ABA_{soil\ lead}$ of 30% and a $ABA_{reference}$ of lead in diet as lead acetate of 50% (i.e. RBA = 0.3/0.5=0.6).

When evaluating bioavailability research for inorganic elements from soil it is important to understand whether the absolute or relative bioavailability of contaminants is being reported. In terms of the risk assessment calculation presented in Table 2-1, it is the RBA_{soil} (i.e., relative to the $ABA_{reference}$ of the toxicity reference dose) that should be substituted for the AF_{GIT} factor (absorption factor from the gastrointestinal tract (unitless)). A common mistake made by risk assessors is the substitution of the ABA_{soil}, which for many elements would result in an underestimation of potential bioavailability.

2.2.2 *In vitro* Bioaccessibility Research

Although the use of *in vivo* models yield the most representative bioavailability data for contaminated soils, the cost, time constraints and the ethics of using these models at individual contaminated sites is unreasonable and has led to the development of several *in vitro* laboratory extraction tests that attempt to simulate *in vivo* physiochemical conditions of the gastrointestinal tract.

In the mid 1990s, Michael Ruby and his colleagues in the United States initiated work on oral bioaccessibility (*in vitro* studies) of lead and arsenic from soils and household dust collected in the vicinity of a historical copper smelter in Anaconda, MT (Ruby *et. al.*, 1996). The *in vivo* oral bioavailability of arsenic and lead from these samples had been previously determined in rabbits (Freeman et al, 1993) and *cynomolgus* monkeys (Freeman et al, 1995). The aim of this research team was to develop a chemical extraction method that could be used to simulate the results of their bioavailability work.

The *in vitro* chemical extraction was coined the "*physiologically based extraction test*" (PBET) (Ruby *et. al.*, 1996). The PBET method was developed through the selection of parameters for gastric and small intestinal pH, soil mass, fluid volume, stomach mixing and emptying rate, and small intestinal transit time, which were based on child gastric conditions. The initial experiments were conducted varying stomach pH between 1.3

and 2.5, and employed both a 1 hour retention time in simulated gastric conditions and 3.5 hours in simulated intestinal conditions, where the pH was raised to 7.0. A reasonable correlation was found to exist between the *in vivo* animal studies and those derived in the *in vitro* chemical PBET experiments for lead and arsenic in the gastric stage of the PBET extraction.

The advantage of the PBET test was that it was shown to conservatively estimate (i.e., tends not to underestimate) arsenic and lead bioavailability, without being too conservative. From this work the Solubility/Bioavailability Research Consortium was formed and currently their standard operating procedure consists of little more than deionized water, titrated to a pH of 1.8 using concentrated hydrochloric acid and a glycine buffer, referred to as the SBRC method (SBRC, 2002). Although data has not been published to support the use of this method, the SBRC has stated that it is validated for Pb and As with *in vivo* comparison data.

The results of the Ruby (1996) PBET experiment sparked the interest of researchers around the world and has led to many publications of lead and arsenic bioaccessibility using either the PBET method, some derivation thereof, or other simulated gastrointestinal conditions meant to mimic the gastric and/or gastrointestinal environment. In this document the term bioaccessibility will be used to refer to *in vitro* laboratory experiments that are designed to mimic the gastric environment and measure the percent of inorganic elements in a soil sample that should be soluble in the gastrointestinal tract for uptake into the central blood compartment.

In the late 1990s several research groups in the United States published data on *in vitro* methods for determining arsenic and lead bioaccessibilities from soils (for example - Hamel, *et. al*., 1998; Rodriguez *et. al*., 1999). These studies form a large part of the *in vitro* review presented in this report.

It is at this stage in the literature that several key parameters were identified for use in *in vitro* experiments. The first parameter of interest is the gastric pH. The pH of the simulated gastric fluid has proved to be the single most influential variable affecting bioaccessibility results. Several pHs have been employed, ranging from 1.3 to 4.0. The selection of these values were based on both *in vivo* and *in vitro* (aspiration of stomach fluid) tests of child pH that indicate that the mean fasting pH values ranged between 1.7 to 1.8, while gastric pH values rise to greater than 4 following ingestion of food. The exact stomach pH that should be used in conducting *in vitro* experiments remains a contentious issue to this day.

The second important factor in *in vitro* work is whether or not an intestinal phase extraction is warranted. It is commonly accepted that intestinal pH should be set at 7.0; however, opinion on the need for its inclusion varies by researcher. The length of time over which soil samples should be subjected to the intestinal phase also varies.

Another variable that has received much attention is the fluid to soil mass ratio that should be used in the experiments. Ruby *et. al.* (1996) settled on a 100:1 fluid to soil

ratio. This ratio was not based solely on physiology, rather on the fact that leaching experiments conducting using 5:1 or 25:1 ratios were shown to adversely affect dissolution of metals in extraction procedures, most likely due to diffusion-limited dissolution kenetics.

Grain size is also an important issue in the conduct of *in vitro* or *in vivo* experiments (Ruby *et. al.*, 1999). The majority of researchers, including Ruby, have used a grain size fraction of <250 μm. There is debate as to exactly what grain size fraction adheres to children's hands and thus can be transferred to the mouth. However, it has become common practice that <250 μm should be used. Some researchers have used <100 μm.

One issue of general consensus is the duration of gastric extraction. Most researchers have accepted that one hour exposure duration should be used in this phase of the extraction, as it is unlikely that soil would be retained in the stomach for greater periods of time.

The remaining issues surrounding bioavailability experiments revolve around the addition of stomach and intestinal enzymes, other additives such as bile in the experiments, addition of food material, and the filtering and analysis of extracts.

In 2002 the Bioavailability Research Group Europe (BARGE) published a paper summarizing the results of a multi-laboratory comparison study of five *in vitro* bioaccessibility models (Oomen *et. al.*, 2002) used to evaluate the bioaccessibility of lead, arsenic and cadmium, using three different soils. BARGE continues to be an active group in the determination of bioaccessibility of metals from soils and has extended its invited membership to researchers in North America. They are currently working on a standardized method for determining bioaccessibility of metals from soils in Europe.

Although experimentation has been carried out, including validation against animal models, the field of bioaccessibility and bioavailability is still an emerging science. To date most of the work has revolved around lead and arsenic, with limited work conducted on cadmium.

Given the varied number of bioavailability and bioaccessibility tests being undertaken by various groups, the United States National Research Council recently published a critical review of the emerging science of bioavailability of contaminants in soil and sediment. This review entitled *Bioavailability of Contaminants in Soil and Sediment: Processes, Tools, and Applications* (NRC, 2003) concluded that although advances have been made in this field, there is still much scientific progress that needs to be made in refining bioavailability tools and understanding their use in human health and ecological risk assessment.

2.3 Physical-chemical Soil Parameters Influencing Bioavailability

Research has shown that there are several key physical and chemical soil properties that could govern the potential bioavailability or bioaccessibility of inorganic elements from soil. The following are those that have been often referred to as being important indicators of bioavailability:

1. soil grain size
2. chemical fractionation
3. chemical speciation
4. organic carbon content

Inorganic elements are bound in soil matrices in varying degrees of solubility. The manner in which elements are bound in soil is governed by the parent material that forms the basis of contamination. For example, naturally occurring arsenic that is bound in a rock material as the parent mineral arsenopyrite (FeAsS) is insoluble under most environmental conditions and takes geochemical weathering over a geological timeframe to become soluble (Sadiq, 1997). Soluble arsenic trioxide that may be spread over a contaminated site due to smelter or ore roaster stack emissions is relatively soluble and mobile in the environment (Cullen and Reimer, 1989). An understanding of the chemical fractionation of how arsenic is bound in soil is necessary for understanding its relative bioavailability (Ollson, 2003).

The organic carbon content of the soil and the fraction of arsenic that is bound to organic material may also play a significant role in its bioavailability. Organic carbon in soil can be dissolved in relatively weak acid conditions (Henderson *et. al.*, 1998) and thus arsenic bound to the organic material may be more readily liberated into gastric fluid, hence being potentially more bioavailable for uptake into the human bloodstream.

Much of the literature surrounding bioavailability of inorganic elements suggests that grain size may play a significant role in its liberation in the gastrointestinal tract. Theoretically, the smaller the grain size, the greater the surface-sorbed metal (on a μg /g soil basis) would be available for dissolution from the soil matrix (Ruby *et. al.*, 1999). However, this relationship may not be true in all cases.

Physiochemical properties of soil will influence bioaccessibility in two important ways. First, physicochemical properties will influence how inorganic elements are bound in the soil matrix being evaluated. Secondly, the laboratory preparation method used to prepare soil samples becomes important, since varying methods will solubilize, release or otherwise ‘unbind’ inorganics from the soil matrix at different rates or to different degrees depending on how those methods interact with soil properties. The manner in which these parameters have been examined or taken into account during experimentation is discussed in this review.

2.4 Canadian Bioavailability Research and Use in Risk Assessment

There are only a limited number of laboratories in Canada that are conducting bioavailability research and even fewer that conduct site specific soil bioaccessibility tests. Canadian sites where site specific oral bioavailability and/or bioaccessibility have been determined and used in risk assessment include; Yellowknife, NT (Ollson, 2003), Port Colborne, ON (OMOE, 2002), and manned lightstation operated by the Department of Fisheries and Oceans (Dodd, 2002). In addition, work is being initiated on the oral bioaccessibility of metal contaminants from soils for a risk assessment being undertaken in Sudbury, ON. The results of arsenic *in vitro* bioaccessibility testing and incorporation into risk assessment are expanded on further in this report.

Researchers and research groups known to be currently undertaking bioavailability or bioaccessibility research in Canada include, but are not limited to (in alphabetical order):

Dr. Matt Dodd - Royal Roads University
Investigating use of physiological based extraction protocols to estimate risks associated with metal contaminated soils and stabilization studies.

Dr. Beverly Hale – University of Guelph
Conducting bioavailability and bioaccessibility research on several metals from soils and foods and lead investigator on Metals in the Human Environment Research Network (MITHE-RN).

Jennifer Kirk – Golder Associates Ltd.
Conducting fee for service bioavailability testing on contaminated soils.

Dr. Guy Mercier – Institut National de la Recherche Scientifique-Eau, Terre et Environment
Developing *in vitro* gastric juice simulation tests for metals in soils.

Dr. Margo Moore – Simon Fraser University
Studying bioavailability of PAHs in the intestinal lumen using a comparison of a cell-free model with Caco-2 cells

Dr. Pat Rasmussen – Health Canada
Conducting research on the bioavailability of metals from indoor dust.

Dr. Steven Siciliano – University of Saskatchewan
Conducting research on the oral bioaccessibility of PAHs from soils and metals from foodstuffs.

Dr. Kenneth Reimer, Dr. Iris Koch, Dr. Christopher Ollson – Royal Military College of Canada
Investigation of oral bioavailability/bioaccessibility and speciation of metal contaminants in soils and foods for incorporation into human and ecological risk assessment.

The aforementioned list of researchers is not meant to be exhaustive; in addition, there are several environmental consulting firms, government organizations and companies exploring the use of bioavailability and its application in contaminated sites risk assessment. A meeting of Canadian's conducting bioavailability research took place in Toronto in August 2005. The result of this meeting was the formation of "*Bioavailability Research Canada (BARC)*", which includes members from academia, government and consultants.

3.0 BIOAVAILABILITY RESEARCH OF ARSENIC IN CONTAMINATED SOIL

The toxicity of environmental contaminants is a function of the route of exposure, dose administered, the concentration that reaches the target organ(s), and in the case of arsenic, its chemical species (form) (Casarett and Doulls, 1991). Arsenic is found in several chemical forms in the environment, which differ markedly in their bioavailability and toxicity (Cullen and Reimer, 1989). For example, arsenobetaine (dominant species of arsenic in shellfish) can be ingested in high doses by humans and although it is absorbed into the bloodstream, it is excreted in the urine unchanged and causes no reported adverse health effects (Vahter, 1994).

However, there is a well documented increased risk of developing an assortment of non-cancerous and cancerous diseases in populations that chronically ingest inorganic arsenic, both in the inorganic arsenite (As(III) and arsenate (As(V)) chemical forms, in their drinking water at concentrations greater than 100 ppb (Smith, 1998). Soluble inorganic arsenic in water is almost 100% absorbed into the bloodstream and is very toxic to humans (US EPA, 1988).

Relatively little is known about the absorbed fraction of arsenic from contaminated soils. Over the past decade several *in vivo* studies have been published involving the relative oral bioavailability of arsenic from contaminated soils. This work involved dosing dogs, monkeys, swine, rabbits, and rats with arsenic.

The following is a summary of the key findings of these studies, which are documented in the accompanying Bioavailability Database.

3.1 Summary of *In vivo* Oral Bioavailability Research of Arsenic Contaminated Soil

Thirteen *in vivo* studies involving the oral administration of arsenic-bearing soil to laboratory animals were uncovered in the literature review. Five different animal models were employed in the studies – rats (3), rabbits (3), dog (1), swine (4), and monkey (2). The premise behind the laboratory animal studies is that the model selected would be representative of the bioavailability of arsenic from soil that would be found in humans. There is much discussion in the literature about the appropriateness of each test species. The fact that there have been several test species used will lead to some variability in the reporting of arsenic bioavailability from soils.

The results of the bioavailability of arsenic from soils is tabulated in Table 3-1. During review of the results of bioavailability experiments it was clear that many of the researchers did not report the physiochemical properties of the soils that are important determinants of arsenic bioavailability – pH, TOC, grainsize, etc. Therefore, for

comparative purposes, only the overall bioavailability of arsenic from soil could be examined.

Regardless of the test species employed, it was consistently found that that arsenic-bearing soil when fed by oral gavage (in all cases) had an absolute oral bioavailability (ABA) less than 100%. As previously stated in Section 2.2.1, the ABA of arsenic from soil is also equivalent to its relative bioavailability (RBA) to be used in human health risk assessment, as the TRV dose is based on arsenic in water which is assumed to have an ABA of 100%. Although never directly measured in the exposed species (humans), soluble As in water near neutral pH would also be 100% soluble at gastric pH, leading to an assumed bioavailiability of As of 100% in the key epidemiological study on which the cancer oral slope factor is based.

There are two common approaches that were selected for determination of the bioavailable fraction of arsenic from soil. The first plotted the blood concentration against time and the area under the curve (AUC) was determined. This involves the collection of blood, typically up to 72 hours after dose administration, and then a calculation of the AUC to establish the oral bioavailability of arsenic from soil. The second method involved a mass balance approach whereby the total dose of arsenic administered was compared to arsenic recovered through collection of faeces, urine, blood and organ tissues. That arsenic that could not be accounted for, or was measured in faeces, was assumed to have been the portion that was not bioavailable. In humans exposed to arsenite (one soluble form in contaminated drinking water, for example) faecal excretion is <5% of ingested dose (see review in ATSDR, 2000). One limiting factor in the majority of animal studies was that they did not report the age of test species.

Knowledge of the chemical form or species of arsenic is critical in understanding its potential toxicity. However, there was very little, and in most cases, no information reported on the chemical species of arsenic that would have been expected in the soil samples used in oral bioavailability testing. It is speculated that the majority of the arsenic in soil samples used in bioavailability tests would have been arsenate (As(V)), as it is the most thermodynamically stable arsenic form in oxygenated soil environments (Cullen and Reimer, 1989).

Table 3-1. Summary of Arsenic Bioavailability (*in vivo*) Studies and Results

Lead Author	Database ID	Year	Test Species	Study / Site	Test Soil ID	Soil Type	Soil Arsenic [ppm]	Soil pH	Soil TOC %	Soil Grainsize µm	% Bioavailable Arsenic
Griffin	85	1991	Rabbits	Tacoma							
Davis	74	1992	New Zealand White rabbits	Butte, Montana	Soil I	composite of 5 mine soils	1380			<250	11
Freeman	73	1993	New Zealand White Rabbits	Anaconda, Montana	ARS-I	residential soil	3900	6.6	7.4	<250	48
Groen	72	1994	Female Beagle - Dogs	Doetinchem, Netherlands	Soil 80 cm	bog-ore	339				8.3 ± 2
Freeman	29	1995	Cynomolgus Monkeys	Anaconda, Montana	ARS-II	residential soil	410	7.8	12	25	13.8
					AHD-I	house dust	170	7.6	42	31	19.2
Lorenzana	78	1996	swine	Residential, near Asarco smelter, Tacoma, Washington	smelter site soil	residential soil	1600				52
					smelter site soil	smelter slag	10100				28
Casteel	86	1997	swine	Aspen		soil (berm)	66.9				62 ± 55
				Aspen		soil (residential)	16.7				98 ± 86
				Bingham Creek		tailings (channel)	149				37 ± 19
				Butte		soil	239				10 ± 5
				Leadville		soil (residential)	203				8 ± 9
				Leadville		soil (Fe-Mn lead oxide	110				28 ± 15
				Leadville		slag (AV)	1050				15 ± 1
				Midvale		slag	591				18 ± 4
				Murray Smelter		slag	695				51 ± 9
				Murray Smelter		soil	310				34 ± 3
				Palmerton		soil (location 2)	110				39 ± 9
				Palmerton		soil (location 4)	134				52 ± 15
				Clark Fork		tailings (GK)	181				49 ± 5

Table 3-1. Summary of Arsenic Bioavailability (*in vivo*) Studies and Results

Lead Author	Database ID	Year	Test Species	Study / Site	Test Soil ID	Soil Type	Soil Arsenic [ppm]	Soil pH	Soil TOC %	Soil Grainsize µm	% Bioavailable Arsenic
Ng	30	1998	male Wistar rats	Canberra, Australia	C1	residential	55			<50	2.31
					C2	residential	32				1.27
					C3	residential	165				2.68
					C4	residential	295				1.44
					C5	residential	67				9.58
					C6	community park	121				2.46
					C7	residential	1597				0.55
					C8	residential	867				0.59
					C9	residential	1325				0.67
					C10	rock	435				1.02
Rodriguez	31	1999	immature swine	Western US - Mine/smelting site	1	calcine	11300	2.6	0.36	<250	2.7
					2	calcine	17500	2.6	0.22	<251	3.3
					3	calcine	13500	3.1	0.58	<252	8.3
					4	calcine	11500	3.1	0.41	<253	22.1
					5	calcine	6250	5.7	0.61	<254	30.1
					6	slag	405	7.4	0.89	<255	bdl
					7	slag	450	7.7	3.13	<256	bdl
					8	slag	1180	7.1	1.58	<257	28.7
					9	slag	5020	7.4	3.38	<258	30.1
					10	slag	4650	7.4	3.22	<259	16.4
					11	soil	331	3.9	0.81	<260	6.2
					12	soil	233	4.6	1.52	<261	42.8
					13	soil	799	7.5	2.28	<262	29.1
					14	soil	1460	7.3	0.23	<263	18.7
					15	soil	401	7.6	4.58	<264	36.5
Lioy	84	1999	Sprague-Dawley rats	NIST 2710	1 Day	SRM	627		3		34
					2 Day	SRM	628		3		37.5

Table 3-1. Summary of Arsenic Bioavailability (*in vivo*) Studies and Results

Lead Author	Database ID	Year	Test Species	Study / Site	Test Soil ID	Soil Type	Soil Arsenic [ppm]	Soil pH	Soil TOC %	Soil Grainsize μm	% Bioavailable Arsenic
					3 Day	SRM	629		3		39.8
					4 Day	SRM	630		3		34.8
Casteel	87	2001	swine	Still waiting	six samples	residential					18-45%
Roberts	54	2001	Cebus apella - monkey	Florida	Electrical substation		312			<250	14.6 ± 5.1
					Cattle dip site		189			<250	24.7 ± 3.2
					Pesticide site #1		743			<250	10.7 ± 4.9
					Woodtreatment site		101			<250	16.3 ± 6.5
					Pesticide site #2		329			<250	17 ± 10
Elliskson	60	2001	see Lioy								
Ng	83	2003	male Wistar rats	Australia - Arsenic from Cattle Dips	average of 16 soils		1136				27.5 ± 13.2

3.1.1 Summary Statistics of *In Vivo* Oral Bioavailability of Arsenic from Soil

A total of 55 soil samples were subjected to bioavailability experiments on an average soil concentration of arsenic of 1950 ± 3760 ppm. The average percent bioavailability of arsenic was 23 ± 20%. Therefore, on average less than 25 % of arsenic subjected to bioavailability studies would have been available for uptake into the bloodstream. This represents the absorbed dose of arsenic in terms of the exposure dose that the animals would have received. Thus, in conducting a human health risk assessment one might assume that arsenic bioavailability would have been 1/4 of that of the traditional approach, assuming 100 % bioavailability in the study upon which the CSF was based, and assuming that the arithmetic average for all bioavalability studies combined was appropriate to apply to a risk assessment.

As with any experimentation there was a range on the average concentration of bioavailable arsenic recorded. Arsenic bioavailability was shown to be less than 5 % in 13 of the 55 soils tested. However, there were four soils where the bioavailable fraction of arsenic from soils was greater than 50%, and ranged as high as 98%. Given the variability in the measured arsenic bioavailability in test species, it suggests that it may not be appropriate to apportion a standard fraction of bioavailability from the literature to individual contaminated sites.

3.1.2 Soil Properties Governing Oral Bioavailability of Arsenic from Soil

Unfortunately, the majority of the *in vivo* bioavailability studies did not report the physiochemical properties of the soils tested, other than the total arsenic concentration. An inspection of the written description of soils tested indicates that they range from likely low organic carbon content (mine soil / slag) to relatively high organic carbon content (residential soils).

An examination of the influence of the total arsenic content in soil and its resulting bioavailability was conducted using a linear regression of the data. Both the total arsenic concentrations in soil and the percent bioavailable arsenic data sets were log transformed to provide normalized datasets.

The linear regression indicates that there is no correlation between the total arsenic concentration in the soil samples and the percent of arsenic that was bioavailable (Figure 3-1). The slope of the regression line was not found to be significant ($p>0.05$) and the very low r^2 value of 0.0029 reveals that arsenic bioavailability cannot be predicted from the total arsenic concentration in the soil sample.

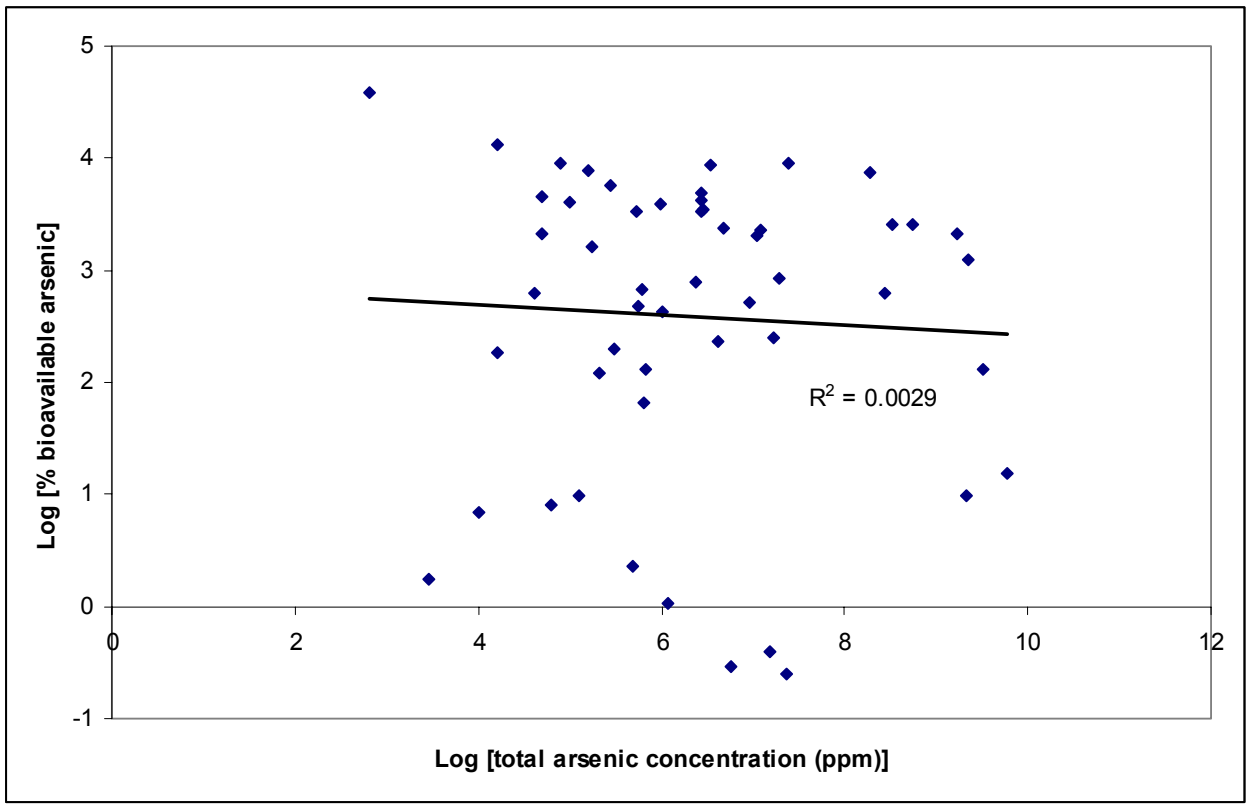


Figure 3-1. Linear regression of arsenic bioavailability against total arsenic concentration in soil sample (ppm). An r^2=0.0029 (p>0.05) indicates that the % of arsenic that was shown to be bioavailable is independent of the total arsenic concentration in the soil sample.

Only a limited number of samples (n=16) published information on the soil percent total organic carbon content. TOC (%) data from the four studies (Freeman 1993, Freeman, 1995 and Rodriguez, 1999) was log transformed to provide a normal distribution of data. The linear regression of % bioavailable arsenic and % TOC indicates that there is reasonable correlation between the two variables (r^2=0.32), thus suggesting that there was a relationship between the sample organic carbon content and the percent of arsenic from the soils that was bioavailable (Figure 3-2).

In general as the percent TOC increases in the soil samples there was a corresponding increase in the percent of arsenic that was determined to be bioavailable.

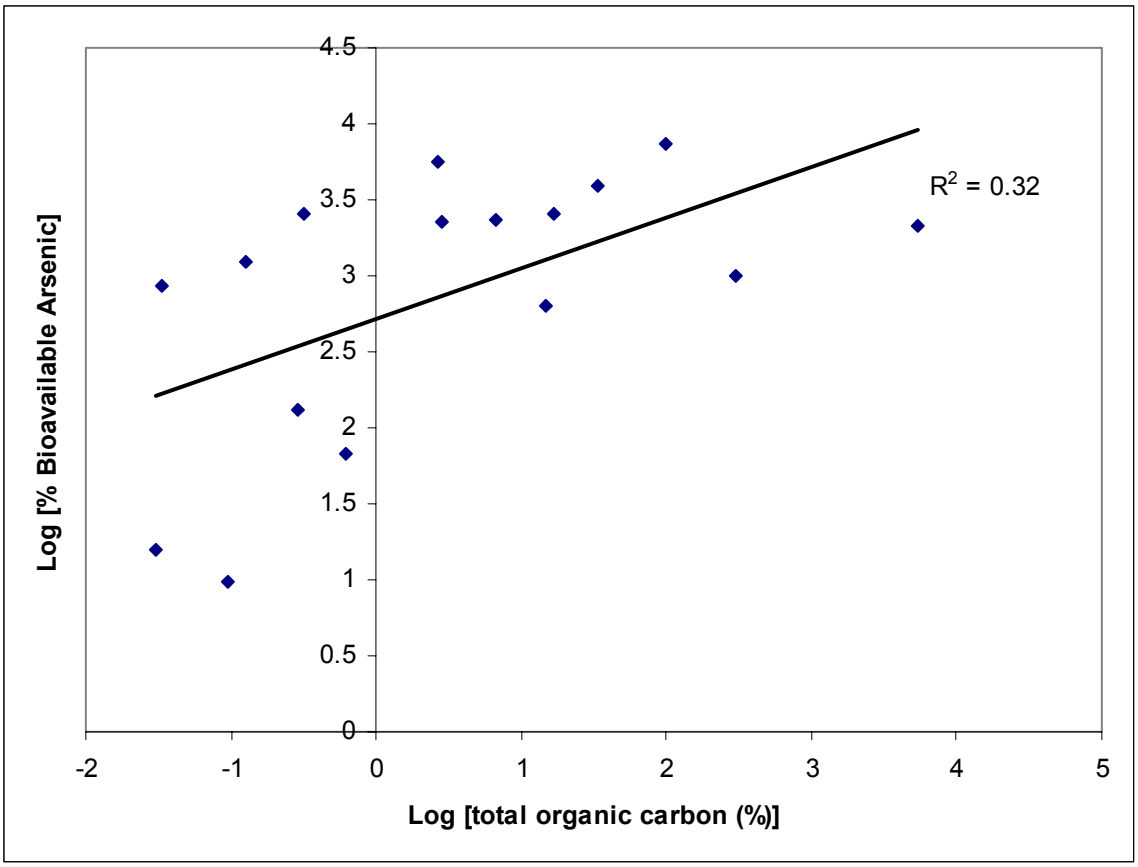


Figure 3-2 Linear regression of arsenic bioavailability against organic carbon content. An r^2=0.36 ($p<0.05$) indicates that the % of arsenic that was shown to be bioavailable in these studies was at least partially dependent on the organic carbon content of the soils.

A stepwise multiple regression (generalized linear model) was run on the sixteen sample points that reported TOC, total arsenic concentration and the percent bioavailability of arsenic after oral administration of arsenic impacted soil. The multiple regression indicates that the total arsenic concentration in the soil sample had no significant impact on the percent bioavailable, while the percent bioavailable arsenic was significantly linked to the TOC of the soil samples (Table 3-2).

Table 3-2. Multiple regression results that indicate TOC has a significant influence on bioavailability of arsenic from soil samples.

Effect	Coefficient	Std Error	Std Coef	Tol	t	P (2 tail)
Constant	2.717	0.198	<0.0001		13.716	<0.0001
Log TOC	0.334	0.130	0.566	1	2.570	0.022
Source	**Sum of squares**	**df**	**Mean square**	**F-ratio**	**P**	
Regression	3.738	1	3.738	6.607	0.022	
Residual	7.920	14	0.566			

3.1.3 Effect of Selected Test Species on Arsenic Bioavailability

When multiple animal test species have been used in generating bioavailability values for inorganic contaminants from soils there are always questions about the most appropriate animal model to select.

In the case where oral toxicity reference values for use in human health risk assessment have been derived from a specific test species, then it would be most appropriate to use that test species in the development of oral bioavailability of contaminants from soil. For example, the United States Environmental Protection Agency (US EPA) oral reference dose (RfD) used in the toxicity assessment of oral dose of nickel from soils of 20 μg/kg-d, was derived from the no observed adverse effect level (NOAEL) from a feeding study of rats with nickel dosed food (US EPA, 1987). The RfD is used as an administered dose and thus the oral bioavailability is accounted for in the administered dose. Therefore, subsequent bioavailability studies of nickel from soil should employ a similar rat model so that administered doses could be compared.

However, the arsenic cancer slope factor (CSF), used by both Health Canada (Health Canada, 2004b) and the US EPA (1988), was derived from the oral ingestion of arsenic contaminated drinking water by a human population. Therefore, bioavailability studies of arsenic from soil should mimic the oral bioavailability of the human gastrointestinal tract for cancer risk assessments.

The test species that have been used to develop oral bioavailability of arsenic from soils were rats, rabbits, dogs, swine, and monkeys. Therefore, it is important to understand the inter-species variability in the arsenic bioavailability reported for each of these test species. Ideally the total organic carbon content of arsenic from soils would have been reported for each experiment. This was not the case. However, an examination of the relative oral bioavailabilities can be conducted given the range of different soil types that were examine by test species.

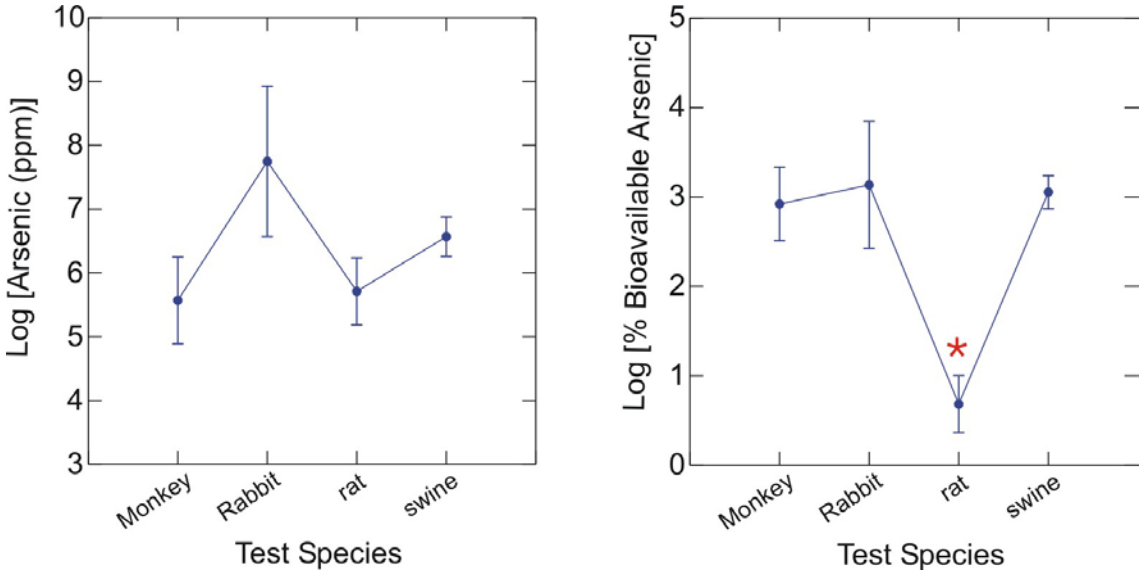
The total arsenic concentration in soil (ppm) and the corresponding percent fraction of arsenic that was reported as being bioavailable were log transformed to normalize the datasets. The data was then categorized by test species and analysis of variance (ANOVA) statistical tests were performed with resulting matrix of pairwise probability comparisons being reported in Figure 3-3.

There was no statistical difference ($p>0.697$) between the average arsenic soil concentrations used for the four test species experiments (Figure 3-3a). There was also no statistical difference in the percent oral bioavailability of arsenic between the monkey, rabbit and swine test species. However, the arsenic oral bioavailability studies conducted using rats had oral bioavailabilities that were significantly lower ($p<0.05$) than the other three test species (Figure 3-3b).

Of the three studies involving rats, it is the Ng *et. al.* (1998) study of Canberra Australia residential soils that skews the bioavailability results to being lower than those for the

other three species of animals tested. It was reported that the soils were residential soils, likely topsoil, collected from around Canberra. Interestingly, despite the expected higher TOC content anticipated for topsoil, less than 10% of the soil arsenic was found to be bioavailable.

Although not conclusive evidence to rule out the use of rat studies for conducting *in vivo* arsenic bioavailability experiments, it does suggest that they may not be the best model and that one of the other test species may be more appropriate for use, as they provide a more conservative estimate of oral bioavailability.



Matrix of pairwise comparison probabilities

	Monkey	Rabbit	Rat	Swine
Monkey	1.000			
Rabbit	0.697	1.000		
Rat	1.000	0.728	1.000	
Swine	1.000	1.000	1.000	1.000

	Monkey	Rabbit	Rat	Swine
Monkey	1.000			
Rabbit	1.000	1.000		
Rat	0.001	0.018	1.000	
Swine	1.000	1.000	0.000	1.000

Figure 3-3 ANOVA of total arsenic concentration and the % bioavailable arsenic across test species. There was no statistical difference in arsenic concentrations in soils, while rats had significantly less bioavailable arsenic than other test species (denoted by *, p<0.05).

3.1.4 Summary of *In Vivo* Arsenic Oral Bioavailability Experiments

It is apparent from a review of the oral bioavailability database that regardless of soil type or area of the world it was collected, it is unlikely that the oral bioavailability of arsenic in humans exposed to contaminated soils would be 100%.

3.2 *In Vitro* Oral Bioaccessibility Research For Arsenic in Soil

Seventeen *in vitro* studies were uncovered in the literature review that report chemical extraction tests meant to mimic the oral bioaccessibility of arsenic in the human GI tract. Nine different extraction methods were employed by researchers, however, the majority are essentially a derivative of the PBET method.

Table 3-3 shows the results of bioaccessibility experiments that have been published either in the scientific literature or in government documents. The basic components of the varying methodology used for *in vitro* tests are provided in Table 3-4. Similar to the *in vivo* experiments, many of the researchers did not report the physiochemical properties of the soils that are potentially important determinants of arsenic bioaccessibility – pH, TOC, grainsize, etc. Therefore, for comparative purposes, only overall bioaccessibility of arsenic from soil could be examined.

The majority of researchers did clearly document the laboratory methodology used in the bioaccessibility experiments. Therefore, the effects of liquid to solid ratio, gastric pH, effect of gastric and intestinal pH, and the total arsenic concentration in soil samples was examined. Regardless of the experimental method employed, it was consistently reported that the absolute bioaccessibility of arsenic from soils was less than 100%.

Similar to the *in vivo* laboratory animal experiments, there was little to no information provided on the chemical speciation of arsenic in the soil samples or the resulting extracts.

Table 3-3 Summary of Arsenic Bioaccessibility (*in vitro*) Studies and Results

Author	Database ID	Year	Study / Site	Test Soil ID	Soil Type	Soil arsenic [ppm]	Soil pH	Soil TOC (%)	Soil grainsize	*In Vitro* Test Method	Liquid: Solid Ratio (mL:g)	pH stomach	pH Intestine	% Bioaccessible Arsenic		
														Stomach	Intestine	overall
Ruby	58	1996	Anaconda, Montana	ARS-I	residential	3900	6.6	7.4	19	PBET	100:1	1.3	7.0	55	50	50
				ARS-I	residential	3900	6.6	7.4	19	PBET	100:1	2.5	7.0	40	30	32
				ARS-II	residential	410	7.8	12	25		100:1	1.3	7.0	49	44	44
				ARS-II	residential	410	7.8	12	25		100:1	2.5	7.0	45	32	31
				AHD_I	house dust	170	7.6	42	31		100:1	2.5	7.0	34	32	34
Hamel	64	1998	Jersey City	Jersey City	chromate ore processing	1120			<250	US Pharm	100:1		NA	4.5 ± 0.8		
			Jersey City	Jersey City	chromate ore processing	1120			<250	US Pharm	200:1		NA	6.0 ± 0.9		
			Jersey City	Jersey City	chromate ore processing	1120			<250	US Pharm	500:1		NA	7.7 ± 0.9		
			Jersey City	Jersey City	chromate ore processing	1120			<250	US Pharm	1000:1		NA	13 ± 4		
			Jersey City	Jersey City	chromate ore processing	1120			<250	US Pharm	2000:1		NA	18 ± 8		
			Jersey City	Jersey City	chromate ore processing	1120			<250	US Pharm	5000:1		NA	25 ± 9		
Hamel	64	1998	NIST Standard	NIST 2710	Reference Material	626		3	<74	US Pharm	100:1			41 ± 18		
			NIST Standard	NIST 2710	Reference Material	626		3	<74	US Pharm	200:1			56 ± 21		
			NIST Standard	NIST 2710	Reference Material	626		3	<74	US Pharm	500:1			44 ± 6		
			NIST Standard	NIST 2710	Reference Material	626		3	<74	US Pharm	1000:1			48 ± 3		
			NIST Standard	NIST 2710	Reference Material	626		3	<74	US Pharm	2000:1			46 ± 5		
			NIST Standard	NIST 2710	Reference Material	626		3	<74	US Pharm	5000:1			48 ± 4		
Williams	48	1998	Ron Phibun, Thailand	RP6		2123			<150	PBET	100:1	2.5	7.0	12.50	14.40	
				RP11		1406			<150	PBET	100:1	2.5	7.0	10.00	20.60	
				MW		20000			<150	PBET	100:1	2.5	7.0	10.90	27.90	
				calcine	calcine	12010	3.42	0.436	<250	IVG	150:1	1.8	5.5	3.66	3.52	
				calcine	calcine	12010	3.42	0.436	<250	PBET	100:1	2.0	7.0	1.44	1.47	
				slag	slag	4428	7.367	3.1089	<250	IVG	150:1	1.8	5.5	24.80	22.70	
				slag	slag	4428.889	7.367	3.1089	<250	PBET	100:1	2.0	7.0	13.90	12.00	
				slag/soil	slag and soil samples	2063.833	6.625	2.3433	<250	IVG	150:1	1.8	5.5	24.80	21.90	
				slag/soil	slag and soil samples	2063.833	6.625	2.3433	<250	PBET	100:1	2.0	7.0	18.30	12.50	
Hamel	40	1999	NIST	NIST 2710	SRM	626	4.5		<74	artificial biofluid	2000:1					41 ± 2
			residential soil	residential soil	organic soil	163	6		<125	artificial biofluid	2000:1					66 ± 8
Ellickson	60	2001	NIST	NIST 2710	Reference Material	626	4.5		<74	artificial biofluid	2000:1					65.9 ± 5.2
Oomen	59	2002	various	Flanders		72	6	3.3	<200	SBET	100:1	1.5	NA			50 ± 0.2
						77	6	3.3	<200	DIN	50:1	2.0	7.5			44 ± 3
						77	6	3.3	<200	DIN-WM	50:1	2.0	7.5			30 ± 1
						55	6	3.3	<200	RIVM	225:1	1.1	7.8			95 ± 10
						82	6	3.3	<200	SHIME	2.5:1	4.0	6.5			6 ± 0.5
						74	6	3.3	<200	TIM	25:1	varying	7.2			52 ± 1

Table 3-3 Summary of Arsenic Bioaccessibility (*in vitro*) Studies and Results

Author	Database ID	Year	Study / Site	Test Soil ID	Soil Type	Soil arsenic [ppm]	Soil pH	Soil TOC (%)	Soil grainsize	*In Vitro* Test Method	Liquid: Solid Ratio (mL:g)	pH stomach	pH Intestine	% Bioaccessible Arsenic		
														Stomach	Intestine	overall
Oomen	59	2002	various	Oker 11		213	5.9	5.1	<1000	SBET	100:1	1.5	NA			11 ± 2
						235				DIN	50:1	2.0	7.5			18 ± 3
						235				DIN-WM	50:1	2.0	7.5			11 ± 1
						206				RIVM	225:1	1.1	7.8			19 ± 1
						227				SHIME	2.5:1	4.0	6.5			1 ± 0.02
						236				TIM	25:1	varying	7.2			15 ± 3
Oomen	59	2002	various	Montana 2711	Reference Material	81		2	<74	SBET	100:1	1.5	NA			59 ± 2
						95		2	<74	DIN	50:1	2.0	7.5			50 ± 1
						95		2	<74	DIN-WM	50:1	2.0	7.5			41 ± 2
						88		2	<74	RIVM	225:1	1.1	7.8			59 ± 1
						105		2	<74	SHIME	2.5:1	4.0	6.5			10 ± 0.4
						103		2	<74	TIM	25:1	varying	7.2			50 ± 1
Palumbo	94	2002	Metal Mines Wales, UK	WLS1		21.15	3.45	19.18	<250	SBET	100:1	1.5	na			24.00
				WLS2		22.75	4.84	17.11	<250	SBET	100:1	1.5	na			11.00
				WLS3		90.12	5.61	3.47	<250	SBET	100:1	1.5	na			19.00
				WLS4		23.48	3.59	2.21	<250	SBET	100:1	1.5	na			5.00
				WLS6		12.5	2.83	2.02	<250	SBET	100:1	1.5	na			12.00
				WLS7		16.09	4.48	2.02	<250	SBET	100:1	1.5	na			16.00
				WLS8		8.47	3.98	0.68	<250	SBET	100:1	1.5	na			16.00
				WLS9		20.42	3.98	0.84	<250	SBET	100:1	1.5	na			7.00
Cave	93	2002		all sites						SBET	100:1	2.5	7.0			13.80
				Bere Alston							100:1	2.5	7.0			10.60
				DGC							100:1	2.5	7.0			21.70
				Drakewalls							100:1	2.5	7.0			15.50
				Wellingborough							100:1	2.5	7.0			8.94
Dodd		2002	B.C. Lightstation	Lightstation soils	53 topsoil samples	30 ± 17			<250	PBET	100:1	1.5		30 ± 17		30 ± 17
Ontario MOE	63	2002	Port Colborne, On	1	ground soil	31.8					100:1	1.8	7.0	27.00	26.00	
				2		50.1					100:1	1.8	7.0	43.00	46.00	
				3		30.5					100:1	1.8	7.0	29.00	30.00	
				4		20.4					100:1	1.8	7.0	22.00	35.00	
				5		49					100:1	1.8	7.0	27.00	22.00	
				6		29.2					100:1	1.8	7.0	19.00	17.00	
				7		35.3					100:1	1.8	7.0	28.00	19.00	
				8		30.3					100:1	1.8	7.0	31.00	17.00	
				9		37.2					100:1	1.8	7.0	51.00	36.00	
				10		34.4					100:1	1.8	7.0	33.00	29.00	
													7.0			
				1	Sieved Soil	46					100:1	1.8	7.0	27.00	20.00	
				2		28.9					100:1	1.8	7.0	48.00	46.00	

Table 3-3 Summary of Arsenic Bioaccessibility (*in vitro*) Studies and Results

Author	Database ID	Year	Study / Site	Test Soil ID	Soil Type	Soil arsenic [ppm]	Soil pH	Soil TOC (%)	Soil grainsize	*In Vitro* Test Method	Liquid: Solid Ratio (mL:g)	pH stomach	pH Intestine	% Bioaccessible Arsenic		
														Stomach	Intestine	overall
				3		38.9					100:1	1.8	7.0	28.00	8.50	
				4		19.3					100:1	1.8	7.0	26.00	56.00	
				5		46.7					100:1	1.8	7.0	37.00	36.00	
				6		42.5					100:1	1.8	7.0	23.00	12.00	
				7		20.9					100:1	1.8	7.0	35.00	9.20	
				8		31.8					100:1	1.8	7.0	37.00	19.00	
				9		29.1					100:1	1.8	7.0	47.00	29.00	
				10		33.4					100:1	1.8	7.0	39.00	22.00	
Reimer	91	2003	Yellowknife Giant Townsite	1	organic topsoil	62			<80	GFE	100:1	1.8	7.0			73.10
				2	organic topsoil	52			<80	GFE	100:1	1.8	7.0			66.00
				3	organic topsoil	784			<80	GFE	100:1	1.8	7.0			37.90
				4	sand/organic	68			<80	GFE	100:1	1.8	7.0			31.40
				5	organic topsoil	68			<80	GFE	100:1	1.8	7.0			22.90
				6	sand	272			<80	GFE	100:1	1.8	7.0			18.70
				7	gravel/cobble	1345			<80	GFE	100:1	1.8	7.0			18.7
				8	gravel fill	1605			<80	GFE	100:1	1.8	7.0			11.2
				9	gravel fill	1997			<80	GFE	100:1	1.8	7.0			10.4
				10	gravel fill	2630			<80	GFE	100:1	1.8	7.0			10.4
				11	gravel fill	3705			<80	GFE	100:1	1.8	7.0			9.3
				12	clay	1760			<80	GFE	100:1	1.8	7.0			8.6
				13	gravel fill	2498			<80	GFE	100:1	1.80	7.0			6.8
				14	silt gravel	2650			<80	GFE	100:1	1.80	7.0			6.3
				15	sand	2897			<80	GFE	100:1	1.80	7.0			5
Kientz	33	2003	Puerto Rico	B1	sandy loam	47	8	7.1	<250	US Pharm	1000:1	1.2				9
				B2	loamy sand	27	7.6	7.1	<250	US Pharm	1000:1	1.2				4.5
				B3	sandy loam	38	7.9	9.3	<250	US Pharm	1000:1	1.2				7
Juhasz	80	2003	South Australia Railway	18 soils	not stated					PBET						6 to 43
										IVG						5 to 38
Ollson	88	2003	Yellowknife	rock	rock	3180		0.88		GFE	100:1	1.8	7			5
				tailings	tailings	4580		1.6		GFE	100:1	1.8	7			2.9
				mine organic	mine organic	850		38		GFE	100:1	1.8	7			20
				residential	residential	142		47		GFE	100:1	1.8	7			31

Table 3-4. *In Vitro* Test Method Parameters for Arsenic Bioaccessibility

	Specifics	PBET	SBET	DIN	RIVM	SHIME	TIM	IVG	Artificial Biofluid	GFE
Input	**Amount of Soil Added**	0.4 g dry weight	1.0 g dry weight	2.0 g dry weight	0.6 g dry weight	10 g dry weight	10 g dry weight	4 g dry weight	50 mg dry weight	0.2 g dry weight
General	**Model type**	Static gastro-intestinal	Static stomach	Static gastro-intestinal	Static gastro-intestinal	Static gastro-intestinal	Dynamic gastro-intestinal	Static gastro-intestinal		Static gastro-intestinal
	Temperature	37°C	37°C	37°C	37°C	37°C	37°C	37°C	37°C	37°C
	Mechanical treatment	Bubbling argon	End-over-end rotation 30 rpm	Agitator 200 rpm	End-over-end rotation 55 rpm	Mechanical stiring at 150 rpm	Peristaltic movements	Stirring 100 rpm		275 rpm on temp control shaker table
	Food components	No	No	Whole mil powder 50 g/L	No	Cream (18g/L) and Nutrilon plus (15 g/L) in stomach	No	200g dough	No	No
Oral Cavity	**Saliva**	No	No	No	Yes	No	Yes	No	Yes	No
	Volume of saliva				9.0 mL		50 mL		8 mL	
	pH				6.5		5.0		1.4	
	Incubation time				5 minutes		5 min		With gastric	
Stomach	**Gastric compartment**	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	Volume of gastric juice	40 mL	100 mL	100 mL	13.5 mL	25 mL	250 mL	600 mL	100 mL	20 mL
	pH	1.5	1.5	2.0	1.1	4.0	5.0 decreasing to 2.0	1.8	1.4	1.8
	Gastric secretion components	Pepsin, citrate, malate, lactic acid, acetic acid	No	Pepsin, mucin	Pepsin, mucin, BSA	Pectin, mucin, cellobiose, proteose peptone, starch	Lipase, pepsin	NaCl, pepsin	NaCl, pepsin	Pepsin, citrate, malate, acetic acid
	Incubation time	1 hour	1 hour	2 hours	2 hours	3 hours	1.5 hours	1 hour	2 hours	1 hour
Intestine	**Intestinal component**	Yes	No	Yes	Yes	Yes	Yes (3 sections)	Yes	Yes	Yes
	Volume of intestinal juice	40 mL unchanged		100 mL	36 mL	15 mL	3 x 70 mL	600 mL (unchanged)	100 mL	20 mL (unchanged)
	Intestinal pH	7		7.5	7.8	6.5	6.5 to 7.2	5.5	6.5	7.0
	Intestinal secretion components	Pancreatin, bile salts		Bile, porcine, chime, trypsin, pancreatine	Bile, duodenal juice, chime, pancreatin, lipase	Pancreatine	Bile, porcine, pancreatine	Pancreatin, bile extract		Bile extract, pancreatine
	Incubation time	3 hours		6 hours	2 hours	5 hours	6 hours	Not specifiied	2 hours	4 hours
Output	**Centrifugation**	Not specified	No	7000 g	3000g	7000 g	No	10 000 rpm	1000 rpm	3700 rpm
	Filtration	Not specified	0.45 μm cellulose acetate disk filter	No	No	No		0.45 μm filter	No	0.45 μm filter
	Special treatment		pH filtrate with 0.5 units of starting pH	Supernatant decanted			Hollow fiber membrane for filtration			
	Acid digestion of fluid	Not specified	Acid digested hotplate	Acid digested hotplate	Acid digestion microwave	Acid digestion microwave	Acid digestion hotplate	Acidified	No	Acid digestion hotplate
	Analytical method	ICP AES	ICP AES	AAS	ICP MS	ICP AES	HAAS	ICP-HG	ICP MS	AAS / ICP MS

3.2.1 Summary Statistics of *In Vitro* Oral Bioaccessibility of Arsenic from Soil

The data set compiled from individual studies on oral bioaccessibility of arsenic consists of either individual soil sample results or in some cases averages where researchers did not report individual sample results. This adds some complexity and uncertainty into statistical analysis of the data set. It was determined that the best approach would be to use data as reported by researchers to conduct statistical analysis. In addition, some researchers report data for both gastric and intestinal phases, while others publish an overall bioaccessibility. It was determined that the total bioaccessibility reported by the researchers would be used in reporting summary statistics in this assessment.

A total of 102 soil arsenic bioaccessibility results were reported on an average soil concentration of arsenic of 1204 $\pm$ 2775 ppm. The average percent of arsenic that was bioaccessible was 25.6 $\pm$ 19.0%. Therefore, on average less than 26 % of arsenic subjected to bioaccessibility studies would have been soluble and thus available for uptake into the bloodstream. Given that soluble arsenic is almost 100% absorbed into the bloodstream, the bioaccessibility results represent the relative proportion of As in soils that is available to be absorbed (i.e., the RBAF that might be used in risk assessment calculations). Thus, in conducting a human health risk assessment one might assume that arsenic bioavailability would have been 1/4 of that of the traditional approach, assuming 100 % bioavailability in the study upon which the CSF was based, and assuming that the arithmetic average for all bioaccessibility studies combined was appropriate to apply to a risk assessment.

The bioaccessible fraction of arsenic from soil ranged from <1% to as high as 95%. Given the variability in the measured arsenic bioaccessibility in *in vitro* tests, it suggests that it may not be appropriate to apportion a standard fraction of bioaccessibility from the literature to individual contaminated sites.

However, the 95th percentile of percent arsenic bioaccessibility was 59%, which suggests that a reasonable maximum oral bioavailability/bioaccessibility of arsenic from soils of, say, 60% might be appropriate for use in screening level risk assessments.

3.2.2 Effect of Liquid to Solid Ratio on Oral Bioaccessibility of Arsenic from Soil

One of the factors that has been shown, at least in some experiments, to affect the bioaccessibility of arsenic from soil samples is the difference in ratio of liquid to solid that have been used by the various research groups. The most commonly employed liquid to solid ratio is 100:1, based on the PBET method (n=68), with 12 analyses having been conducted using <100:1 and 22 conducted using >100:1.

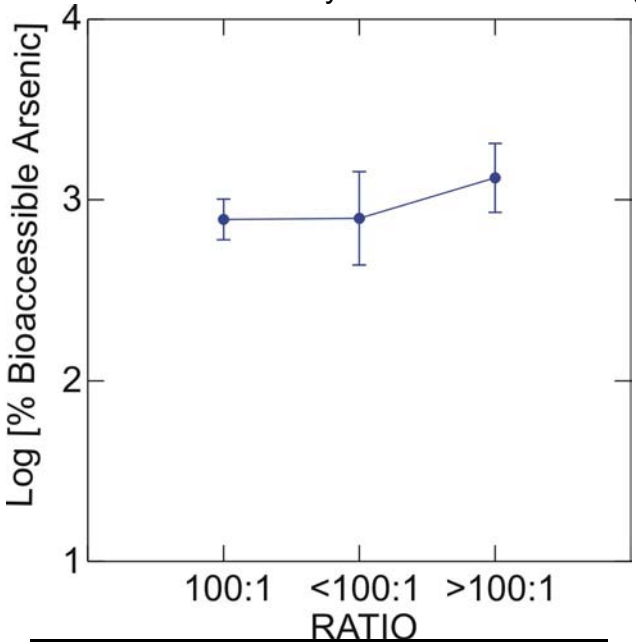
Hamel *et. al.* (1998) demonstrated that in the case of one soil sample, the higher the ratio used, there was an increase in arsenic bioaccessibility with a corresponding increase in the ratio of liquid to soil for PBET extraction. This has lead to additional

research in the area and Hamel et al. (1998) has suggested using a ratio of 1000:1 for bioaccessibility experimentation.

Building on the work of Hamel *et. al.* (1998), a series of fluid to solid ratios of 100:1 to 5000:1 were carried out by Ollson (2003) to ascertain the influence of this parameter on bioaccessibility of As in soil and tailings samples from Yellowknife, NT. This method was coined gastric fluid extraction (GFE). The GFE method built on both the PBET (Ruby, 1995) and IVG (Rodriguez, 1999) using a liquid to solid ratio of 100:1, gastric pH of 1.8 and intestinal pH of 7.0, with the largest divergence being that experiments were conducted using 50mL centrifuge tubes on a temperature controlled shaker table (37^{o}C) in order to increase the number of samples that could be run at one time.

Of the eight soil samples analyzed by Ollson at varying ratios (including a standard reference material (SRM)) there was no statistical difference in the bioaccessibility of arsenic from any of the soil samples for liquid to soil rations of 100:1 to 5000:1. Therefore, a liquid to solid ratio of 100:1 was carried forward in the remaining experiments.

To examine the relationship between liquid:soil ratio and bioaccessiblity across all published stuidies, the bioaccessibility results for arsenic from soil samples were categorized into 3 groups: <100 mL:1 g, 100 mL:1 g, and >100 mL:1g. The data was log transformed to provide for normalcy and an ANOVA was run to examine differences amongst the varying ratios. Figure 3-4 illustrates that there was no statistical difference between the percent bioaccessible arsenic yielded using varying ratios, although Figure 3-4 is suggestive of increased bioaccessibility at ratios >100 mL:1 g.



	100:1	<100:1	>100:1
100:1	1.000		
<100:1	1.000	1.000	
>100:1	0.903	1.000	1.000

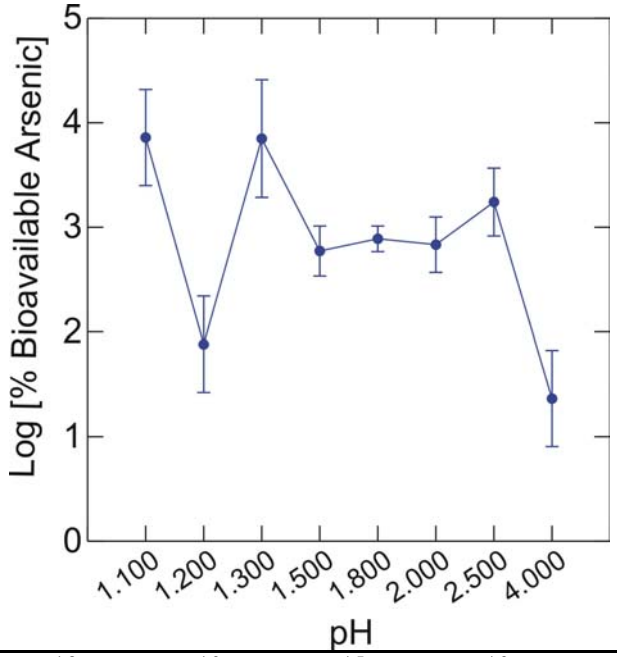
Figure 3-4 Effect of liquid to solid ratios on arsenic bioaccessibility from soil. ANOVA results indicated that there was no statistical difference (p>0.05) across the varying ratios.

However, data limitations, including potential influences of unmeasured confounders between studies, precludes any clear or unambiguous interpretation of these data. The discrepancies between the results from Hamel et al. (1998) and Ollson (2003) suggest this is an area that should be given future consideration for researcher.

3.2.3 Effect of Gastric pH Oral Bioaccessibility of Arsenic from Soil

Another parameter that varies across *in vitro* extraction tests for arsenic bioaccessibility is the gastric pH used. Figure 3-5 indicates that there was no statistical difference in bioaccessibility results for methods that use a gastric pH of 2.5 or less. Given the narrow range of variability in results from pH 1.5 to 2.0 it is suggested that this would be a suitable range of pH for conducting further experiments to assess the oral bioaccessibility from specific sites.

The SHIME method reported in Oomen *et. al.* (2002) uses a stomach pH of 4.0 and returns arsenic bioaccessibility concentrations significantly lower than at pH 2.5 and less (see Figure 3-5). It is not recommended that this method be employed in Canada for bioaccessibility testing.



	1.1	1.2	1.3	1.5	1.8	2.0	2.5	4.0
1.1	1.000							
1.2	0.092	1.000						
1.3	1.000	0.239	1.000					
1.5	1.000	1.000	1.000	1.000				
1.8	1.000	1.000	1.000	1.000	1.000			
2.0	1.000	1.000	1.000	1.000	1.000	1.000		
2.5	1.000	0.513	1.000	1.000	1.000	1.000	1.000	
4	0.007	1.000	0.029	0.233	0.057	0.202	0.038	1.000

Figure 3-5 Effect of stomach acid pH variable on percent bioaccessibility. ANOVA results report that the use of a pH of 4.0 resulted in significantly less ($p<0.05$) arsenic bioaccessibility.

3.2.4 Difference Between Gastric and Intestinal Phase Oral Bioaccessibility of Arsenic from Soil

Another aspect of arsenic bioaccessibility tests that is often examined is the difference in bioaccessibility if gastric or intestinal phases and experiments are carried out. Several researchers have reported arsenic bioaccessibility for simulation of a gastric condition only and then the result if the experiment was carried forward by raising the pH to equivalent to that of the intestine (pH=7.0) and extended the retention time for approximately four hours.

Statistical analysis determined that there was no significant difference in arsenic bioaccessibility between gastric and intestinal conditions (t-test, df=66, p=0.20). This is likely due to the fact that once the arsenic has been liberated under the gastric pH it remains in solution when the pH is raised to 7.0 and that no additional arsenic is leached from the soil samples. Therefore, for arsenic bioaccessibility the intestinal phase is likely redundant, hence the reason SBRC has eliminated it from its standard operating procedure (SOP).

However, it should be noted that previous work on lead (Ruby, 1996) and more recent work (Ollson *et. al.*, 2003a) has shown that for divalent cations such as lead, copper, and cobalt the intestinal phase is important in determining bioaccessibility. As the pH is raised to 7.0 there is a significant decrease in the percent bioaccessibility measured. This is likely due to the chemical nature and reactions at varying pHs for divalent cations. Unlike these divalent cations, however, arsenic is in the As(V) and As(III) forms and its solubility is not affected by change in pH.

3.2.5 Effect of TOC on Oral Bioaccessibility of Arsenic from Soil

There was only a limited number of studies that reported the % TOC in samples analyzed. Given the relatively narrow range of TOC reported statistical analysis could not be performed on the dataset. This is something that should be evaluated further. However, in general as the TOC in soil samples increased there was a corresponding increase in the percent of arsenic that was shown to be bioaccessible.

Ollson (2003) reported a significant difference ($p<0.05$) for arsenic bioaccessibility with respect to TOC (%) in soil samples. There was a positive linear correlation between arsenic bioaccessibility and TOC. This suggests that knowledge of TOC is critical for evaluating site specific oral bioaccessibility, at least for As. This finding is consistent with the *in vivo* results presented in Section 3.1.2.

3.2.6 Effect of Grain Size on Arsenic Oral Bioaccessibility

Unfortunately the effect that grain size has on the oral bioaccessibility of soils can not be determined from the data published. In most cases the experiments have been conducted on soils that were sieved to less than 250 μm, and the bioaccessibility of As from other particle size fractions has not been reported.

Initial work conducted by Ollson (2003) indicated that there was no statistical difference between the bioaccessibility of arsenic at varying grain sizes from <250 μm to <63 μm of soils collected in Yellowknife. However, reviews by Ruby (1999) and Richardson *et al.* (2005) have indicated that grain size could be an important factor in bioaccessibility.

3.2.7 Effect of Total Arsenic Concentration in Soil on Bioaccessibility

Similar to the results of the *in vivo* work there was a weak but significant negative correlation between total arsenic concentration in the soil sample and the percent that was determined to be bioaccessible (r^2=0.069, p<0.05; Figure 3-6).

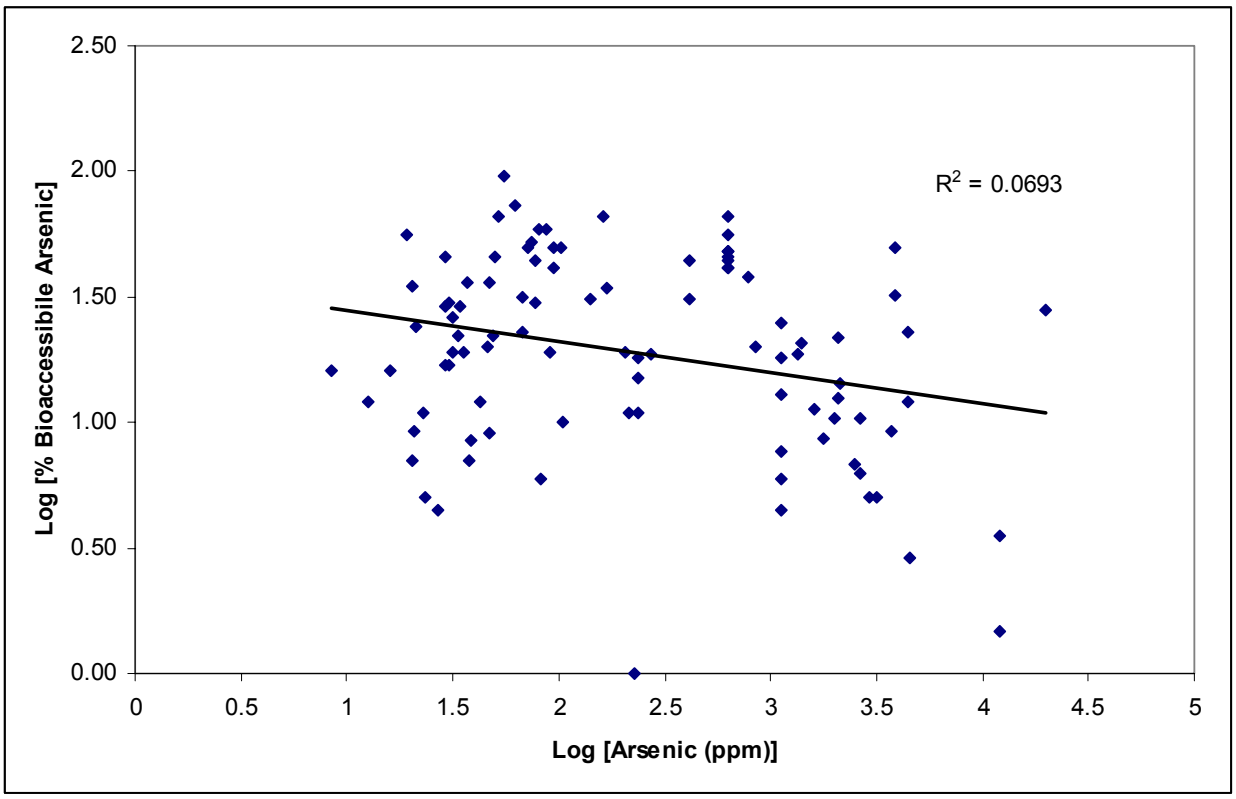


Figure 3-6 Linear regression of arsenic bioaccessibility against total arsenic concentration in soil sample (ppm). An r^2=0.069 indicates that the % of arsenic that was shown to be bioavailable is independent of the total arsenic concentration in the soil sample.

3.2.8 Summary of *In Vitro* Arsenic Oral Bioaccessibility Experiments

Examination of the bioaccessibility testing that has been reported in the literature indicates that regardless of the soil type or area of the world from which it was collected, it is unlikely that the oral bioaccessibility of arsenic would be 100%. The reported As bioaccessibility ranges from <1% to 95% and indicates that arsenic bioaccessibility varies considerably by site.

4.0 BIOAVAILABILITY RESEARCH OF LEAD IN CONTAMINATED SOIL

Lead has long been an environmental contaminant of concern, both from releases at industrial facilities and mine sites, but also as a residential exposure through the use of lead based paints. Considerable research has been conducted on the issue of child exposure to lead through lead based paints, and the contamination of residential soils.

Until 1978, lead paint was commonly used on the interiors and exteriors of North American homes. The US Department of Housing and Urban Development (HUD) estimates that about 38 million homes in the US still contain some lead paint. Low level exposure to lead has been demonstrated to affect fetal and childhood development (ATSDR, 1997).

Absorption of lead in the GI tract has been shown to vary markedly between infants and adults. The WHO provides a summary that suggests that absorption of lead acetate and dietary exposure ranges from 25% to 50%, while adult normally only absorb typically around 10% of dietary lead (WHO, 1986). Baseline risk assessments in the United States for lead contaminated sites assume a default relative bioavailability (RBA) of Pb in soil of 60%, which is used by the Integrated Exposure and Uptake Biokinetic (IEUBK) model for lead in children (US EPA, 1994).

The IEUBK uses absorption parameters (*e.g.*, the fraction of lead absorbed from water) as well as intake and exposure rates to estimate blood lead levels for children (6 months to 7 years of age). The estimated blood-lead concentration is then benchmarked against the EPA and the Centers for Disease Control and Prevention (CDC) childhood PbB benchmark concentration of 10 micrograms of Pb per deciliter of blood (µg Pb/dL).

Given the prevalence of lead as a soil contaminant of concern at superfund sites, mining sites, and residential properties, work has been done to determine the *in vivo* bioavailable fraction of lead from soil. *In vitro* laboratory experiments to mimic bioavailability of lead from contaminated soils have also been conducted.

4.1 Summary of *In Vivo* Oral Bioavailability Research of Lead Contaminated Soil

This section deals specifically with *in vivo* soil lead bioavailability experiments using mammalian test species. There have been a number of studies and research that has dealt with the use of the Integrated Exposure and Uptake Biokinetic (IEUBK) model for lead in children (US EPA, 1994), which will not be reviewed in this assessment.

Although there have been a number of papers that report the results of in vivo testing of soils for lead bioavailability, a number of these are reports of earlier studies and interpretation of the data from other studies. A comprehensive review of many lead *in*

vivo studies was reported by Ruby *et. al.* (1999) in their review paper. Only a limited number of additional studies were retrieved as part of this review.

Male swine studies have been conducted for 22 soil samples, rat studies for 4 soil samples, and one soil sample was used in a human feeding trial. It is likely that there may have been additional in vivo studies that could not be located during this review. As with arsenic the premise behind laboratory animal sampling is that the animal model selected would be representative of the human GI tract.

The results of the bioavailability of lead from soils is tabulated in Table 4-1. During review of the results of these bioavailability experiments it was clear that many of the researchers did not report the physiochemical properties of the soils that are important determinants of lead bioavailability – pH, TOC, grainsize, etc. Therefore, for comparative purposes, only the overall bioavailability of lead from soil could be examined.

Regardless of the test species employed, it was consistently found that that lead-bearing soil when fed by oral gavage (in all cases except human trial) had a relative oral bioavailability less than 100%. Unlike arsenic, less than 100% of the lead ingested from aqueous reagent testing is bioavailable. There are likely two reasons behind this:

1. Not all of the orally administered lead (usually as lead acetate) is solubilized in the GI tract; and,
2. It is unlikely that all of the lead that is soluble in the GI tract is absorbed across the intestinal wall.

Therefore, the studies report a relative bioavailable fraction of lead that is based on correcting for the bioavailability of orally administered lead acetate. In some cases lead acetate was administered as part of the study, whereas in other cases it was corrected using the assumed 50% absolute bioavailability reported for lead acetate in humans.

As discussed in Section 2.2.1 the lead bioavailability reported in this document are as the relative bioavailable fraction or RBA. The RBA is generated using the following equation:

$$ABA_{soil} = ABA_{lead\ acetate} * RBA_{soil}$$

Where:

ABA_{soil}	absolute bioavailability of lead in soil
$ABA_{lead\ acetate}$	absolute bioavailability of lead acetate (default assumed 50%)
RBA_{soil}	relative bioavailability of lead in soil to lead acetate

This equation can also be rearranged to provide the relative bioavailability of lead from soil as:

$$RBA_{soil} = ABA_{soil} / ABA_{lead\ acetate}$$

Although a number of studies reported the RBA_{soil} based on a variety of tissue analysis and blood, for consistency of examination of the data in this report the RBA_{soil} was determined through the use of the area under the curve (AUC) from the blood concentration versus time plot.

Although knowledge of the chemical form of lead is less critical in understanding its potential toxicity, it is important in determination of its bioavailability. However, there was very little, and in most cases no, information reported on the chemical form of lead that would have been expected in the soil samples used in oral bioavailability testing.

Table 4-1. Summary of Lead Relative Bioavailability (*in vivo*) Studies and Results

Author	Database ID	Year	Test Species	Study / Site	Test Soil ID	Soil Type		Soil Doses				% Relative Bioavailability Lead			
							Test Group	Lead [ppm]	standard deviation	Lead Dose [ppm]	dose (mg/kg-d)	Lead (Blood)	Lead (Liver)	Lead (Kidneys)	Lead (Bone)
Freeman	124	1992	Sprague-Dawley rats	Butte, MT	Test Soil I	Composite residential soil	2%-male	810	21	16.2 pm	1.475	21.9	2.45		9.3
					Test Soil I	Composite residential soil	5%-male	810	21	40.5 ppm	3.76	12.95	8.45		5.45
					Test Soil III	Composite residential soil	2%-male	3908	31	78.2 ppm	6.26	23.2	10.35		10.4
					Test Soil III	Composite residential soil	5%-male	3908	31	195 ppm	17.65	21.8	8.65		10.4
Freemen	114	**1996**	rats	California Gulch NPL Site	Cont. soil+control (LO)	reference material		8440		17.6		15			
				California Gulch NPL Site	Cont soil +control (ME)	reference material		8440		42.8		4.6			
				California Gulch NPL Site	Cont Soil +control (HI)	reference material		8400		127		2.3			
Maddalioni	101	**1998**	Human	New York	Bunker Hill Soil	residential	average	2924	36		1.22	52.4			
				New York	Bunker Hill Soil	residential	Fed -7	2924	36		1.22	5.0			
Ellickson	102	**2001**	rats		NIST SRM 2710	reference material	Day 1	5532	80		1.46	0.5	ND	ND	0.9
Schroder	103	2004	male swine	various	1	Sieved soil		1590				33	33	21	21
					2	Sieved soil		8600				22	9	13	13
					3	Sieved soil		11200				1	0	1	1
					4	Sieved soil		10800				56	92	50	55
					5	Sieved soil		4050				78	110	77	70
					6	Sieved soil		6940				82	66	50	94
					7	Sieved soil		7510				71	92	91	62
					8	Sieved soil		4320				87	96	124	84
					9	Sieved soil		10600				20	11	10	18
					10	Sieved soil		1270				6	5	4	0.04
					11	Sieved soil		7895				20	8	8	9
					12	Sieved soil		11500				55	37	44	61
					13	Sieved soil		3200				67	87	102	63
					14	Sieved soil		8350				82	85	70	63
					15	Sieved soil		3230				74	50	42	47
					16	Sieved soil		2150				58	54	34	39
					17	Sieved soil		14200				56	86	68	72
					18	Sieved soil		3870				58	74	74	68
Casteel		**various**	male swine	Joplin	No 1			4300				60			
			male swine	Joplin	No 3			5100				67			
			male swine	Joplin	No 8			5200				64			
			male swine	Bingham Creek	tailings (channel)	tailings		6330				28			

4.1.1 Summary Statistics of *In Vivo* Oral Bioavailability of Lead from Soil

A total of 27 soil samples were subjected to relative bioavailability experiments on an average soil concentration of lead of 6067 ± 3521 ppm. The average percent relative bioavailability of lead was 46 ± 27%. Therefore, on average less than 50 % of lead subjected to bioavailability studies would have been available for uptake into the bloodstream, relative to lead acetate.

This suggests that the average absolute bioavailable fraction of lead in the testing was 23%. This represents the absorbed dose of lead in terms of the exposure dose that the animals would have received. Thus, in conducting human health risk assessment one would assume that the relative bioavailable fraction of lead (against that of lead acetate) would have been 1/2 of that of the traditional approach of assuming 100 % relative bioavailability in Canada.

The US EPA traditionally uses a RBA_{soil} of 60% in risk assessment, both in the IEUBK model and contaminated site risk assessment dose calculations. The 60% RBA_{soil} lead is based on the assumption that lead bound in soil has a 'reasonable maximum' absolute bioavailability of 30%, while lead as lead acetate (using in TRV development by WHO and IEUBK modeling by US EPA) has an ABA of 50%. Thus giving the RBA_{soil} lead of 60% (0.3/0.5 = 0.6 or 60%).

As with any experimentation there was a range of average RBA_{soil} lead values reported. The relative bioavailability was shown to range from 0.5% to as high as 87%. In nine out of the 27 soil samples evaluated the RBA was greater than the US EPA RBA_{soil} default value of 60%. The 95^{th} percentile RBA_{soil} from the studies reviewed was 82%, well over the accepted value used by the US EPA in human health dose calculations for risk assessment.

4.1.2 Soil Properties Governing Oral Bioavailability of Lead from Soil

Unfortunately, the majority of the bioavailability studies did not report the physiochemical properties of the soils tested, other than the total lead concentration. An inspection of the written description of soils tested indicates that they range from likely low organic carbon content (mine soil / slag) to only a few samples with relatively high organic carbon content (residential soils).

An examination of the influence of the total lead content in soil on relative bioavailability was conducted using a linear regression of the data. Both the total lead concentrations in soil and the relative percent bioavailable lead data sets were log transformed to provide normalized datasets.

Similar to arsenic tests, the linear regression indicates that there was no correlation between the total lead concentration in the soil samples and the relative percent of lead that was bioavailable (Figure 4-1). The slope of the regression line was not found to be

significant (p>0.05) and the very low r^2 value of 0.0009 reveals that lead bioavailability cannot be predicted from the total lead concentration in the soil sample.

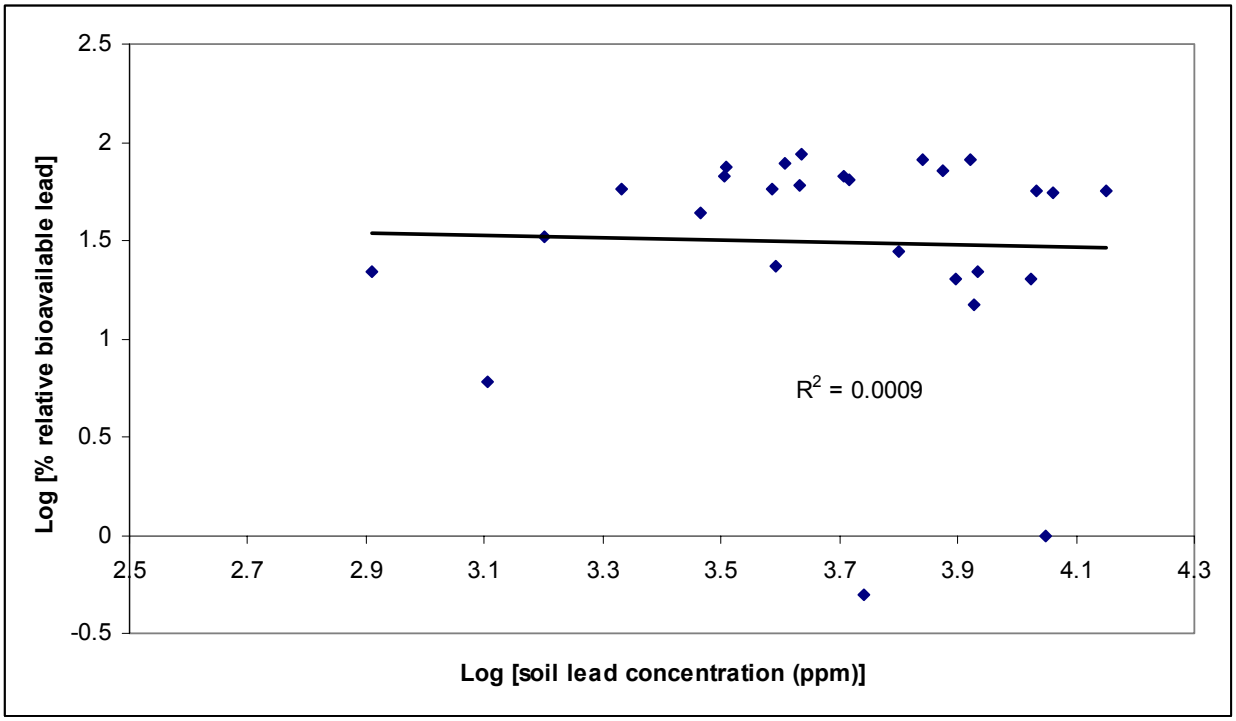


Figure 4-1. Linear regression of lead relative bioavailability against total lead concentration in soil sample (ppm). An r^2=0.0009 (p>0.05) indicates that the % of lead that was shown to be bioavailable is independent of the total lead concentration in the soil sample.

4.1.3 Effect of Selected Test Species on Lead Relative Bioavailability

There were three animal models used in the determination of lead RBA_{soil} - male swine (22 soils), rats (4), and human (1). The average human RBA_{soil} was reported as 52% for fasted administration or close to that of the average RBA_{soil} reported of 46%. Given that only one soil sample was used in the human experiment, it was not carried forward in the statistical analysis presented in this section. As previously discussed for arsenic (Section 2.1.2), the test species employed for bioavailability testing should be able to mimic the relative bioavailability used in the development of the oral RfD (oral reference dose) or the CSF (cancer slope factor).

A t-test was conducted on relative bioavailability results of lead from both male swine and rat studies. There was no statistical difference (p=0.23) between the soil lead concentrations used in the male swine study (6460 ± 3600 ppm) and the rat study (4670 ± 3180 ppm). However, the relative bioavailable fraction of lead from the rat study (15 ± 10%) was significantly lower (p=0.001) than the RBA_{soil} lead determined from male swine (52±25%). The adult human single soil RBA test for lead provided an average of 52% and thus was much closer to the value returned in the male swine studies.

Health Canada's oral tolerable daily intake of lead (TDI) is 0.0036 mg/kg-d (HC, 2004b), based on the World Health Organization provisional tolerable weekly intake (PTWI) for children of 25 μg/kg, equivalent to approximately 3.6 μg/kg/day from all sources (WHO 1986). The PTWI is considered sufficiently low to protect against net accumulation of Pb in blood, and assumes a 50% bioavailability of lead from the original dose. This PTWI was based on the results of metabolic studies in infants and was also used to establish Canadian drinking water standards for lead (CCME, 1999). WHO (1993) has more recently extended this PTWI to all age groups to protect other sensitive population groups, such as women of child-bearing age. The provisional tolerable weekly intake (PTWI) of 0.025 mg/kg was maintained at the fifty-third meeting of the Joint FAO/WHO Expert Committee on Food Additives (WHO,1999).

Given that the TDI is based on a human dose response relationship to blood lead from food spiked with lead acetate, the preferred animal model would be one that closely mimics bioavailability of lead in children. From the two animal models used to determine the RBA_{soil} for lead, the swine model would appear to provide a much closer value to the very limited human dataset.

The fact that the rat relative oral bioavailability of lead from soil was much lower than other studies, and that the oral TRV is based on human absorption of lead, it is not likely a good test species to use when conducting oral bioavailability experiments for lead. This is consistent with the findings for in vivo studies of arsenic bioavailability reported in Section 3.1.3 where rat models also returned significantly lower oral bioavailabilities.

Although not conclusive evidence to rule out the use of rat studies for conducting *in vivo* lead bioavailability experiments, it does suggest that they may not be the best model and that a different test species (such as swine) may be more appropriate for use, as a more conservative estimate of human oral bioavailability.

4.1.4 Summary of *In Vivo* Lead Oral Bioavailability Experiments

It is apparent from a review of the oral bioavailability database for Pb that regardless of soil type or area of the world it was collected, it is unlikely that the oral bioavailability of lead in humans exposed to contaminated soils would be 100%.

4.2 *In vitro* Oral Bioaccessibility Research for Lead in Soil

Fourteen *in vitro* studies were uncovered in the literature review that report chemical extraction tests meant to mimic the oral bioaccessibility of lead in the human GI tract. Thirteen different extraction methods were employed by researchers; however, the majority are essentially a derivative of the PBET method.

Table 4-2 shows the results of lead bioaccessibility experiments that have been published either in the scientific literature or in government documents. The basic components of the varying methodology used for *in vitro* tests are provided in Table 4-3. Similar to the *in vivo*

experiments for lead RBA, many of the researchers did not report the physiochemical properties of the soils that are potentially important determinants of lead bioaccessibility – pH, TOC, grainsize, etc. Therefore, for comparative purposes, only overall bioaccessibility of lead from soil could be examined.

The majority of researchers did clearly document the laboratory methodology used in bioaccessibility experiments. Therefore, effects of liquid to solid ratio, gastric pH, effect of gastric and intestinal pH, and the total lead concentration in soil samples was examined. Regardless of the experimental method employed, it was consistently reported that the bioaccessibility of lead from soils was less than 100%.

Table 4-2. Summary of Lead Bioaccessibility (*in vitro*) Studies and Results

Author	Database ID	Year	Study / Site	Test Soil ID	Soil Type	Soil arsenic [ppm]	Soil pH	Soil TOC (%)	Soil grainsize	Liquid: Solid Ratio (mL:g)	pH stomach	pH Intestine	% Bioaccessible Lead		
													Stomach	Intestine	overall
Davis	113	1992	Butte, MT	Soil I	smelter site soils	3900				14:1	1.3	7.0			0.18
Ruby et al	135	**1992**	Butte Montana	1	soil mixed with mine waste rock	3900			<250	14:1	1.3				20
				2	soil mixed with mine waste rock	3900			<250	14:1	2				40
Ruby	115	1993	Butte, MT	BMW-1	composite mine-waste samples	3900	3.7		<250	10:1	1.3	7.0			4.00
				BMW-2	composite mine-waste samples	1030	2.8		<250	10:2	1.3	7.0			0.50
				BMW-3	composite mine-waste samples	5820	3.8		<250	10:3	1.3	7.0			6.00
				BMW-4	composite mine-waste samples	1790	2.6		<250	10:4	1.3	7.0			2.00
Ruby	100	1996	Butte, MT	BMW-II	composite mine-waste samples	3908	3.7	4.1	42	160:1	2.5	7.0	6	5	22.5
			Bartlesville, OK	BVS	residential	1388	7	12.8	23	160:1	1.3	7.0	68	16	35.0
			Bartlesville, OK	BVS	residential	1388	7	12.8	23	160:1	2.5	7.0	22	13	35.0
			Salt Lake City, UT	SCS	residential	2090	7.5			160:1	1.3	7.0	72	27	41.0
			Salt Lake City, UT	SCS	residential	2090	7.5			160:1	2.5	7.0	11	8±2	41.0
			Copperton, UT	CT-1	tailings	7220	2.4	0.6	38	160:1	1.3	7.0	12	1.7	14.7
			Copperton, UT	CT-1	tailings	7220	2.4	0.6	38	160:1	2.5	7.0	6.8	0.2	14.7
			Copperton, UT	CT-2	tailings	6890	2.8	1.8	23	160:1	1.3	7.0	8	0.4	8.7
			Copperton, UT	CT-2	tailings	6890	2.8	1.8	23	160:1	2.5	7.0	4	1	8.7
			Bingham Creek Channel, UT	CT-3	stream channel sample	10230	4.9	2.9	21	160:1	1.3	7.0	39	8	36.0
			Bingham Creek Channel, UT	CT-3	stream channel sample	10230	4.9	2.9	21	160:1	2.5	7.0	22	7	36.0
Hamel	116	1998	Jersey City	Jersey City	chromate ore processing	972			<250	100:1			22		.
			Jersey City	Jersey City	chromate ore processing	972			<250	200:1			30		.
			Jersey City	Jersey City	chromate ore processing	972			<250	500:1			35		.
			Jersey City	Jersey City	chromate ore processing	972			<250	1000:1			46		.
			Jersey City	Jersey City	chromate ore processing	972			<250	2000:1			58		.
			Jersey City	Jersey City	chromate ore processing	972			<250	5000:1			38		.
			NIST Standard	NIST 2710	Reference Material	5532		3	<74	100:1			36		.
			NIST Standard	NIST 2710	Reference Material	5532		3	<74	200:1			46		.
			NIST Standard	NIST 2710	Reference Material	5532		3	<74	500:1			29		.
			NIST Standard	NIST 2710	Reference Material	5532		3	<74	1000:1			34		.
			NIST Standard	NIST 2710	Reference Material	5532		3	<74	2000:1			35		.
			NIST Standard	NIST 2710	Reference Material	5532		3	<74	5000:1			42		.
Hamel et al	126	**1999**	various	Jersey City	slag materail	1163	8.2		<125	2000:1					39
				Montana SRM 2710	reference material	5532	4.5		<74	2000:1					62
				Residential Soil	organic soil	68	6		<125	2000:1					69
				Bunker Hill soil	reference material	2924	4		<125	2000:1					70
Ellickson,	102	**2001**	Butte, Montana	NIST SRM 2710	reference material	5532	5.2	5.4	<74 µm	2160:1	1.4	6.5	76.1	10.7	10.7
Yang et al	132	**2002**	Jasper City Superfund Site	Plot 1: 0-5 cm	residential (untreated)	5572	7.13			100:1					36.8

Table 4-2. Summary of Lead Bioaccessibility (*in vitro*) Studies and Results

Author	Database ID	Year	Study / Site	Test Soil ID	Soil Type	Soil arsenic [ppm]	Soil pH	Soil TOC (%)	Soil grainsize	Liquid: Solid Ratio (mL:g)	pH stomach	pH Intestine	% Bioaccessible Lead		
													Stomach	Intestine	overall
				Plot 1: 5-10 cm	residential (untreated)	5924	7.32			100:1					66.5
				Plot 1: 10-20 cm	residential (untreated)	6843	7.51			100:1					70.2
				Plot 2: 0-5 cm	residential (untreated)	2154	6.87			100:1					52.5
				Plot 2: 5-10 cm	residential (untreated)	3020	7.08			100:1					42.4
				Plot 2: 10-20 cm	residential (untreated)	2623	7.12			100:1					65.6
				Plot 2: 20-30 cm	residential (untreated)	2434	7.19			100:1					33.7
				Plot 3: 0-5 cm	residential (untreated)	3101	6.93			100:1					28.6
				Plot 3: 5-10 cm	residential (untreated)	3002	7.15			100:1					26.7
				Plot 3: 10-20 cm	residential (untreated)	3047	7.31			100:1					46.0
				Plot 3: 20-30 cm	residential (untreated)	4589	7.37			100:1					17.9
				Plot 4: 0-5 cm	residential (untreated)	4102	6.53			100:1					30.5
				Plot 4: 5-10 cm	residential (untreated)	4905	7.04			100:1					38.3
				Plot 4: 10-20 cm	residential (untreated)	4553	7.17			100:1					44.8
				Plot 4: 20-30 cm	residential (untreated)	5365	7.46			100:1					33.6
Oomen et al	112	**2003**	Soils near pottery	A	Pottery flakes	11000				98:1	1.07	7.8			0.3
				A-1	Soil sample	220				98:1	1.07	7.8			64
				A-2	Soil sample	250	7.2	2.6	75.3 µm	98:1	1.07	7.8			66
				A-3	Soil sample	340				98:1	1.07	7.8			42
				B-1	Soil sample	430				98:1	1.07	7.8			59
				B-2	Soil sample	470	7.7	4	70.9 µm	98:1	1.07	7.8			55
				B-3	Soil sample	820				98:1	1.07	7.8			28
				C-1	Soil sample	1200	7.4	2	75.9 µm	98:1	1.07	7.8			60
				C-2	Soil sample	1400				98:1	1.07	7.8			59
				C-3	Soil sample	2400	7.6	4.1	64.6 µm	98:1	1.07	7.8			54
				D-1	Soil sample	50				98:1	1.07	7.8			46
				D-2	Soil sample	280				98:1	1.07	7.8			55
				D-3	Soil sample	350	8.2	4.2	56.8 µm	98:1	1.07	7.8			51
				D-4	Soil sample	660				98:1	1.07	7.8			53
				E-1	Soil sample	730	7.8	1.7		98:1	1.07	7.8			73
Oomen et al	108	**2004**	3 reference soils + OECD standardized artificial soil	Flanders - ox bile (sigma)	reference material	612				100:1	1	8			66
				Flanders - ox bile (ICN)	reference material	612				100:1	1	8			63
				Flanders - pig bile	reference material	612				100:1	1	8			66
				Flanders - chicken bile	reference material	612				100:1	1	8			80
				Oker -11 - ox bile (sigma)	reference material	5454				100:1	1	8			28
				Oker -11 - ox bile (ICN)	reference material	5454				100:1	1	8			29
				Oker -11 - pig bile	reference material	5454				100:1	1	8			23
				Oker - 11 - chicken bile	reference material	5454				100:1	1	8			33
				Montana 2710 - ox bile (sigma)	reference material	5532				100:1	1	8			28
				Montana 2710 - ox bile (ICN)	reference material	5532				100:1	1	8			30

Table 4-2. Summary of Lead Bioaccessibility (*in vitro*) Studies and Results

Author	Database ID	Year	Study / Site	Test Soil ID	Soil Type	Soil arsenic [ppm]	Soil pH	Soil TOC (%)	Soil grainsize	Liquid: Solid Ratio (mL:g)	pH stomach	pH Intestine	% Bioaccessible Lead		
													Stomach	Intestine	overall
				Montana 2710 - pig bile	reference material	5532				100:1	1	8			30
				Montana 2710 - chicken bile	reference material	5532				100:1	1	8			35
				Montana 2711 - ox bile (sigma)	reference material	1162				100:1	1	8			10
				Montana 2711 - ox bile (ICN)	reference material	1162				100:1	1	8			12
				Montana 2711 - pig bile	reference material	1162				100:1	1	8			19
				Montana 2711 - chicken bile	reference material	1162				100:1	1	8			60
Schroder	103	2004	Various	1 - With dough	Sieved soil	1590			<250	150:1	1.8	5.5	19.7	0.54	
				2 - With dough	Sieved soil	8600			<250	150:1	1.8	5.5	5.9	0.17	
				3 - With dough	Sieved soil	11200			<250	150:1	1.8	5.5	0.7	0.02	
				4 - With dough	Sieved soil	10800			<250	150:1	1.8	5.5	27.8	1.16	
				5 - With dough	Sieved soil	4050			<250	150:1	1.8	5.5	31.6	1.06	
				6 - With dough	Sieved soil	6940			<250	150:1	1.8	5.5	34.3	0.95	
				7 - With dough	Sieved soil	7510			<250	150:1	1.8	5.5	26.4	0.47	
				8 - With dough	Sieved soil	4320			<250	150:1	1.8	5.5	35	0.8	
				9 - With dough	Sieved soil	10600			<250	150:1	1.8	5.5	8.24	0.04	
				10 - With dough	Sieved soil	1270			<250	150:1	1.8	5.5	4.74	0.18	
				11 - With dough	Sieved soil	7895			<250	150:1	1.8	5.5	13.8	0.06	
				12 - With dough	Sieved soil	11500			<250	150:1	1.8	5.5	22.3	0.57	
				13 - With dough	Sieved soil	3200			<250	150:1	1.8	5.5	31.6	0.32	
				14 - With dough	Sieved soil	8350			<250	150:1	1.8	5.5	29.6	0.84	
				15 - With dough	Sieved soil	3230			<250	150:1	1.8	5.5	31.1	0.59	
				16 - With dough	Sieved soil	2150			<250	150:1	1.8	5.5	36.3	0.87	
				17 - With dough	Sieved soil	14200			<250	150:1	1.8	5.5	23.3	0.66	
				18 - With dough	Sieved soil	3870			<250	150:1	1.8	5.5	31	0.73	
				1 - Without dough	Sieved soil	1590			<250	150:1	1.8	5.5	21.1	2.79	
				2 - Without dough	Sieved soil	8600			<250	150:1	1.8	5.5	6.81	0.48	
				3 - Without dough	Sieved soil	11200			<250	150:1	1.8	5.5	1.4	0.32	
				4 - Without dough	Sieved soil	10800			<250	150:1	1.8	5.5	55.2	1.66	
				5 - Without dough	Sieved soil	4050			<250	150:1	1.8	5.5	64.4	0.49	
				6 - Without dough	Sieved soil	6940			<250	150:1	1.8	5.5	58.8	2.22	
				7 - Without dough	Sieved soil	7510			<250	150:1	1.8	5.5	41	1.93	
				8 - Without dough	Sieved soil	4320			<250	150:1	1.8	5.5	53	1.95	
				9 - Without dough	Sieved soil	10600			<250	150:1	1.8	5.5	7.5	0.09	
				10 - Without dough	Sieved soil	1270			<250	150:1	1.8	5.5	6.71	0.18	
				11 - Without dough	Sieved soil	7895			<250	150:1	1.8	5.5	6.85	0.03	
				12 - Without dough	Sieved soil	11500			<250	150:1	1.8	5.5	24.7	0.05	
				13 - Without dough	Sieved soil	3200			<250	150:1	1.8	5.5	51.9	0.07	
				14 - Without dough	Sieved soil	8350			<250	150:1	1.8	5.5	36.9	1.01	

Table 4-2. Summary of Lead Bioaccessibility (*in vitro*) Studies and Results

Author	Database ID	Year	Study / Site	Test Soil ID	Soil Type	Soil arsenic [ppm]	Soil pH	Soil TOC (%)	Soil grainsize	Liquid: Solid Ratio (mL:g)	pH stomach	pH Intestine	% Bioaccessible Lead		
													Stomach	Intestine	overall
				15 - Without dough	Sieved soil	3230			<250	150:1	1.8	5.5	32.2	0.75	
				16 - Without dough	Sieved soil	2150			<250	150:1	1.8	5.5	36.3	0.36	
				17 - Without dough	Sieved soil	14200			<250	150:1	1.8	5.5	37.7	1.43	
				18 - Without dough	Sieved soil	3870			<250	150:1	1.8	5.5	36.2	3.23	
Ontario MOE	63 (as)	**2002**	Port Colborne	1	ground soil	187				100:1	1.8	7	61	8.4	
				2		239				100:1	1.8	7	69	14	
				3		120				100:1	1.8	7	78	3.1	
				4		231				100:1	1.8	7	76	6	
				5		383				100:1	1.8	7	68	6	
				6		209				100:1	1.8	7	64	2	
				7		343				100:1	1.8	7	70	5	
				8		1030				100:1	1.8	7	66	3.6	
				9		838				100:1	1.8	7	34	1.7	
				10		911				100:1	1.8	7	81	8.7	
				1	Sieved Soil	232				100:1	1.8	7	72	3.8	
				2		211				100:1	1.8	7	79	5.7	
				3		190				100:1	1.8	7	79	3.7	
				4		222				100:1	1.8	7	80	9.5	
				5		430				100:1	1.8	7	73	4.8	
				6		389				100:1	1.8	7	86	3.7	
				7		320				100:1	1.8	7	79	4	
				8		1210				100:1	1.8	7	84	4	
				9		973				100:1	1.8	7	50	1.3	
				10		511				100:1	1.8	7	77	3.9	
Oomen	59	2002	various	Flanders		634	6	3.3	<200	100:1	1.50	NA			91.00
						730	6	3.3	<200	50:1	2.00	7.50			40.00
						730	6	3.3	<200	50:1	2.00	7.50			31.00
						612	6	3.3	<200	225:1	1.10	7.80			66.00
						725	6	3.3	<200	2.5:1	4.00	6.50			4.00
						616	6	3.3	<200	25:1	varying	7.20			13.00
				Oker 11		6380	5.9	5.1	<1000	100:1	1.50	NA			56.00
						5742				50:1	2.00	7.50			23.00
						5742				50:1	2.00	7.50			16.00
						5454				225:1	1.10	7.80			29.00
						6230				2.5:1	4.00	6.50			1.00
						6230				25:1	varying	7.20			4.00
				Montana 2711	NIST SRM 2711	1069		2	<74	100:1	1.50	NA			90
						1082		2	<74	50:1	2.00	7.50			68

Table 4-2. Summary of Lead Bioaccessibility (*in vitro*) Studies and Results

Author	Database ID	Year	Study / Site	Test Soil ID	Soil Type	Soil arsenic [ppm]	Soil pH	Soil TOC (%)	Soil grainsize	Liquid: Solid Ratio (mL:g)	pH stomach	pH Intestine	% Bioaccessible Lead		
													Stomach	Intestine	overall
						1082		2	<74	50:1	2.00	7.50			46
						1022		2	<74	225:1	1.10	7.80			11
						1162		2	<74	2.5:1	4.00	6.50			3
						1070		2	<74	25:1	varying	7.20			17
Dodd		**2002**	B.C. Lightstations	Lightstation soils	53 topsoil samples	866				100:1	1.5		70		

Table 4-3. *In Vitro* Test Method Parameters for Lead Bioaccessibility

	Specifics	Artificial Biofluid	Modified PBET	RIVM + cell culture exp.	Modified Artificial Biofluid	Modified Artificial Biofluid	Simple Chemical Extraction	Modified PBET	Kinetics Test	Modified PBET	Artificial Biofluid I	Artificial Biofluid II	IVG	RIVM
Author		**Ellickson, 2000**	**Fendorf, 2004**	**Oomen, 2003**	**Hamel et al, 1998**	**Hamel et al, 1999**	**Yang et al, 2002**	**Brown et al , 2003**	**Ruby et al, 1992**	**Ruby 1996**	**Davis 1992**	**Ruby 1993**	**Schroder et al, 2004**	**Oomen et al, 2003, 2004**
Input	**Amount of Soil Added**	50 mg dry weight	0.5g dry weight	0.6 g dry weight	5mg and 50 mg dry weight	50 mg dry weight	0.4 g dry weight	2.5 g	20 g	0.4 g dry weight	10 g dry weight	4.2 g dry weight	4 g dry weight	0.6 g dry weight
General	**Model type**		Dynamic stomach	Static gastro-intestinal			Static stomach	Dynamic stomach	Dynamic stomach	Static gastro-intestinal	Static gastro-intestinal	Static gastro-intestinal	Static gastro-intestinal	Static gastro-intestinal
	Temperature	37°C	35.6	37°C	37°C	37°C	37	37	37	37°C	37°C	37°C	37°C	37°C
	Mechanical treatment		rotating horizontal armature at 12 rpm	End-over-end rotation 55 rpm	wrist-action shaker	wrist-action shaker	End-over-end rotation 30 rpm	Stirred with magnetic stir bars	Mixed by wrist action shaker, 3 degrees amplitude, 3 s frequency	Bubbling argon	wrist-action shaker	wrist-action shaker	Stirring 100 rpm	End-over-end rotation 55 rpm
	Food components	No	No	No	No	No	No	No	No	No	10 g high fiber rabbit chow	1.0g rabbit chow	200g dough	No
Oral Cavity	**Saliva**	Yes	No	Yes		Yes	No	No	No	No	No	No	No	Yes
	Volume of saliva	8 mL		9.0 mL		8 mL								9.0 mL
	pH	1.4		6.5		5.5								6.5
	Incubation time	With gastric		5 minutes		With gastric								5 minutes
Stomach	**Gastric compartment**	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	Volume of gastric juice	100 mL	50 mL	13.5 mL	100 mL	100 mL	40 mL	250 mL	280 mL	40 mL	140 mL	40 mL	600 mL	13.5 mL
	pH	1.4	3	1.1	not specified	1.4		2.0 (1.5, 2.3)	1.3, 2.0	1.5, 2.5, 4.0	1.3	1.3	1.8	1.07
	Gastric secretion components	NaCl, pepsin	1 *M* Glycine	Pepsin, mucin, BSA	NaCl, pepsin	NaCl, pepsin		Pepsin, citrate, malate, lactic acid, acetic acid, deionized water, HCl)		Pepsin, citrate, malate, lactic acid, acetic acid	No	pepsin, acetate, citrate, malate, lactate	NaCl, pepsin	Pepsin, mucin, BSA
	Incubation time	2 hours	1hour	2 hours	2 hours	2 hours	1 hour	1 hour	2 hours	1 hour	2 hours	2 hours	1 hour	2 hours
Intestine	**Intestinal component**	Yes	No	Yes	No	Yes	No	No	No	Yes	Yes	Yes	Yes	Yes
	Volume of intestinal juice	100 mL		36 mL		100 mL				40 mL unchanged	140 mL	40 mL	600 mL (unchanged)	27 mL
	Intestinal pH	6.5		7.8		6.5				7	7	7	5.5	7.8
	Intestinal secretion components			Bile, duodenal juice, chime, pancreatin, lipase						Pancreatin, bile salts		Pancreatin, bile salts	Pancreatin, bile extract	Bile, duodenal juice, chime, pancreatin, lipase
	Incubation time	2 hours		2 hours		2 hours				3 hours	Not specifiied	2 hours	Not specifiied	2 hours
Output	**Centrifugation**	1000 rpm	Yes - time not specified.	3000g	3400 rpm	1000 rpm	No	No		2100 rpm	2500 rpm	2100 rpm	10 000 rpm	3000g
	Filtration	No		No	Whatman 51 filter papers	0.45 µm filtration disk	0.2 µm filter	whatman #40 filter papers	0.22 µm nylon filter	Not specified	No	No	0.45 mm filter	No
	Special treatment			Chyme was diluted with 1 part DNEM. Caco-2 cells were exposed to the chyme mix.				No			Samples removed at 2,4,6,8,11,23 and 31 h			
	Acid digestion of fluid	No			HNO_3	HNO_3		No	HNO_3	Not specified	Acidified	Not specified	Acidified	Acid digestion microwave
	Analytical method	ICP MS	ICP-OES	ICP MS	ICP MS	ICP MS	absoption spectro-photometry	AAS	AAS	ICP AES	AAS	AAS	ICP-AES	ICP MS

4.2.1 Summary Statistics of *In Vitro* Oral Bioaccessibility of Lead from Soil

The data set compiled from individual studies on oral bioaccessibility of lead consists of either individual soil sample results or in some cases averages where researchers did not report individual sample results. This adds some complexity and uncertainty into statistical analysis of the data set. It was determined that the best approach would be to use data as reported by researchers to conduct statistical analysis. In addition, some researchers report data for both gastric and intestinal phases, while others publish an overall bioaccessibility. It was determined that the total bioaccessibility reported by the researchers would be used in reporting summary statistics in this assessment.

A total of 86 discrete soil lead bioaccessibility results were reported on an average soil concentration of lead of 3180 $\pm$ 3285 ppm. The percent of lead that was bioaccessible varied dramatically between the stomach phase and intestinal phase. The average bioaccessibility of lead in the stomach phase from all tests was 51 $\pm$ 26% (n=86), and decreased to only 4.2 $\pm$ 5.0% (n=45) in the intestinal phase. The significance of this result is discussed in Section 4.2.2.

Unlike arsenic, only 50% of soluble lead is absorbed across the intestinal wall (WHO, 1986). In addition, the ABA of lead acetate (as dietary lead) is reported as 50%. In order to make direct comparisons between the in vivo RBA lead results and the bioaccessible results a conversion of lead bioaccessibility is required. The bioaccessible lead concentrations can be converted to an ABA_{soil} lead by multiplying concentrations by the 50% (0.5) absorption factor of soluble lead in the GI tract. To derive a RBA_{soil} bioaccessible equivalent one then divides the ABA_{soil} lead by the oral RfD $ABA_{lead\ acetate}$, which is also 50% (0.5). Thus, the two conversion factors cancel each other out and the reported lead bioaccessibilities are actually equivalent to the *in vivo* RBA for lead. Thus bioaccessible lead values reported can be directly applied as the RAF in risk assessment.

The bioaccessible fraction of lead from soil ranged from <1% to as high as 86%. Given the variability in the measured lead bioaccessibility in *in vitro* tests, it suggests that it may not be appropriate to apportion a standard fraction of bioaccessibility from the literature to individual contaminated sites.

However, the 95th percentile of reported lead bioaccessibility values from the stomach phase was 80%, which suggests that a reasonable maximum oral bioavailability/bioaccessibility of arsenic from soils of 80% might be appropriate for use in screening level risk assessments.

4.2.2 Difference Between Gastric and Intestinal Phase Oral Bioaccessibility of Lead from Soil

Of the 86 discrete soil samples that were assessed for their lead bioaccessibility from soil, 45 reported bioaccessibilities for both stomach and intestinal phases. Unlike for

arsenic (Section 3.2.4) there was a significant difference between the results of the two phases. The average bioaccessibility of lead in the stomach phase from all tests (n=86) was 51 ± 26%, and decreased significantly (t-test, df=47, p<0.001) to only 4.2 ± 5.0% in the intestinal phase.

It has been reported by Ruby (1996) and others (Schroder, 2004) that the significant drop in bioaccessible lead concentrations between stomach and intestinal phases is likely a function of lead solubility at the increased pH of the intestinal phase. Although lead is absorbed through the intestinal epithelium, the comparison of gastric bioaccessibility studies to in vivo bioavailability studies (i.e., their general agreement; 46% versus 51% for in vivo versus in vitro) suggests that the bioavailability-controlling step in oral lead absorption is dissolution in the stomach.

Given that the *in vivo* RBA was 46 ± 27% and that lead bioaccessibility values are equivalent to an RBA, as reported by others the best method for estimating the RBA of lead in chemical laboratory tests appears to be through the use of the stomach phase only. This is the reason the SBRC has adopted a stomach phase only approach for lead bioaccessibility.

It should be noted that the applicability of the stomach phase only approach to bioaccessibility for all metals can not be assumed. A recent validation of nickel bioaccessibility against *in vivo* bioavailability results in rats (Jacques Whitford, unpublished data) suggests that the intestinal phase may be a better indicator of RBA for nickel.

4.2.3 Effect of Liquid to Solid Ratio on Oral Bioaccessibility of Lead from Soil

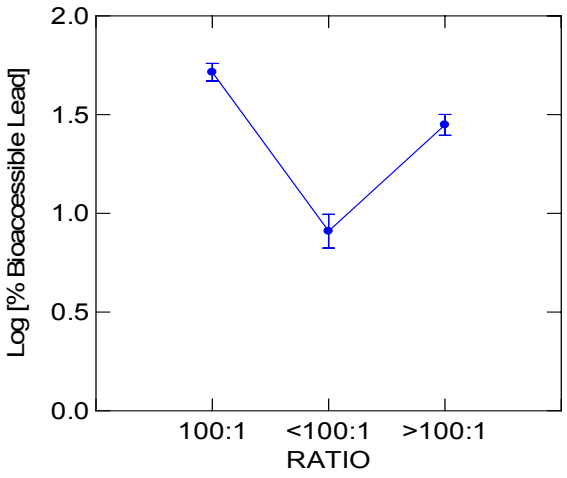
Unlike arsenic, there has not been a reported increase of lead bioaccessibility with liquid to solid ratio above a 100:1 ratio. However, Ruby et al. (1999) reported a decrease in the amount of lead that was found to be bioaccessible for liquid:solid ratios of <100:1. This was one factor in establishing a 100:1 ratio in the SBRC methodology.

The results of the bioaccessibility of arsenic from soil samples were divided into groupings of liquid to solid ratios of <100:1, 100:1, and >100:1. The data was log transformed to provide for normalcy and an ANOVA was run to examine differences amongst the varying ratios.

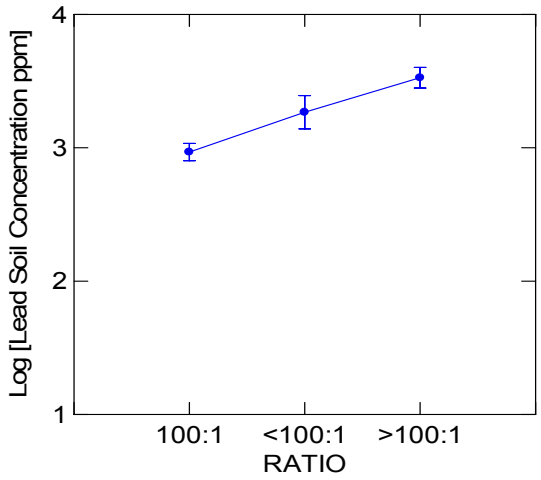
From the ANOVA analysis it appears that lead bioaccessibility was statistically different amongst all three ratio groups, with the highest percent bioaccessible lead being recovered in the 100:1 ratio experiments (Figure 4-2). This is not consistent with Hamel (1998), who reported no statistical difference amongst varying ratios of 100:1 to 5000:1 for lead in two soil types.

As is shown in Section 4.2.7 there is a weak decreasing trend of lead bioaccessibility with increasing total lead soil concentration. However, further examination of the data

sets suggests that there was significantly less lead in the <100:1 group and in the 100:1 ratio experiments than those conducted with a liquid:solid ratio >100:1 (Figure 4-2). Therefore, the results of this analysis can not be attributed to the liquid: soil ratio alone. There was not enough data to perform a stepwise multiple regression (generalized linear model). Given the inconsistency of this finding it suggests this is an area that should be given future consideration by reserchers.



	100:1	<100:1	>100:1
100:1	1.000		
<100:1	<0.001	1.000	
>100:1	0.001	<0.001	1.000



	100:1	<100:1	>100:1
100:1	1.000		
<100:1	0.106	1.000	
>100:1	<0.001	0.238	1.000

Figure 4-2. Effect of liquid to solid ratios on lead bioaccessibility from soil. ANOVA results indicated that there was a statistical difference ($p<0.05$) across the varying ratios. However, there was also a significant difference in soil lead concentrations amongst experiments ($p<0.05$).

4.2.4 Effect of Gastric pH on Oral Bioaccessibility of Lead from Soil

There was insufficient variation in stomach pH used in the experiments to draw meaningful conclusions about the effect of pH on the oral bioaccessibility of lead. The majority of researchers have now adopted a gastric pH of 2.0 or less for bioaccessibility assays of Pb in soil. It is unlikely that modifying pH between 1.5 to 2.0 will have an impact on lead bioaccessibility. Based on the results of Oomen (2004), gastric pH in the range of 4.0 did result in a marked decrease in lead bioaccessbility.

4.2.5 Effect of TOC on Oral Bioaccessibility of Lead from Soil

There was only a limited number of studies that reported the % TOC in samples analyzed. Given the relatively narrow range of TOC reported statistical analysis could not be performed on the dataset. This is something that should be evaluated further.

However, in general as the TOC in soils samples increased a corresponding increase in the percent of lead that was shown to be bioaccessible was observed. The biological and statistical significance of this relationship, however, can not be ascertained with the limited available data.

4.2.6 Effect of Grain Size on Oral Bioaccessibility of Lead

The effect that grain size has on the oral bioaccessibility of soils can not be determined from the data published. In most cases the experiments have been conducted on soils that were sieved to less than 250 μm and the bioaccessibility of Pb from other particle size fractions has not been reported.

4.2.7 Effect of Total Lead Concentration in Soil on Bioaccessibility

There appears to be a negative logarithmic relationship between the total soil lead concentration and the percent of lead that was bioaccessible. This relationship has been previously discussed by other researchers in the absolute bioavailability of lead from substrates such as lead acetate (WHO, 1986) and to a limited extent soils. However, such a marked relationship has not previously been reported for lead soil bioaccessibility results. (Figure 4-3; r^2=0.34, p<0.05).

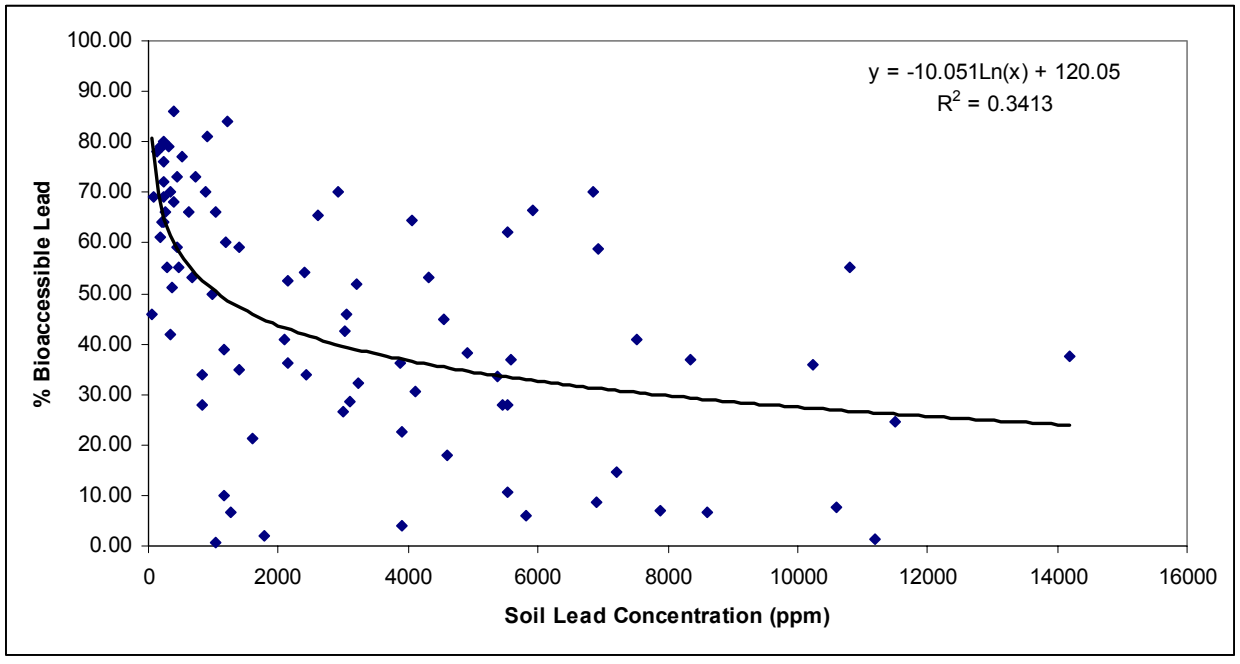


Figure 4-3. Linear regression of lead bioaccessibility against total lead concentration in soil sample (ppm). An r^2=0.34 indicates that the % of lead that was shown to be bioaccessible was inversely logarithmically correlated with total lead concentration in the soil sample.

4.2.8 Lead Bioaccessibility and Soil pH

For 36 of the samples that reported lead bioaccessibilty, pH of the soil samples was also reported. As shown in Figure 4-4 the bioaccessibility of lead from soil was positively correlated with an increase in soil pH. However, those soils with a lower measured pH appear to have been predominately mine tailings samples, while those with a higher pH were from residential soil samples. Therefore, the confounding influence of TOC and Pb speciation cannot be isolated. Residential soils would be expected to have higher organic carbon content and have lead contamination in a more environmentally available form.

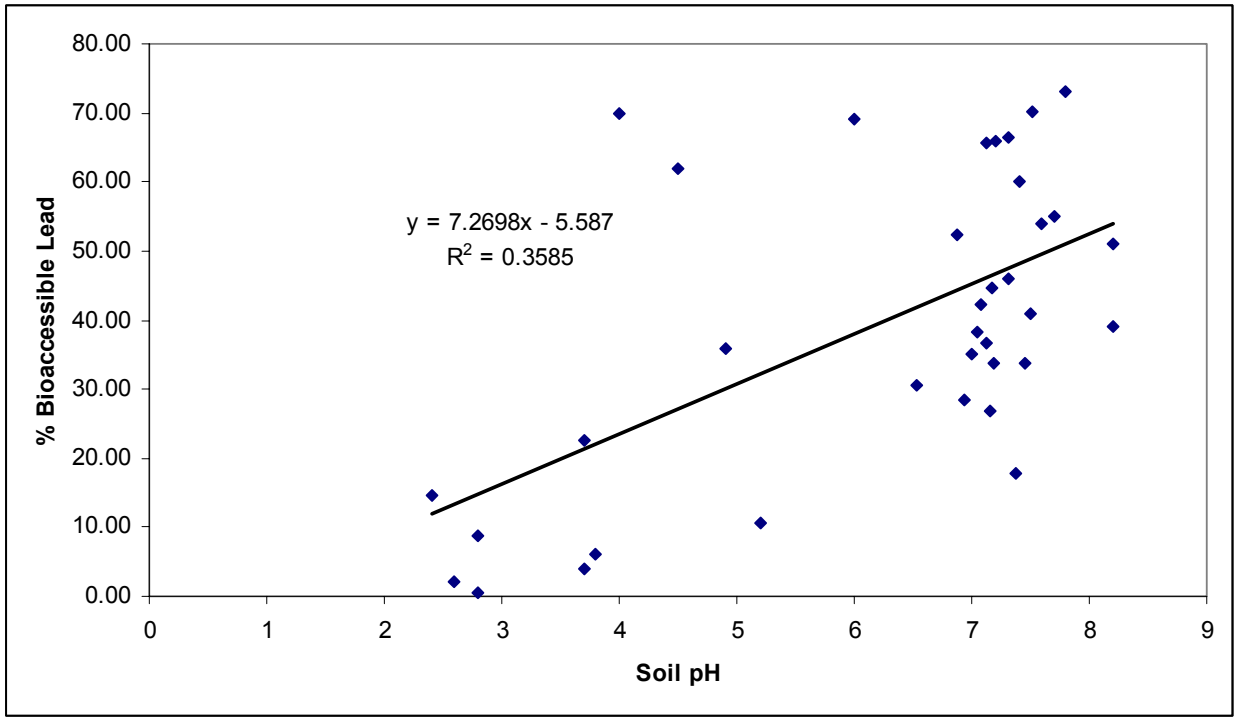


Figure 4-4 Linear regression of lead bioaccessibility against soil pH. A r^2=0.36 indicates that the % of lead that was shown to be bioaccessible was positively correlated to some extent with the pH of the soil sample.

4.2.9 Summary of *In Vitro* Lead Oral Bioaccessibility Experiments

Examination of the bioaccessibility testing that has been reported in the literature indicates that regardless of the soil type or area of the world it was collected, it is unlikely that the oral bioaccessibility of lead would be 100%. Statistical analysis and trends presented have posed numerous questions than can only be answered by further research.

5.0 *IN VIVO* ORAL BIOAVAILABILITY RESEARCH OF CADMIUM CONTAMINATED SOIL

Cadmium (Cd) is present in the environment as a result of both natural processes (including forest fires, volcanic emissions and weathering of soil, till, and bedrock) and human activities. Anthropogenic sources of cadmium entry to the environment include metal production (particularly base metal smelting and refining), stationary fuel combustion (power generation and heating), transportation, solid waste disposal, and sewage sludge application (CCME, 1999).

The oral toxicity reference value (RfD) for cadium from IRIS is based on either exposure to cadium in drinking water 5E-4 mg/kg/day (water) or food 1E-3 mg/kg/day. These TRVs assume an absolute bioavailability cadium of either 2.5% absorption of Cd from food (Foulkes, 1986) or 5% from water (US EPA,1985). The Health Canada oral TDI 8.0E-4 mg/kg-d, is similar to the provisional TDI (PTDI) of 1.0E-3 mg/kg-d (adopted from WHO, 1992c), used by CCME when deriving the soil quality guideline. The joint FAO/WHO Expert Committee on Food Additives (JECFA) set the PTDI based on an assumed absorption rate of 5% for dietary cadmium (similar to the US EPA) and a daily excretion rate of 0.005% of the total body burden (WHO,1992).

Cadmium has also been classified a Group II “probably carcinogenic to humans” through inhalation exposure, however there is no evidence that the oral exposure route poses a carcinogenic risk to humans. Although cadmium is a contaminant of concern on many Canadian contaminated sites, and is reported in 433 of the US National Priorities List hazardous waste sites (Schroder *et. al.*, 2003), very little *in vivo* or *in vitro* soil bioavailability testing has been conducted on cadmium.

The following sections provide a qualitative summary of *in vivo* and *in vitro* cadmium bioavailability studies, as there is not enough data to provide any statistical interpretation of results beyond those already presented in the individual papers.

5.1 Summary of Cadmium Soil *In Vivo* Bioaccessibility Studies

Only two studies were uncovered that studied the oral relative bioavailability of cadmium from soils to a reference material. In 1997, Schilderman *et. al.* orally administered a cadmium chloride ($CdCl_2$) contaminated spiked soil or dissolved cadmium as $CdCl_2$ in a saline solution (Table 5-1) to eight week old male Lewis rats. Only 4.0% of the soluble Cd salt was seen to be absolutely bioavailable to the rats, whereas 1.7% of the Cd spiked soil was found to be absolutely bioavailable.

Table 5-1. Summary of Cadmium Relative Bioavailability (*in vivo*) Studies and Results

Author	Database ID	Year	Test Species	Study / Site	Test Soil ID	Soil Type	Soil	Soil	Soil	Soil	% Relative Bioavailable Cadmium
							Cadmium [ppm]	pH	TOC (%)	Grainsize um	Blood
Schilderman	100	1997	Lewis rats	N/A	Cd-soil	artificial	0.65	5.8	9.5	< 500	43
Schroder	99	2003	male swine	various	1	sieved soil	465			<250	55.4
					2	sieved soil	43			<250	29.9
					3	sieved soil	26.6			<250	73.4
					4	sieved soil	188			<250	53.6
					5	sieved soil	139			<250	63.3
					6	sieved soil	29.9			<250	10.4
					7	sieved soil	23.8			<250	56.8
					8	sieved soil	195			<250	94.2
					9	sieved soil	319			<250	116
					10	sieved soil	47.4			<250	80.6

The results of this study indicate that the relative bioavailable fraction (RBA_{soil}) of cadmium compared to the cadmium soluble salt (water) was 43% (Schilderman *et. al.*, 1997). Therefore, risk assessment calculations being conducted using 100% RBA_{soil} and the water oral TRV from the US EPA or Health Canada would result in almost two fold unnecessary risk being calculated.

Schroder *et. al.* (2003) conducted an *in vivo* cadmium experiment in order to validate an *in vitro* bioaccessibility method for cadmium contaminated soils. Male swine (5-6 weeks old), weighing 10-12 kg were dosed for 15 days with either control solution, $CdCl_2$ solution or one of 10 Cd contaminated soils. The percent relative bioavailable Cd contaminated soil (based on $CdCl_2$), from the juvenile swine model ranged from 10.4 % to 116%, with an average RBA_{soil} of 63%.

Given the limited data set of these two experiments it is difficult to draw conclusions of the differences of using immature swine models versus a rat model to determine the oral bioavailability of cadmium from soil. However, it is interesting that like arsenic and lead, that even with limited findings the rat model did return an RBA_{soil} 20% less than that in the swine study.

5.2 *In vitro* Oral Bioaccessibility Research for Cadmium in Soil

Three *in vitro* cadmium oral bioaccessibility experiments were found in the literature (Hamel, *et. al.*, 1998; Schroder, *et. al.*, 2003; Oomen, *et. al.*, 2004). In 1998, Hamel *et. al.* conducted the previously reported study to assess the effect of solid to liquid ratio on a number of inorganic contaminants from two soils. The NIST 2710 standard reference material soil contains 21.8 mg/kg cadmium. Using the U.S. Pharmacopeia method (Table 5-3), and a range of solid to liquid ratios of 1:100 to 1:5000 the bioaccessible fraction of cadmium was determined to be 50 $\pm$ 5.8%. No statistical difference was seen between all five liquid to solid ratios in the experiment (Hamel *et. al.*, 1998) (Table 5-2).

The second stage of the Schroder *et. al.* (2003) study was to determine the effectiveness of the Rodriguez (1999) IVG *in vitro* method (Table 5-3) in predicting the relative bioavailable fraction of soil from immature swine studies. The IVG method was conducted both with dough (food) and no dough (without food) with only the gastric phase and the inclusion of an intestinal phase (Table 5-2). In both experiments the cadmium from soil “crashed out” when the pH was raised to 7 in the intestinal phase. This was reported to have been a result of the reduced solubility of Cd in the high pH conditions.

The IVG method in the gastric phase using dough had an overall Cd bioaccessibility of 38.2%, while the IVG gastric phase without dough had an overall Cd mean bioaccessibility of 63%. Linear regressions of *in vitro* phases against the percent relative bioavailable fraction of cadmium in soil were reported. Overall, the IVG method without dough at the gastric phase indicated the strongest relationship to the *in vivo* results (r=0.86). It was concluded that the IVG method showed promise in predicting the relative bioavailability of cadmium from soil.

Table 5-2. Summary of Cadmium Bioaccessibility (*in vitro*) Studies and Results

Author	Database ID	Year	Study / Site	Test Soil ID	Soil Type	Test Group	Soil						*In Vitro* Test Method	Liquid: Solid Ratio (mL:g)	pH stomach	pH Intestine	% Bioaccessible Arsenic		
							Cadmium [ppm]	standard deviation	pH	TOC (%)	grainsize	Minerology					Stomach	Intestine	overall
Hamel	105	1998	NIST Standard	NIST 2710	Reference Material		21.8			3	<74		US Pharm	100:1			51.4±18.5		
			NIST Standard	NIST 2710	Reference Material		21.8			3	<74		US Pharm	200:1			61±19.6		
			NIST Standard	NIST 2710	Reference Material		21.8			3	<74		US Pharm	500:1			50.9±14.6		
			NIST Standard	NIST 2710	Reference Material		21.8			3	<74		US Pharm	1000:1			49.5±11		
			NIST Standard	NIST 2710	Reference Material		21.8			3	<74		US Pharm	2000:1			45.7±8		
			NIST Standard	NIST 2710	Reference Material		21.8			3	<74		US Pharm	5000:1			45±13		
Schroder	99	2003	Various	1	Sieved soil	With dough	465				<250		IVG	150:1	1.8	5.5	34	11.2	
				2	Seived soil	With dough	43				<250		IVG	150:1	1.8	5.5	11.7	4.05	
				3	Seived soil	With dough	26.6				<250		IVG	150:1	1.8	5.5	37.9	9.81	
				4	Seived soil	With dough	188				<250		IVG	150:1	1.8	5.5	28.7	11.2	
				5	Seived soil	With dough	139				<250		IVG	150:1	1.8	5.5	46.8	17.9	
				6	Seived soil	With dough	29.9				<250		IVG	150:1	1.8	5.5	40.4	6.69	
				7	Seived soil	With dough	23.8				<250		IVG	150:1	1.8	5.5	42.9	16.2	
				8	Seived soil	With dough	195				<250		IVG	150:1	1.8	5.5	46.1	16.1	
				9	Seived soil	With dough	319				<250		IVG	150:1	1.8	5.5	46.1	19.5	
				10	Seived soil	With dough	47.4				<250		IVG	150:1	1.8	5.5	47.5	16.6	
Schroder	99	2003	Various	1	Seived soil	Without dough	465				<250		IVG	150:1	1.8	5.5	54.8	43.2	
				2	Seived soil	Without dough	43				<250		IVG	150:1	1.8	5.5	21.3	15	
				3	Seived soil	Without dough	26.6				<250		IVG	150:1	1.8	5.5	75.6	42.9	
				4	Seived soil	Without dough	188				<250		IVG	150:1	1.8	5.5	53.2	33.5	
				5	Seived soil	Without dough	139				<250		IVG	150:1	1.8	5.5	69	55	
				6	Seived soil	Without dough	29.9				<250		IVG	150:1	1.8	5.5	42.1	25.6	
				7	Seived soil	Without dough	23.8				<250		IVG	150:1	1.8	5.5	75	49.2	
				8	Seived soil	Without dough	195				<250		IVG	150:1	1.8	5.5	75.2	38.1	
				9	Seived soil	Without dough	319				<250		IVG	150:1	1.8	5.5	95.9	40.8	
				10	Seived soil	Without dough	47.4				<250		IVG	150:1	1.8	5.5	68.1	47.9	
Oomen et al	108	**2004**	3 reference soils + OECD standardized artificial soil	Flanders - ox bile (sigma)	reference material		14	1				RIVM	100:1	1	8				78
				Flanders - ox bile (ICN)	reference material		14	1				RIVM	100:1	1	8				75
				Flanders - pig bile	reference material		14	1				RIVM	100:1	1	8				78
				Flanders - chicken bile	reference material		14	1				RIVM	100:1	1	8				85
				Oker -11 - ox bile (sigma)	reference material		24	1				RIVM	100:1	1	8				50
				Oker -11 - ox bile (ICN)	reference material		24	1				RIVM	100:1	1	8				52
				Oker -11 - pig bile	reference material		24	1				RIVM	100:1	1	8				50
				Oker - 11 - chicken bile	reference material		24	1				RIVM	100:1	1	8				55
				Montana 2710 - ox bile (sigma)	reference material		21.8	0.2				RIVM	100:1	1	8				55
				Montana 2710 - ox bile (ICN)	reference material		21.8	0.2				RIVM	100:1	1	8				62
				Montana 2710 - pig bile	reference material		21.8	0.2				RIVM	100:1	1	8				61
				Montana 2710 - chicken bile	reference material		21.8	0.2				RIVM	100:1	1	8				64
				Montana 2711 - ox bile (sigma)	reference material		41.7	0.25				RIVM	100:1	1	8				40
				Montana 2711 - ox bile (ICN)	reference material		41.7	0.25				RIVM	100:1	1	8				41
				Montana 2711 - pig bile	reference material		41.7	0.25				RIVM	100:1	1	8				41
				Montana 2711 - chicken bile	reference material		41.7	0.25				RIVM	100:1	1	8				60

Table 5-3. . *In Vitro* Test Method Parameters for Cadmium Bioaccessibility

	Specifics	IVG	Modified Artificial Biofluid	RIVM
Author		**Schroder 2003**	**Hamel et al, 1998**	**Oomen et al, 2004**
Input	**Amount of Soil Added**	4 g dry weight	5mg and 50 mg dry weight	0.6 g dry weight
General	**Model type**	Static gastro-intestinal		Static gastro-intestinal
	Temperature	37°C	37°C	37°C
	Mechanical treatment	Stirring 100 rpm	wrist-action shaker	End-over-end rotation 55 rpm
	Food components	200g dough	No	No
Oral Cavity	**Saliva**	No		Yes
	Volume of saliva			9.0 mL
	pH			6.5
	Incubation time			5 minutes
Stomach	**Gastric compartment**	Yes	Yes	Yes
	Volume of gastric juice	600 mL	100 mL	13.5 mL
	pH	1.8	not specified	1.07
	Gastric secretion components	NaCl, pepsin	NaCl, pepsin	Pepsin, mucin, BSA
	Incubation time	1 hour	2 hours	2 hours
Intestine	**Intestinal component**	Yes	No	Yes
	Volume of intestinal juice	600 mL (unchanged)		27 mL
	Intestinal pH	5.5		7.8
	Intestinal secretion components	Pancreatin, bile extract		Bile, duodenal juice, chime, pancreatin, lipase
	Incubation time	1 hour		2 hours
Output	**Centrifugation**	10 000 rpm	3400 rpm	3000g
	Filtration	0.45 mm filter	Whatman 51 filter papers	No
	Special treatment			
	Acid digestion of fluid	Acidified	HNO_3	Acid digestion microwave
	Analytical method	ICP-AES	ICP MS	ICP MS

In 2004, Oomen *et. al.* published their findings on how four different animal bile preparations impacted on the bioaccessibility of a number of inorganic and organic contaminants (Table 5-2). The bioaccessibility experiments were conducted using the RIVM (Table 5-3) method and four different standard reference materials – NIST 2710, NIST 2711, Oker 11, and Flanders. No significant difference was found using the four different bile types across all soil types. The average bioaccessible fraction of Cd from soil was found to be 79% (Flanders), 52% (Oker 11), 60% (NIST 2710) and 46% (NIST 2711). The overall mean percent bioaccessibility of cadmium from the four soils and all four bile treatments was 59 $\pm$ 14%.

Overall, the bioaccessibility and bioavailability testing that has been reported in the literature for cadmium indicates that it is unlikely that the oral bioaccessibility/bioavailability of cadmium from soil would be 100%. From the in vivo experimentation it appears that somewhere on the order of 50% of soil cadmium is bioavailable relative to the TRV based cadmium in water.

6.0 SUMMARY AND CONCLUSIONS

Over the past ten years there has been considerable research effort around the world on the oral bioavailability and bioaccessibility. The focus of this review was the research that was specifically conducted for arsenic, lead and cadmium bioavailability from soils. The overarching conclusion of both the animal studies (*in vitro*) and the laboratory bioaccessibility tests (*in vitro*) was that the oral bioavailability of arsenic, lead and cadmium from soils from any given contaminated site would not be 100%. Thus, there is merit to considering the bioavailability / bioaccessibility of arsenic from soil when conducting site specific risk assessment in Canada.

However, questions still remain:

Do in vivo animal experiments need to be conducted on a site specific basis?
The cost, significant time requirement (greater than six months) and potential ethical issues surrounding animal testing make it prohibitive to conduct *in vivo* bioavailability experiments on a routine site specific basis.

Has the science of in vitro bioaccessibility experiments advanced to the point where they can be used to provide a valid representation of bioavailability that can be used in risk assessment?
In general the use of *in vitro* tests would appear to be a valid approach for approximating the absolute oral bioavailability of arsenic and the relative oral bioavailability of lead and cadmium from soils. However, more data will be required before *in vitro* methods are routinely applied and accepted in Canada.

Can literature default values be used in human health risk assessment in lieu of site specific soil testing.
Given the number published papers available on the oral bioavailability and bioaccessibility of arsenic and lead from soils, it has led to inconsistent use of values being incorporated into risk assessment across Canada. In addition, it was demonstrated that site specific soil properties do influence bioavailable concentrations. Therefore, use of published data from other sites would not be the preferred method for incorporation of bioavailability into risk assessment.

In the coming years, these and other questions will be the challenge of Bioavailability Research Canada (BARC) and international organizations like Bioavailability Research Group Europe (BARGE). Although a standardized *in vitro* method that would be applicable to all inorganic elements (and organics) at contaminated sites would be ideal, there is likely numerous years of study ahead to develop methods that can be used routinely in human and ecological risk assessment. At this point methods that have consistently demonstrated that they have been validated against *in vivo* models should be considered for use in Canadian risk assessments.

7.0 APPLICATION OF BIOAVAILABILITY IN FEDERAL RISK ASSESSMENT

The following section is an excerpt from the Health Canada $SSRA_{CHEM}$ document, which is in preparation (Meridian Environmental Inc). It also contains information that Health Canada has provided in recent past (by Dr. G. Mark Richardson) with respect to Health Canada's minimum data requirements to be provided if site specific bioaccessibility values are being generated for use in human health risk assessment on sites under federal jurisdiction.

1. A range of soil particle size fractions (such as <45 µm; <125 µm; <250 µm) should be assayed to determine if soil particle size significantly influences measured bioaccessibility at the site in question. If it does not, then soil characteristics based on the <250 µm size fraction, including bioaccessibility, soil-borne contaminant concentration (and other factors) can be employed in estimating exposures and risks. However, in situations where soil particle size is influential, data for the smallest size fraction should be used in the risk assessment.

2. For a subset of assayed soil samples, a range of ratios of simulated gastric fluid (mL) to soil mass (g) must be employed, ranging from 100:1 to perhaps 5,000:1, and possibly up to 10,000:1 if analytical detection limits are not limiting. If measured bioaccessibility is not significantly influenced by this ratio, then further assays for that site can proceed with the standard 100:1 (or other) methodology. However, in situations where measured bioaccessibility is influenced by these ratios, and particularly where bioaccessibility increases as the ratio of simulated gastric fluid volume to soil mass increases, then bioaccessibility adjustments within the risk assessment should be based on assays employing the maximum ratio possible, up to 10,000:1 where feasible.

3. Further to requirement 2, above, statistical analysis of the bioaccessibility data should confirm that contaminant solubility is not confounding the measure of bioaccessibility. Except in rare instances where a ratio of 10,000 mL of gastric fluid to 1 g of soil can be employed, the final data used to determine site-specific absolute bioaccessibility should demonstrate the independence of measured bioaccessibility and the concentration or mass of contaminant in soil being assayed.

4. For application as a bioavailability adjustment factor (BAF) in risk assessment, the absolute bioaccessibility (as directly measured in a bioaccessibility assay) must be adjusted *relative* to the (likely) bioavailability of the contaminant in the key toxicological or epidemiological study upon which the toxicological reference value (e.g. tolerable daily intake or cancer slope factor) was derived. To facilitate the determination of relative bioaccessibility, Health Canada is currently compiling oral bioavailabilities in key toxicological studies upon which Canadian federal TRVs have been established.

CLOSURE

This report has been prepared for the sole benefit of Health Canada. Any use which a third party makes of this report, or any reliance on decisions made based on it, are the responsibility of such third parties. Jacques Whitford accepts no responsibility for damages, if any, suffered by any third party as a result of decisions made or actions taken based on this report.

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This study was undertaken exclusively for the purpose outlined herein and was limited to those substances, exposure pathways, receptors, and related uncertainties specifically referenced in this report. This work was specific to arsenic, lead and cadmium bioavailability/bioaccessibility from soils described herein. The report cannot be used or applied under any circumstances to another location or situation or for any other purpose without further evaluation of the data and related limitations.

If any conditions become apparent that differ significantly from our understanding of conditions as presented in this report, we request that we be notified immediately to reassess the conclusions provided herein.

Yours truly,

JACQUES WHITFORD LIMITED

Original Signature on File	***Original Signature on File***
Siobhan Sutherland, B.Sc., M.A. Risk Assessor	Dr. Christopher Ollson Senior Risk Assessor

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