Attachment 18

- Review on bioavailability of metals and in vitro methods to predict RBA by Alloy, LLC (December 2017)
- Summary of responses to questions based on Alloy LLC review, prepared by Eurometaux

Alloy LLC
All the right elements

Bioavailability of Metals from Soil: Review of In Vitro Methods to Predict RBA

Prepared for:

Eurometaux

Prepared by:

Alloy, LLC
Yvette Wieder Lowney
Boulder, CO

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ACRONYMS

ABA	absolute bioavailability
Alloy	Alloy, LLC
BAc	bioaccessibility
BARGE	Bioaccessibility Research Group of Europe
DIN	Deutsches Institut fur Normung [European and international standards]
IVBA	in vitro bioaccessibility
IVG	in vitro gastrointestinal [test]
IVIVC	in vitro to in vivo correlation
NIST	National Institute of Standards and Technology [US]
PBET	physiologically based extraction test
RBA	relative bioavailability
RBALP	relative bioavailability leaching procedure
RIVM	National Institute for Public Health and the Environment [the Netherlands]
SBRC	Solubility Bioavailability Research Consortium
SGET	simple gastrointestinal extraction test
SHIME	simulator of the human intestinal microbial ecosystem
SRM	standard reference material
TCLP	toxicity characteristic leaching procedure
TIM	TNO gastrointestinal model [Triskelion in vitro dynamic model]
UBM	unified bioaccessibility method

DEFINITIONS OF KEY TERMS

Bioavailability:
"Refers to the portion of the total quantity of a chemical present that is absorbed by a living organism (Klassen 2013), and reaches the central (blood) compartment, whether exposures occur via the GI tract, skin, or lungs (NEPA 2000)" (From ITRC 2017)

Absolute Bioavailability (ABA):
Absolute bioavailability is assessed in animal studies and can be reported in many ways *(e.g., area under the curve (AUC))*. It refers to the fraction of an administered dose of a chemical that is absorbed into systemic circulation following exposure. Below are several definitions used by various organizations.

- "… the fraction of the applied does that is absorbed and reaches the systemic circulation (and can never be greater than 100%)" (NRC 2003).
- "…the fraction (or percentage) of an ingested (or dermally applied, as applicable) dose that is absorbed and reaches systemic circulation" (ITRC 2017).
- "… the ratio of the amount [of chemical] absorbed, compared to the amount ingested: ABA = Absorbed dose/Ingested dose" (EPA 2007 – document refers only to ingestion exposures).
- "…the fraction or percentage of a compound which is ingested, inhaled, or applied on the skin surface that is absorbed and reaches the systemic circulation" (TSERAWG, 2003).
- "…the rate and extent to which a substance can be taken up by an organism and is available for metabolism or interaction with biologically significant receptors. Bioavailability (biological availability) involves both release from a medium (if present) and absorption by an organism (IPCS 2004)" (ECHA Guidance, 2015).
- "…the fraction of an administered dose that reaches the systemic circulation or is made available at the site of physiological activity" (OECD 2008).

Relative Bioavailability (RBA):
The concept of RBA is meant to express the bioavailability of a chemical from one matrix, relative to another matrix; for example, absorption of a metal from soil or alloy relative to the absorption of soluble forms of metal or pure metal. Below are definitions used by various organizations.

- "Refers to the ratio of the absorption of a chemical from soil, relative to the absorption in the exposure medium used in the critical toxicity study (the study that forms the basis for the cancer slope factor or reference dose)." (ITRC 2017 – note document is focused on exposures from soil)
- "A measure of the difference in extent of absorption among two or more forms of the same chemical (e.g., lead carbonate vs. lead acetate), different vehicles (e.g., food, soil, water), or different doses. In the context of environmental risk assessment, relative

bioavailability is the ratio of the absorbed fraction from the exposure medium in the risk assessment (e.g., soil) to the absorbed fraction from the dosing medium used in the critical toxicity study. (TSERAWG, 2003)

- "Represents a comparison of absorption under two different sets of conditions – for example from a soil sample vs. food – and can be greater than or less than 100 percent." (NRC 2003).
- "The ratio of the absolute bioavailability of lead present in some test material compared to the absolute bioavailability of lead in some appropriate reference material: RBA = [ABA test material]/[ABA reference material]" (EPA 2007).

Bioaccessibility:

The term "bioaccessibility" refers to the solubility of a chemical under physiological conditions. It most commonly is used to express solubility of a chemical under simulated physiological conditions (e.g., simulated gastrointestinal fluid), and referred to interchangeably in the published literature as "bioaccessibility," "bioelution," or "in vitro bioaccessibility." It is most commonly reported as the fraction of the total mass of chemical present in the test material that is soluble under the test conditions (i.e., [mass soluble]/[total mass in test material] and cannot be higher than 100%). The bioaccessible fraction may vary depending on test conditions; for example, the bioaccessibility of metals in simulated gastric fluid may be significantly different from the bioaccessibility in simulated intestinal fluid. Some definitions provided in existing documents include:

- "Refers to the results from chemical extraction tests that have been developed to try to simulate or predict the RBA of chemicals from soil. In this context "bioaccessibility" is interchangeable with "in vitro extraction testing," wherein experimental systems have been developed to assess the potential for human exposure to chemicals in soil by capturing a critical component that affects bioavailability." (ITRC 2017)
- "A term for the fractional dissolution of a metal from soil in an in vitro study." (TSERAWG, 2003)
- "The fraction of [chemical] which solubilizes in an in vitro system is referred to as in vitro bioaccessibility (IVBA). These methods are based on the concept that the rate and/or extent of solubilization in gastrointestinal fluid is likely to be an important determinant of bioavailability in vivo…" (EPA 2007).

Relative Bioaccessibility:

Some studies report their findings in terms of "relative bioaccessibility." In these cases, the solubility of the chemical in simulated gastrointestinal fluid is reported in comparison to the solubility from a solid material (e.g., pure metal or alloy), or in comparison to the recovery of a soluble form of the same chemical following processing in simulated gastrointestinal fluid.

Sources for Definitions:

Some references that may be useful for more information:

- Bioavailability of Contaminants in Soils and Sediments: Processes, Tools and Applications. Developed by the Committee on Bioavailability of Contaminants in Soil and Sediments, Water Science and Technology Board, Division on Earth and Life Studies. United States National Research Council of the National Academies. The National Academies Press. Washington, DC. 2003.
- U. S. EPA 2007. Estimation of relative bioavailability of lead in soil and soil-like materials using in vivo and in vitro methods. Office of Solid Waste and Emergency Response. OSWER 9285.7-77.
- Guide for Incorporating Bioavailability Adjustments into Human Health and Ecological Risk Assessments at U.S. Department of Defense Facilities, Prepared for the Tri-Service Ecological Risk Assessment Workgroup (TSERAWG). June 2003
- Bioavailability of Contaminants in Soil: Considerations for Human Health Risk Assessment. Interstate Technology & Regulatory Council, 2017. www.itrcweb.org.
- ECHA Guidance on the Application of the CLP Criteria, version 4.1, June 2015, section 1.3.1.
- OECD Guideline for the Testing of Chemicals, 2008

FIGURES

Figure 1	Schematic representation of data from animal studies of RBA from soil
Figures 2a–2b	Using IVIVC to predict RBA from IVBA data
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TABLES

Table 1	Literature on IVIVC for metals from soil: Studies with in vivo data
Table 2	Literature on IVIVC for metals from soil: Comparison of in vitro methods
Tables 3a–3g	Literature on IVIVC for metals from soil: Antimony, arsenic, cadmium, cobalt, lead, mercury, nickel
Table 4	Summary of data for studies reporting IVBA for NIST standard reference material soils
Table 5	Relative bioavailability and bioaccessibility data extracted from Denys et al. 2012
Table 6	Composition and parameters utilized in common in vitro bioaccessibility assays

ATTACHMENTS

Attachment 1	Cobalt Institute: Submission from REACH dossier
Attachment 2	Synthesis of nickel studies: NiPERA

INTRODUCTION

Alloy, LLC (Alloy) has been retained by Eurometaux to conduct a review of available information to understand the relationship between in vivo bioavailability and in vitro bioaccessibility for metals. Bioavailability is measured based on results from animal (i.e., in vivo) studies. Studies performed in vitro (i.e., bioaccessibility or bioelution tests) can provide a measure of the solubility of metals under specific laboratory conditions and have been evaluated for their ability to provide an estimate of bioavailability. While bioavailability and bioaccessibility data can be used in many different applications, there is a broad base of research to understand the bioavailability of metals from soil, because this information forms a part of the risk assessment process that dictates the need for remedial action at contaminated sites. Findings from these studies of soil, including evaluation of in vitro extraction testing to predict bioavailability, could significantly advance our understanding of how bioelution can predict (or provide a conservative estimate of) the bioavailability of metals from alloys. Incorporating considerations of bioavailability into the human health risk assessment process allows for less uncertainty in understanding the potential for adverse health risks.

The goals of this work were, first, to provide an evaluation of the available data for evidence on the nature of the relation between in vitro extraction tests and in vivo bioavailability of metals for studies of paired samples, and second, understanding what components or conditions of in vitro methods affect the results of extraction testing, with the goal of understanding what extraction conditions provide conservative estimates of bioavailability. For this effort, 38 peer-reviewed studies published in the open literature were reviewed, of which 21 contained data for evaluations conducted both in vivo and in vitro and an additional 17 presented data from in vitro extraction testing of one or more soils. Some additional information from unpublished reports is included and indicated as such.

Background

A focus of bioavailability research for contaminated soils is to understand how well metals are absorbed from soil relative to absorption of the soluble metal compound dosed in the toxicity study that forms the basis of health-based criteria—such as slope factors for oral carcinogens and reference doses for noncancer systemic endpoints of toxicity. The results of this research are expressed as relative bioavailability (RBA) adjustment factors, in that they specifically describe the relative nature of absorption from soil in comparison to absorption of the soluble dose from toxicity studies.

$$RBA = \frac{absorption\ from\ soil}{absorption\ from\ soluble\ metal\ compound\ dosed\ in\ toxicity\ study}$$

The data that support RBA values are generated using animal studies—most commonly of swine or rodents, and occasionally of primates. Figure 1 provides a schematic representation of the type of data from animal studies that might be used to derive an RBA.

However, studies of RBA conducted with animals are expensive, time consuming, and may raise ethical concerns. Therefore, laboratory methods have been developed to provide a more efficient and inexpensive mechanism to estimate RBA. These methods rely on laboratory extraction testing of soil to assess the fraction (or percent) of total chemical released, and the results are referred to interchangeably in the published literature as

- bioelution,
- bioaccessibility (BAc), and
- in vitro bioaccessibility (IVBA).

These in vitro approaches include many different methods, generally intended to mimic conditions of the gastrointestinal tract (i.e., physiologically-based), and generally report results as the percent of metal released from the test substrate. For regulatory acceptance, research and development generally fall into one of three categories:

- in vitro extraction methods that are argued to be most physiologically representative of the human gastrointestinal tract,
- methods that are argued to include components that make them worst-case estimates of RBA, or
- methods that include a "validation" against animal data, where paired sets of soils are studied in animals as well as under laboratory extraction conditions (i.e., in vitro to in vivo correlation [IVIVC]).

In vitro bioaccessibility test protocols attempt to predict the extent to which metals are released into solution and hence become available for uptake, but are not capable of mimicking other processes that affect absorption. Therefore, in the IVIVC process (depicted schematically in Figure 2a), paired samples of contaminated soil are fed to animals to establish RBA and are also tested with in vitro extraction methods. The data are then compared to determine whether a predictive relationship exists, generally through regression analysis. The goal of such an effort is to optimize the extraction method to create an IVBA that most closely forms a predictive relationship with RBA (generally measured as a linear correlation). If such a relationship can be established, then in vitro methods can be considered to be representative, or predictive, of RBA, and the regression equation is used to "translate" IVBA data into a prediction of RBA (Figure 3). Many regulatory jurisdictions rely on this IVIVC approach to assess the validity of IVBA methods, including in the United States, Canada, Australia, and within Europe (EPA 2017; Health Canada 2006; Naidu et al. 2015; BARGE 2016). A more complex approach to developing an IVIVC incorporates variability in both the measured IVBA and RBA values, in which case the variability can be incorporated into the model to derive prediction limits for the IVIVC. Examples of this are provided in EPA (2007), Denys et al. (2012), and Diamond et al. (2016). An excerpt from Diamond et al. 2016 is included herein (Figure 2b).

Because an IVIVC is based on a regression analysis, it does not assume a 1:1 relationship between RBA and IVBA. If a 1:1 relationship between RBA and IVBA exists, then the slope of the regression line is unity (i.e., slope = 1). However, a very strong predictive relationship can be established for data where the slope of the line diverges from unity; a slope <1 indicates that, on average, the IVBA estimate is higher than the RBA estimate. Conversely, a slope >1 indicates that, on average, the RBA values exceed the IVBA values[1]. Therefore, the IVBA data may not be used directly to predict RBA, but rather the equation that explains the regression line is used to "translate" IVBA data into a prediction of RBA. This process is depicted in Figure 3.

It is important to note, however, that while the goal of the IVIVC process is to identify the IVBA method that is *most predictive* of RBA, it may rely on an IVBA method that does not necessarily result in the *most conservative* results. Therefore, in other circumstances (e.g., where animal testing is not considered ethically appropriate, or where RBA data are not available for a particular chemical), IVBA methods may be developed with the specific intention of generating worst-case conditions that are then assumed to overestimate bioavailability from human exposures.

[1] Note: this assumes that the in vitro data are plotted on the x-axis, and the in vivo data are plotted on the y-axis.

In summary:

$$IVBA = estimate\ of\ the\ soluble\ fraction\ that\ may\ be\ available\ for\ absorption$$

- ✓ *many methods exist*
- ✓ *various levels of complexity*
- ✓ *may or may not be physiologically-based*
- ✓ *may or may not be validated against animal data.*

Review and Report

The primary focus of this review was on those studies with data both on in vivo bioavailability (i.e., RBA) and in vitro extraction test results (i.e., IVBA) for the same soils. Comparison of RBA and IVBA provides an indication of how well the extraction tests predict RBA for target metals (e.g., bioavailability of metal from soil compared to bioavailability of metal from a water-soluble metal compound). In some instances, it may also be possible to assess whether the extraction tests provide a conservative estimate of absolute bioavailability (ABA; percent of ingested dose that is absorbed).

A second component of this evaluation was to spotlight insights that might be gleaned from studies comparing results for different IVBA methods. This form of comparison allows identification of factors that affect dissolution under simulated conditions of the human gastrointestinal tract. Factors that are commonly investigated include pH, temperature, contact time, agitation, particle size, the ratio of soil to extraction fluid, and presence of surfactants or other extraction fluid components.

Together, RBA and IVBA studies were reviewed to assess whether they could provide information to address specific questions for the target metals of interest:

- Are data available regarding the relationship between in vivo bioavailability and IVBA?
- What is the nature of this relationship, if present?
- Does IVBA provide a conservative (i.e., overpredicted) estimate of bioavailability?
- Are there instances where IVBA underestimates RBA?
- How does extraction into simulated gastric fluid compare to extraction into simulated saliva or intestinal fluid?
- What does research tell us with regard to bioaccessibility (in simulated gastric fluid) of a metal from an alloy relative to that of a pure metal?
- In simulated gastric fluid, does bioaccessibility of a metal from one sample relative to a standard (e.g., soluble metal compound) reflect RBA in vivo?

The attached tables provide a synthesis of the information extracted from the literature and include details on the study authors, methods, and results. In some instances, data from a published study was extracted by Alloy, to allow for evaluations beyond what is provided in the publication (in such cases, the respective tables or figures indicate the source of the data). Many, if not all, of the individual studies have some limitations; however, the effort undertaken herein was not intended to provide a detailed critical review of each study. Instead, Alloy mined each study for what each can provide regarding the ability to predict bioavailability of metals from rapid laboratory extraction tests. Collectively, these studies provide a weight of evidence with regard to conclusions that can be made about the role of IVBA methods in predicting RBA.

This evaluation focused on specific target metals: **arsenic, cadmium, cobalt, lead, mercury, nickel, selenium, beryllium, and antimony**. The majority of studies included in this review have evaluated metal compounds (as opposed to pure metals), either as the compounds are present in contaminated soils, or as pure compounds. Metals in soils will reflect the source as well as geochemical characteristics of the soil. Bioavailability of metals from soil can depend on several factors, including the mineralogic form of the metal present, particle size, and matrix effects (Ruby et al. 1999). Tables 1 and 2 provide a summary of the various studies reviewed, organized by those that include data from animal studies (Table 1), and those that include only data from in vitro extraction testing (Table 2). Tables 3a–3g provide a summary of information for each of the target metals except selenium and beryllium, for which no studies were identified that provided relevant data.

In addition to the detailed summary tables, figures have been provided that illustrate the findings of some specific studies, including excerpts or reanalysis of data.

Some studies include data from evaluation of the same standard reference material (SRM). These SRMs are soils for which concentration data are standardized and reported as certified values. When tested in different laboratories and under different conditions, data from extraction testing of SRMs provide information that can be compared across studies for a particular soil. These data are compiled and presented in Table 4. The relationship between IVBA and the pH of the extraction fluid used in these studies, depicted in Figure 4, suggests that low pH provides worst-case extraction conditions.

The tables and figures included in this report form the majority of the project work product. A further description of how the tables are organized, a brief explanation of what is reported in the tables, and general trends that emerge from this information are included in the sections below.

DATA SUMMARIES FOR IN VIVO AND IN VITRO STUDIES OF BIOAVAILABILITY OF METALS FROM SOIL

Alloy extracted information from 39 studies, summarized in Tables 1 and 2, including whether the study included animal data, what specific metals were included in each study, what type of IVBA extraction methods were used, the pH of the various IVBA extraction fluids, the nature of the predictive relationship between RBA and IVBA, and answers to the questions bulleted above. Of the 39 studies reviewed, 22 included information on both RBA as measured in animals (mouse, rat, and swine animal models) and IVBA measured in laboratory extraction tests (Table 1). The IVIVC relationships investigated in the studies are indicated in the table, and may include comparisons based on one or several IVBA methods. Occasionally, data from IVBA extraction tests are compared to results of feeding trials performed with human volunteers. These are also listed in Table 1; however, no IVIVC is provided because these studies do not include enough test soils to support a statistically meaningful relationship between RBA and IVBA. Table 2 contains summaries of 17 studies that only provide data from in vitro extraction testing.

Studies That Include Animal Data

Data from the 22 reviewed studies that included assessment of RBA from soil in animal studies is summarized in Table 1. Overall, there is an abundance of information on the RBA and IVBA for both lead and arsenic, including the evaluation of IVIVC relationships and the comparison of how different IVBA methods perform. Eight studies summarized here included arsenic in soil, and nine studies included lead in soil. There is a focus on arsenic and lead (arsenic is a metalloid, but will be referred to as a metal in this document) because these two chemicals drive risk-based remedial decisions at many large contaminated sites. Fewer studies are available for other metals; nickel is evaluated in seven studies and cadmium is evaluated in four, including two studies that included them in a broad set of metals investigated.

More details regarding what conclusions can be drawn from these studies are provided below, in the discussion of specific metals. More generally, across all studies and all metals evaluated, the data form strong evidence that soil- and site-specific factors exert control on the bioavailability of metals from soil relative to bioavailability of soluble forms. Strong predictive relationships between RBA and IVBA emerge for lead and arsenic, but there are fewer available data for the other metals.

The current paradigm for validating in vitro methods for predicting RBA of chemicals from soil is very specific. This is discussed above, and Figure 3 illustrates the concept. The goal in the IVIVC process is to find a statistical relationship between in vivo and in vitro data, and define that

relationship so that it can be used to translate in vitro data and predict RBA. As described above and depicted in Figures 2a and 2b, this is generally done through application of linear regression (simple or weighted to account for variability in IVBA and RBA). By definition, several individual points will fall above the regression line, and several will fall below it. Assuming that IVBA is on the *x*-axis and RBA is on the *y*-axis, data points above the line mean that RBA is greater than IVBA. The inverse is true for points below the line.

For studies listed in Table 1 where an IVIVC and regression equation are provided, the slope of the regression line is nearly always less than unity (i.e., slope is <1.0). This indicates that, on average across the data set for which the IVIVC was developed, the IVBA methods that were used extract a higher percentage of metal from the soil than is absorbed when the same soil is fed to an animal (not in absolute terms but relative to what is absorbed from soluble metal compounds). For predictive purposes, the regression relationship allows for use of IVBA to predict RBA. For precautionary purposes, what is of greater interest is whether IVBA exceeds RBA consistently, irrespective of whether a more refined predictive relationship exists. This would be represented by data points from paired samples falling below the "line of unity" depicted in Figure 2a.

The relationship between IVBA and ABA is not directly evaluated in this literature. ABA depends on the extent of absorption of soluble forms of each metal. RBA explains absorption relative to an index, but does not provide information about ABA[2]. For example, the ABA of lead is assumed to be approximately 50% of an orally administered dose (EPA 1994). Therefore, an RBA for lead of 100% means an ABA for lead from that sample of 50%, and an RBA for lead of 50% means an ABA for lead from that sample of 25%. The ABA of soluble metals from the gastrointestinal tract varies considerably depending on the particular metal. For example, estimates for absorption of soluble arsenic are near 100% (Roberts et al. 2007), while estimates of cadmium absorption after oral exposures range from 1% to 10.6% (ATSDR 2012).

The results summarized here are expressed as reported in the published studies. Values reported as RBA have not been adjusted to reflect ABA.

Table 1 includes an assessment of whether the IVBA methods evaluated provide a conservative estimate of RBA (i.e., IVBA > RBA if directly compared, rather than interpreted through the regression relationship). What the studies indicate is that even if IVBA is predictive of RBA for

[2] Conceptually, an RBA *could* be higher than 100%. This would occur if the form administered is more bioavailable than the form of metal dosed in the critical toxicity study. For metals this is very unlikely, because soluble forms of metals are used in the toxicity tests. An RBA higher than 100% may also indicate a study design problem with the reference dose group; for example, this has been observed when the soluble control dose was administered as a mixture of metals that caused precipitation and hence an inaccurate estimate of the administered dose in the reference dose group.

most soils, in nearly all instances—for all metals, all animal research models, and all in vitro methodologies evaluated—there are individual soils for which RBA exceeds IVBA. The frequency with which RBA exceeds IVBA varies considerably between metals and between studies, and is discussed in the metal-specific summaries provided in Tables 3a–3g.

This is illustrated by results from research published by Denys et al. (2012). This research is cited by the Bioaccessibility Research Group of Europe (BARGE) as providing in vivo validation of the unified bioaccessibility method (UBM) in vitro extraction method for predicting the RBA of arsenic, cadmium, lead, and antimony from soils (BARGE 2016). The RBA of the four target metals was evaluated in swine based on several measurement endpoints (including measurement of metals in kidney and liver tissue, bone, and urine). Although the publication by Denys et al. only provides example plots of IVIVC, both RBA and IVBA data are available for each metal evaluated; therefore, data have been extracted from the study (Table 5) and plots of bioaccessibility vs. RBA as reported by Denys et al. (2012) for lead, cadmium, arsenic, and antimony, respectively, have been plotted by Alloy (Figures 5a–5d). The line best fit by simple regression is indicated in the figures, and the line of unity is superimposed on the data. All points above the line of unity indicate samples for which RBA > IVBA, using the UBM for in vitro evaluations. The results indicate that, generally, it is reasonably conservative to assume that IVBA is predictive of RBA for most soils represented in this study. As depicted in the publication by Denys et al. (2012), and in the summary of data presented in Table 5 of this report, taking into account variability in RBA and IVBA estimates further supports the IVBA data as reasonable estimates of RBA in this study.

The summaries provided in Table 1 and Tables 3a–3g include information regarding how often RBA exceeds IVBA, if at all.

Another general finding from review of the full collection of studies in Table 1 is that there is only one published study where the RBA or IVBA of an element in metallic form was evaluated. In this study, the absorption (i.e., RBA) or dissolution (i.e., IVBA in water or saline) of pure nickel metal and nickel compounds were compared (Ishimatsu et al., 1995). Consequently, this database will not significantly inform our understanding of how methods compare for metals in complex materials vs. pure metal forms.

While metals in soils or various metal compounds were included in studies, no studies identified characterization of metal alloys. It might be expected that IVBA would be as predictive of the RBA of metal from alloys as from soils, since oral absorption of metals from alloys or soil depends on the bioaccessible fraction. Additionally, taken together, the results from in vitro extraction testing indicate that extraction fluids with low pH (e.g., in simulated gastric fluids) nearly always result in dissolution of metal from soil that is equal to or greater than dissolution at higher pH (e.g., in simulated intestinal fluid); only mercury demonstrated

evidence of higher dissolution with IVBA methods that use higher pH (Safruk et al. 2015, Welfringer and Zagury, 2009). This pH dependence is particularly strong for lead and less pronounced for arsenic. This was also observed in studies that included no animal data, as discussed further below.

Studies That Include Only In Vitro Data

Several studies were identified that do not provide an IVIVC with animal data, but do contain information from in vitro testing of soils, which may be informative for understanding how various in vitro methods perform and what components of the IVBA methods affect extraction of metals from soil. Additionally, while IVBA data do not directly address a biological effect, they can provide an estimate of relative bioaccessibility of metals from different materials, and hence allow for a refinement of the bulk concentration of a metal in a mixture by considering the fraction that can become bioavailable.

Table 2 provides a summary of the studies with only in vitro data, including information on how many soils were included in each evaluation (the number of samples investigated in an individual study ranged from 1 to 110), the different in vitro extraction methods employed in each study, which metals were evaluated (some include only one metal, while other studies report results for up to eight metals), and any major conclusions that can be drawn. Table 6 provides a summary of the components of several different IVBA methods that are used in these assessments.

Some studies report findings for one in vitro method applied to many soils and/or many metals, while others compare different in vitro methods for one or more soils and one or more metals. Of course, these studies provide no information regarding the ability of an IVBA to predict (or overpredict) results from animal studies. A clear trend that emerges from these studies is that extraction testing performed at lower pH values, such as found in simulated gastric fluid, generally results in higher measures of the bioaccessible fraction of metals from soil. Some exceptions occur, particularly for arsenic and mercury; for arsenic, one study reports that IVBA is higher after extraction with higher-pH simulated intestinal fluid (Meunier et al, 2010). This contradicts several other studies that indicate that the solubility of arsenic is either higher at lower pH, or similar irrespective of the pH of the extraction fluid. For mercury, only two studies report IVBA, and both of these indicate higher bioaccessibility in simulated intestinal fractions (Safruk et al. 2015, Welfringer and Zagury, 2009), but offer no explanation of why mercury would behave differently from other metals in this regard.

This trend for higher bioaccessibility with lower pH is well demonstrated by Koch et al. (2013). This study investigates only one soil (National Institute of Standards and Technology SRM 2710) but includes results from extraction testing by several different laboratories, using a multitude

of IVBA methods, including both simulated gastric and intestinal phases. A summary of some of the data reported in this study has been excerpted and provided in Figure 6, which plots the effect of pH vs. measured bioaccessibility for arsenic, cadmium, copper, lead, and zinc, and the associated linear regression line for each metal. Koch et al. interpret their findings to indicate that the "negative correlations, meaning that percent bioaccessibility decreases with increasing pH, were confirmed for all elements," and that "the strongest correlations (highest R values) were statistically significant and occurred for lead, zinc, and cadmium." The magnitude of the negative relationship between bioaccessibility and pH (i.e., how much bioaccessibility increases as pH decreases) was most pronounced for lead and cadmium, and slightly less pronounced for arsenic, copper, and zinc.

This is further demonstrated by Koch et al. (2013), as presented in Figure 7, which includes data for measured arsenic and lead bioaccessibility for each IVBA method, organized by whether the data are from gastric- or gastrointestinal-phase extractions. Relative bioaccessibility in Koch et al. represents bioaccessibility corrected for recovery (i.e., the ability of the laboratory to recover soluble metal spiked into the extraction fluid). This figure shows how many of the IVBA methods generate bioaccessibility values that underestimate the relative oral bioavailability measured in animals (represented by horizontal lines). Importantly, the figure also illustrates that simple extraction methods that use hydrochloric acid at low pH can yield the most conservative measures of bioaccessibility. The highest reported bioaccessibility values are for either the relative bioaccessibility leaching procedure (RBALP) method (pH 1.5 HCl, buffered with 0.4 M glycine) or the Health Canada method (HC-HCL, which is simply 0.07 M HCl at pH 1.5) (Koch et al. 2013).

All of the summary tables (i.e., Tables 1–3) include a column specifically identifying whether a particular study addresses the question of how bioaccessibility in gastric fluid compares to other fluids.

As discussed above, an additional source of information about the effect of pH on measured bioaccessibility comes from data on SRMs (soils and dusts). SRMs have been included in several studies and hence have generated data when assessed under a variety of extraction methods. Different SRMs are included in different studies, and no study evaluated all SRMs. Table 4 provides a summary of the studies that provide data on one or more SRM, including the metals reported and the pH of the extraction method used. Figure 4 provides a graphical representation of these data and illustrates the clear trend of the inverse relationship between IVBA and pH for all metals considered (i.e., bioaccessibility decreases as pH increases).

Studies Organized by Specific Metal

Tables about specific metals are provided in this report to summarize studies for each of the following metals: antimony, arsenic, cadmium, cobalt, lead, mercury, and nickel (Tables 3a–3g). Beryllium and selenium were identified as metals of interest for this project, but no relevant data were identified, so they are not included among the metal-specific tables. Studies that include several metals are noted separately on each relevant metal-specific table. The level of detail of the available data depends on several factors, including importance in contaminated site investigations, analytical challenges (e.g., mercury), and the availability of animal models. The available data on lead and arsenic far exceed the amount available for other metals. A summary of studies for each metal is presented alphabetically, below and in Tables 3a–3g.

Antimony

The studies that provide information on antimony are summarized in Table 3a. As indicated there, only two of the reviewed studies include data for antimony; one with that includes RBA data from in vivo evaluations (Denys et al. 2012), and one that investigated the extraction of metals from dust samples with a simple HCl-based extraction (LeBot et al. 2010). When assessed for RBA, most measurement endpoints did not provide reliable data. The strongest RBA data were based on urinary excretion measurement endpoint, and suggest very low RBA of antimony for all soils tested. Bioaccessibility values were similarly low, but no strong predictive relation was identified (as assessed by R^2 <0.3). This is depicted in Figure 5a. Thus, the information available on antimony is too limited to yield a good understanding of the ability of IVBA to predict RBA.

Arsenic

In contrast to antimony, many of the reviewed studies provide information on the RBA and IVBA of arsenic in soil, eight of which include animal data, and 12 with data from IVBA extraction tests under a variety of conditions (Table 3b). Over 100 soils have been evaluated for arsenic RBA and IVBA (an exact count is difficult to decipher, because several of the studies investigate the same soils, either using different methods or as part of meta-analyses). Unlike most of the metals considered in IVBA investigations, the results from gastric extractions can be similar to results from extractions using gastric+intestinal phases. Figure 5b depicts the finding of similar IVBA results for arsenic in gastric or gastric+intestinal fluid extractions performed by Denys et al. (2012). One study (Qin et al. 2016) report higher IVBA using gastric+intestinal, but this study uses only two soils, and are in contrast to results from Koch et al. (2013, presented in Figure 6), and Brattin et al. (2013), which demonstrate higher IVBA at lower pH across several soils. Brattin et al. do show that the magnitude of the pH effect can be very high for some soils (IVBA changes from 90% to 20% when pH changes from 1.5 to 7.5), but very subtle for others (IVBA varies only slightly across the pH range of 1.5 to 7.5). Importantly, it is IVBA methods at

low pH that appear to have a more consistent predictive relationship for RBA of arsenic. Two studies (Li et al. 2015; Juhasz et al. 2009) performed IVIVC evaluations using several IVBA methods, each conducted at low (gastric) and high (intestinal) pH. Results from Li et al. indicated that the correlation between IVBA and RBA was better at low pH for four of the five IVBA systems investigated, and results from Juhasz et al. indicate the correlation is better at low pH for three of the four IVBA methods investigated. Another pattern that emerges from the summary of studies is that simple extraction fluids (e.g., those that consist of a simple glycine-buffered system, or even an unbuffered HCl solution) tend to result in higher measured IVBA than systems that include additional components to make the extraction fluid mimic physiological conditions more closely. This point was illustrated in the research by Koch et al. (2013), as discussed above (Figure 7). But similar results were reported in Juhasz et al. (2009) and Meunier et al. (2010).

Cadmium

For cadmium, the reviewed studies provide information on RBA in three studies, and IVBA in a total of seven studies (Table 3c). Although Koch et al. (2013) present some RBA data from swine and mouse studies, those are limited to lead and arsenic, and do not include RBA data for cadmium. The RBA studies include data from evaluations conducted using mouse and swine research models. Four studies report a clear trend of higher IVBA for gastric-phase extractions when compared to extraction in gastric+intestinal–phase IVBA systems. Two studies (Denys et al. 2012; Kang et al. 2016) report similar IVBA for gastric or gastric+intestinal conditions, including a report of slightly higher IVBA in the higher pH for one soil (Kang et al. 2016). The cadmium data from Denys et al (2012) are plotted in Figure 5C. Taken together, the studies suggest that lower pH extraction conditions provide a more conservative evaluation of IVBA. For assessing whether IVBA is a conservative assessment of RBA, the study results disagree. The weight of evidence would suggest that IVBA is a reasonably conservative predictor of RBA (i.e., based on the 26 soils studied by Denys et al. [2012] and Schroder et al. [2003] vs. the results for one soil reported by Kang et al. [2016]).

Cobalt

Very limited data are available regarding the RBA or IVBA of cobalt from soils (Table 3d). Only one published study provided data on cobalt (Koch et al. 2013); and although this study investigated seven IVBA methods for other metals, for cobalt it included IVBA testing in the gastric phase with five methods and only one method for gastric+intestinal–phase IVBA. The limited data from this study suggest that for cobalt, gastric-phase extraction results in IVBA that is approximately twice that found with methods using a higher, intestinal-phase pH.

Unpublished data from the Cobalt Institute have been submitted as part of a “Registration, Evaluation, Authorisation and Restriction of Chemicals” (REACH) dossier (Attachment 1).

Information provided includes a bioaccessibility study of four cobalt compounds, evaluation of oral RBA for $CoCl_2$ and Co_3O_4, and acute toxicity data for cobalt, $CoCl_2$, Co_3O_4, and CoS. Taken together, the data indicate that the bioaccessibility of cobalt compounds can vary considerably (from 0.2% to 86%) and that this solubility appears to be reflected in bioavailability and associated toxicity. The compound Co_3O_4 had 0.2% bioaccessibility relative to $CoCl_2$, and 1% RBA relative to $CoCl_2$. Acute toxicity was also reduced for the less soluble compounds, but the exact relationship cannot be deciphered because the dosing was truncated at high (not acutely toxic) doses.

Lead

Several of the reviewed studies included data for lead, including eight studies that assessed both RBA and IVBA, and an additional seven that compare IVBA methods (Table 3e). Nearly all studies identified that gastric-phase IVBA was higher than intestinal-phase IVBA performed at higher pH, and that lead dissolution from soil appears particularly sensitive to pH, even in comparison to other metals. This pH dependence is also illustrated in Figure 6, where lead demonstrates the strongest effect of pH on IVBA of all the metals investigated by Koch et al (2013). Four studies listed in Table 3e include RBA and IVBA data for multiple soils (Denys et al. 2012; Dong et al. 2016; Juhasz et al. 2016, and Li et al. 2014). Of these, Li et al. and Juhasz et al. conclude that extraction with low pH provides a conservative estimate of RBA, Denys indicates that extraction with low pH provides a reasonable or conservative prediction of RBA (data also plotted and presented in Figure 5d), and the meta-analysis by Dong et al. indicates that IVBA extraction at pH 1.5 overestimates RBA for about 50% of soils.

Mercury

Only limited data are available from the studies that investigated the RBA or IVBA of mercury from soils (Table 3f). Only one study includes any data from in vivo studies (Safruk et al. 2015), and that study compares IVBA on 50 soils against limited biomonitoring data, with no paired data for soils texted in vitro and in vivo. Two additional studies provide data for IVBA testing; the first evaluates only a gastric-phase extraction and concludes that data from sequential extraction fractions can represent the bioaccessible fraction (Hagan et al. 2014). A second study presents IVBA data for two extraction methods (Welfringer and Zagury 2009), used to assess five soils. Results are too limited to draw broad conclusions, but do indicate that IVBA is higher following intestinal-phase extractions in comparison to gastric-phase. Schoof and Nielsen (1997) provided a review paper that suggested a research program that would provide meaningful data to assess RBA and IVBA of mercury in soils. Despite subsequent research, many of their recommendations remain unaddressed, and the available literature is too limited to draw firm conclusions regarding the ability of IVBA to predict RBA for mercury in soil.

Nickel

Eleven studies were identified that provide information on the RBA or IVBA of nickel, and are summarized in Table 3g. Seven studies provide data from animal studies, of which six provide information on both animal and IVBA investigations. An additional four provide only IVBA data. Information on whether IVBA provides a conservative estimate of RBA are mixed. A study by Kang et al. (2016) suggests that IVBA under predicts RBA, however this study is limited to a single soil and appears to suffer from some issues in design or interpretation. A poster presented by Dutton et al. (2016) suggests that IVBA significantly overpredicts RBA, but details of the study are lacking. Vasiluk et al. (2011) indicates that IVBA is a conservative estimate of RBA for about half of the soils investigated. Two companion studies by Henderson (2012a, 2012b) provide an investigation of the IVBA and acute toxicity of 12 samples, including 11 nickel compounds. The results indicate that the solubility of the samples ranged widely (from <1% to >90% after two hours in simulated gastric fluid), that dissolution in simulated gastric fluid (pH 1.5) was much higher than dissolution in simulated intestinal fluid (pH 7.4) for all samples, and that LD50 values were clearly and negatively correlated with IVBA (i.e., higher LD50 for samples with lower IVBA). Ishimatsu et al. (1995) similarly establish a relationship between nickel solubility and the absorbed fraction of nickel, based on eight forms of nickel compounds. In this study, solubility was investigated in saline or distilled water. In all cases, solubility was higher in saline than distilled water, absorbed dose in rats reflects the nickel solubility, and the authors conclude that “a correlation between the estimated absorbed fraction of each Ni compound and its solubility was observed.” The results from the publications by Henderson et al. and Ishimatsu et al. have been compiled by the Nickel Producers Environmental Research Association (NiPERA) into an unpublished report (Attachment 2), allowing a comparison of nickel releases in gastric and intestinal fluids with oral absorption values.

Based in the information provided, the exact relationship between RBA and IVBA cannot be fully deciphered. Taken together, however, the available information on nickel indicates that the solubility of nickel is predictive of an absorbed dose, including for saline and simulated gastric fluid. The studies consistently identify that gastric extraction conditions result in higher IVBA estimates when compared to extractions conducted at higher pH.

SUMMARY

This review provides a summary of 39 studies from the published literature regarding the bioavailability and bioaccessibility of metals from soil. Some unpublished results of quality data are also included. These studies were evaluated to assess how they might inform an understanding of the relation between in vitro and in vivo estimates of bioavailability, what conditions of an in vitro method favor higher dissolution of metals from soil, and whether in vitro data can provide a conservative estimate of bioavailability. It is important to differentiate between ABA and RBA. There are very few studies that have assessed the ability of IVBA to predict in vivo ABA (e.g., see Appendix 2); the majority of the studies reviewed here have reported on the relationship between IVBA and RBA.

This review does not provide a critical evaluation of strengths and limitations of each study, but rather focuses on identifying useful information from each study, and the author's conclusions regarding bioavailability and bioaccessibility of target metals. Each of the studies, individually have limitations. One of the goals in providing a broad review is to assess whether, taken together, the studies provide adequate "weight of evidence" to derive conclusions on broad trends that appear regarding the nature of the relationship between in vitro and in vivo estimates of bioavailability.

This effort included 22 studies that contain data on both RBA as measured in animal studies and IVBA as measured with in vitro methods. Seventeen additional studies investigated in vitro measures for a variety of metals, soils, and in vitro extraction methods. These data sets provide a representation of the information available to inform the questions of interest for this project.

The attached tables and figures provide summary of relevant information from each paper, with a focus on information that addresses target questions:

- Are data available regarding the relationship between in vivo bioavailability and IVBA?
- What is the nature of this relationship, if present?
- Does IVBA provide a conservative (i.e., overpredicted) estimate of bioavailability?
- Are there instances where IVBA underestimates RBA?
- How does extraction into simulated gastric fluid compare to extraction into simulated saliva or intestinal fluid?
- What does research tell us with regard to bioaccessibility (in simulated gastric fluid) of a metal from an alloy relative to that of a pure metal?
- In simulated gastric fluid, does bioaccessibility of a metal relative to a standard reflect RBA in vivo?

Additionally, the summary allows for identification of data gaps that may exist for specific metals.

Details of the evaluation are provided in the attached tables, and primary conclusions are discussed above, organized by the specific metals investigated. A few broad conclusions can also be identified from assessing the information as a whole.

- Information is completely absent for two of the metals of interest, beryllium and selenium.
- The database on arsenic and lead include many studies that assess both RBA and IVBA for paired soil samples, allowing for identification of a strong predictive relationship (assessed via linear regression). On average, across soils tested, IVBA tends to overpredict RBA (as indicated by a regression line with slope <1), but for individual soils, IVBA may not provide a conservative estimate of RBA. For other metals, few studies are available that directly compare results for RBA and IVBA of paired samples.
- For all metals evaluated, in nearly all studies reviewed, there are instances where the average IVBA under predicts average RBA, for at least some samples[3].
- For cadmium, cobalt, lead, and nickel, higher measures of IVBA are achieved with lower pH conditions, and this effect of pH outweighs other components of the extraction fluid that are intended to create a more "physiological" environment. For arsenic, solubility from soil appears to have a lower dependence on pH, although for some soils, low pH is associated with higher IVBA, and the low pH extraction methods are more closely correlated with RBA when studied with paired soils. Conversely, the IVBA of mercury is higher at higher pH.
- These studies provide no information regarding bioavailability of metals from soils in comparison to bioavailability from pure metals.
- Nearly all studies, conducted in vivo or in vitro, provide a comparison of RBA or IVBA against a water-soluble standard. Individual studies fail to include this basis of comparison, but they are generally included because data on the soluble forms of the metals are required in order to assess *relative* bioavailability, or to establish good analytical recovery under in vitro conditions.

As described in the introduction and in the metal-specific discussions above, there is a large body of evidence supporting a strong predictive relationship between RBA and IVBA for lead and arsenic. Regulatory agencies have used the available information to provide formal guidance (e.g., EPA 2017; CalEPA 2017) or technical reports (e.g., BARGE 2016; Health Canada

[3] This does not necessarily imply that IVBA under predicts ABA (e.g., see discussion on page 7).

2006; New Zealand Ministry for the Environment 2016; RIVM 2017) on the use of IVBA methods to predict the RBA of these contaminants in soil. Hence, IVBA data are already being used in the context of soil remediation based on human health risk assessment for lead and arsenic. Some reports and regulatory jurisdictions also support the use of IVBA methods for other metals such as cadmium and antimony (e.g., BARGE 2016; Health Canada 2006; New Zealand Ministry for the Environment 2016). In each case, a review of the available information was conducted; specific methods were proposed for IVBA testing and data interpretation, but generally there is increasing acceptance of IVBA methods to provide an efficient and effective method for predicting the bioavailability of metals.

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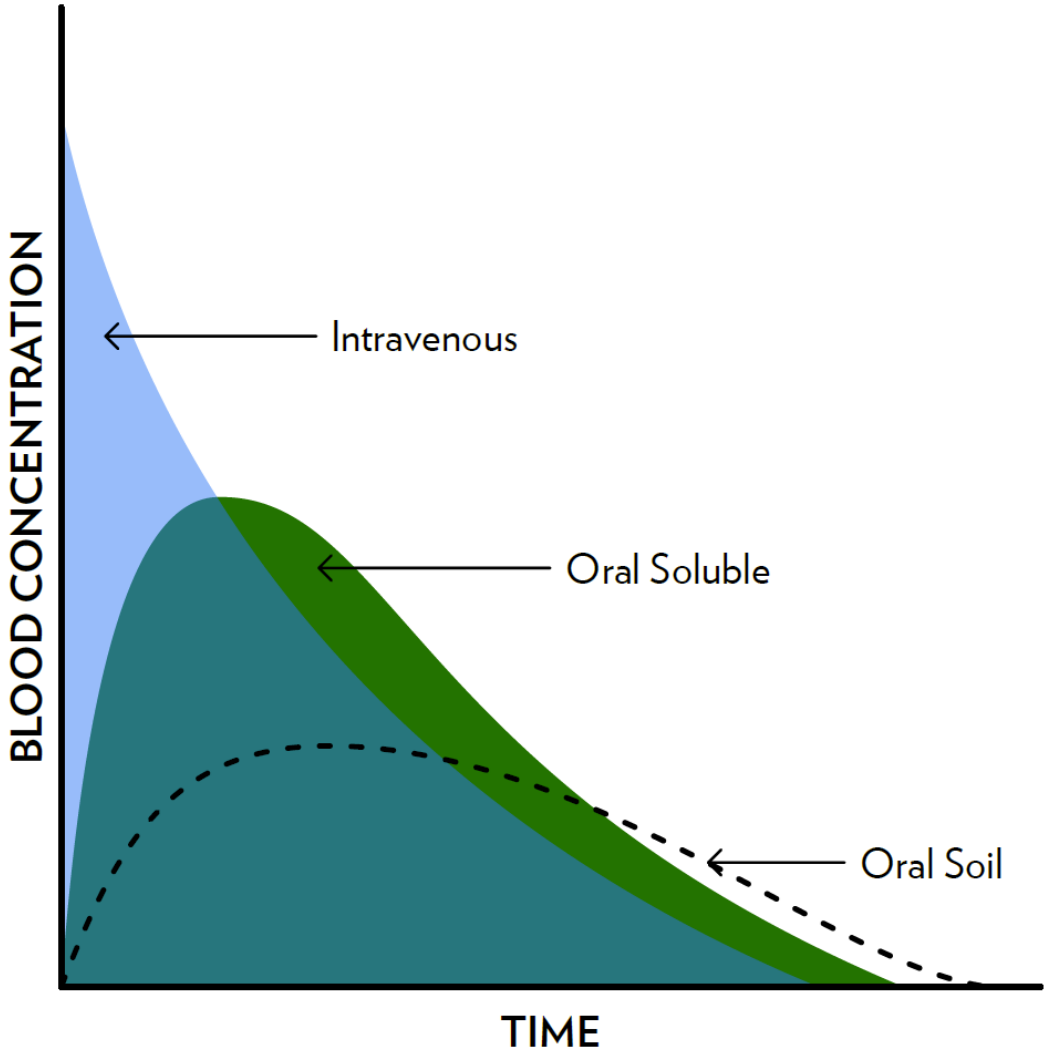
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Figure 1: Schematic representation of data from animal studies of RBA from soil



Area under the curve (AUC) from plot of time vs. concentration for contaminant in biological medium (e.g., blood, plasma, urine, etc.) is used to assess absorbed dose. Relative oral bioavailability (RBA) is calculated as the ratio of the AUC following an oral dose of soil divided by the AUC from an oral dose of chemical in the form administered in the critical toxicity test that forms the basis of toxicity factors such as Cancer Slope Factor or Reference Dose, which is usually a soluble form of the chemical.

Figure 2a. IVIVC Comparing RBA and IVBA
(based on data reported in Diamond et al., 2016)

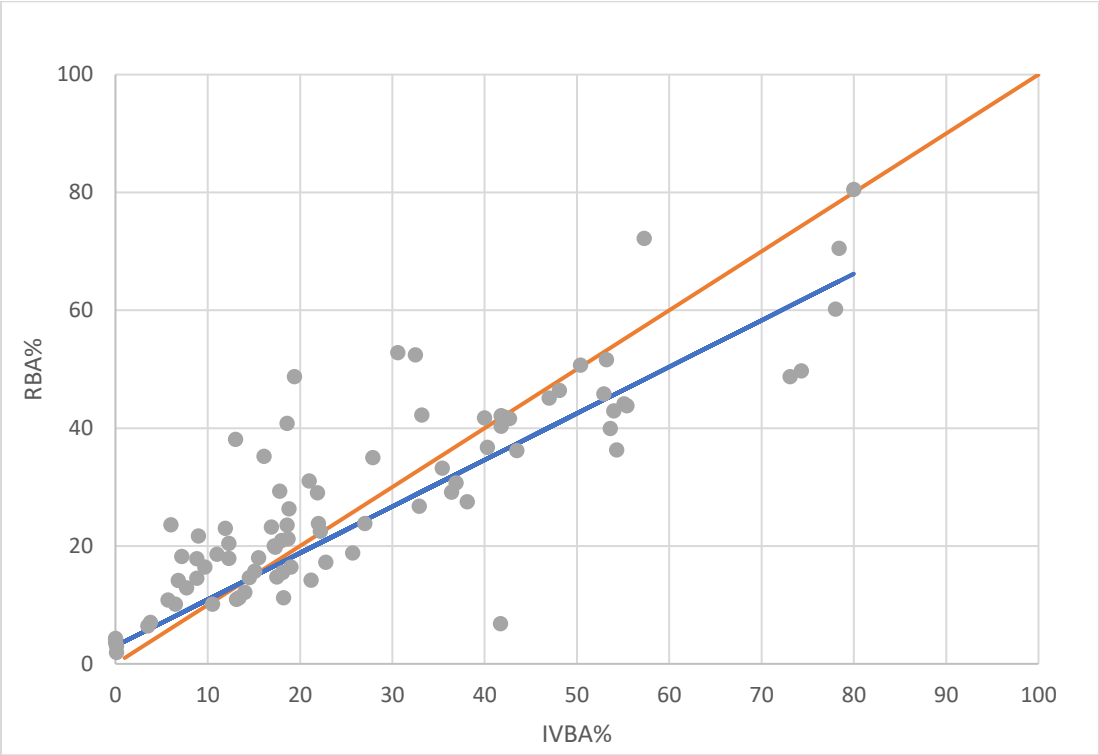


Figure 2a demonstrates the general concept of developing an in vitro to in vivo correlation (IVIVC). The in vitro bioaccessibility data (IVBA) is plotted on the x-axis and relative oral bioavailability (RBA) data are plotted on the y-axis. The blue line represents the regression line, and represents the "best fit" for predicting the relationship between IVBA and RBA. In this figure, the orange line is the "line of unity." If IVBA were exactly equal to RBA, then all values would fall on this line. Points below the orange line represent samples for which IVBA>RBA. Points above the orange line represent samples for which RBA>IVBA.

Notes:
IVBA – in vitro bioaccessibility
RBA – Relative oral bioavailability (assessed in animal studies)

Figure 2b. IVIVC Comparing RBA and IVBA
(From Diamond et al., 2016)

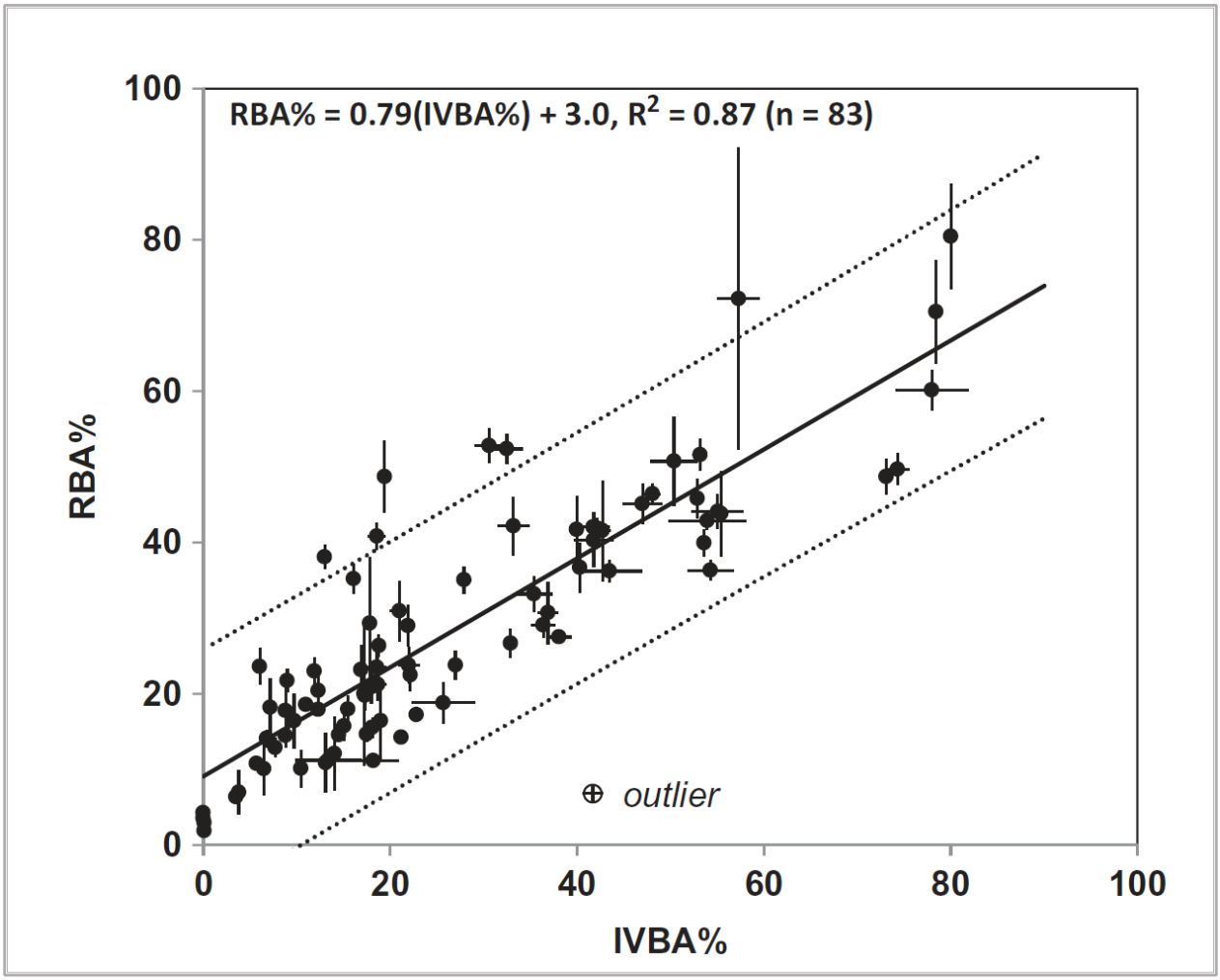
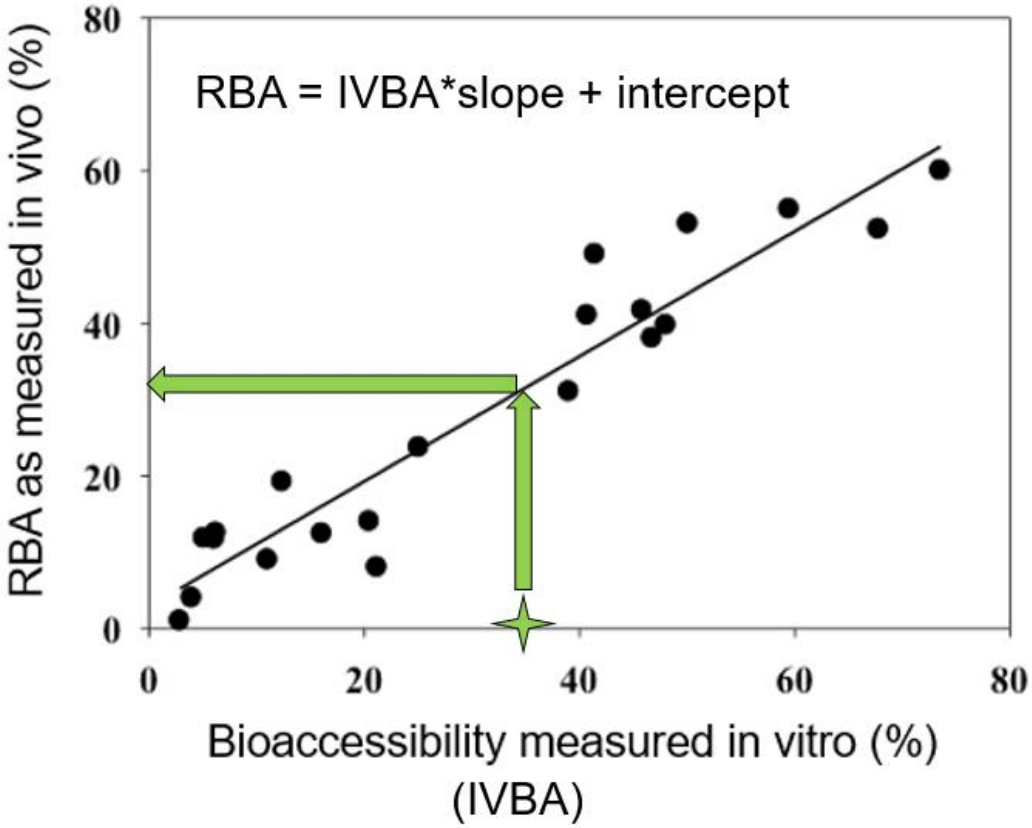


Figure 2b demonstrates the process of developing an in vitro to in vivo correlation (IVIVC). In this instance, data for in vitro bioaccessibility data (IVBA) and relative oral bioavailability (RBA) of arsenic from soil are each plotted with their associated variability. The IVIVC is reflected in the regression equation provided at the top of the figure. With this equation, IVBA data can be used to estimate RBA. Full derivation is provided in Diamond et al., 2016.

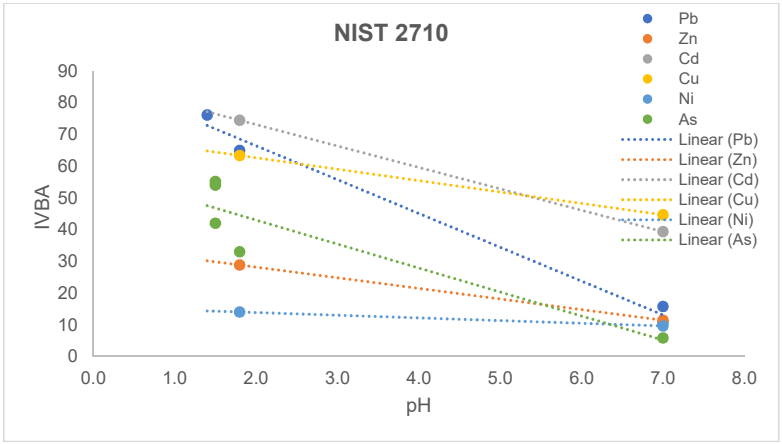
Notes:
IVBA – in vitro bioaccessibility
RBA – Relative oral bioavailability (assessed in animal studies)
Solid Line represents the weighted regression model
Dotted lines are 95% prediction limits for the weighted model.

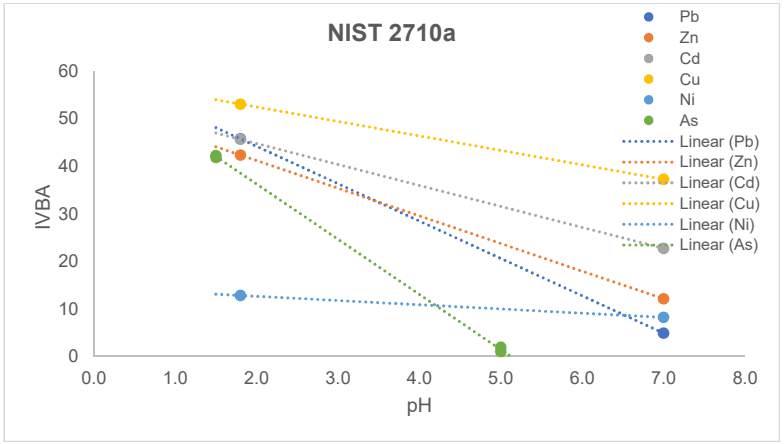
Figure 3: Example of use of IVIVC to predict RBA from IVBA data

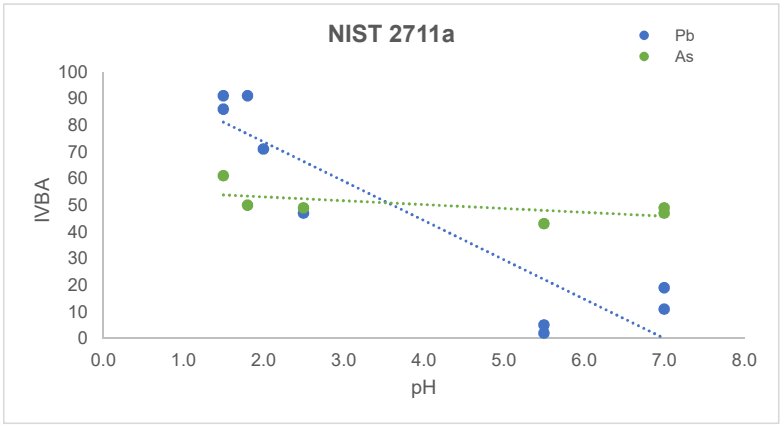


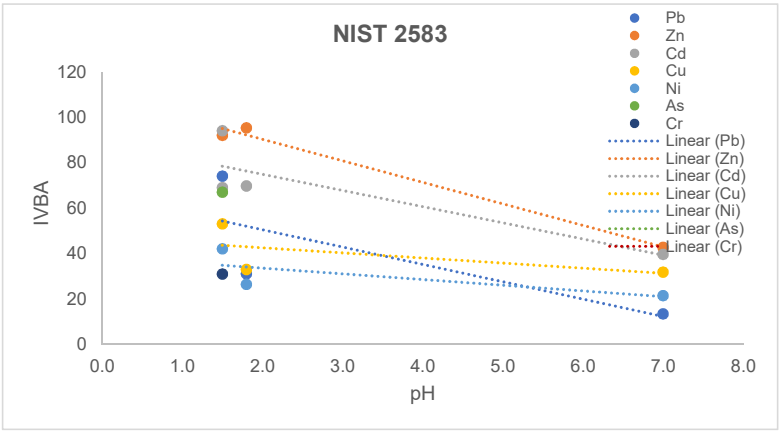
Understanding the regression relationship between in vitro bioaccessibility and relative oral bioavailability as measured in animals allows one to predict RBA from IVBA by using the equation that explains the regression line. For this use, it is important that IVBA be on the x-axis and RBA be on the y-axis.

Figure 4. Effect of pH on IVBA for NIST standard reference material soils.









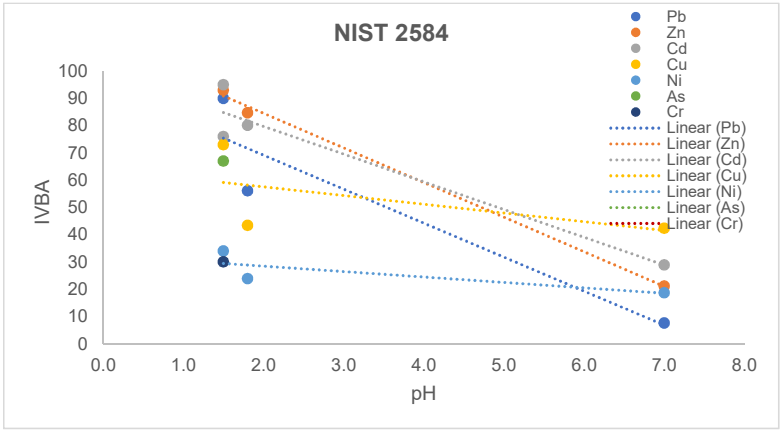
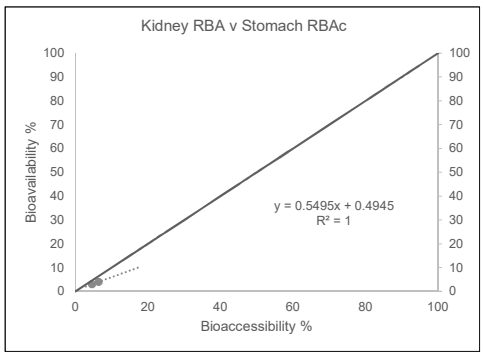
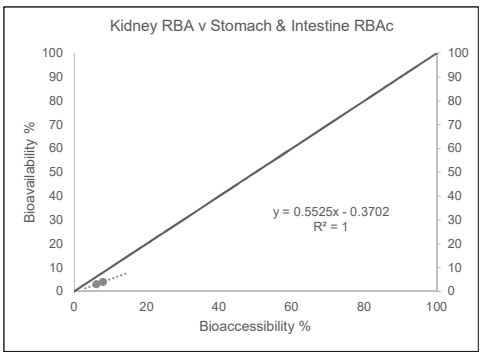
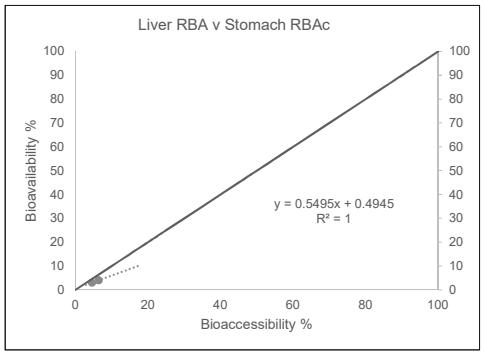
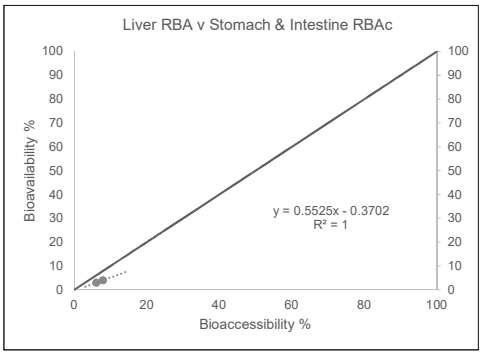


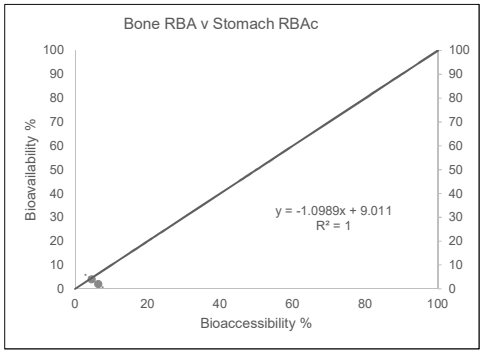
Figure 5a. Plots of IVBA vs. RBA extracted from Denys et al. 2012 - ANTIMONY

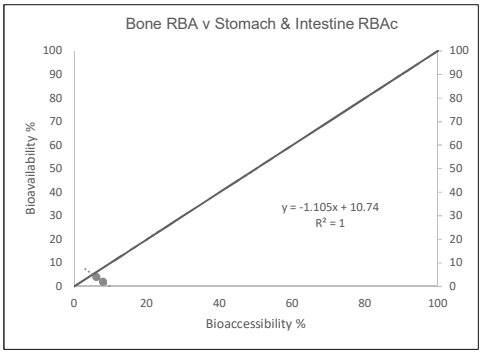


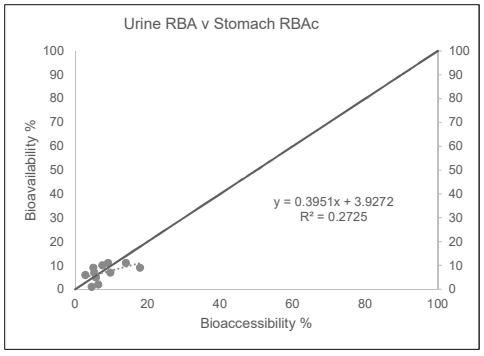


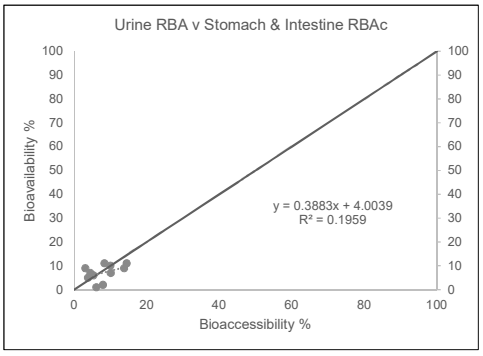






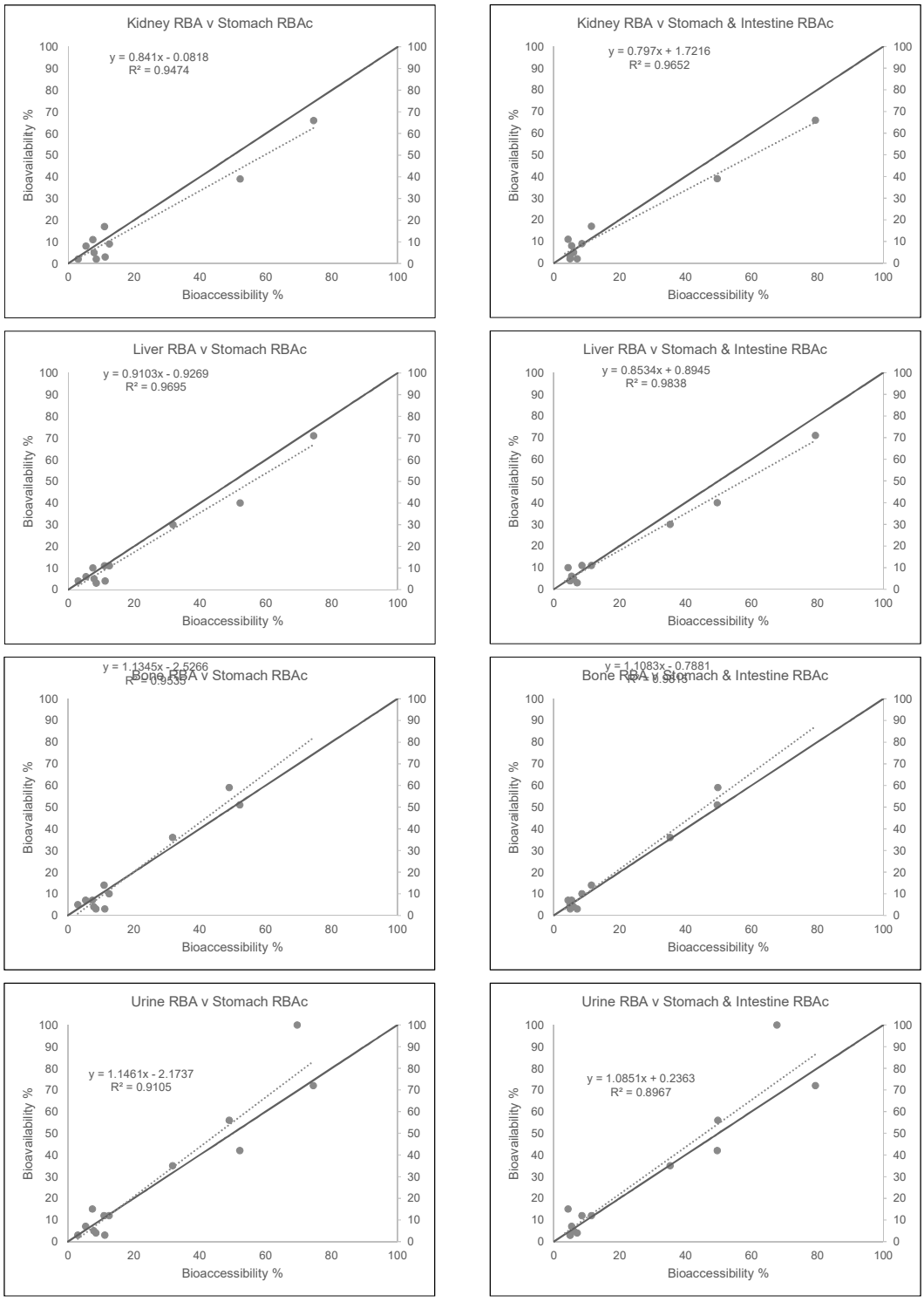






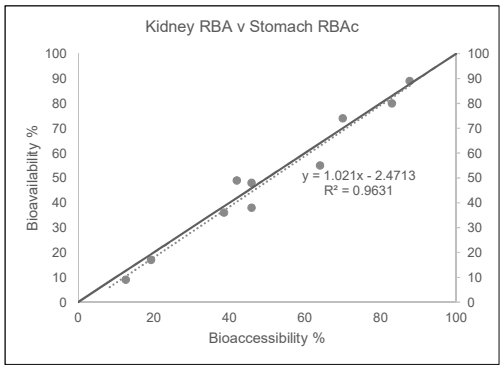
Notes:
Data as provided in supplemental information.
Plots all RBA measurement endpoints vs. IVBA (gastric or gastric & intestinal, as indicated).
Dashed line represents best fit simple regression.
Solid line represents line of unity.
For samples above the line of unity, RBA > IVBA.

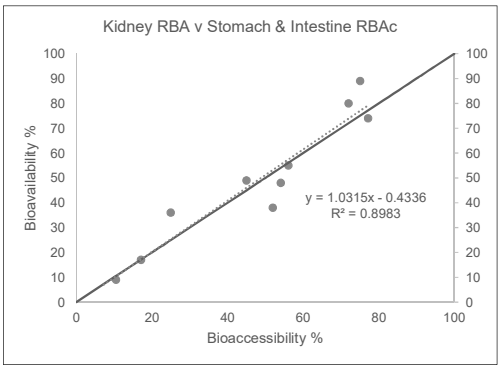
Figure 5b. Plots of IVBA vs. RBA extracted from Denys et al. 2012 - ARSENIC

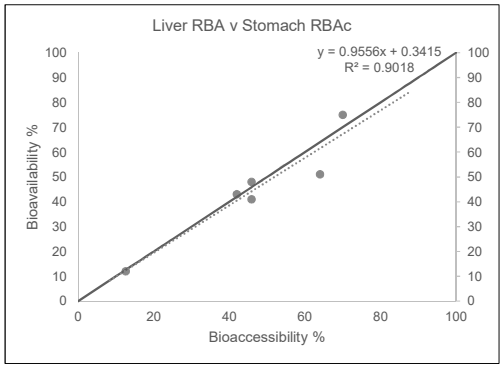


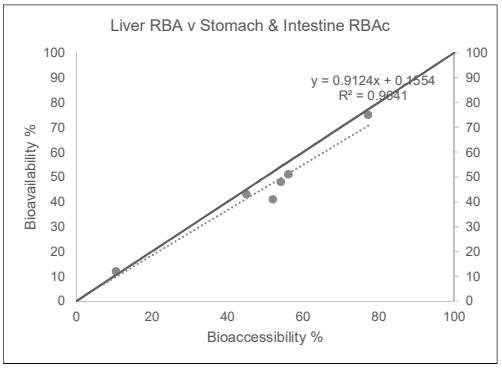
Notes:
Data as provided in supplemental information.
Plots all RBA measurement endpoints vs. IVBA (gastric or gastric & intestinal, as indicated).
Dashed line represents best fit simple regression.
Solid line represents line of unity.
For samples above the line of unity, RBA > IVBA.

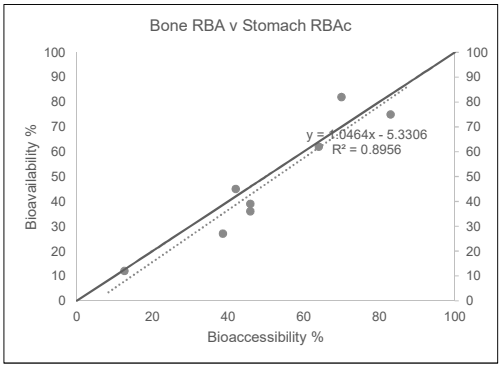
Figure 5c. Plots of IVBA vs. RBA extracted from Denys et al. 2012 - CADMIUM

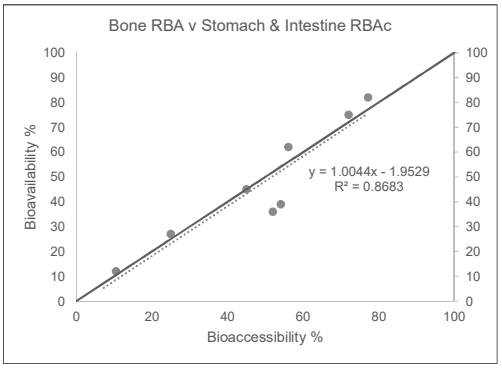


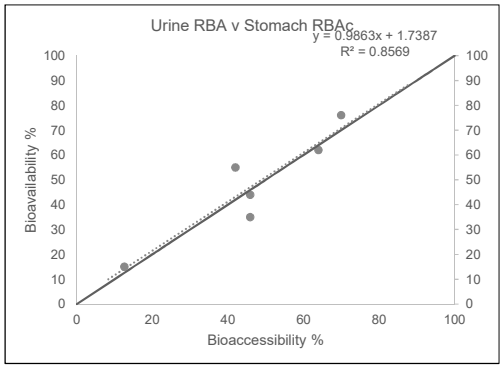


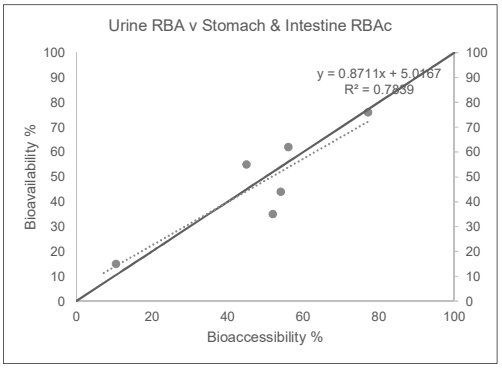






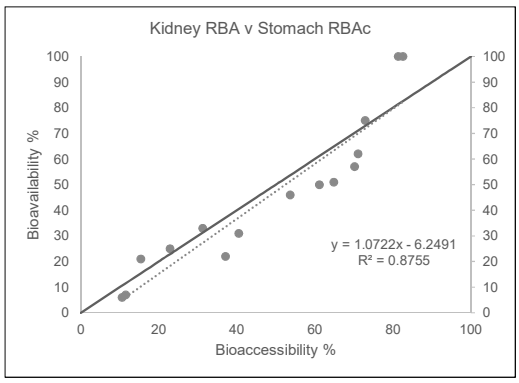


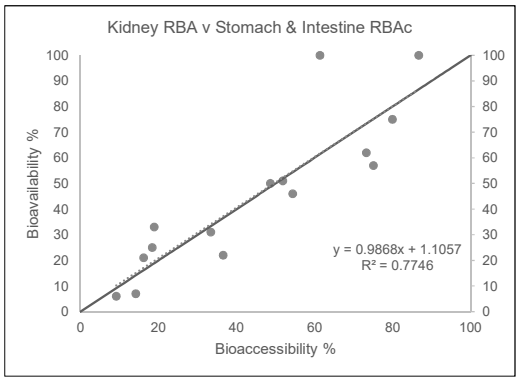


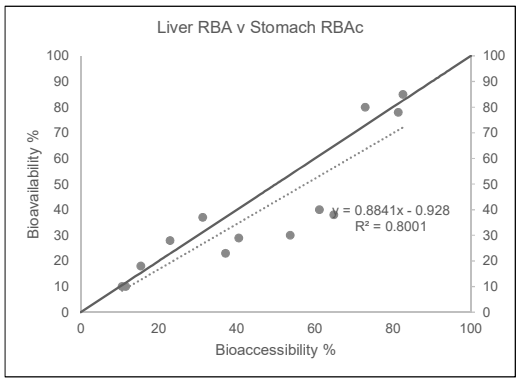


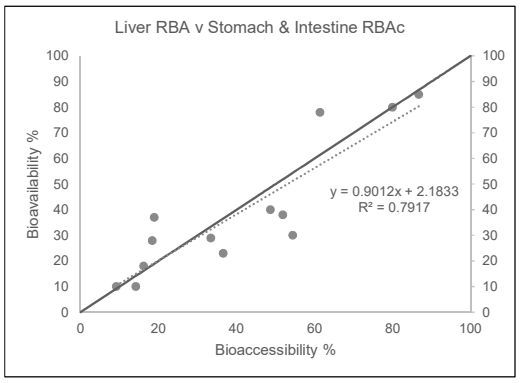
Notes:
Data as provided in supplemental information.
Plots all RBA measurement endpoints vs. IVBA (gastric or gastric & intestinal, as indicated).
Dashed line represents best fit simple regression.
Solid line represents line of unity.
For samples above the line of unity, RBA > IVBA.

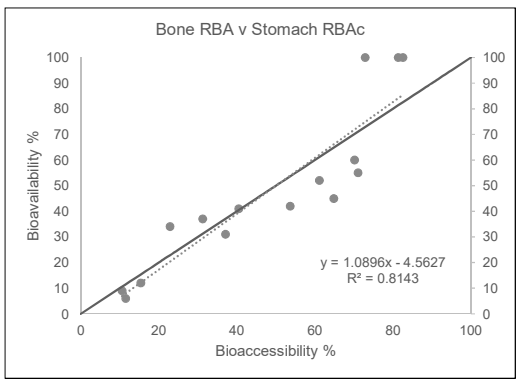
Figure 5d. Plots of IVBA vs. RBA extracted from Denys et al. 2012 - LEAD

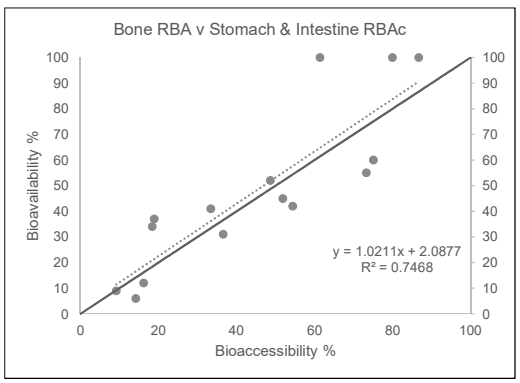


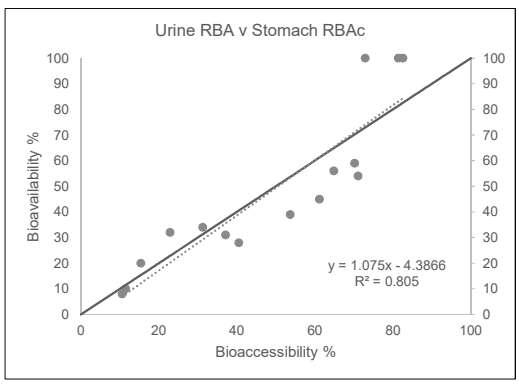


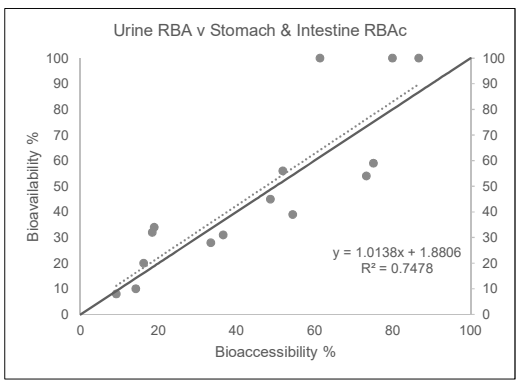






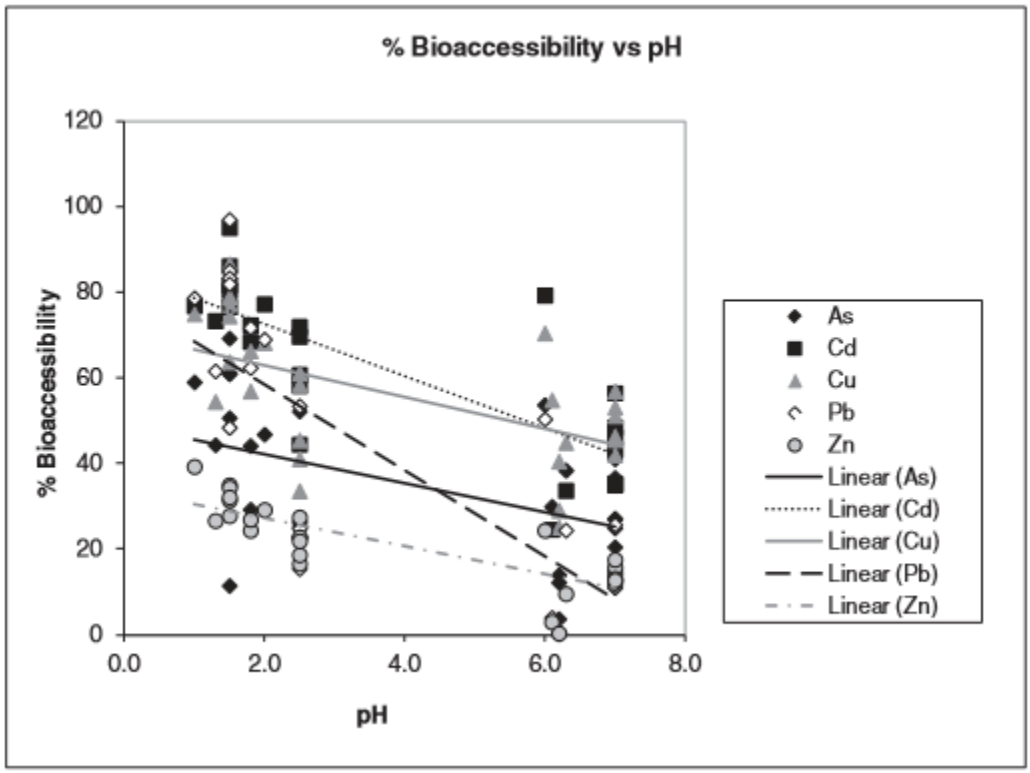






Notes:
Data as provided in supplemental information.
Plots all RBA measurement endpoints vs. IVBA (gastric or gastric & intestinal, as indicated).
Dashed line represents best fit simple regression.
Solid line represents line of unity.
For samples above the line of unity, RBA > IVBA.

Figure 6. Percent bioaccessibility vs. pH (gastric and gastric+intestinal phase extractions) for some elements of interest. (Figure excerpted from Koch et al. (2013)).



The study published by Koch et al. investigates the IVBA of one soil using multiple IVBA methods. The methods include several pH conditions, and reported data for 5 metals. This Figure from Koch et al. depicts the general negative relationship between pH and bioaccessibility as measured by a variety of IVBA methods, when applied to NIST SRM 2710. Koch et al. report these as: "negative correlations, meaning that percent bioaccessibility decreases with increasing pH, were confirmed for all elements," and that the "strongest correlations (highest R values) were statistically significant and occurred for lead, zinc, and cadmium, and were not as pronounced for arsenic, copper and zinc, in particular arsenic."

Figure 7. Comparison of IVBA to RBA for lead and arsenic (Excerpted from Koch et al. 2013)

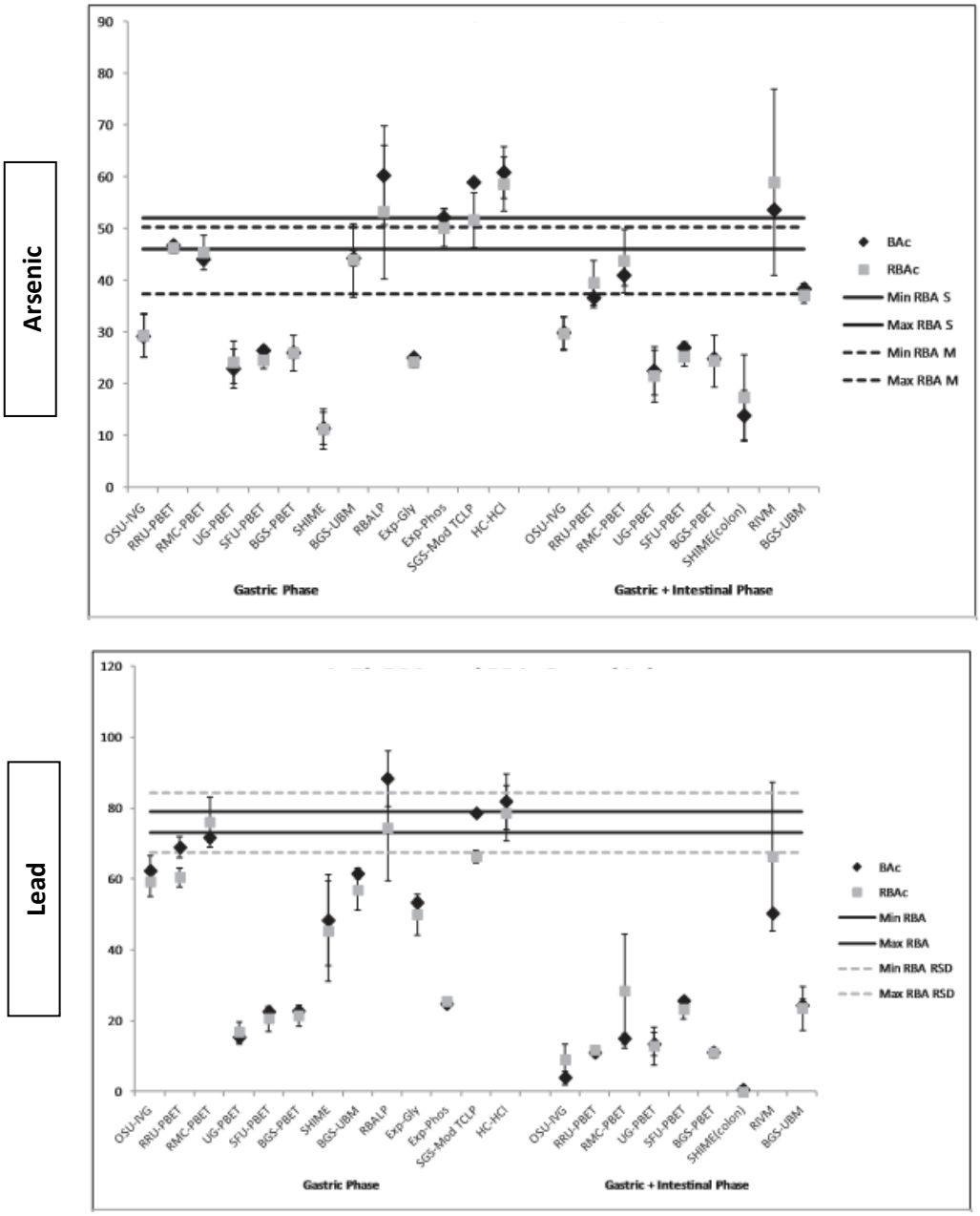


Figure 7 provides data on IVBA for NIST SRM soil 2710, using a diversity of extraction methods and test laboratories. The top panel presents data for arsenic, and the bottom panel is data for lead. "Gastric Phase" extractions are aggregated to the left of each panel, and the results from "Gastric + Intestinal" extractions aggregated to the right. For both lead and arsenic, gastric phase extractions resulted in higher IVBA, with the highest values observed for simple acid extractions at low pH (pH 1.5).

- Diamonds are raw data for IVBA.
- Squares represent IVBA corrected for recovery of soluble spike in specified in vitro method
- For arsenic, horizontal lines represent RBA as measured in swine (solid line) or mouse (dashed line)
- For lead, horizontal lines represent RBA as measured in swine, with minimum and maximum in black lines, and RSD in dashed lines.

Table 1. Literature on IVIVC for Metals from Soil: Studies with in vivo data

Author	Date	in vivo data?	in vitro data?	As	Be	Cd	Co	Cu	Cr	Hg	Mn	Ni	Pb	Sb	Se	Zn	IVBA Method	Saliva pH	Gastric pH	Intestinal pH	# Soils	Specific Questions for Target Metals of Interest: IVIVC exist?	Regression Equation for IVIVC	R2	Is IVBA always a conservative estimate of RBA?	Are there instances where RBA>IVBA?	How do gastric vs other fluids compare?	Are there data on IVBA of metal from alloy vs. pure metal?	Comments
Studies that include animal data (sorted by metal)																													
Kang et al.	2016	mouse	Yes			X		X	X			X	X				Modified PBET		1.5	7.0	1	NA	NA	NA		NA		No	Investigation of 1 soil: RBA of metals in soil with mouse model and comparison to IVBA. Authors conclude that gastric>intestinal for Pb, Cu, Cr, Ni, but not Cd. IVBA <RBA for all metals, by significant fraction -- from Figure 4, RBA appears to be higher than IVBA by more than 2X for all metals. "poor correlation between IVBA and RBA" and that "RBA provides more accurate information..." Note: averagedRBA values reported were >100% for 2 metals, and >100% for individual animals for 4 of 5 metals evaluated. Suggest interpretation issues.
						X																			No	Yes	I>G	No	
								X																	No	Yes	G>I	No	
									X																No	Yes	G>>I	No	
												X													No	Yes	G>>I	No	
													X												No	Yes	G>>I	No	
Koch et al.	2013	swine	Yes	X		X	X	X	X		X	X	X			X					1	Yes	Not Provided -- 1 soil			No	Yes (all)	G>G+I for all metals	No	Provides interlaboratory comparison of extraction testing on NIST 2710. Good comparison of different methods. Demonstrates general trend to lower IVBA at higher pH. Compares extraction results to RBA for lead and arsenic. Data indicate that most IVBA methods tested underpredict RBA for arsenic and lead.
		mouse	Yes														IVG		1.8	6.1									
																	PBET		1.8-2.5	7.0									
																	RBALP		1.5	--									
																	TCLP		1.0	--									
																	RIVM		1.5	6.0									
																	UBM		1.2	6.3									
																	Health Canada 1:2000		1.5	--									
Denys et al.	2012	swine	Yes	X		X							X	X			UBM	6.5	1.2	6.3	16	Yes	Not Provided	NA					Validation effort to assess BARGE UBM extraction method. Uses 16 soils from mining/smelting areas and assesses RBA based on mulitple measurement endpoints relative to soluble salts. IVBA uses gastric and gastric+intestinal phases. For antimony, data are out of range for IVIVC. Reports "Relative Bioaccessibility" in comparison to IVBA for soluble salts. (Note: data extracted from SI and plotted for Eurometaux analysis)
																						Data from study plotted by Alloy. IVIVC for every RBA endpoint and IVBA phase presented.							
				X																		Yes	Not Provided		Depends on endpoint	Yes	similar	No	
						X																Yes	Not Provided		Depends on endpoint	Yes	similar	No	
													X									Yes	Not Provided		Depends on endpoint	Yes	similar	No	
														X								Yes	Not Provided		Depends on endpoint	Yes	similar	No	
Ellickson et al.	2001	rat	Yes	X									X				Sequential Extraction				1								Studies the RBA and IVBA of lead and arsenic from NIST 2710. Only one soil, so no regression relation for IVIVC. In vitro included sequential saliva, gastric, and intestinal phase. Bioavailability values are NOT reported as RBA, and based on % dose recovered. No IV or soluble dose reported.
				X														unspecfied	1.4	6.5		No	NA	NA	Yes	No	similar	No	
													X					unspecfied	1.4	6.5		No	NA	NA	Yes	No	G>GI	No	
Diamond et al.	2016	swine	Yes	X													RBALP		1.5		83	Yes	RBA=0.79(IVBA)+3	0.87	43% of soils	57% of soils	NA	No	Meta-analysis of data for 83 soils from 3 studies using mice or swine and IVBA extraction using RBALP. Data have been extracted and presented in a separate figure.
Li et al.	2015	mouse	Yes	X													UBM Gastric		1.1		12	Yes	RBA=0.97(IVBA)-4.40	0.66	Many soil samples where RBA exceeds IVBA, for every method tested. Results for gastric fluid similar or slightly more conservative than G+I fluid	Yes, many	Gastric approx = G+I	No	IVIVC for arsenic in 12 soils from 8 cities in China. RBA from mouse vs 5 IVBA methods. Figure 5 shows IVIVC for all methods with G and GI separate. Conclude IVG provides best prediction of RBA, but "UBM and SBRC ... also suitable." Slope of regression line very close to 1 for all methods. GI provided good correlation, but Gastric more conservative.
																	UBM G+I			6.3			RBA=1.09(IVBA)-4.43	0.80		Yes, many			
																	SBRC Gastric		1.5				RBA=0.96(IVBA)-0.60	0.67		Yes, many			
																	SBRC G+I			7.0			RBA=1.35(IVBA)+8.22	0.57		Yes, many			
																	IVG Gastric		1.8				RBA=1.29(IVBA)-1.06	0.83		Yes, many			
																	IVG Intestinal			5.5			RBA=1.35(IVBA)+2.28	0.81		Yes, many			
																	DIN Gastric		2.0				RBA=0.79(IVBA)+12.1	0.61		Yes, many			
																	DIN Intestinal			7.5			RBA=1.36(IVBA)+4.52	0.53		Yes, many			
																	PBET Gastric		2.5				RBA=1.08(IVBA)+8.59	0.56		Yes, many			
																	PBET Intestinal			7.0			RBA=1.04(IVBA)+9.46	0.50		Yes, many			

Table 1. (cont.)

Author	Date	in vivo data?	in vitro data?	As	Be	Cd	Co	Cu	Cr	Hg	Mn	Ni	Pb	Sb	Se	Zn	IVBA Method	Saliva pH	Gastric pH	Intestinal pH	# Soils	Specific Questions for Target Metals of Interest: IVIVC exist?	Regression Equation for IVIVC	R2	Is IVBA always a conservative estimate of RBA?	Are there instances where RBA>IVBA?	How do gastric vs other fluids compare?	Are there data on IVBA of metal from alloy vs. pure metal?	Comments
Brattin et al.	2013	swine	Yes	X													RBALP		1.5	--	20	Yes	RBA=.62(IVBA)+19.7	0.72	20% of soils	65% of soils	G>I	No	IVIVC investigation for 20 soils. Investigated the effect of pH, temp, extraction time, PO4, hydroxylamine. Found clear negative trend with pH, slight increase with temp (range of 20-37 degrees) small effect of extraction time (for most samples), increase with addition of PO4, and little effect of hydroxylamine.
		primate	Yes	X																									
Juhasz et al.	2009	swine	Yes	X													SBRC		1.5	7.0	12	Yes	RBA=.99(SBRCg)+1.66	0.75	50% of soils	50% of soils	G>I for most soils	No	Study of 12 soils from Australia. Demonstrates G generally higher than Intestinal phase extractions, but not for all soils. RBA>IVBA for all IVBA method for 6 of 12 soils. SBRC gastric generally highest IVBA, and had highest correlation. Figure 3 shows that most samples RBA>IVBA. Note: table provides regression equations, but figures have axes flipped for predictive equation.
																	IVG		1.8	5.5			RBA=0.85(IVGg)+14.3	0.57	Nearly Never	Nearly all soils	Similar		
																	DIN		2.0	7.5			RBA=1.8(DINg)+5.7	0.54	Nearly Never	Nearly all soils	Similar		
																	PBET		2.5	7.0			RBA=1.1(PBETg)+10.1	0.64	Nearly Never	Nearly all soils	Similar		
Juhasz et al.	2007	swine	Yes	X													SBET		1.5		12	Yes	RBA=.68(IVBA) + 5.67	0.69	Not Reported	Not Reported	Not Reported	No	Study of 12 soils from Australia. Evaluated for IVBA using SBET (method specified in subsequent paper), and for RBA using swine. Regression equation not provided, but extracted from Brattin et al. Also attempted to correlate RBA with soil properties, demonstrating relatively strong relationship based on arsenic and iron concentration.
Schroder et al.	2003	swine	Yes	X													IVG		1.8		10	Yes	IVBA=0.60(RBA)+25.3	0.74	for 6 of 10 soils	Yes	Gastric>G+I	No	Conducted RBA evaluation of 10 soils from hazardous wast sites vs. cadmium chloride. Also performed in vitro extraction using IVG method at pH 1.8 and 5.5. Conlude that gastric>GI, and that the presence of food decreases IVBA. Suggest gastric extraction for screening of site-specific RBA.
																	IVG			5.5		Yes	IVBA=0.21(RBA)+25.8	0.54	for 1 of 10 soils	Yes	Gastric>G+I	No	
Cobalt Institute, Unpublished. Included as Attachment 1 to this report.		Yes	Yes		X												Stopford 2003		1.5			No	Not provided	NA	approximate	approximate	NA	Co forms and Co metal included	Cobalt Institute unpublished data provided as part of REACH Dossier, Attachment 1 of this report. Includes IVBA, acute toxicity, and 28-day oral toxicity data for 4 cobalt compounds (CoCl2, CO, Co3O4, and CoS), and relative bioavailability data for CoCl2 (soluble) and Co3O4 (insoluble). Results indicate that the IVBA in simulated gastric fluid is very high (86-98%) for soluble forms tested (CoCl2 and Co), and very low (<1.0%) for insoluble forms (Co3O4 and CoS). Results from toxicity and bioavailability testing demonstrated similar results, with low bioavailability and low toxicity for the cobalt forms with low solubility IVBA. Correlation across samples is precluded by the number of forms tested.
Safruk et al.	2015	human	Yes							X							PBET modified		1.8	7.0	50	No	NA		NA	NA	G+I>G by factor of 1.5-6	No	Compared bioaccessible mercury as measured by modified PBET with urinary inorganic mercury. Report no relation between soil Hg and urinary Hg in biomonitoring. Found G+I>G by factor of 1.5 to 6.

Table 1. (cont.)

																						Specific Questions for Target Metals of Interest						
Author	Date	in vivo data?	in vitro data?	As	Be	Cd	Co	Cu	Cr	Hg	Mn	Ni	Pb	Sb	Se	Zn	IVBA Method	Saliva pH	Gastric pH	Intestinal pH	# Soils	IVIVC exist?	Regression Equation for IVIVC	R2	Is IVBA always a conservative estimate of RBA?	Are there instances where RBA>IVBA?	How do gastric vs other fluids compare?	Are there data on IVBA of metal from alloy vs. pure metal?	Comments
Dutton et al.	2016	rat	Yes									X					PBET		2.5	7.0	21	Yes	RBA=0.01(IVBA)-0.0004	0.526	Yes	No	Not Reported	No	RBA and IVBA of 21 soils from Port Colborne. Urinary Ni in rats, 2% excreted across dose levels. Small range in RBA, wide range in IVBA.
Henderson et al	2012a	Yes	No									X											No	NA	Refer to Henderson 2012b	NA	NA	No	Acute toxicity study of 16 samples, including 9 Ni compounds, 2 complexes and 3 mattes. Conducted to comply with requirements of REACH. Report LD50 values ranging from 310 mg/kg to >5000 mg/kg. Refers to companion publication, Henderson et al 2012b to establish that toxicity associated with bioavailability. Information from Henderson 2012a and 2012b has been summarized by NiPERA and interpreted with regard to IVBA estimates on bioavailability and toxicity, and is included as Attachment 2 to this report.
Henderson et al	2012b	Yes	Yes									X					Stopford 2003	NA	1.5	7.4		IVBA vs. LD50	Not Provided	0.91	NA IVBA vs. LD50	NA	GI>Intestinal, generally by >2X	No	Investigation of bioaccessibility of 12 samples of 11 nickel compounds, conducted to fill data gap for nickel substances and investigate use of read-across for nickely substances and toxicitieis. All very fine particles (<100 um average). Conducted extraction in simulated gastric and simulated intestinal fluids, at several time points (2-72 hour extractions). Results indicate much higher IVBA in gastric solution than intestinal. Also demonstrates much higher IVBA for water-soluble forms of nickel. Gastric (2-hour) IVBA showed strongest relation with LD50. Plot of IVBA vs. LD50 shows strong relationship between toxicity and IVBA -- those with low IVBA also have low LD50. This relationship is not linear. Dissolution in simulated gastric fluid at 2 hours was higher than at 72 hours in simulated intestinal fluid. 5 Ni forms match those tested for RBA, presented in Ishimatsu 1995. Information from Henderson 2012a and 2012b has been summarized by NiPERA and interpreted with regard to IVBA estimates on bioavailability and toxicity, and is included as Attachment 2 to this report.
Ishimatsu et al.	1995	Yes	Yes									X					Water or Saline						not provided	NA	not stated	only evaluated saline and water	No. Does include metal compounds and metalic nickel		Study of tissue distribution of nickel after oral exposure by rats, for 8 nickel compounds (NiO, Ni-M, Ni3S2 ,NiS, NiSO4, NiCl2, Ni(NO3)2). Reported "absorbed fraction" correlated with solubility. Solubility in saline was greater than in distilled water for all metals with data for both solutions, generally by a factor of less than 2X. Authors report that the absorbed fraction (sum of organ, blood and urine mass divided by administered dose) ranged from less than 1% (nickel oxides and nickel metal) to 33.8% (nickel nitrate). No data on solubility under gastrointestinal conditions, but data establish that absorbed dose is related to aqueous solubility. Information from this study is included in a summary prepared by NiPERA, included as Attachment 2.

Table 1. (cont.)

Author	Date	in vivo data?	in vitro data?	As	Be	Cd	Co	Cu	Cr	Hg	Mn	Ni	Pb	Sb	Se	Zn	IVBA Method	Saliva pH	Gastric pH	Intestinal pH	# Soils	IVIVC exist?	Regression Equation for IVIVC	R2	Is IVBA always a conservative estimate of RBA?	Are there instances where RBA>IVBA?	How do gastric vs other fluids compare?	Are there data on IVBA of metal from alloy vs. pure metal?	Comments
				Specific Questions for Target Metals of Interest																									
Vasiluk et al.	2011	rat	Yes									X					PBET (modified)		2.5	7.0	2	No			75% of soils	25% of soils	Very close.	No	Conducted study in vitro and in vivo (rat) for 2 soils and 2 particle sizes. Found little difference in IVBA between G and G+I extracts. No clear relation between RBA and IVBA for the 4 samples (2 soils) evaluated. Bioavailability estimates highly variable. State that "in vitro…will be predictive of bioavailability to the degree that mobilization from the soil matrix in GI solution limts oral bioavailability."
Dong et al.	2016	swine rat	Yes									X					Statiscial evaluation with aggregate data from RBALP, SBRC, UBM and RIVM.		1.5		252	Yes	RBA=0.87(IVBA)+4.7	NS	about 50% of soils	Yes	Only used gastric	No	RBA and IVBA from 252 paired soils from 9 publications. Meta-analysis. Provides statistical analysis of aggregate data from multiple IVBAs and RBA endpoints. Used gastric phase data only. Comment that IVIVC slopes are all <1. This analysis does not tell us which IVBA method is best predictor, but Figure 2 shows that many samples have RBA>IVBA.
Liu et al.	2016	See Dong et al., 2016										X					See Dong et al., 2016												Same as Dong, 2016
Juhasz et al.	2016	mouse	Yes									X					SBRC		1.5	7.0	3	Yes	RBA=0.16(IVBA)-1.74	0.84	Yes	Yes	G>>G+I	No	IVIVCa for 3 phosphate-amended soils using 5 IVBA methods. Generally demonstrates that extraction with gastric phase IVBA provides conservative estimate of RBA. For G+I, RBA was generally and significantly underestimated.
Li et al.	2014	mouse	Yes									X					SBRC		1.5	7.0	15	Yes	RBA=0.61(SBRCg)+3.15	0.68	Yes (SBRC. Not others)	No (SBRC)	Gastric>>G+I	No	Investigates RBA and IVBA of lead from 15 dust samples from China. Gastric always>Intestinal. Intestinal phase always underpredicts RBA. Includes 4 IVBA methods, each at G and GI. SBRC>IVB>DIN>PBET.
																	IVG		1.8	5.5			RBA=0.48(IVGg)+14.3	0.56	All but one soil	1 of 15 samples	Gastric>>G+I		
																	DIN		2.0	7.5			RBA=0.67(DINg)+17.4	0.85	No	>50% of soils	Gastric>>G+I		
																	PBET		2.5	7.0			RBA=0.69(PBETg)+20	0.52	No	>50% of soils	Gastric>>G+I		
Van de Wiele et al.	2007	human	Yes									X					PBET		2.5	7.0	1	Yes	NA Only one soil	NA	No	Yes	G>G+I for fasted, and generally for "fed" conditions	No	Assessed IVBA using 5 in vitro methods on the one Bunker Hill soil fed to humans to assess bioavailability (by Maddaloni). When food added, "fed" IVBA always > than fed RBA. Without food, IVBA>RBA 3 times, IVBA=RBA 3 times, and IVBA<RBA 3 times.
																	DIN		2.0	7.5					No	Yes			
																	RIVM	6.5	1.1	7.0					Yes	Yes (intestinal)			
																	SHIME	5.2	2.0 or 4.0	6.5					No	Yes			
																	TIM	1.8 or 2	6.5	6.8 to 7.2					Yes for fasted condition	Yes (fed)			

Table 2. Literature on IVIVC for Metals from Soil: Comparison of in vitro methods

Author	Date	in vivo data?	in vitro data?	As	Be	Cd	Co	Cu	Cr	Hg	Mn	Ni	Pb	Sb	Se	Zn	IVBA Method	Saliva pH	Gastric pH	Intestinal pH	G+I sequentially	# Soils	Specific Questions for Target Metals of Interest: Is IVBA always a conservative estimate of RBA?	Are there instances where RBA>IVBA?	How do gastric vs other fluids compare?	Are there data on IVBA of metal from alloy vs. pure metal?	Are there data on IVBA vs. soluble or insoluble forms?	Comments
Other studies (sorted by metal)																												
Boros et al.	2017	No	Yes			X X		X X	X X			X X X	X X			X X	PBET		1.8	7.0	Yes	33	NA	NA	G=3xG+I G=1.1xG+I LOD G=1.3xG+I G=8xG+I G=4xG+I	No	Yes	Study using dust from 33 homes to investigate which IVBA method is more conservative, using Gastric vs Gastric+Intestinal PBET. Find that G>GI by a factor of 1.1x to 8x, for all metals except Chromium. Report that use of Gastric extraction is more conservatieve. Pb, Cu, Cd strongly influenced by pH, Cu and Ni less so.
Cave et al.	2016	No	Yes	X				X	X			X	X			X	UBM -G Dynamic	6.5	1.2		Yes	1	NA	NA	NA	NA	NA	Provide a comparison of dynamic extraction vs. Static UBM on solubility of metals from one soil. For most metals dynamic and batch results were similar, except for lead, which is much lower from the batch method. The authors suggest this is due to readsorption to soil in the static reaction vessel as the pH increases.
Qin et al.	2016	No	Yes	X		X		X	X		X	X	X			X	UBM	6.5	1.2	6.3	Yes	2	NA	NA	G>G+I except arsenic	no	No	2 soils. Investigation of the effect of particle size on IVBA with UBM. Particle size had mixed effect. G>G+I except for arsenic.
LeBot et al.	2010	No	Yes	X		X		X	X		X		X	X			1.4% HCl		1.5			dust from 13 homes	NA	NA	NA	No	NA	Presents extraction testing of dust samples from 13 homes. No IVIVC, but presents data on SRM 2583 and SRM 2584
Eckert et al.	2014	No	Yes					X								X	Various pH w appropriate buffer.		3.0 4.0 5.0 6.0 7.0 8.0			NA	NA	NA	Higher solubility at lower pH.	No	No	Testing effects of peptides on solubility from mineral salts. No soils included. All ions showed increased solubility at lower pH. At high pH, presence of peptides increased solubility.
Rosende et al.	2014	No	Yes	X		X		X	X			X	X			X	UBM-G	6.5	1.1	NA		2	NA	NA	Gastric assumed to be "worst case." No difference with saliva or mucin. Temp has effect	No	No	Evaluation of UBM-like extraction in dynamic format, using 2 soils. Results show little effect of mucin or saliva, but effect of temp on extraction.
Li et al.	2013	No	Yes	X				X					X			X	PBET SGET		2.5 1.1	7.0 7.0	Yes Yes	3	NA	NA	Gastric>G+I for Pb and Zn. Less so for Copper	No	No	Investigated the function of digestive enzymes on relase of copper, zinc, and lead from 3 soil samples from China, using two IVBA methods. Reports Gastric, and Gastric+Intestinal. Concluded that lead and zinc solubility is pH dependent. Found no change on copper or lead when pepsin added to PBET. Pepsin has mixed effect on zinc when added to PBET or SGET. Consistent increase when pepsin added to GI phase of SGET.

Table 2. (cont.)

Author	Date	in vivo data?	in vitro data?	As	Be	Cd	Co	Cu	Cr	Hg	Mn	Ni	Pb	Sb	Se	Zn	IVBA Method	Saliva pH	Gastric pH	Intestinal pH	G+I sequentially	# Soils	Specific Questions for Target Metals of Interest: Is IVBA always a conservative estimate of RBA?	Are there instances where RBA>IVBA?	How do gastric vs other fluids compare?	Are there data on IVBA of metal from alloy vs. pure metal?	Are there data on IVBA vs. soluble or insoluble forms?	Comments
Meunier et al.	2010	No	Yes	X													SBRC		1.5	7.0	Yes	19	NA	NA	G+I>G for glycine	No	Yes, Pyrite	Study of 19 substrates for IVBA of arsenic, including soil amendments, NIST soils, and arsenopyrite. Compared two IVBA methods and the effect of higher fluid volume. Found that Glycine system had higher IVBA at higher fluid volume. Also showed that G+I provided higher estimate of IVBA in glycine buffer, but mixed in more physiologically-based system.
																	IVG (mod)		1.8	7.0	Yes							
Broadway et al.	2010	No	Yes						X								UBM	6.5	1.2	6.3	Yes	27	NA	NA	G>G+I by factor of 1.5	No	No	Investigated the IVBA of chromium from 27 soils. Reported both gastric and gastrointestinal phase results separately. Found that G>GI by factor of 1.5
Bradley et al.	2017	No	Yes							X							Not Specified					NA	NA	NA	NA	No		Investigation of the bioavailability of mercury from fish. Demonstrates reduced bioaccessibility from cooking. States need for IVIVC.
Hagan et al.	2014	No	Yes							X							RBALP	--	1.5	--	NA	60	NA	NA	NA	No	No	Compares RBALP to Hg sequential extraction tests for 61 samples of brick and 49 samples of dirt floors in Peru. Authors propose that F1+F2+F3 fractions represent bioaccessible mercury.
Welfringer and Zagury	2009	No	Yes						X								IVG	--	1.8	6.5	Yes	5	NA	NA	G+I>Gastric	No	HgS and HgCl2	Studied 5 soils from mining or industrial sites in Canada, plus two CRMs (CRM 025-050 and ERM-CC580. Found that extraction in IVG>CDM method. Not able to tell whether that is due to consituents, pH, or temperature. Bioaccessibility from soil is very low relative to soluble standard (IVBA range from 1.5-7.5%).
																	CDM		2.5	5.5	Yes							
Zagury et al.	2009	No	Yes						X								More data presented in Welfringer and Zagury 2009.											Studied three soils from Canada and two CRMs. More data from same study and additional soils are presented in Welfringer and Zagury 2009.
Yan et al.	2016	No	Yes								X						RBALP		1.5	--		10	NA	NA	G>G+I	No	No	Uses 10 soils from mining and smelter locations in Australia. Conducts IVBA extractions and compares findings of different IVBA methods. Found soil:fluid ratio has effect for RIVM method. Figure 1 shows significant correlation between all extraction methods. Concludes that Gastric>GI for lead, and extraction is sensitive to rotation, pH, and soil:fluid ratio. For lead, pH effects are very strong. Lower pH resulted in higher IVBA. Rotation>shaking, and soil:fluid ratio mattered.
																	SBRC		1.5	6.5	Yes							
																	PBET		2.0	6.5	Yes							
																	UBM	6.5	1.1	7.4-8.0	Yes							
																	RIVM	6.5	1.1	7.8-8.0	Yes							

Table 2. (cont.)

																									Specific Questions for Target Metals of Interest						
Author	Date	in vivo data?	in vitro data?	As	Be	Cd	Co	Cu	Cr	Hg	Mn	Ni	Pb	Sb	Se	Zn	IVBA Method	Saliva pH	Gastric pH	Intestinal pH	G+I sequentially	# Soils	Is IVBA always a conservative estimate of RBA?	Are there instances where RBA>IVBA?	How do gastric vs other fluids compare?	Are there data on IVBA of metal from alloy vs. pure metal?	Are there data on IVBA vs. soluble or insoluble forms?	Comments
Kastury et al.	2017	No	No	X		X							X			X	Review. No new data					NA			NA			Summary of literature on bioavailability and bioaccessibility of metals from particulate matter and dust. Useful summary of literuature, but no useful IVIVC or comparison of methods. Compare siumulated lung fluid, cell culture, and gamble solution.
Ng et al.	2015	No	No	X									X				Review. No new data					NA	NA	NA	NA	NA	NA	Conclude that there is little evidence to support a single IVBA method provides discussion of chemicals added to IVBA and basis for inclusion.
Schoof and Nielsen	1997	No	No							X							Review. No new data						NA	NA	NA	No	HgS and HgCl2	Provides a review of method for assessing mercury absorption, and suggestions for approaches to evaluating bioavailability for less available forms that occur in soils. Suggests that MgCl2 has approximately 20% bioavailability, but that even as a salt, HgS may be 30-60-fold less bioavailable than HgCl2.

Table 3a. Literature on IVIVC for Metals from Soil: Antimony

										Specific Questions for Target Metals of Interest								
Author	Date	in vivo data?	in vitro data?	IVBA Method	Saliva pH	Gastric pH	Intestinal pH	G+I sequentially	# Soils	IVIVC exist?	Regression Equation for IVIVC	R2	Is IVBA conservative estimate of RBA?	Are there instances where RBA>IVBA?	How do gastric vs other fluids compare?	Are there data on IVBA of metal from alloy vs. pure metal?	Are there data on IVBA vs. soluble or insoluble forms?	Comments
Denys et al.	2012	swine	Yes	UBM	6.5	1.2	6.3	Yes	16	Yes	Not Provided	NA	Depends on measurement endpoint	Yes. About 50% of soils for urine endpoint	Similar	No	Yes	Validation effort to assess BARGE UBM extraction method. Uses 16 soils from mining/smelting areas and assesses RBA based on mulitple measurement endpoints relative to soluble salts. IVBA uses gastric and gastric+intestinal phases. For antimony, data are out of range for IVIVC. Reports "Relative Bioaccessibility" in comparison to IVBA for soluble salts. (Note: data extracted from SI and plotted for Eurometaux analysis)
LeBot et al.	2010	No	Yes	1.4% HCl		1.5				No	NA	NA	NA	NA	NA	No	NA	Presents extraction testing of dust samples from 13 homes. No IVIVC, but presents data on SRM 2583 and SRM 2584

Table 3b. Literature on IVIVC for Metals from Soil: Arsenic

									Specific Questions for Target Metals of Interest							
Author	Date	in vivo data?	in vitro data?	IVBA Method	Saliva pH	Gastric pH	Intestinal pH	G+I sequentially	# Soils	IVIVC exist?	Regression Equation for IVIVC	R2	Is IVBA conservative estimate of RBA?	Are there instances where RBA>IVBA?	How do gastric vs other fluids compare?	Are there data on IVBA of metal from alloy vs. pure metal?	Are there data on IVBA vs. soluble or insoluble forms?	Comments
Koch et al.	2013	swine mouse	Yes Yes	 IVG PBET RBALP TCLP RIVM UBM Health Canada 1:2000		 1.8 1.8-2.5 1.5 1.0 1.5 1.2 1.5	 6.1 7.0 -- -- 6.0 6.3 --	 Yes Yes No No Yes Yes No	1	Yes	Not Provided -- 1 soil	NA	Yes, for three of the methods investigated, including RBALP, RIVM, and a simple HCl extraction. Other methods underpredicted RBA from mouse or swine	Yes	G approx = GI For arsenic, there was little difference between IVBA measured in "gastric" or "intestinal" fluid. Simple extractions appear to give highest IVBA	NO	Yes	Provides interlaboratory comparison of extraction testing on NIST 2710. Good comparison of different methods. Demonstrates general trend to lower IVBA at higher pH. Compares extraction results to RBA for lead and arsenic. Data indicate that most IVBA methods tested underpredict RBA for arsenic and lead.
Denys et al.	2012	swine	Yes	UBM	6.5	1.2	6.3	Yes	16	Yes	Not Provided	NA	IVBA was greater than or equal to RBA for 7 of 15 soils, when comparison is based on urinary excretion measurement endpoint. Other measurement endpoints had less reliable data (e.g., non-detect values for more soils)	Yes	G approx = GI Results were generally very similar for the "stomach" and "stomach & intestine" extractions.	No	Yes	Validation effort to assess BARGE UBM extraction method. Uses 16 soils from mining/smelting areas and assesses RBA based on mulitple measurement endpoints relative to soluble salts. IVBA uses gastric and gastric+intestinal phases. Reports "Relative Bioaccessibility" in comparison to IVBA for soluble salts. (Note: data extracted from SI and plotted for Eurometaux analysis)
Ellickson et al.	2001	rat	Yes	Sequential Extraction	unspecified	1.4	6.5	Yes	1	No	NA	NA	Yes	No	for arsenic, gastric and G+I were similar	No	No	Studies the RBA and IVBA of lead and arsenic from NIST 2710. Only one soil, so no regression relation for IVIVC. In vitro included sequential saliva, gastric, and intestinal phase. Bioavailability values are NOT reported as RBA, and based on % dose recovered. No IV or soluble dose reported.
Diamond et al.	2016	swine mouse	Yes	RBALP		1.5		NA	83	Yes	RBA=0.79(IVBA)+3	0.87	43% of soils	57% of soils	NA	No	Yes, NaAsO4	Meta-analysis of data for 83 soils from 3 studies with RBA from studies with mice or swine and IVBA extraction using RBALP. Data have been extracted and presented in a separate figure.
Li et al.	2015	mouse	Yes	UBM Gastric		1.1			12	Yes	RBA=0.97(IVBA)-4.40	0.66	33% of soils	Yes, many	Gastric approx = G+I, but gastric slightly more conservative	No	Yes, IVBA. Data not presented	IVIVC for arsenic in 12 soils from 8 cities in China. RBA from mouse vs 5 IVBA methods. Figure 5 shows IVIVC for all methods with G and GI separate. Conclude IVG provides best prediction of RBA, but "UBM and SBRC ... also suitable." Slope of regression line very close to 1 for all methods. GI provided good correlation, but Gastric more conservative.
				UBM G+I			6.3				RBA=1.09(IVBA)-4.43	0.80	33% of soils	Yes, many				
				SBRC Gastric		1.5					RBA=0.96(IVBA)-0.60	0.67	42% of soils	Yes, many				
				SBRC G+I			7.0				RBA=1.35(IVBA)+8.22	0.57	91% of soils	Yes				
				IVG Gastric		1.8					RBA=1.29(IVBA)-1.06	0.83	50% of soils	Yes, many				
				IVG Intestinal			5.5				RBA=1.35(IVBA)+2.28	0.81	83% of soils	Yes				
				DIN Gastric		2.0					RBA=0.79(IVBA)+12.1	0.61	50% of soils	Yes, many				
				DIN Intestinal			7.5				RBA=1.36(IVBA)+4.52	0.53	58% of soils	Yes, many				
				PBET Gastric		2.5					RBA=1.08(IVBA)+8.59	0.56	67% of soils	Yes, many				
				PBET Intestinal			7.0				RBA=1.04(IVBA)+9.46	0.50	75% of soils	Yes				
Brattin et al.	2013	swine primate	Yes Yes	RBALP		1.5			20	Yes	RBA=.62(IVBA)+19.7	0.72	20% of soils	65% of soils	G>I	No	Yes, NaAsO4	IVIVC investigation for 20 soils. Investigated the effect of pH, temp, extraction time, PO4, hydroxylamine. Found clear negative trend with pH, slight increase with temp (range of 20-37 degrees) small effect of extraction time (for most samples), increase with addition of PO4, and little effect of hydroxylamine.
Juhasz et al.	2009	swine	Yes	SBRC		1.5			12	Yes	RBA=0.99(SBRCg)+1.66	0.75	50% of soils	50% of soils	G>I for most soils	No	unstated spike	Study of 12 soils from Australia. Same soils as reported in 2007 publication. Demonstrates G generally higher than Intestinal phase extractions, but not for all soils. RBA>IVBA for all IVBA method for 6 of 12 soils. SBRC gastric generally highest IVBA, and had highest correlation. Figure 3 shows that most samples RBA>IVBA. Note: table in publication provides regression equations, but figures have axes flipped for predictive equation.
							7.0				RBA = 1.6(SBRCi))+5.6	0.65						
				IVG		1.8					RBA=0.85(IVGg)+14.3	0.57	Nearly Never	Nearly all soils	Similar or G>I			
							5.5				RBA=1.11(IVGi)+13.9	0.57						
				DIN		2.0					RBA=1.8(DINg)+5.7	0.55	Nearly Never	Nearly all soils	Similar or G>I			
							7.5				RBA=1.46(DINi)+9.2	0.53						
				PBET		2.5					RBA=1.1(PBETg)+10.1	0.64	Nearly Never	Nearly all soils	Similar or G>I			
							7.0				RBA=1.76(PBETi)+5.7	0.67						
Juhasz et al.	2007	swine	Yes	SBET		1.5			12	Yes	RBA=.68(IVBA) + 5.67	0.69	Not Reported	Not Reported	Not Reported	No	No	Study of 12 soils from Australia. Evaluated for IVBA using SBET (method specified in subsequent paper), and for RBA using swine. Regression equation not provided, but extracted from Brattin et al. Also attempted to correlate RBA with soil properties, demonstrating relatively strong relationship based on arsenic and iron concentration.

Table 3b. (cont.)

										Specific Questions for Target Metals of Interest							
Author	Date	in vivo data?	in vitro data?	IVBA Method	Saliva pH	Gastric pH	Intestinal pH	G+I sequentially	# Soils	IVIVC exist?	Regression Equation for IVIVC	R2	Is IVBA conservative estimate of RBA?	Are there instances where RBA>IVBA?	How do gastric vs other fluids compare?	Are there data on IVBA of metal from alloy vs. pure metal?	Are there data on IVBA vs. soluble or insoluble forms?	Comments
Cave et al.	2016	No	Yes	UBM -G Dynamic	6.5	1.2 <1.5		Yes	1	No	NA	NA	NA	NA	NA	NA	NA	Provide a comparison of dynamic extraction vs. Static UBM on solubility of metals from one soil. For arsenic, the extraction in the dynamic method was very similar, although slightly higher than then extraction in the batch mode.
Qin et al.	2016	No	Yes	UBM	6.5	1.2	6.3	Yes	2	NA	NA		NA	NA	G<GI	No	No	Investigation of the effect of particle size on IVBA with UBM, for two soils. Particle size had mixed effect. For arsenic, higher bioaccessibility observed in G+I phase extractions. This contrasts to observation for other metals
LeBot et al.	2010	No	Yes	1.4% HCl		1.5			Dust from 13 homes	No	NA	NA	NA	NA	NA	No	NA	Presents extraction testing of dust samples from 13 homes. No IVIVC, but presents data on SRM 2583 and SRM 2584
Meunier et al.	2010	No	Yes	SBRC modified PBET/IVG		1.5 1.8	7.0 7.0	Yes Yes	19	NA	NA	NA	NA	NA	G+I>G for glycine-based system. Less so for physiologically-based system	No	Yes, Pyrite and soluble spikes	Study of 19 substrates for IVBA of arsenic, including soil amendments, NIST soils, and arsenopyrite. Compared two IVBA methods and the effect of higher fluid volume. Found that Glycine system had higher IVBA at higher fluid volume. Also showed that G+I provided higher estimate of IVBA in glycine buffer, but mixed in more physiologically-based system.
Kastury et al.	2017	No	No	Review. No new data					NA	NA	NA		NA	NA	NA	NA	NA	Summary of literature on bioavailability and bioaccessibility of metals from particulate matter and dust. Useful summary of literuature, but no useful IVIVC or comparison of methods. Compare siumulated lung fluid, cell culture, and gamble solution.
Ng et al.	2015	No	No	Review. No new data					NA	NA	NA		NA	NA	NA	NA	NA	Conclude that there is little evidence to support a single IVBA method provides discussion of chemicals added to IVBA and basis for inclusion.
U.S. EPA	2012	summary	No	Review. No new data					NA	NA	NA		NA	NA	NA	No	Yes, NaAsO4	Provides detailed review of available data regarding the bioavailabiltiy of arsenic from soils.

Table 3c. Literature on IVIVC for Metals from Soil: Cadmium

									Specific Questions for Target Metals of Interest								
Author	Date	in vivo data?	in vitro data?	IVBA Method	Saliva pH	Gastric pH	Intestinal pH	G+I sequentially	# Soils	IVIVC exist?	Regression Equation for IVIVC	R2	Is IVBA conservative estimate of RBA?	Are there instances where RBA>IVBA?	How do gastric vs other fluids compare?	Are there data on IVBA of metal from alloy vs. pure metal?	Are there data on IVBA vs. soluble or insoluble forms?	Comments
Kang et al.	2016	mouse	Yes	Modified PBET		1.5	7.0	No? not clearly stated	1	NA	NA	NA	No	Yes Reported RBA was greater than the sum of G+I extractions for the one soil tested. Note, RBA of Cd extended >100% for some individual animals, suggesting interpretation errors.	Similar. For Cd, intestinal phase was slightly higher than gastric, but within range of error	No	Yes: note, soluble forms of metal administered in mixed solution. Some metals may have precipitated.	Investigation of 1 soil: RBA of metals in soil with mouse model and comparison to IVBA. Authors conclude that gastric>intestinal for Pb, Cu, Cr, Ni, but not Cd. IVBA <RBA for all metals, by significant fraction -- from Figure 4 in the publication, RBA appears to be higher than IVBA by more than 2X for all metals. Authors conclude that there is "poor correlation between IVBA and RBA" and that "RBA provides more accurate information..." Note: averagedRBA values reported were >100% for 2 metals, and >100% for individual animals for 4 of 5 metals evaluated. Suggest interpretation issues.
Koch et al.	2013	swine	Yes						1	Yes	Not Provided -- 1 soil		no animal data for Cd	NA	G>G+I Clear trend noted, see Figure 3 and Table 3 of publication	No	Yes Soluble forms used to assess recovery from extraction fluid	Provides interlaboratory comparison of extraction testing on one soil: NIST 2710. Good comparison of different methods. Demonstrates general trend to lower IVBA at higher pH. Compares extraction results to RBA for lead and arsenic, but does not present RBA data for cadmium.
		mouse	Yes	IVG		1.8	6.1											
				PBET		1.8-2.5	7.0											
				RBALP		1.5	--											
				TCLP		1.0	--											
				RIVM		1.5	6.0											
				UBM		1.2	6.3											
				Health Canada 1:2000		1.5	--											
Denys et al.	2012	swine	Yes	UBM	6.5	1.2	6.3 Data from study plotted by Alloy. IVIVC for every RBA endpoint and IVBA phase presented.	Yes	16	Yes	Not Provided	NA	Depends on endpoint. For most measurement endpoints reported for Cd, IVBA is a reasonable predictor of RBA. In instances where RBA>IVBA, it is by a small margin.	Few	Similar	No	Yes Soluble forms administered	Validation effort to assess BARGE UBM extraction method. Uses 16 soils from mining/smelting areas and assesses RBA based on mulitple measurement endpoints relative to soluble salts. IVBA uses gastric and gastric+intestinal phases. Reports "Relative Bioaccessibility" in comparison to IVBA for soluble salts. (Note: data extracted from SI and plotted for Eurometaux analysis)
Schroder et al.	2003	swine	Yes	IVG		1.8	5.5	Yes	10	Yes	IVBA=0.60(RBA)+25.3	0.74	for 6 of 10 soils	Yes	Gastric>G+I For all 10 soils, G>G+I, by a factor of 1.3x to 2.3x	No	Yes, CdCl2	Conducted RBA evaluation of cadmium in 10 soils from hazardous wast sites vs. cadmium chloride. Also performed in vitro extraction using IVG method at pH 1.8 and 5.5. Conlude that gastric>GI, and that the presence of food decreases IVBA. Suggest gastric extraction for screening of site-specific RBA.
											IVBA=0.21(RBA)+25.8	0.54	for 1 of 10 soils					
Boros et al.	2017	No	Yes	PBET		1.8	7.0	Yes	33	No	NA	NA	NA	NA	G>G+I For NIST Soils, G > G+I by approx. 2x to 3x. For home dust samples, median G was 3-fold higher than median G+I.	No	Yes	Study using dust from 33 homes to investigate which IVBA method is more conservative, using Gastric vs Gastric+Intestinal PBET. Also investigated 4 NIST SRMs. Report that use of Gastric extraction is more conservative. Pb, Cu, Cd strongly influenced by pH, Cu and Ni less so.
Qin et al.	2016	No	Yes	UBM	6.5	1.2	6.3	Yes	2	NA	NA		NA	NA	G>G+I for both soils in all particle sizes tested.	No	No	2 soils. Investigation of the effect of particle size on IVBA with UBM. Particle size had mixed effect. G>G+I except for arsenic.
LeBot et al.	2010	No	Yes	1.4% HCl		1.5				No	NA	NA	NA	NA	NA	No	NA	Presents extraction testing of 13 French dust samples. No IVIVC, but presents data on SRM 2583 and SRM 2584
Kastury et al.	2017	No	No	Review. No new data					NA						NA			Summary of literature on bioavailability and bioaccessibility of metals from particulate matter and dust. Useful summary of literuature, but no useful IVIVC or comparison of methods. Compare siumulated lung fluid, cell culture, and gamble solution.

Table 3d. Literature on IVIVC for Metals from Soil: Cobalt

									Specific Questions for Target Metals of Interest								
Author	**Date**	**in vivo data?**	**in vitro data?**	**IVBA Method**	**Saliva pH**	**Gastric pH**	**Intestinal pH**	**G+I sequentially**	**# Soils**	**IVIVC exist?**	**Regression Equation for IVIVC**	**R2**	**Is IVBA conservative estimate of RBA?**	**Are there instances where RBA>IVBA?**	**How do gastric vs other fluids compare?**	**Are there data on IVBA of metal from alloy vs. pure metal?**	**Are there data on IVBA vs. soluble or insoluble forms?**	**Comments**
Koch et al.	2013	swine	Yes						1	Yes	Not Provided -- 1 soil		No	NA	G>G+I. Limited data (only one method used G+I for cobalt) suggest gastric IVBA is about 2x G+I phase.	No	Yes	Provides interlaboratory comparison of extraction testing on NIST 2710. Good comparison of different methods. Demonstrates general trend to lower IVBA at higher pH. Compares extraction results to RBA for lead and arsenic, but no animal data for cobalt. IVBA data for cobalt are limited, including 5 methods assessed for gastric phase IVBA, but only one method for G+I phase IVBA.
		mouse	Yes	IVG		1.8	6.1							No animal data provided for cobalt			Soluble forms assessed for recovery from IVBA fluids.	
				PBET		1.8-2.5	7.0											
				RBALP		1.5	--											
				TCLP		1.0	--											
				RIVM		1.5	6.0											
				UBM		1.2	6.3											
				Health Canada 1:2000		1.5	--											
Cobalt Institute, Unpublished. Included as Attachment 1 to this report.		Yes	Yes	Stopford 2003		1.5		NA		No	Not provided	NA	approximate	approximate	NA	Co forms and Co metal included	Yes	Cobalt Institute unpublished data provided as part of REACH Dossier, see Attachment 1 of this report. Includes IVBA, acute toxicity, and 28-day oral toxicity data for 4 cobalt compounds (CoCl2, CO, Co3O4, and CoS), and relative bioavailability data for CoCl2 (soluble) and Co3O4 (insoluble). Results indicate that the IVBA in simulated gastric fluid is very high (86-98%) for soluble forms tested (CoCl2 and Co), and very low (<1.0%) for insoluble forms (Co3O4 and CoS). Results from toxicity and bioavailability testing demonstrated similar results, with low bioavailability and low toxicity for the cobalt forms with low solubility IVBA. Correlation across samples is precluded by the number of forms tested.

Table 3e. Literature on IVIVC for Metals from Soil: Lead

												Specific Questions for Target Metals of Interest				
Author	**Date**	**in vivo data?**	**in vitro data?**	**IVBA Method**	**Saliva pH**	**Gastric pH**	**Intestinal pH**	**G+I sequentially**	**# Soils**	**IVIVC exist?**	**Regression Equation for IVIVC**	**R2**	**Is IVBA conservative estimate of RBA?**	**Are there instances where RBA>IVBA?**	**How do gastric vs other fluids compare?**	**Are there data on IVBA of metal from alloy vs. pure metal?**	**Are there data on IVBA vs. soluble or insoluble forms?**	**Comments**
Kang et al.	2016	mouse	Yes	Modified PBET		1.5	7.0	No?	1	NA	NA	NA	No Only one soil. Figure 4 of paper indicates that IVBA is about 20% of RBA, however it also indicates RBA averaging 100% across animals, suggesting design or interpretation errors.	Yes	G>>I Figure 1 of paper indicates that gastric phase extraction is about 4x intestinal phase.	No	Yes: note, soluble forms of metal administered in mixed solution. Some metals may have precipitated.	Investigation of 1 soil: RBA of metals in soil with mouse model and comparison to IVBA. Authors conclude that gastric>intestinal for Pb, Cu, Cr, Ni, but not Cd. IVBA <RBA for all metals, by significant fraction -- from FIgure 4, RBA appears to be higher than IVBA by more than 2X for all metals. "poor correlation between IVBA and RBA" and that "RBA provides more accurate information..." Note: averagedRBA values reported were >100% for 2 metals, and >100% for individual animals for 4 of 5 metals evaluated. Suggest interpretation issues.
Koch et al.	2013	swine mouse	Yes Yes	IVG PBET RBALP TCLP RIVM UBM Health Canada 1:2000		1.8 1.8-2.5 1.5 1.0 1.5 1.2 1.5	6.1 7.0 -- -- 6.0 6.3 --		1	Yes	Not Provided -- 1 soil		No	Yes. All G+I extractions were less than RBA. Several G-phase IVBA also <RBA. Low pH, simple extraction fluids give conservative estimate of RBA	G>G+I Data presented indicates that gastric phase extraction is about 3x higher than intestinal phase. Higher variability with G+I observed as well. pH dependence stronger for lead than other metals (higher pH results in lower IVBA).	No	Yes. Soluble forms used to correct for recovery from IVBA fluids.	Provides interlaboratory comparison of extraction testing on NIST 2710. Good comparison of different methods. Demonstrates general trend to lower IVBA at higher pH. Compares extraction results to RBA for lead and arsenic. Data indicate that most IVBA methods tested underpredict RBA for arsenic and lead. Simple methods of HCl buffered at low pH provided highest estimates of IVBA
Denys et al.	2012	swine	Yes	UBM	6.5	1.2	6.3	Yes	16	Yes	Not Provided	NA	Generally Depends on measurment endpoint for establishing RBA.	Yes RBA >IVBA for 20-30% of soils tested. IVBA is fairly close to RBA for most soils.	G>G+I	No	Yes	Validation effort to assess BARGE UBM extraction method. Uses 16 soils from mining/smelting areas and assesses RBA based on mulitple measurement endpoints relative to soluble salts. IVBA uses gastric and gastric+intestinal phases. Reports "Relative Bioaccessibility" in comparison to IVBA for soluble salts. (Note: data extracted from SI and plotted for Eurometaux analysis)
								Data from study plotted by Alloy. IVIVC for every RBA endpoint and IVBA phase presented.										
Ellickson et al.	2001	rat	Yes	Sequential	unspecified	1.4	6.5	Yes	1	No	NA	NA	Yes	No RBA values reported for lead are all <1%. IVBA ranged from 10% to 76%.	G>>G+I Gastric phase IVBA is more than 7x intestinal phase.	No	No	Studies the RBA and IVBA of lead and arsenic from NIST 2710. Only one soil, so no regression relation for IVIVC. In vitro included sequential saliva, gastric, and intestinal phase. Bioavailability values are NOT reported as RBA, and based on % dose recovered. No IV or soluble dose reported.
Dong et al.	2016	swine rat	Yes	Statistical		1.5			252	Yes	RBA=0.87(IVBA)+4.7	NS	about 50% of soils	Yes	Only used data from gastric phase extractions.	No	No	RBA and IVBA from 252 paired soils from 9 publications. Meta-analysis. Provides statistical analysis of aggregate data from multiple IVBAs and RBA endpoints. Used gastric phase data only. Comment that IVIVC slopes are all <1. This analysis does not tell us which IVBA method is best predictor, but Figure 2 shows that many samples have RBA>IVBA.
Liu et al.	2016	See Dong et al., 2016		See Dong et al., 2016														Same as Dong, 2016
Juhasz et al.	2016	mouse	Yes	SBRC		1.5	7.0		3	Yes	RBA=0.16(IVBA)-1.74	0.84	Yes	Yes	G>>G+I	No	No	IVIVCa for 3 phosphate-amended soils using 5 IVBA methods. Generally demonstrates that extraction with gastric phase IVBA provides conservative estimate of RBA. For G+I, RBA was generally and significantly underestimated.

Table 3e. (cont.)

										Specific Questions for Target Metals of Interest								
Author	**Date**	**in vivo data?**	**in vitro data?**	**IVBA Method**	**Saliva pH**	**Gastric pH**	**Intestinal pH**	**G+I sequentially**	**# Soils**	**IVIVC exist?**	**Regression Equation for IVIVC**	**R2**	**Is IVBA conservative estimate of RBA?**	**Are there instances where RBA>IVBA?**	**How do gastric vs other fluids compare?**	**Are there data on IVBA of metal from alloy vs. pure metal?**	**Are there data on IVBA vs. soluble or insoluble forms?**	**Comments**
Li et al.	2014	mouse	Yes	SBRC		1.5	7.0		15	Yes	RBA=0.61(SBRCg)+3.15	0.68	Yes (SBRC. Not others)	None (SBRC)	Gastric>>G+I	No	Yes	Investigates RBA and IVBA of lead from 15 dust samples from China. Gastric always>Intestinal. Intestinal phase always underpredicts RBA. Includes 4 IVBA methods, each at G and GI. SBRC>IVB>DIN>PBET.
				IVG		1.8	5.5				RBA=0.48(IVGg)+14.3	0.56	All but one soil	1 of 15 samples	Gastric>>G+I			
				DIN		2.0	7.5				RBA=0.67(DINg)+17.4	0.85	No	>50% of soils	Gastric>>G+I			
				PBET		2.5	7.0				RBA=0.69(PBETg)+20	0.52	No	>50% of soils	Gastric>>G+I			
Van de Wiele et al.	2007	human	Yes	PBET		2.5	7.0		1	Yes	NA Only one soil	NA	Yes,, Gastric	Yes, G+I	G>G+I for fasted, and generally for "fed" conditions.	No	No	Assessed IVBA using 5 in vitro methods on the one Bunker Hill soil fed to humans to assess bioavailability (by Maddaloni). When food added, "fed" IVBA always > than fed RBA. Without food, IVBA>RBA 3 times, IVBA=RBA 3 times, and IVBA<RBA 3 times.
				DIN		2.0	7.5						Not for fasted	Yes, fasted				
				RIVM	6.5	1.1	7.0						Yes	No				
				SHIME	5.2	2.0 or 4.0	6.5						Not for fasted	Yes, fasted				
				TIM	1.8 or 2	6.5	6.8 to 7.2						Yes	No				
Boros et al.	2017	No	Yes	PBET		1.8	7.0	Yes	33	No	NA	NA	NA	NA	G=8xG+I	No	Yes	Study using dust from 33 homes to investigate which IVBA method is more conservative, using Gastric vs Gastric+Intestinal PBET. Find that G>GI by a factor of 1.1-8, for all metals except Chromium. Report that use of Gastric extraction is more conservatieve. Pb, Cu, Cd strongly influenced by pH, Cu and Ni less so.
Cave et al.	2016	No	Yes	UBM -G Dynamic	6.5	1.2		Yes	1	No	NA	NA	NA	NA	NA	NA	NA	Provide a comparison of dynamic extraction vs. Static UBM on solubility of metals from one soil. For most metals dynamic and batch results were similar, except for lead, which is much lower from the batch method. The authors suggest this is due to readsorption to soil in the static reaction vessel as the pH increases.
Qin et al.	2016	No	Yes	UBM	6.5	1.2	6.3	Yes	2	NA	NA		NA	NA	G>G+I Comparing figures suggests extraction in gastric phase was about 4x observed in intestinal phase	No	No No CRM, no blanks, no spikes.	Using 4 particle sizes for each of two crushed soils, Investigated the effect of particle size on IVBA with UBM. Investigated many metals. Particle size had mixed effect. G>G+I for all metals except for arsenic.
LeBot et al.	2010	No	Yes	1.4% HCl		1.5				No	NA	NA	NA	NA	NA	No	NA	Presents extraction testing of 13 French dust samples. No IVIVC, but presents data on SRM 2583 and SRM 2584
Rosende et al.	2014	No	Yes	UBM-G	6.5	1.1	NA		2	No	NA		NA	NA	Gastric assumed to be "worst case." No difference with saliva or mucin. Temp has effect	No	No	Evaluation of UBM-like extraction in dynamic format, using 2 soils. Results show little effect of mucin or saliva, but effect of temp on extraction.
Li et al.	2013	No	Yes	PBET		2.5	7.0	Yes	3	No	NA		NA	NA	Gastric>G+I for Gastric > G+I For both methods, Gastric IVBA was at least 2x G+I.	No	No	Investigated the function of digestive enzymes on relase of copper, zinc, and lead from 3 soil samples from China, using two IVBA methods. Reports Gastric, and Gastric+Intestinal. Concluded that lead and zinc solubility is pH dependent. Found no change on copper or lead when pepsin added to PBET. Pepsin has mixed effect on zinc when added to PBET or SGET. Consistent increase when pepsin added to GI phase of SGET.
				SGET		1.1	7.0	Yes										
Yan et al.	2016	No	Yes	RBALP		1.5	--		10	No	NA	NA	NA	NA	G>G+I gastric IVBA varied directly with pH. Intestinal IVBA always much lower than gastric.	No	No	Uses 10 soils from mining and smelter locations in Australia. Conducts IVBA extractions and compares findings of different IVBA methods. Found soil:fluid ratio has effect for RIVM method. Figure 1 shows significant correlation between all extraction methods. Concludes that Gastric>GI for lead, and extraction is sensitive to rotation, pH, and soil:fluid ratio. For lead, pH effects are very strong. Lower pH resulted in higher IVBA. Rotation>shaking, and soil:fluid ratio mattered.
				SBRC		1.5	6.5	Yes										
				PBET		2.0	6.5	Yes										
				UBM	6.5	1.1	7.4-8.0	Yes										
				RIVM	6.5	1.1	7.8-8.0	Yes										

Table 3e. (cont.)

										Specific Questions for Target Metals of Interest								
Author	Date	in vivo data?	in vitro data?	IVBA Method	Saliva pH	Gastric pH	Intestinal pH	G+I sequentially	# Soils	IVIVC exist?	Regression Equation for IVIVC	R2	Is IVBA conservative estimate of RBA?	Are there instances where RBA>IVBA?	How do gastric vs other fluids compare?	Are there data on IVBA of metal from alloy vs. pure metal?	Are there data on IVBA vs. soluble or insoluble forms?	Comments
Kastury et al.	2017	No	No	Review. No new data					NA						NA			Summary of literature on bioavailability and bioaccessibility of metals from particulate matter and dust. Useful summary of literuature, but no useful IVIVC or comparison of methods. Compare siumulated lung fluid, cell culture, and gamble solution.
Ng et al.	2015	No	No	Review. No new data					NA	NA	NA		NA	NA	NA	NA	NA	Conclude that there is little evidence to support a single IVBA method provides discussion of chemicals added to IVBA and basis for inclusion.

Table 3f. Literature on IVIVC for Metals from Soil: Mercury

				If IVIVC Presented										Specific Questions for Target Metals of Interest								
Author	Date	in vivo data?	in vitro data?	slope	intercept	axes correct?	R2	Instance where RBA>IVBA?	IVBA Method	Saliva pH	Gastric pH	Intestinal pH	G+I sequentially	# Soils	IVIVC exist?	Regression Equation	R2	Is IVBA conservative estimate of RBA?	Are there instances where RBA>IVBA?	How do gastric vs other fluids compare?	Are there data on IVBA of metal from alloy vs. pure metal?	Are there data on IVBA vs. soluble or insoluble forms?	Comments
Safruk et al.	2015	human	Yes	NA	NA	NA	NA	NA	PBET modified		1.8	7.0	Yes	50	No	NA		NA	NA	G+I>G Assessed for 4 neighvorhoods, and summary statistics provided. Mean G+I IVBA was greater than gastric alone by a factor of 1.5-6.	No	No	Compared bioaccessible mercury as measured by modified PBET with urinary inorganic mercury. Report no relation between soil Hg and urinary Hg in biomonitoring of children. Found G+I>G by factor of 1.5to 6.
Bradley et al.	2017	No	Yes	NA	NA	NA	NA	NA	Not Specified					NA	No	NA		NA	NA	NA	No		Investigation of the bioavailability of mercury from fish. Demonstrates reduced bioaccessibility from cooking. States need for IVIVC.
Hagan et al.	2014	No	Yes	NA	NA	NA	NA	NA	RBALP	--	1.5	--	NA	60	No	NA		NA	NA	NA	No	No	Compares RBALP to Hg sequential extraction tests for 61 samples of brick and 49 samples of dirt floors in Peru. Authors propose that F1+F2+F3 fractions represent bioaccessible mercury.
Welfringer and Zagury	2009	No	Yes	NA	NA	NA	NA	NA	IVG CDM	-- 	1.8 2.5	6.5 5.5	Yes Yes	5	No	NA		NA	NA	G+I>Gastric G+I>Gastric	No	HgS and HgCl2	Studied 5 soils from mining or industrial sites in Canada, plus two CRMs (CRM 025-050 and ERM-CC580. Found that extraction in IVG>CDM method. Not able to tell whether that is due to consituents, pH, or temperature. Bioaccessibility from soil is very low relative to soluble standard (IVBA range from 1.5-7.5%).
Zagury et al.	2009	No	Yes						More data presented in Welfringer and Zagury 2009.														Studied three soils from Canada and two CRMs. More data from same study and additional soils are presented in Welfringer and Zagury 2009.
Schoof and Nielsen	1997	No	No	NA	NA	NA	NA	NA	Review. No new data						NA	NA		NA	NA	NA	No	HgS and HgCl2	Provides a review of method for assessing mercury absorption, and suggestions for approaches to evaluating bioavailability for less available forms that occur in soils. Suggests that MgCl2 has approximately 20% bioavailability, but that even as a salt, HgS may be 30-60-fold less bioavailable than HgCl2.

Table 3g. Literature on IVIVC for Metals from Soil: Nickel

										Specific Questions for Target Metals of Interest								
Author	Date	in vivo data?	in vitro data?	IVBA Method	Saliva pH	Gastric pH	Intestinal pH	G+I sequentially	# Soils	IVIVC exist?	Regression Equation for IVIVC	R2	Is IVBA conservative estimate of RBA?	Are there instances where RBA>IVBA?	How do gastric vs other fluids compare?	Are there data on IVBA of metal from alloy vs. pure metal?	Are there data on IVBA vs. soluble or insoluble forms?	Comments
Kang et al.	2016	mouse	Yes	Modified PBET		1.5	7.0	No?	1	NA	NA	NA	No	Yes RBA appears to be more than 2x IVBA, but data limited to one soil. RBA approximately 30%	G>>I Indicates gastric IVBA is more than 2x intestinal IVBA	No	No	Investigation of 1 soil: RBA of metals in soil with mouse model and comparison to IVBA. Authors conclude that gastric>intestinal for Pb, Cu, Cr, Ni, but not Cd. IVBA <RBA for all metals, by significant fraction -- from Figure 4, RBA appears to be higher than IVBA by more than 2X for all metals. Authors state "poor correlation between IVBA and RBA" and that "RBA provides more accurate information..." Note: averagedRBA values reported were >100% for 2 metals, and >100% for individual animals for 4 of 5 metals evaluated. Suggest interpretation issues.
Koch et al.	2013	swine mouse	Yes Yes	 IVG PBET RBALP TCLP RIVM UBM Health Canada 1:2000		 1.8 1.8-2.5 1.5 1.0 1.5 1.2 1.5	 6.1 7.0 -- -- 6.0 6.3 --		1	Yes	Not Provided -- 1 soil		No	NO	G>G+I. For Ni, Gastric IVBA was more than 2x G+I for both mean and maximum reported across labs.	No	Yes. Soluble forms used to correct for recovery from IVBA fluids.	Provides interlaboratory comparison of extraction testing on NIST 2710. Good comparison of different methods. Demonstrates general trend to lower IVBA at higher pH. Compares extraction results to RBA for lead and arsenic. Data indicate that most IVBA methods tested underpredict RBA for arsenic and lead.
Dutton et al.	2016	rat	Yes	PBET		2.5	7.0	Yes	21	Yes	RBA=0.01(IVBA)-0.0004	0.526	Yes	No	Not Reported Poster	No	NiSO4	RBA and IVBA of 21 soils from Port Colborne. Urinary Ni in rats, 2% excreted across dose levels. Small range in RBA, wide range in IVBA.
Henderson et al	2012a	Yes	No								No	NA	Refer to Henderson 2012b	NA	NA	No	Various nickel forms tested	Acute toxicity study of 16 samples, including 9 Ni compounds, 2 complexes and 3 mattes. Conducted to comply with requirements of REACH. Report LD50 values ranging from 310 mg/kg to >5000 mg/kg. Refers to companion publication, Henderson et al 2012b to establish that toxicity associated with bioavailability. Information from Henderson 2012a and 2012b has been summarized by NiPERA and interpreted with regard to IVBA estimates on bioavailability and toxicity, and is included as Attachment 2 to this report.
Henderson et al	2012b	Yes	Yes	Stopford 2003	NA	1.5	7.4	no		IVBA vs. LD50	Not Provided	0.91	NA IVBA vs. LD50	NA	GI>Intestinal, generally by >2X	No	Six water-soluble forms and six water insoluble forms	Investigation of bioaccessibility of 12 samples of 11 nickel compounds, conducted to fill data gap for nickel substances and investigate use of read-across for nickely substances and toxicitieis. All very fine particles (<100 um average). Conducted extraction in simulated gastric and simulated intestinal fluids, at several time points (2-72 hour extractions). Results indicate much higher IVBA in gastric solution than intestinal. Also demonstrates much higher IVBA for water-soluble forms of nickel. Gastric (2-hour) IVBA showed strongest relation with LD50. Plot of IVBA vs. LD50 shows strong relationship between toxicity and IVBA -- those with low IVBA also have low LD50. This relationship is not linear. Dissolution in simulated gastric fluid at 2 hours was higher than at 72 hours in simulated intestinal fluid. 5 Ni forms match those tested for RBA, presented in Ishimatsu 1995. Information from Henderson 2012a and 2012b has been summarized by NiPERA and interpreted with regard to IVBA estimates on bioavailability and toxicity, and is included as Attachment 2 to this report.
Vasiluk et al.	2011	rat	Yes	PBET (modified)		2.5	7.0	Yes	2	No	NA	NA	50% to 75% of soils IVBA is greater than reported RBA for 3 of 4 soils tested, but RBA for one is negative number. So likely unknown for that sample.	25% to 50% of soils	Very close.	No	NiSO4	Conducted study in vitro and in vivo (rat) for 2 soils and 2 particle sizes for smelter-contaminated soil. Found little difference in IVBA between G and G+I extracts. No clear relation between RBA and IVBA for the 4 samples (2 soils) evaluated. Bioavailability estimates highly variable. State that "in vitro…will be predictive of bioavailability to the degree that mobilization from the soil matrix in GI solution limts oral bioavailability."

Table 3g. (cont.)

										Specific Questions for Target Metals of Interest								
Author	**Date**	**in vivo data?**	**in vitro data?**	**IVBA Method**	**Saliva pH**	**Gastric pH**	**Intestinal pH**	**G+I sequentially**	**# Soils**	**IVIVC exist?**	**Regression Equation for IVIVC**	**R2**	**Is IVBA conservative estimate of RBA?**	**Are there instances where RBA>IVBA?**	**How do gastric vs other fluids compare?**	**Are there data on IVBA of metal from alloy vs. pure metal?**	**Are there data on IVBA vs. soluble or insoluble forms?**	**Comments**
Ishimatsu et al.	1995	Yes	Yes	Water or Saline							not provided	NA	not stated		only evaluated saline and water	No. Does include metal compounds and metalic nickel	Yes. Forms of various solubility tested.	Study of tissue distribution of nickel after oral exposure by rats, for 8 nickel compounds (NiO, Ni-M, Ni3S2 ,NiS, NiSO4, NiCl2, Ni(NO3)2). Reported "absorbed fraction" correlated with solubility. Solubility in saline was greater than in distilled water for all metals with data for both solutions, generally by a factor of less than 2X. Authors report that the absorbed fraction (sum of organ, blood and urine mass divided by administered dose) ranged from less than 1% (nickel oxides and nickel metal) to 33.8% (nickel nitrate). No data on solubility under gastrointestinal conditions, but data establish that absorbed dose is related to aqueous solubility. Information from this study is included in a summary prepared by NiPERA, included as Attachment 2.
Boros et al.	2017	No	Yes	PBET		1.8	7.0	Yes	33	No	NA	NA	NA	NA	G>G+I Gastric IVBA exceeded G+I by factor of 1.2 to 1.6	No	Yes	Study using dust from 33 homes to investigate which IVBA method is more conservative, using Gastric vs Gastric+Intestinal PBET. Find that G>GI by a factor of 1.1-8, for all metals except Chromium. Report that use of Gastric extraction is more conservative. Report that Ni IVBA is less sensitive to pH than Pb, Cu, and Cd.
Cave et al.	2016	No	Yes	UBM -G Dynamic	6.5	1.2		Yes	1	No	NA	NA	NA	NA	NA	NA	NA	Provide a comparison of dynamic extraction vs. Static UBM on solubility of metals from one soil. For most metals dynamic and batch results were similar, except for lead, which is much lower from the batch method. The authors suggest this is due to readsorption to soil in the static reaction vessel as the pH increases.
Qin et al.	2016	No	Yes	UBM	6.5	1.2	6.3	Yes	2	NA	NA		NA	NA	G>G+I Figure indicates slightly higher IVBA in Gastric phase. Less than 2x.	no	No	Investigate IVBA of 4 particle size fractions of two soils. Investigation of the effect of particle size on IVBA with UBM. Particle size had mixed effect. G>G+I for Nickel
Rosende et al.	2014	No	Yes	UBM-G	6.5	1.1	NA		2	No	NA		NA	NA	Gastric assumed to be "worst case." No difference with saliva or mucin. Temp has effect	No	No	Evaluation of UBM-like extraction in dynamic format, using 2 soils. Results show little effect of mucin or saliva, but effect of temp on extraction. No intestinal phase included because gastric assumed to represent "worst case" conditions.

Table 4. Summary of data for studies reporting IVBA for NIST standard reference material soils.

			IVBA (%)						
SRM	**Study**	**pH**	**As**	**Pb**	**Zn**	**Cd**	**Cu**	**Ni**	**Cr**
NIST 2710									
As RBA= 44 in swine (Casteel), 42.9 in mice (Bradham) and 38 in rat (Ellickson)									
	Boros 2017	7		15.7	11.4	39.3	44.6	9.6	
	Boros 2017	1.8		64.9	28.8	74.4	63.3	14	
	Ellickson 2001	1.4		76.1					
	Ellickson 2001	7		10.7					
	Brattin 2013	1.5	55.1						
	Brattin 2013	7	5.8						
	Diamond 2016	1.5	54						
	Meunier 2012	1.5	42						
		1.8	33						
	Koch 2013	Figure 3 from Koch et al., 2013 demonstrate similar							
NIST 2710a									
As RBA= 42 in swine (EPA 2012) & 42.1 in mice (Bradham)									
	Boros 2017	7.0		4.9	12.1	22.7	37.2	8.2	
	Boros 2017	1.8		45.7	42.3	45.6	53	12.8	
	Brattin 2013	1.5	42.2						
		5.0	1.0						
		7.0	1.9						
	Diamond 2016	1.5	41.8						
NIST 2711a									
Pb RBA= 37 mice (Li 2014)									
	EPA 2017	1.5		86					
	Li 2014, 2015	1.5	61	91					
		1.8	50.0	91					
		2.0		71					
		2.5	49	47					
		5.5	43	5					
		7.5		2					
		7	47	11					
		7	49	19					
NIST 2583									
	Boros 2017	1.8		31.1	95.3	69.7	33	26.4	
	Boros 2017	7		13.4	42.7	39.6	31.8	21.4	
	Dodd (from Boros)	1.5			92	94	53	42.0	
	Le Bot 2010	1.5	67.0	74		69			31
NIST 2584									
	Boros 2017	1.8		56.1	84.7	80.1	43.4	23.9	
		7		7.6	21.1	28.9	42.3	18.7	
	Dodd (from Boros)	1.5			93	95	73	34.0	
	Le Bot 2010	1.5	40.0	90		76			30

Table 5. Relative bioavailability data and bioaccessibility data extracted from Denys et al. 2012

		Pb RBA (%)												Pb RBAc (%)	
		95% CI			95% CI			95% CI			95% CI				
Site	Soil	*Lower*	*Upper*	**Kidney**	*Lower*	*Upper*	**Liver**	*Lower*	*Upper*	**Bone**	*Lower*	*Upper*	**Urine**	Stomach	Stomach & Intestine
Site 1 & 2 (mining)	1	*2*	*10*	6	*6*	*14*	10	*5*	*11*	9	*4*	*12*	8	10.6	9.2
	2	*4*	*10*	7	*7*	*13*	10	*2*	*10*	6	*7*	*13*	10	11.5	14.2
	3	*19*	*23*	21	*15*	*21*	18	*7*	*17*	12	*18*	*22*	20	15.4	16.2
	4	*23*	*27*	25	*26*	*30*	28	*32*	*36*	34	*30*	*34*	32	22.9	18.4
	5	*27*	*39*	33	*33*	*41*	37	*32*	*42*	37	*27*	*41*	34	31.25	18.9
	6	*16*	*28*	22	*19*	*27*	23	*27*	*35*	31	*25*	*37*	31	37.1	36.6
	7	*48*	*52*	50	*38*	*42*	40	*50*	*54*	52	*43*	*47*	45	61.2	48.7
	8	*54*	*60*	57			NE	*59*	*62*	60	*57*	*61*	59	70.2	75.1
	9	*60*	*64*	62			NE	*53*	*57*	55	*52*	*56*	54	71.1	73.3
Site 3 (smelting)	A	*28*	*34*	31	*27*	*31*	29	*39*	*43*	41	*26*	*30*	28	40.5	33.4
	B	*43*	*49*	46	*26*	*34*	30	*38*	*46*	42	*33*	*45*	39	53.7	54.4
	C	*47*	*55*	51	*35*	*41*	38	*42*	*48*	45	*53*	*59*	56	64.9	51.9
	D	*62*	*88*	75	*70*	*90*	80	*94*	*106*	100	*93*	*107*	100	72.9	80.0
	E	*87*	*113*	100	*66*	*80*	78	*94*	*106*	100	*93*	*107*	100	81.4	61.4
	F	*87*	*113*	100	*77*	*93*	85	*94*	*106*	100	*93*	*107*	100	82.6	86.7

		Cd RBA (%)												Cd RBAc (%)	
		95% CI			95% CI			95% CI			95% CI				
Site	Soil	*Lower*	*Upper*	**Kidney**	*Lower*	*Upper*	**Liver**	*Lower*	*Upper*	**Bone**	*Lower*	*Upper*	**Urine**	Stomach	Stomach & Intestine
Site 1 & 2 (mining)	1			ND			ND			ND			ND	8.37	7.25
	2	*3*	*15*	9	*6*	*18*	12	*6*	*18*	12	*10*	*20*	15	12.6	10.5
	3	*43*	*53*	48	*42*	*54*	48	*35*	*43*	39	*36*	*52*	44	45.9	54.1
	4			ND			ND			ND			ND	54.6	49.2
	5	*30*	*42*	36			ND	*27*	*32*	27			ND	38.6	25.0
	6	*51*	*59*	55	*45*	*57*	51	*55*	*69*	62	*55*	*69*	62	64.0	56.1
	7	*9*	*25*	17			ND			ND			ND	19.3	17.1
	8	*45*	*53*	49	*36*	*50*	43	*37*	*53*	45	*50*	*60*	55	42.0	45.0
	9	*31*	*45*	38	*33*	*49*	41	*29*	*43*	36	*29*	*41*	35	45.9	52.0
Site 3 (smelting)	A			ND			ND			ND			ND	79.8	75.1
	B	*71*	*107*	89			ND			ND			ND	87.7	75.1
	C			ND			ND			ND			ND	65.2	60.5
	D	*61*	*99*	80			ND	*53*	*97*	75			ND	83.0	72.0
	E			ND			ND			ND			ND	36.9	28.2
	F	*71*	*77*	74	*73*	*77*	75	*79*	*85*	82	*73*	*79*	76	70.0	77.2

Table 5. (cont.)

		As RBA (%)												As RBAc (%)	
		95% CI			95% CI			95% CI			95% CI				
Site	Soil	*Lower*	*Upper*	**Kidney**	*Lower*	*Upper*	**Liver**	*Lower*	*Upper*	**Bone**	*Lower*	*Upper*	**Urine**	Stomach	Stomach & Intestine
Site 1 & 2 (mining)	1	*0.5*	*3.5*	2	*2*	*6*	4	*3*	*7*	5	*0.5*	*5.5*	3	3.00	4.95
	2	*1*	*5*	3	*2*	*6*	4	*1*	*5*	3	*1*	*5*	3	11.2	5.00
	3	*10*	*24*	17	*2*	*20*	11	*4*	*24*	14	*0.5*	*23.5*	12	11.0	11.4
	4	*0.2*	*3.8*	2	*0.3*	*5.7*	3	*0.4*	*5.6*	3	*0.6*	*7.4*	4	8.51	7.06
	5			ND			ND			ND			ND	4.48	3.41
	6	*5*	*13*	9	*7*	*15*	11	*5*	*15*	10	*7*	*17*	12	12.5	8.52
	7	*1*	*9*	5	*1*	*9*	5	*1*	*7*	4	*1*	*9*	5	7.85	6.00
	8	*5*	*11*	8	*3*	*9*	6	*4*	*10*	7	*3*	*11*	7	5.39	5.39
	9	*7*	*15*	11	*6*	*14*	10	*3*	*11*	7	*10*	*20*	15	7.46	4.35
Site 3 (smelting)	A			ND			ND	*19*	*99*	59	*16*	*96*	56	49.0	49.8
	B	*59*	*73*	66	*63*	*78*	71			NC	*60*	*84*	72	74.5	79.4
	C			ND	*19*	*41*	30	*14*	*58*	36	*20*	*50*	35	31.8	35.3
	D			ND			ND			ND			ND	10.8	8.54
	E			ND			ND			ND	*87*	*113*	100	69.6	67.7
	F	*34*	*44*	39	*34*	*46*	40	*40*	*62*	51	*36*	*48*	42	52.2	49.6

		Sb RBA (%)												Sb RBAc (%)	
		95% CI			95% CI			95% CI			95% CI				
Site	Soil	*Lower*	*Upper*	**Kidney**	*Lower*	*Upper*	**Liver**	*Lower*	*Upper*	**Bone**	*Lower*	*Upper*	**Urine**	Stomach	Stomach & Intestine
Site 1 & 2 (mining)	1	*1*	*5*	3	*1*	*5*	3	*2*	*6*	4	*0.2*	*1.8*	1	4.56	6.10
	2	*1.5*	*6.5*	4	*2*	*6*	4	*0.6*	*3.4*	2	*0.8*	*3.2*	2	6.38	7.91
	3			ND			ND			ND			ND	4.64	5.18
	4			ND			ND			ND	*2.6*	*11.4*	7	5.29	4.47
	5			ND			ND			ND			ND	7.94	7.55
	6			ND			ND			ND	*3*	*15*	9	17.9	13.8
	7			ND			ND			ND	*3.8*	*8.2*	6	2.85	5.32
	8			ND			ND			ND	*1.1*	*20.9*	11	14.0	14.5
	9			ND			ND			ND	*2.8*	*11.2*	7	9.72	10.1
Site 3 (smelting)	A			ND			ND			ND	*4.3*	*15.7*	10	7.50	10.0
	B			ND			ND			ND	*5.6*	*16.4*	11	9.11	8.33
	C			ND			ND			ND	*2.2*	*7.8*	5	5.73	3.84
	D			ND			ND			ND			ND	6.19	9.44
	E			ND			ND			ND			ND	6.55	9.86
	F			ND			ND			ND	*2.9*	*15.1*	9	5.05	3.07

Table 5. (cont.)

Source:
Denys et al. (2012) In vivo validation of the Unified BARGE Method to assess the bioaccessibility of arsenic, antimony, cadmium, and lead in soils

Notes:
As - Arsenic
Cd - Cadmium
CI - Confidence interval
ND - Not determined because values of metals in tissue/fluid were inferior to the limit of quantification
NE - Not estimated because dose-response curves were not adjusted by linear model
Pb - Lead
RBA - Relative bioavailability
RBAc - Relative bioaccessibility
RSD - Relative standard deviation
Sb - Antimony

Table 6. Composition and parameters utilized in common in vitro bioaccessibility assays

Method /	ASTM D5517-4	BARGE UBM	DIN	EN 71-3	IVG	PBET	RBALP	SBRC
phase		**Saliva–gastric Saliva (pH 6.5±0.5):**		**"Maceration" for oily or waxy samples**				
In vitro parameters								
Composition (g l−1)		0.896 g KCl, 0.888 g NaH2PO4, 0.2 g KSCN, 0.57 g Na2SO4, 0.298 g NaCl, 1.8 ml of 1 M NaOH, 0.2 g urea, 0.145 g amylase, 0.05 g mucin, 0.015 g uric acid		water				
pH		1.2		not specified				
Soil/ solution ratio		0.6:22.5		1:25				
Extraction time (h)		1		1 min, follow by gastric				
	Gastric	**Gastric phase**	**Gastric**	**Gastric**	**Gastric**	**Gastric**	**Gastric**	**Gastric**
In vitro parameters								
Composition (g l−1)	0.7N HCl	2.752 g NaCl, 0.266 g NaH2PO4, 0.824 g KCl, 0.4 g CaCl2, 0.306 g NH4Cl, 8.3 ml of 37 % HCl, 0.65 g glucose, 0.02 mg glucuronic acid, 0.085 g urea, 0.33 g glucosaminehydrochloride, 1 g bovine serum albumin, 3 g mucin, 1 g pepsin	1 g pepsin, 3 g mucin, 2.9 g NaCl, 0.7 g KCl, 0.27 g KH2PO4	0.7N HCl	10 g pepsin, 8.77 g NaCl	1.25 g pepsin, 0.5 g sodium malate, 0.5 g sodium citrate, 420 µl lactic acid, 500 µl acetic acid	0.4M Glycine	0.4M Glycine
pH	1.0 to 1.5	1.2	2.0	1.0 to 1.5	1.8	2.5	1.5	1.5
Soil/ solution ratio	1:50	0.6:22.5	1:50	1:50	1:150	1:100	1:100	1:100
Extraction time (h)	1shaken, 1 stationary	1	2	1shaken, 1 stationary	1	1	1	1
		Gastric–intestinal Duodenal phase (pH 7.4±0.2)	**Intestinal**		**Intestinal**	**Intestinal**	**Intestinal**	**Intestinal**
In vitro parameters								
Composition (g l−1)		7.012 g NaCl, 5.607 g NaHCO3, 0.08 g KH2PO4, 0.564 mg KCl, 0.05 g MgCl2, 0.18 ml of 37 % HCl, 0.1 g urea, 0.2 g CaCl2, 1 g bovine serum albumin, 3 g pancreatin, 0.5 g lipase	9.0 g bile, 9.0 g pancreatin, 0.3 g trypsin, 0.3 g urea, 0.3 g KCl, 0.5 g CaCl2, 0.2 g MgCl2		3.5 g bile, 0.35 g pancreatin	1.75 g bile, 0.5 g pancreatin		1.75 g bile, 0.5 g pancreatin
pH		6.5	7.5		5.5	7.0		7.0
Soil/ solution ratio		0.6:58:5	1:100		1:150	1:100		1:100
Extraction time (h)		4	6		1	4		4
		Bile phase (pH 8.0±0.2)						
In vitro parameters								
Composition (g l−1)		5.259 g NaCl, 5.785 g NaHCO3, 0.376 g KCl, 0.18 ml of 37 % HCl, 0.25 g urea, 0.222 CaCl2, 1.8 g bovine serum albumin, 6 g bile						
pH		6.5						
Soil/ solution ratio		0.6:58:5						
Extraction time (h)		4						

ASTM: International Association for Testing Materials
BARGE UBM: Bioaccessibility Research Group Europe, Unified BARGE Method
DIN: Deutsches Institut fur Normunge (2000)
EN 71-3: European Committee for Standarization
IVG: In Vitro Gastrointestinal (Rodriquez 1999)
PBET: Physiologically-Based Extraction Test (Ruby 1996)
RBALP: Relative Bioavailability Leaching Procedure (Drexler & Brattin, 2007)
SBRC: Solubility Bioavailability Research Consortium (Ruby 1999)

ATTACHMENT 1. COBALT INSTITUTE: SUBMISSION FROM REACH DOSSIER

Relationship between bioaccessibility in gastric fluid and in vivo bioavailability and toxicity

1. Bioaccessibility of Co from various Co substances in gastric fluid. These substances are in powder/particulate/crystal form, and were added at a loading of 2 g/L. The methods were as described in Stopford et al (2003).

Table 1. Bioaccessibility of Co from various Co substances in gastric fluid

Substance	Particle Size D50	duration [h]	Co content in sample (%)	Co % release of Co content	Co release (µg/mL)	Co release (mg Co/g substance)
$CoCl_2$	(Crystal)	2	24.80	86	432.10	216.05
Co	4.88 µm	2	99.98	98	1980.14	990.07
Co_3O_4	6.65 µm	2	72.40	0.2	2.92	1.46
CoS	26.24 µm	2	62.95	0.6	7.45	3.72

2. In vivo bioavailability of Co from Co dichloride and tricobalt tetraoxide measured by determination of Co plasma levels after i.v. or p.o. bolus dose

Table 2. In vivo bioavailability of Co from $CoCl_2$ and Co_3O_4

Test item	Dose level (substance) mg/kg bw	Co in dose (mg Co/kg bw)	Admin. route	AUC(0-inf) [h*ug/L], adjusted to theoretical dose of 1 mg/kg bw	% Relative bioavailability based on $CoCl_2$ (i.v.)	% Relative bioavailability based on $CoCl_2$ (p.o.)	Co release in gastric fluid (mg Co/g substance) [% of Co content released]
$CoCl_2$	0.1	0.0248	i.v.	356.85	100		
$CoCl_2$	10	2.48	p.o.	17.34	6.24	100	216.05 [86%]
Co_3O_4	300	213.9	p.o.	0.16	0.06	0.96	1.46 [0.2%]

3) Acute oral toxicity of Co substances

Co substances (Co, Co_3O_4, CoS) were tested in acute toxicity studies according to OECD guideline 425 in female rats, as previous studies indicated that females are frequently more sensitive to cobalt than males. Testing followed the "Up and Down Procedure", where dosing begins with the limit dose of 5000 mg test substance/kg bw in one female, and proceeds to 2 additional females at the same dose and higher (depending on toxicity), or proceeds to the main test (175, 550 and 1750 mg/kg) if toxicity is observed at the Limit Dose. Animals were observed for 14 days following exposure for mortality, signs of gross toxicity and behavioral changes at least once a day. Body weights were recorded prior to exposure, at day 7 and day 14. Necropsies were performed on all animals. LD50 were determined.

The LD50 of $CoCl_2$ is based on Speijers et al (1982) and Llobet & Domingo (1983).

Table 3. In vivo acute toxicity data from various Co substances

Substance	LD50 (mg test item/kg bw)	Co release in gastric fluid bioelution test (mg Co/g substance)
$CoCl_2$	766	216.05
Co	550	990.07
Co_3O_4	>5000	1.46
CoS	>5000	3.72

4) 28-day oral toxicity of Co substances

Co, Co_3O_4 and CoS were tested in an OECD 422 guideline compliant study in rats (Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test by p.o. exposure). From these studies, the NOAEL of the F0 generation is shown in the below table. Males were exposed to the test item beginning 2 weeks before mating, continuing during the mating period and approx. 2 weeks post mating until the minimum total dosing period of 28 days was completed. Females were dosed beginning 2 weeks before mating and continuing up to and including day 3 postpartum or the day before sacrifice.

$CoCl_2$ was tested in a 28-day range finder for an OECD 408 guideline compliant study in rats (Repeated Dose 90-day Oral Toxicity Study in Rodents by p.o. exposure).

Table 4. In vivo repeated exposure toxicity data from various Co substances

Substance	28-day NOAELs (equivalent substance in mg Co/kg bw/day)	Bioaccessibility in gastric fluid Co release (mg Co/g substance)	Bioaccessibility in gastric fluid (% of Co content released)
$CoCl_2$	10	216.05	86
Co	30	990.07	98
Co_3O_4	1000	1.46	0.2
CoS	1000	3.72	0.6

Data presented in Table 1, above, indicates that the mass of cobalt released from a gram of Co3O4 is approximately 150-fold lower than the mass released from a gram of CoCl2. Similarly, Table 2 demonstrates that the bioavailability of cobalt from Co3O4 is 104-fold lower than CoCl2, indicating that bioaccessibility in simulated gastric fluid predicts the same order of magnitude difference in bioavailability as it was found in the rat studies. This was also consistent with the NOAELs for oral repeated exposure that differ by 100-fold (Table 4). Results could not be evaluated quantitatively for acute oral toxicity since the LD50 for Co_3O_4 was above the limit dose of 5000 mg/kg (Table 3); nevertheless, it followed the same trend as for bioavailability and NOAELs.

Conclusions: in vitro bioaccessibility in gastric fluid correlates with the Co substances' systemic bioavailability, acute oral toxicity and repeated dose oral toxicity.

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ATTACHMENT 1. COBALT INSTITUTE: SUBMISSION FROM REACH DOSSIER

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Relationship between Oral Bioaccessibility and In vivo Oral Bioavailability for Nickel Compounds

Bioaccessibility data in fluids relevant to the oral route of exposure can provide important information regarding the potential bioavailability and subsequent toxicity for oral systemic endpoints including acute and chronic oral toxicity and reproductive toxicity.

To investigate if relative bioaccessibility in oral fluids could be used to predict relative systemic bioavailability and toxicity of nickel, several nickel substances were subjected to bioaccessibility testing in various oral fluids (Henderson et al., 2012a). Twelve nickel-containing samples were extracted in two relevant synthetic biological fluids, gastric and intestinal (**Tables 1** and **2**). In general, nickel release in synthetic intestinal fluid (24h) was much lower than that in synthetic gastric fluid (2h), even for the water-soluble substances. For each substance where nickel ion release in the intestinal fluid was >1%, the substance had already released 82-91% of its available nickel ion after the first 2 hours in gastric fluid, suggesting that any contribution to systemic nickel ion levels from intestinal dissolution would be minimal. A detailed description of the samples tested, testing protocol, fluid composition and bioaccessibility results is provided in Henderson et al. (2012a).

In vivo Verification Studies: oral bioavailability

Two types of studies have looked at the bioavailability and absorption of Ni after oral intake of nickel compounds. The studies of Nielsen et al. (1999) and others examined the oral absorption of Ni (II) ion in human volunteers when ingested as a water soluble nickel compound added to drinking water or food. Presence of food decreased nickel absorption compared to fasting (2.5 – 26%). The study of Ishimatsu et al. (1995) looked at the absorption of Ni in rats after oral administration of a variety of Ni-containing substances. While the results from the Nielsen et al. (1999) study permit an assessment of the range of oral bioavailability and Ni absorption values that can be obtained in humans depending on their fasting status, the Ishimatsu et al. (1995) study provides an indication of the relative absorption (and bioavailability) of Ni from a variety of Ni compounds, under the same ingestion conditions, in rats. Absorption data in rats are relevant because the oral acute toxicity classification is based on LD_{50} data from rat studies. Therefore, the bioaccessibility data in gastric and intestinal fluids can be compared to the bioavailability data (in rats) for several nickel compounds (**Table 1**).

Table 1. Oral bioaccessibility and in vivo absorption and relative bioavailability of nickel compounds

Test Substance	Gastric Bioaccessibility after 2 hours (% Ni content released)	Intestinal Bioaccessibility after 24 hours (% Ni content released)	Absorbed Fraction after 24 hours (%)[a]	Relative Bioavailability after 24 hours (%)
Ni sulphate	90.55	58.00	11.12	100
Ni chloride	89.85	38.45	9.8	88
Ni oxide (black)	29.6	0.32	0.04	0.36
Ni oxide (green)	9.75	0.12	0.01	0.09
Ni subsulphide	22.65	0.25	0.47	4.2
Ni sulphide	9.75	0.18	2.12	19

[a] Ishimatsu et al. (1995).

The data show a reasonable correlation between bioaccessibility in gastric or intestinal fluids and *in vivo* oral bioavailability, particularly when considering that these *in vitro* and *in vivo* tests were performed with different samples of the nickel compounds (**Figure 1**). Variations in particle size distribution and

surface properties of different samples of the same water insoluble substance can affect both the in vitro and in vivo results. Given that this finding is based on a limited number of samples (6), these data should be considered together with other available information in a weight-of-evidence approach (e.g., toxicity data as discussed below). In looking at the graph, two main groups of nickel compounds can be preliminarily identified: one group corresponding to the 2 water soluble nickel compounds (e.g., chloride and sulphate) and another one corresponding to the less water soluble compounds (e.g., sulphides and oxides).

Figure 1.
Correlation between bioavailability in rats and *in vitro* bioaccessibility

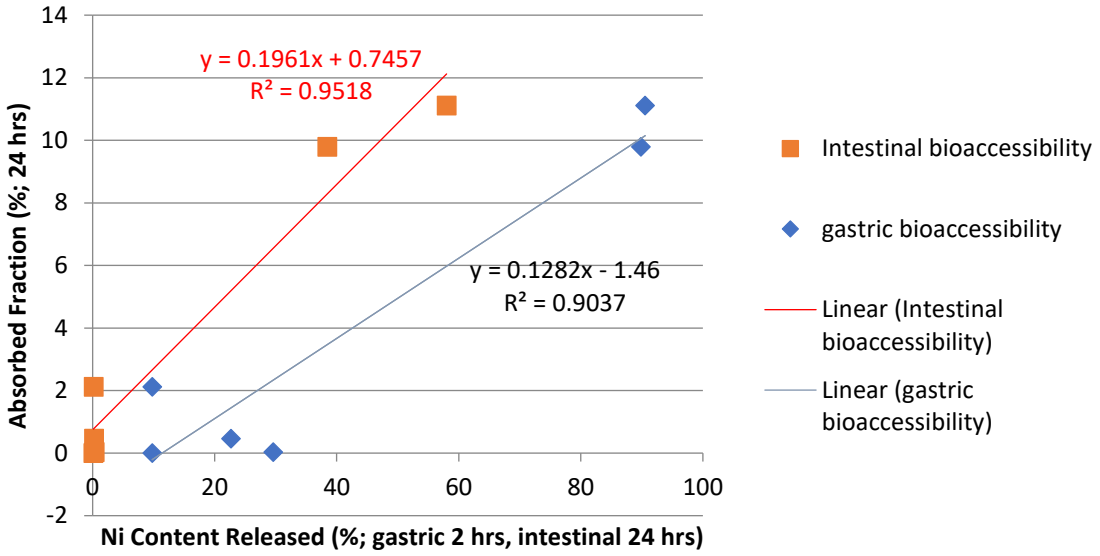
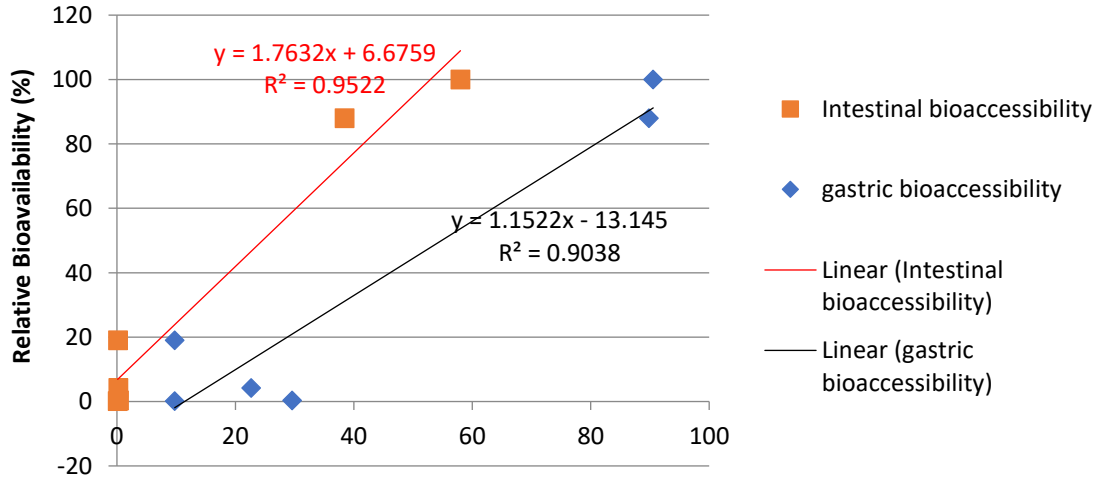


Figure 2 shows the relative bioavailability of nickel from various nickel compounds (relative to nickel sulphate, 100%) as a function of gastric or intestinal bioaccessibility. (Based on data from Ishimatsu et al., 1995 and Henderson et al., 2012a)

Figure 2.
Relative oral bioavailability versus oral bioaccessibility of nickel compounds



These graphs are important because they show that although the relative bioavailability values can be higher than the relative bioaccessibility ones (e.g., for the intestinal fluid in Figure 2), the absolute values are generally lower than the bioaccessible values (Table 1).

In Vivo Verification: Acute Oral Toxicity Studies

To further verify that the relative bioaccessibility data provide reasonable estimations of relative systemic bioavailability and hence toxicity, acute oral toxicity studies in rats were carried out for all the same nickel substances samples tested in the bioaccessibility studies except for Ni sulphide (Henderson et al., 2012b). **Table 2**, corresponding to Table 4 of Henderson et al. (2012a), summarizes the gastric bioaccessibility and oral acute toxicity data.

Table 2. Gastric bioaccessibility and oral acute toxicity

Test Substance	Sample ID	Gastric Bioaccessibility- 2 hours (µg Ni/g sample)[a]	Gastric Bioaccessibility- 2 hours (% Ni content released)[b]	Acute Toxicity (oral LD_{50}; mg/kg/bw)
Ni sulphate hexahydrate[d]	N58	201,670	90.55	362
Ni chloride hexahydrate	N98	215,632	89.85	500
Ni acetate tetrahydrate	N103	209,810	88.50	550
Ni hydroxycarbonate	N109	408,690	84.30	2,000
Ni sulfamate tetrahydrate	N104	147,783	83.40	1,098
Ni fluoride tetrahydrate	N111	264,930	82.35	310
Ni oxide (black)	N105	222,328	29.60	9,990
Ni dihydroxide	N106	142,693	26.30	5,000
Ni subsulphide[d]	N18	158,185	22.65	>11000
Ni sulphide	N97	57,485	9.75	NC
Ni oxide (green)[d]	N9, N46[e]	2,540	0.33	>11000
Ni oxide (green)[d]	N112	<25	<LOD[f]	>11000

NC, not completed.

a. Bioaccessibility reported as µg Ni released per gram of sample. Reported values are mean values from duplicate experiments.

b. Bioaccessibility reported as the percent of available Ni content released in synthetic gastric fluid after 2 hours. Reported values are mean values from duplicate experiments. Shading indicates a possible pre-grouping of the samples based solely on % Ni content released in gastric fluid after 2 hours.

c. Source substance for purposes of read-across.

d. The N9 sample was used in bioaccessibility studies and the N46 sample in the *in vivo* studies.

e. LOD, limit of detection equal to 25.0 µg Ni/g sample. Substances releasing Ni ion below the LOD are considered to have 0.00% bioaccessibility.

Relationship Between Bioaccessibility And Oral Toxicity Data

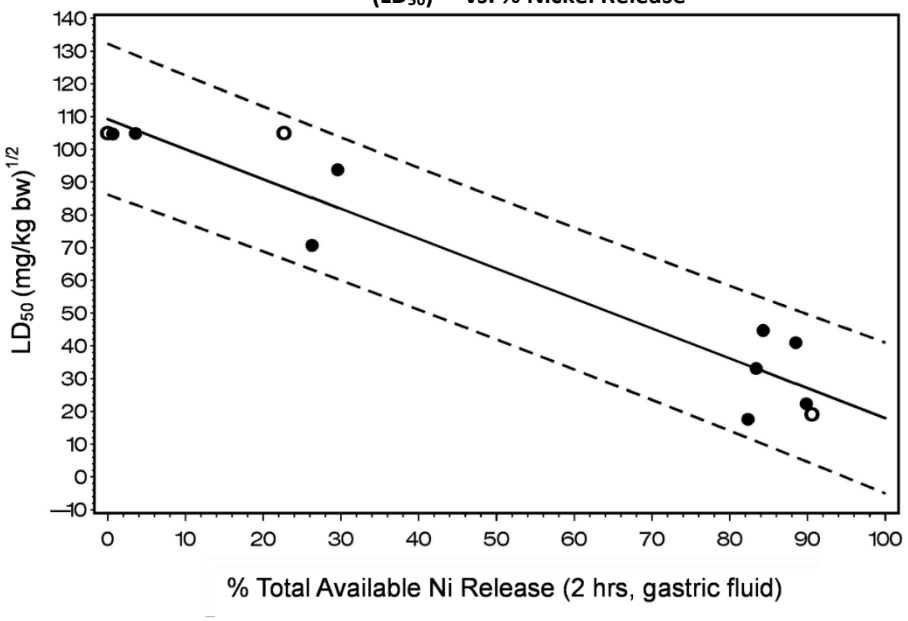
The bioaccessibility data expressed as percent Ni released from available nickel in gastric fluid after 2 hours revealed two main categories of substances: those that released above 80% of available Ni and those that released less than 30% of available nickel. Statistical correlations between bioaccessibility

data in various fluids and *in vivo* toxicity data were explored by an independent statistician (Seilkop, 2009). A good fit was found for bioelution data in gastric fluid (2 hrs) and the calculated oral LD_{50} (the dose at which 50% of animals are expected to survive). The LD_{50} data (mg/kg bw) were found to be well predicted by the nickel release data based on the model:

$$LD_{50}^{1/2} = 109.2 - 0.9123 \text{ Nickel Release (\%)}.$$

The fit and predictive power of the regression line were very good with 91% explained variance (R^2=0.91). In addition, as shown in **Figure 3**, the residual (observed – predicted) values are of similar magnitude across the nickel release prediction range (Henderson et al., 2012a). The regression fit was only marginally improved by considering both gastric and intestinal release in a sequential manner (R=-0.96, R^2 =0.93) (Henderson et al., 2012a).

Figure 3.
$(LD_{50})^{1/2}$ vs. % Nickel Release



Taken together these bioelution, toxicokinetic and toxicity oral studies demonstrate that oral fluids bioaccessibility data can be used to assess the relative oral bioavailability and toxicity of nickel compounds.

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<u>Responses to questions prepared by Euromteaux</u>

The main objective of the review carried out by Alloy LLC was to evaluate whether the existing RBA and IVBA studies in the literature could help with providing responses to the following questions:

- ***Are data available regarding the relationship between in vivo bioavailability and IVBA?***
- ***What is the nature of this relationship, if present?***
- ***Does IVBA provide a conservative (i.e., overpredicted) estimate of bioavailability?***
- ***Are there instances where IVBA underestimates RBA?***
- ***How does extraction into simulated gastric fluid compare to extraction into simulated saliva or intestinal fluid?***
- ***What does research tell us with regard to bioaccessibility (in simulated gastric fluid) of a metal from an alloy relative to that of a pure metal?***
- ***In simulated gastric fluid, does bioaccessibility of a metal from one sample relative to a standard (e.g., soluble metal compound) reflect RBA in vivo?***

This annex aims at providing responses for each of the questions. It has been prepared by Eurometaux on the basis of the work done by Yvette Lowney, Alloy LLC.

1. Are data available regarding the relationship between in vivo bioavailability and IVBA?

The literature search has identified 39 studies, 38 of which are peer-reviewed studies published in the open literature (summarized in tables 1 and 2 of the report):

- 22 contained data for evaluations conducted both in vivo and in vitro, i.e. information on both Relative Bioavailability (RBA) as measured in animals (mouse, rat, and swine animal models) and on in vitro bioaccessibility (IVBA) measured in laboratory extraction tests. For the metals investigated, RBA refers to the bioavailability of metal from soil compared to the bioavailability of metal from a water-soluble metal compound
- An additional 17 studies present data from in vitro extraction testing of one or more soils, which may be informative for understanding how various in vitro methods perform and what components of the IVBA methods affect extraction of metals from soil.
- Some additional information on cobalt and nickel (published and unpublished reports) is considered as well.

More precisely, the literature search has revealed an abundance of information on the RBA and IVBA for both lead (N=9) and arsenic (N=8), including the evaluation of in vitro to in vivo correlation (IVIVC) relationships and the comparison of how different IVBA methods perform. The focus on arsenic and lead can be explained by the fact that these two chemicals drive risk-based remedial decisions at many large contaminated sites.

Fewer studies are available for other metals: nickel is evaluated in seven studies and cadmium is evaluated in four, including two studies that included them in a broad set of metals investigated. There is no information available on beryllium and selenium.

2. What is the nature of this relationship, if present? And

3. Does IVBA provide a conservative (i.e. overpredicted) estimate of bioavailability?

The primary focus of the review was on those studies with data both on in vivo bioavailability (RBA) and in vitro extraction test results (IVBA) for the same soils. In the in vitro to in vivo correlation process (IVIVC), paired samples of contaminated soil are fed to animals to establish RBA and are also tested with in vitro extraction methods. The data are then compared to determine whether a predictive relationship exists, generally through regression analysis.

The ultimate goal of the IVIVC is to optimize the extraction method to create an IVBA that most closely forms a predictive relationship with RBA. If such a relationship can be established, then in vitro methods can be considered to be representative, or predictive, of RBA, and the regression equation is used to “translate” IVBA data into a prediction of RBA (further depicted in Figures 2 and 3 of the report).

Whether IVBA provides a conservative (i.e. overpredicted) estimate of absolute bioavailability cannot be answered with the information provided in the papers reviewed here. The papers reviewed generally do not provide information on absolute bioavailability but rather on bioavailability of metals from soil relative to that of a soluble metal compound. In other words, these compounds may be fully soluble under the conditions of the IVBA extraction but still be only partially bioavailable, since not all bioaccessible metal gets absorbed systemically.

Because an IVIVC is generally based on a regression analysis, a very strong predictive relationship can be established for data where the slope of the line diverges from unity:

- a slope <1 indicates that, on average, the IVBA estimate is higher than the RBA estimate
- a slope >1 indicates that, on average, the RBA values exceed the IVBA values

Therefore, the IVBA data may not be used directly to predict RBA, but rather the equation that explains the regression line is used to "translate" IVBA data into a prediction of RBA.

For the studies listed in Table 1 where an IVIVC and regression equation are provided, the slope of the regression line is nearly always less than unity (i.e., slope is <1.0). This indicates that, on average across the data set for which the IVIVC was developed, the IVBA methods that were used extract a higher percentage of metal from the soil than is absorbed when the same soil is fed to an animal (not in absolute terms but relative to what is absorbed from soluble metal compounds). This means that for predictive purposes, the regression relationship allows for the use of IVBA to predict RBA.

It should be noted, that the goal of the IVIVC process is to identify the IVBA method that is *most predictive* of RBA, even if in some cases the extraction results from the IVBA underpredicts or overpredicts the RBA results (this does not mean that it underpredicts the absolute bioavailability results). Therefore, in other circumstances (e.g. where animal testing is not considered ethically appropriate, or where RBA data are not available for a particular chemical), IVBA methods may be developed with the specific intention of generating worst-case conditions that are then assumed to always overestimate absolute bioavailability from human exposures.

4. Are there instances where IVBA underestimates RBA?

To address this question, it is of interest to know whether for some metals IVBA exceeds RBA consistently, irrespective of whether a more refined predictive relationship exists.

Table 1 of the report includes an assessment of whether the evaluated IVBA methods provide a conservative estimate of RBA (i.e.: IVBA > RBA if data are directly compared, not interpreted through the regression relationship). The studies indicate that even if IVBA is predictive of RBA for most soils, in nearly all instances—for all metals, all animal research models, and all in vitro methodologies evaluated—there are **individual soils** for which RBA exceeds IVBA. The frequency with which RBA exceeds IVBA varies considerably between metals and between studies. The summaries provided in Table 1 and Tables 3a–3g include information regarding how often RBA exceeds IVBA. However, if the **variability** in RBA and IVBA estimates is taken into account (see e.g. publications by Denys et al. 2012 and the summary of data presented in Table 5), it can be concluded that IVBA data provide reasonable estimates of RBA.

5. ***How does extraction into simulated gastric fluid compare to extraction into simulated saliva or intestinal fluid?***

The review has also extracted from the available studies the factors identified as possibly affecting dissolution under simulated conditions of the human gastrointestinal tract. Factors that were commonly investigated in the studies include pH, temperature, contact time, agitation, particle size, the ratio of soil to extraction fluid, and presence of surfactants or other extraction fluid components.

The results from in vitro extraction testing indicate that extraction fluids with **low pH** (e.g., in simulated gastric fluids) nearly always result in dissolution of metal from soil that is equal to or greater than dissolution at higher pH (e.g., in simulated intestinal fluid); only mercury demonstrated evidence of higher dissolution with IVBA methods that use higher pH.

For cadmium, cobalt, lead, and nickel, higher measures of IVBA are achieved with lower pH conditions, and this effect of pH outweighs other components of the extraction fluid that are intended to create a more “physiological” environment. For arsenic, solubility from soil appears to have a lower dependence on pH, although for some soils, low pH is associated with higher IVBA, and the low pH extraction methods are more closely correlated with RBA when studied with paired soils.

This trend for higher bioaccessibility with lower pH is well demonstrated by Koch et al. 2013. This study investigates only one soil (National Institute of Standards and Technology SRM 2710) but includes results from extraction testing by several different laboratories, using a multitude of IVBA methods, including both simulated gastric and intestinal phases. A summary of some of the

data reported in this study has been excerpted and is provided in Figure 6 of the report, which plots the effect of pH vs. measured bioaccessibility for arsenic, cadmium, copper, lead, and zinc, and the associated linear regression line for each metal. Koch et al. describe their findings as follows: "negative correlations, meaning that percent bioaccessibility decreases with increasing pH, were confirmed for all elements," and "the strongest correlations (highest R values) were statistically significant and occurred for lead, zinc, and cadmium." The magnitude of the negative relationship between bioaccessibility and pH (i.e., how much bioaccessibility increases as pH decreases) was most pronounced for lead and cadmium, and slightly less pronounced for arsenic, copper, and zinc. Their work also illustrates that simple extraction methods that use hydrochloric acid at low pH can yield the most conservative measures of bioaccessibility. The highest reported bioaccessibility values are for either the relative bioaccessibility leaching procedure (RBALP) method (pH 1.5 HCl, buffered with 0.4 M glycine) or the Health Canada method (HC-HCL, which is simply 0.07 M HCl at pH 1.5) (figure 7 of the report).

6. ***What does research tell us with regard to bioaccessibility (in simulated gastric fluid) of a metal from an alloy relative to that of a pure metal?***

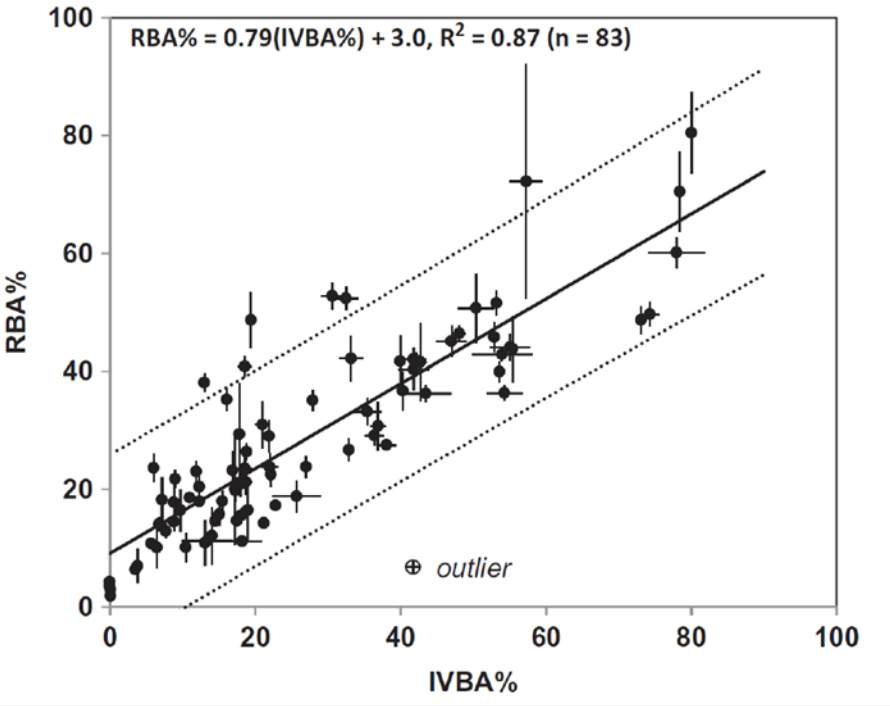
The studies collected in Table 1 reveal that there is only one published study that evaluates the RBA or IVBA of different pure chemical forms of a metal (nickel) (Ishimatsu et al., 1995). Consequently, the available database can only partly inform our understanding of how methods compare for metals in complex media vs. pure metal forms.

No studies investigating IVBA vs. RBA of metal alloys could be identified. However, it might be expected that IVBA would be as predictive of the RBA of metal from alloys as from soils, since oral absorption of metals from alloys or soil depends on the bioaccessible fraction. It is therefore estimated that while IVBA data do not directly address a biological effect, they can provide an estimate of relative bioaccessibility of metals from different materials, and hence allow for a refinement of the bulk concentration of a metal in a mixture by considering the fraction that can become bioavailable. Once an IVBA to RBA relationship has been established for a particular metal based on oral ingestion of some metal-containing substances (e.g., compounds or soils), this relationship is likely to be valid for other substances containing that same metal (e.g., alloys or M^o).

7. ***In simulated gastric fluid, does bioaccessibility of a metal from one sample relative to a standard (e.g., soluble metal compound) reflect RBA in vivo?***

Nearly all studies, conducted in vivo or in vitro, provide a comparison of RBA or IVBA against a water-soluble standard. Individual studies fail to include this basis of comparison, but they are generally included because data on the soluble forms of the metals are required in order to assess *relative* bioavailability, or to establish good analytical recovery under in vitro conditions.

Strong predictive relationships between RBA and IVBA emerged for Pb and As, with fewer available data for Sb, Cd, Co, Ni, and Hg, and no data for Be and Se. For example, Diamond et al. (2016) conducted a meta-analysis looking at the relationship between the in vitro bioaccessibility (IVBA) and the in vivo relative bioavailability (RBA) of arsenic from soils. IVBA was assessed with the 0.4 M glycine/pH 1.5 assay and bioavailability was assessed in either C57BL/6 mice or juvenile swines. Two types of regression analyses were performed on the data. A preliminary regression model relied on ordinary least squares linear regression, while the final regression modelling used weighted least squares regression. This approach allowed incorporation of uncertainty in individual IVBA and RBA estimates into the regression model. Figure below corresponds to Figure 1 of Diamond et al. (2016). The solid line represents the weighted regression model and the dotted lines are the 95% prediction limits for the weighted model. The authors conclude that the data from IVBA can predict RBA of arsenic in soil.



Some information linking IVBA with RBA and/or toxicity (in vivo) for different Ni and Co compounds is also available:

- For two cobalt compounds, the RBA and acute oral toxicity data from rat studies were compared to IVBA in gastric fluid. Both RBA and toxicity were lower for the compound with the lower IVBA value, although the exact relationship could not be established because the dosing was truncated at high (not acutely toxic) doses.
- Henderson 2012 (a, b) provide information on the IVBA in gastric fluid and acute oral toxicity in rats for 11 nickel compounds. The solubility of the samples ranged from <1% to >90% in simulated gastric fluid. The LD50 values were clearly and negatively correlated with IVBA in gastric fluid. Ishimatsu et al. (1995) similarly establish a relationship between nickel solubility in saline and the absorbed fraction of nickel, based on eight forms of nickel compounds. The authors conclude that "*a correlation between the estimated absorbed fraction of each Ni compound and its solubility was observed*." This correlation was also present when gastric fluid data from Henderson et al. (2012a) was used. Thus, the available information on nickel indicates that the solubility of nickel in gastric fluid will be predictive of the RBA and relative oral toxicity.

To conclude, there is a large body of evidence supporting a strong predictive relationship between RBA and IVBA for lead and arsenic. Regulatory agencies have used the available information to provide formal guidance (e.g. EPA 2017, CalEPA 2017) or technical reports (e.g., BARGE 2016, Health Canada 2006, New Zealand Ministry for the Environment 2016, RIVM 2017) on the use of IVBA methods to predict the RBA of these contaminants in soil. Hence, IVBA data are already being used in the context of soil remediation based on human health risk assessment for lead and arsenic. Some reports and regulatory jurisdictions also support the use of IVBA methods for other metals such as cadmium and antimony (e.g. BARGE 2016, Health Canada 2006, New Zealand Ministry for the Environment 2016). In each case, a review of the available information was conducted; specific methods were proposed for IVBA testing and data interpretation, but generally there is increasing acceptance of IVBA methods to provide an efficient and effective method for predicting the bioavailability of metals.