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Use of *In Vitro* Bioaccessibility/Relative Bioavailability Estimates for Metals in Regulatory Settings: What Is Needed?

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Abbreviations

%	Percent
ABA	Absolute Bioavailability
As	Arsenic
BARGE	Bioaccessibility Research Group of Europe
BGS	British Geological Survey
Cr	Chromium
Cu	Copper
DMA	Dimethylarsenic
DQO	Data Quality Objective
g	gram
g/kg	gram per kilogram

GI	Gastrointestinal
HERA	Human and Ecological Risk Assessment
HF	Hydrofluoric acid
HHRS	Human Health Risk Assessment
ICCVAM	Interagency Coordinating Committee for Validation of Alternative Methods
IAEA	International Atomic Energy Agency
ISEA	International Society of Exposure Analysis
IVG	*In vitro* gastrointestinal
k	thousand
µg	microgram
mg/kg	milligram per kilogram
MMA	methylarsonic acid
NaAS	Sodium arsenate
Ni	Nickel
NIST	National Institute of Standards and Technology
OSWER	Office of Solid Waste and Emergency Response
Pb	Lead
PBET	Physiologically Based Extraction Technique
ppm	parts per million
PRG	Preliminary Remediation Goal
Q&A	Question and Answer
RA	Risk Assessment
RBA	Relative Bioavailability
RIVM	Rijksinstituut Voor Volksgezondheid En Milieu
SBRC	Solubility/Bioavailability Research Consortium
SERDP	Strategic Environmental Research and Development Program
SOP	Standard Operating Procedure
TIM	TNO Gastrointestinal Model
TRV	Toxicity Reference Value
UEF	Urinary Excretion Fraction
UK	United Kingdom
US EPA	United States Environmental Protection Agency
XANES	X-Ray Absorption Near-Edge Spectroscopy
XRD	X-Ray Diffraction
Zn	Zinc

Introduction

Oral ingestion of soil and dust is a key pathway for human exposures to metal and metalloid contaminants. It is widely recognized that the site-specific bioavailability of metals in soil and dust may be reduced relative to the metal bioavailability in media such as water and food, and adjustments for oral relative bioavailability are becoming more accepted. Both animal models and *in vitro* bioaccessibility models have been used to estimate relative bioavailability of metals in soil and dust. Although animal models are often considered the "gold standard", they may be costly or otherwise prohibitive at certain sites and may not be sensitive enough to test

environmentally relevant samples for all contaminants. Routine application of *in vitro* metal bioaccessibility models in regulatory settings is being held up by different perceptions of what is required of these models in terms of validation.

This symposium provided the opportunity for international experts to exchange their views on methods for assessing relative bioavailability/bioaccessibility for application in risk assessments at contaminated sites. The symposium speakers presented recent developments in animal models, new *in vitro* models, the role of mineralogical analyses in assessing relative bioavailability, and the application of physiologically based models as research tools. In addition, two panel discussions addressed specific research questions and discussed future research needs in this area. Recognizing the multi-disciplinary nature of exposure assessment, this symposium included representation from many disciplines including risk assessment, toxicology, environmental geochemistry, geology, soil, and analytical chemistry from the U.S., Europe, and Canada.

Proceedings

Introduction and Overview

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A. Yeow; U.S. Environmental Protection Agency, OSRTI, Washington, DC
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R. A. Schoof; Integral Consulting, Inc., Mercer Island, WA.
M. R. Cave; British Geological Survey, Nottingham, UNITED KINGDOM.

Karen Bradham provided definitions and background for the discussions. Oral bioavailability of metals is important for Human Health Risk Assessment (HHRA) of metals and decision-making. Absorption from the gastrointestinal tract (GI) depends on the metal, chemical, and physical form, as well as biological factors. Karen acknowledged the many definitions of bioavailability, but she asked attendees to consider the definition in the Guidance for Evaluating the Oral Bioavailability of Metals in Soils for Use in Human Health Risk Assessment (OSWER 9285.7-80): "*the fraction of an ingested dose that crosses the gastrointestinal epithelium and becomes available for distribution to internal target tissues and organs*" Absolute bioavailability (ABA) and relative bioavailability (RBA) are also important definitions. ABA: *ratio of the amount of metal absorbed compared to the amount ingested*. RBA: *the ratio of the bioavailability of a metal in one exposure context (i.e., physical chemical matrix or physical chemical form of the metal) to that in another exposure context*. Bioaccessibility is a related term, typically referring to *a measure of the physiological solubility of the metal at the portal of entry into the body*. Bioavailability information can be used to improve the accuracy of risk calculations and inform decisions at hazardous waste sites. Karen provided an overview of the three forms of studies: *in vivo*, *in vitro*, and mineralogical/speciation studies. Highlights from the 2006 ISEA symposium: general agreement that criteria are needed to assess *in vivo* and *in vitro* methods; some countries are allowing limited site-specific adjustments; and few are comfortable with allowing existing site-specific methods to be

universally applied. Karen reviewed the panel discussion questions for the audience, which were disseminated to the panelists prior to the symposium.

Q&A: No time for questions

Evaluating the Bioavailability of Soil-Borne Contaminants at Waste Sites
M. Beringer; U.S. Environmental Protection Agency, Kansas City, KS.
A. Yeow; U.S. Environmental Protection Agency, OSRTI, Washington, DC.

Mike Beringer discussed EPA's new guidance on using bioavailability studies to gather site-specific information at Superfund sites. EPA developed this guidance because there was a need for a consistent basis for approaching sites and evaluating new bioavailability methods. The bioavailability guidance is limited in scope to oral ingestion of metals at hazardous waste sites for HHRA. The assessment of bioavailability is consistent with existing EPA guidance. The new guidance provides a decision framework for collecting site-specific bioavailability information to make quantitative adjustments. The guidance also recommends using a validated methodology and provides recommended evaluation criteria for the development of new methods. The recommended evaluation criteria are based on the Interagency Coordinating Committee for Validation of Alternative Methods **(**ICCVAM) criteria for method validation and regulatory acceptance. The ICCVAM criteria are widely accepted internationally. Mike noted that the evaluation criteria are not all critical to the validation or acceptance of a methodology. Mike highlighted the importance of establishing a correlation between an *in vitro* method and an *in vivo* method. Mike provided an overview of the methods for evaluating lead bioavailability and bioaccessibility. The juvenile swine model and the *in vitro* bioaccessibility method for lead have been evaluated and accepted for site-specific HHRA by EPA using the ICCVAM criteria. These methods are described in a companion document to the guidance, referred to as the Lead Technical Support Document. Mike noted that the evaluation covered a broad range of metal forms and a range of bioavailability values. The documentation also outlines some limitations of the accepted methodology. Mike listed some future activities to include support for implementation of the guidance and evaluation of arsenic methodologies.

Q&A

- What was the *in vitro* method?
    - Mike responded that the *in vitro* assay published by John Drexler was evaluated (Drexler and Brattin, 2007).
- For the *in vivo* bioavailability: *in vitro* bioaccessibility correlation, how did you derive the confidence interval?
    - Mike responded that this was a prediction interval, not a confidence interval.
- Do you recommend using the prediction interval in the RA?
    - Mike responded that the EPA recommendation suggests that one should use the best-fit line.
- What is the mechanism for using new methods that are similar to the existing method? Do we need to show equivalence or go through the entire process?

- Mike responded that the method described in Drexler and Brattin (2007) is recommended because it has satisfied the ICCVAM criteria and suggested that we discuss the process for similar methods later in the symposium.

Method Development and the Application of Oral Bioavailability Data in U.S. Risk Assessments

R. A. Schoof; Integral Consulting, Inc., Mercer Island, WA.

Rosalind Schoof posed several (5) questions related to EPA's new guidance on bioavailability.

1. How is validation defined?
2. What are validation requirements?
3. What are regulatory acceptance requirements?
4. What is the process for method development and validation?
5. What methods are already considered validated based on history of use?

Rosalind expressed concerns about how strictly the validation process will be defined. Validation requires an understanding of relevance and reliability. Rosalind noted that the ICCVAM criteria were developed for evaluating the alternative toxicity models, not for bioavailability. These may or may not necessarily be applicable to bioavailability methods. Rosalind provided an overview of the process leading from research to acceptance and implementation. Validation occurs when its performance characteristics, advantages, and limitations have been adequately documented for a specific purpose. Rosalind identified three types of tests: definitive, screening, and adjunct. Are bioavailability test methods adjunct? Rosalind offered that some test methods might be considered validated based on history of use (e.g., *in vivo* pharmacokinetic studies of bioavailability). Rosalind noted that validated studies still require scientific interpretation and oversight. GI physiology differs among species, so animal models are predictive, but not perfect. Rosalind expressed concerns related to her perception that EPA is going to require validation for all metals. A number of other metals that are of concern in soil, are not typically found at high enough concentration in soil to allow for animal study and may therefore not be evaluated using the EPA process (cost of method validation is too high). Rosalind ended with four questions:

1. How reliable are the *in vivo* methods?
2. Are the *in vivo* methods considered validated?
3. Should validation of *in vitro* protocols be required on a metal-specific basis?
4. Should results of methods that have not been validated be considered in risk assessment? If not then they may not be developed using site money. If so how?

Q&A

- Related to *in vivo* testing of other metals, I agree that concentrations are too low to establish a correlation. Can you comment on this limitation?
    - Rosalind agreed that this would be difficult for metals other than lead and arsenic.

- The concept of saying that concentrations are too low is not necessarily true. We can develop methods to assess low concentrations. The document EPA developed is not universal to all metals. We need to examine relevant samples.
    - Rosalind agreed and responded that the concern is high background in diet and other sources to distinguish additional exposure from site soil to detect site exposure.

Arsenic Bioaccessibility Testing Using Various Extraction Methods: Results and Relation to Relative Oral Bioavailability as Measured in the Cynomolgus Monkey

Y. W. Lowney; Exponent, Boulder, CO.
S. Roberts; University of Florida, Gainesville, FL.
S. Saikat; UK Environment Agency, Oxfordshire, UNITED KINGDOM.

Yvette Lowney discussed the *in vivo* bioavailability method using the Cynomolgus monkey model and *in vitro* methods using a variety of methods. The Cynomolgus monkey model was used because the oral bioavailability of the monkeys tends to correlate well with humans. Excretion of As into urine is relatively rapid—within 48 hours nearly all of the As has been excreted. The Cynomolgus monkey studies used a low arsenic diet: Soil dose <1 g/kg and As dose <1 mg/kg. Five animals were used to develop an RBA estimate. Urinary and fecal recoveries were very good. Controls (high and low RBA) were used. Sample RBA ranged from 5% to 31%. No RBA was found to be higher than 32%. Soluble NaAs was used and found to have an RBA of nearly 100%. The study (published by Roberts et al., 2007) used a variety of soil types. *In vitro* model development began with the Solubility/Bioavailability Research Consortium (SBRC) method, with some alterations and consideration of The National Institute for Public Health and the Environment (RIVM) and Bioavailability Research Group of Europe (BARGE) methods. The SBRC method had good correlation for some samples, but not all (even when different pH levels were used). It could not be determined why all soils did not have good correlation. Phosphate additions improved the correlation, but did not work for all soil samples. Other alterations to the method were made, but even with hydroxylamine additions correlation was not good enough. When the RIVM and unified BARGE methods were used, none of these methods worked (correlated) well for all soil samples. There is a need to develop a method that is predictive for all soils or to understand which soils will work with the method. Available methods do not provide a 1:1 correlation, but may be predictive. Yvette noted that the soil samples have been extensively studied: mineralogy, speciation, etc. Mathematic modeling may not necessarily work; however, the factors that control arsenic bioavailability can be stated. Research status: data suggests RBA <30% from *in vivo* bioavailability testing, need more work for the *in vitro* model.

Q&A

- What is going on with the soils that affect the correlation? We need to define the parameters for when soil samples will and will not work with the method.
    - Yvette responded that she agrees; however, soil sample source (e.g., mine tailings or orchard samples) and mineralogy cannot explain correlation.
- Were the monkeys fasted?
    - Yvette responded that the monkeys were fasted overnight.

- Can you give background as to why correlation is the standard as opposed to predicting or over predicting *in vitro*?
    - Yvette responded that she agrees that an *in vitro* assay either predicts or over predicts (slope of the line is flatter than it should be). The objective is to have the *in vitro* assay predictive of bioavailability.
- Have you considered reabsorption of the arsenic? Should you consider a different method, perhaps to add some resin beads to absorb the arsenic?
    - Yvette agrees that reabsorption and precipitation could be a problem. Yvette noted that the Hawaii volcanic soil sample had very low recovery.

Assessing Bioavailability Using the Swine Model
S. Casteel; University of Missouri, Columbia, MO.
G. Fent; University of Missouri, Columbia, MO.
C. Weis; US EPA, Denver, CO.
W. Brattin; Syracuse Research Corp, Denver, CO.

Stan Casteel gave an overview of the swine model (*in vivo* bioavailability) used for Pb, As, Cd, and Cr. Juvenile swine were used as a surrogate for children. Naïve animals are used, so background is not typically a concern. The model allows multiple tissue endpoints to assess exposure and absorption. The model is reproducible. Intravenous and oral routes can be used. Most oral exposures are fed to the pig in a dough ball (moistened, powdered feed). Twice daily fasted dosing is typically used for consecutive 12-14 days (subchronic exposure). Three (3) levels of soil sample and three levels of reference standard are used (along with negative controls). For arsenic, urinary arsenic (48 hours) approximates the oral absorption fraction (ABA). After 5 days of exposure, arsenic excretion is a linear function of dose (independent of time). RBA is the ratio of urinary excretion fraction (UEF) of test material to standard. In general 80 ppm is lowest level in soil that they will use—because of the amount of soil that needs to be used to achieve a measurable urinary arsenic concentration. With increasing exposure dose, variability increases (heteroscedascity). Research has found RBAs of 26% to 72% for the test soils.

Q&A

- Is it relevant to do an *in vivo* study when soil arsenic levels are as high as 4000 ppm?
    - Stan said that they probably do not, but that is not his decision.
- Can you explain the variability in the control group?
    - Stan responded that there is some As in the low As feed, but that it is likely due to biological variability.

Assessing Soil Arsenic Bioavailability in the Laboratory Mouse
D. Thomas; US EPA, Research Triangle Park, NC.
M. Hughes; US EPA, Research Triangle Park, NC.
K. Herbin-Davis; US EPA, Research Triangle Park, NC.
P. Seales; US EPA, Research Triangle Park, NC.

Dave Thomas discussed the results of the pilot study in mice. Dave noted that this is a method that is still in development. Goals are to develop a mouse model and to determine whether the mouse is a good model for As absorption in humans. Mice were chosen because they have been well studied, can be manipulated experimentally and a large body of As information is available for this animal model. Dietary exposure was used. Total arsenic absorption (body burden and excretion) was used. Dave noted that pharmacodynamic issues are significant for As (methylated forms are more toxic); in the GI tract methylation and demethylation reactions do occur, as well as thiol forms of As. Similarly in the tissues (post absorption) methylation and thiolation occur. The method permits the evaluation of arsenic forms (inorganic As, methylarsonic acid (MMA), dimethylarsenic (DMA)). Mice are fed purified diet which is non-detect for As (cellulose in the diet is replaced with soil). The diet preparation method needs further development. Eight (8) to 9 days of exposure are used (steady state). Food consumption is not impacted by addition of soil to the diet. One limitation is stress involved with use of the metabolism cage (mice lose weight). Future work will repeat existing research, examine other soils, and refine methods of arsenic speciation, examine the relationship between soil source and patterns of arsenic metabolism, and examine dietary factors that may impact absorption (dietary fat, iron, and copper levels).

Q&A

- Are you also interested in comparing the mouse model with other *in vivo* models?
    - Dave replied that yes, for those soils that have been evaluated by others.
- Why do they lose weight?
    - Dave responded that this is likely the stress of the open bottom metabolism cage.
- Is a loss of 10% of body mass likely to impact the kinetics of absorption?
    - Dave responded that we do not know and we would have to design a study to evaluate that issue.
- How many mice in each study?
    - Dave responded there are three replicates per cage.

Panel Discussion Session I

Question for Stan: at 80 ppm As, how much soil is delivered to the pig?

Stan responded that is approximately 5-6 grams of soil per dose. Food impacts absorption so this is a concern.

Question for Stan: are there different quantities of soil at different dose levels?

Yes.

Question for Stan: do the different quantities impact absorption?

No. However, at higher doses we have an increase in variability, but not a change in absorption.

What is the cost per sample for various methods?

Yvette: monkeys are approximately $90-100k for three soils; *in vitro* assay for 10 soils is approximately $10k.

Stan: swine are approximately $60k for two soils.

Dave: mice are probably several thousand dollars per sample. We have not looked at this yet.

Is the purpose of *in vivo* methods to calibrate the *in vitro* tests or to use data for a site?

Stan: no. the *in vitro* assay plays a role, as well as soil sample characterization (mineralogy).

Aaron: EPA wants something cost effective and quick. We are looking to use *in vivo* models for correlation/calibration to have less reliance on animal models.

Yvette: although *in vitro* methods will allow us to generate data, there are sites where animal studies are warranted.

Which animal model is best? Primate vs. swine vs. mouse vs. human? Which is best for developing a correlation?

Aaron: we have accepted the *in vitro* model for lead based on *in vivo* bioavailability. For As, we have not yet accepted an *in vivo* bioavailability model. Until we compare the animal models we cannot identify an animal model.

Mike added that juvenile swine were used for lead to mimic the child for lead. Monkeys may be good models for adult exposure and for As because As is a carcinogen.

Rosalind agreed. Arsenic absorption may be more applicable to adults.

Question for the panel: How can we determine the better model swine or primates?

Stan replied that the dosing methods are not the same. We are currently evaluating a single soil for comparison. The age issue is pertinent to the discussion. Another consideration is using a naïve animal vs. using an animal that was exposed over and over to the same metal.

Yvette noted that we have not yet cross-dosed different species with the same test soil. That work needs to be done. We need to do this work and discuss the benefits of the various models.

General agreement that this would be helpful.

Kirk Scheckel suggested that this would be a huge investment and an opportunity for EPA to attack the problem. European researchers and regulators agree that this would be helpful.

Mark Cave: what is the best way forward to work together to achieve this goal?

Mike agreed that the available data should be pulled together to identify what needs to be done to fill in the data gaps.

Suggestion to plan the next phase: get real soil, characterize the soil, and compare the *in vivo* bioavailability and *in vitro* results back to the epidemiological data.

Responses noted that the epidemiological data are confounded by background exposures to arsenic exposures in the diet. This complicates the biomarker analysis. The UK Environment Agency is working on this issue and the BARGE group is also considering this.

Question for the Panel: Can the rat data and swine data be used for arsenic? A lot of research has been done with swine. Yvette asked for what chemicals is there a preponderance of data.

Stan responded that he has dosed between 30-50 arsenic soil samples.

Rosalind suggested that we should not leave other animal models behind until we have a correlation between juvenile swine and the adult human.

It was also noted that Toxicity Reference Values (TRV) are from rodents—that should be a consideration for model selection.

Marc Stifelman noted that recoverability and mass balance were good for non-human primates, less so with swine (older Region 10 studies).

Stan replied that the urinary excretion fraction is not affected by total uptake. His laboratory does not collect mass balance data, because they no longer have these difficulties.

Dave Thomas noted that recovery in the mouse model is 80-85% (urinary and fecal, not considering tissue retention, which is expected to account for the other 15%).

Stan noted that this information is necessary for ABA, not for RBA.

Mark Maddaloni noted that discrepancies in mass balance might be a cause for concern because it is these tissue levels that are having the ultimate toxic effect.

Rosalind agreed that pharmacokinetic considerations need to be evaluated in developing an animal model.

Stan agreed and noted that differences in distribution are easily observed with different routes of exposure.

Yvette agreed and stated we need to be careful in selecting a single animal model because our data set is based on a small subset of metals.

Questions for Panel Discussion Session I (evaluation/site application and *in vivo* research):

1 - For regulatory applications such as site clean-up decisions, is an *in vitro* model that is predictive (i.e., correlates with an *in vivo* model) adequate even if we don't know why it is predictive, or is it important to know why a model is predictive?

From the audience, a suggestion to not be closed minded about model acceptance without complete understanding.

Kirk noted that complete understanding of the model may be the ultimate goal to help evaluate long-term stability of contaminants at sites.

Rosalind would prefer not to need to understand mechanism as long as the model is predictive.

Stan agrees. We do not need to know the answer—the utility of the *in vitro* method depends upon consistency in correlation for soil types of interest. How much confidence we have in adding a new soil type depends upon how well we understand the factors controlling the bioavailability.

2 - Given that many animal models require use of soil concentrations exceeding those of public health concern, what efforts are necessary, if any, to show that bioavailability at these concentrations reflect oral absorption at lower environmental concentrations? In other words, how important is it to evaluate whether bioavailability is concentration dependent?

Pat asked that if 80 ppm is the lower limit for arsenic studies in swine, what do we do for soils between 20 ppm (Canadian soil level) and 80 ppm? It was agreed that this is a concern for monkeys, too.

Nick offered that when you are below 100 ppm perhaps you are stuck with *in vitro* assays. Nick noted that the soil type that doesn't work may be the most informative. Why doesn't it work? That is the important piece of information.

Kirk suggested that rather than arsenic, can we look at a tracer.

Mark Maddaloni responded that we have to identify a tracer that we know is very similar to arsenic.

3 - Should a single animal model be specified, or are studies in a variety of different animal models more likely to improve our understanding of relative bioavailability in humans.

No. Agreement to retain various animal models for further evaluation.

4 - Is there a single animal model that will work for all chemicals considering the widely varying toxicokinetic profiles?
Not at this time.

***In-vitro* Bioaccessibility of Soil-borne Contaminants: An Environment Agency Perspective**
S. Saikat; UK Environment Agency, Oxfordshire, UNITED KINGDOM.

Sohel Saikat gave a brief overview of contamination in the United Kingdom. The UK must use *in vitro* methods because of ethical constraints. Five *in vitro* methods that have been used in the UK were evaluated using an inter-laboratory approach (metals tested: As, Pb, and Ni). Labs that used the same methods and same SOP produced consistent results; however, different methods did not produce comparable results. They also investigated the utility of *in vitro* methods for *in vivo* bioavailability for As (the Cynomolgus monkey was used). In general, the results showed that the five *in vitro* methods evaluated are not adequate for these arsenic soils (i.e., poor correlation between *in vivo* and *in vitro*). Sohel also reported that of the three methods tested, none was adequate for all metals (As, Pb, and Ni). Sohel noted that bioavailability and bioaccessibility are considered the same for many UK researchers. Sohel suggested that more work is needed to develop the *in vitro* assay including appropriate validation with an *in vivo* bioavailability model. Sohel suggested furthering the knowledge of geochemical characterization (method screening, geochemical matching, geochemical classification, and biomarkers) to further the *in vitro* method. Sohel suggested focused discussion on data gaps, harmonization of approaches among international regulators, and sharing of data among the research community.

Q&A

- What is a reasonable correlation to move forward?
    - We don't know. It was also noted that the correlation will change (as will p-value) when more data become available.
- Mark Maddaloni noted that the important area is when the bioavailability is low. Sohel agreed.

Measurement of Metal Bioaccessibility in Urban Household Dust and Corresponding Garden Soils
P. E. Rasmussen; Health Canada, Ottawa, ON, CANADA.

Patricia Rasmussen noted high variability of bioaccessible metals in house dust and the many geochemical differences between outdoor soil and indoor dust. Patricia noted that sample preparation (size fraction) and analytical procedures must be consistent for comparisons of outdoor soil and indoor dust. She observed that tracking-in models do not work in the city of Ottawa (indoor dust metal concentrations are not predicted accurately using outdoor soil metal concentrations). Patricia cautioned that dust may differ from outdoor soil (e.g., some metals are

found at higher concentrations and with higher bioaccessibilities; dust also has higher concentrations of organic carbon). Patricia reported data for Zn, Ni, Cu, inorganic carbon, organic carbon and a variety of other metals in dust and corresponding garden soil, and noted that the higher organic content of indoor dust is associated with greater variability in Ni and Zn. Patricia reported that for some metals (e.g. copper) bioaccessibility is affected by particle size, but noted that speciation can override the influence of particle size (likely due to different species of the metal in the various size fractions). She indicated that although smallest size fractions yield important information, she finds it necessary to sieve house dust to larger fractions (e.g. 80 microns or 150 microns) due to the small amount of dust that is typically obtained when sampling inside homes. Patricia noted that in her pilot study, the High Volume Small Surface Sampler (HVS3) vacuum sampler was used (from Rasmussen et al., 2008 [HERA, in press]). She provided an overview of the sources of variability in bioaccessibility (variability in both the numerator and the denominator of the % bioaccessibility equation). Variability in the numerator (bioaccessible metal) may result from differences in mass to volume ratio, pH, other constituents in the sample such as buffers, complexing agents, means of physical mixing and means of separation (filtration vs. centrifugation). Variability in the denominator (total metal concentration) may result from differences in recovery from using alternative methods to determine total metal concentrations (EPA 3051 vs. HF total digestion methods). Patricia suggested qualitative statements (low [<20%], medium [20-59%], and high [≥60%]) could be used to categorize the bioaccessibility of metals for outdoor soil and indoor dust, in light of the many sources of large variability. Patricia suggested that indoor-outdoor ratios need to be determined to understand indoor exposures. She offered that representative indoor dust data is being collected across Canada (Canadian House Dust Study) to develop a national baseline against which site-related measurements can be compared.

Q&A

- What is the German VDI method?
    - Patricia reported that this is a whole house vacuum method.

Assessment of the Use of Dynamic Human Stomach Models for In-vitro Measurement of the Bioaccessibility of Arsenics and Chromium in Soils – Can They Replace Animal Testing?

M. R. Cave; British Geological Survey, Nottingham, UNITED KINGDOM.

Mark Cave reported on his research to develop dynamic *in vitro* human stomach models: TIM-1 and Model Gut. These models have been used for pharmacology research. The TIM-1 system (TIM) includes a stomach phase and a small intestinal phase. TIM seeks to mimic human physiological processes (e.g, temperature, pH changes, peristalsis, secretions, and absorption). While TIM has been validated for glycemic response, there has been limited testing to date using soil samples. Results for soil samples showed good correlation for a single As and Pb soil, but not for Cd. TIM compared well with human subject data for fed/fasted ratio for Pb. The British Geological Survey (BGS) is developing a standard reference material: 104 ppm As, and 79 ppm Pb (BGS 102). Data suggest that the arsenic in this sample is of low bioaccessibility. A chromium (Cr VI) soil sample (approx 3500-ppm total Cr; 1400 ppm Cr VI) was also evaluated. Low bioaccessibility was reported for this sample using BARGE and TIM assays. The dynamic *in vitro* models show promising results, however, these methods are not intended to replace batch

methods (they may be used as reference methods, to characterize factors underlying differences in absorption, and provide an alternative to animal models).

Q&A

- How much soil is used for TIM and how much do they cost?
    - 10 g of soil and a few thousand Euros.
- Does TIM include a mastication process?
    - No, but pH changes over time.
- Is redox state controlled?
    - I am not sure, but I don't think so.

The Use of *In Vitro* Bioavailability Studies in Human Health Risk Assessment: Scientific Research and Application by Policy Makers

A. G. Oomen; RIVM, Bilthoven, THE NETHERLANDS.
W. I. Hagens; RIVM, Bilthoven, THE NETHERLANDS.
J. P. A. Lijzen; RIVM, Bilthoven, THE NETHERLANDS.
E. B. P. Kessels; Actief Bodembeheer de Kempen, Eindhoven, THE NETHERLANDS.
A. J. A. M. Sips; RIVM, Bilthoven, THE NETHERLANDS

Agnes Oomen noted that for most risk assessments oral bioavailability is equal to the bioavailability in the studies underlying the reference toxicity study (default RBA is 100%). Agnes reported that this has changed recently for lead, where RBA for soil lead is 74% (based on 80th percentile value). The intervention value (similar to Preliminary Remediation Goals) in the Netherlands is 530 ppm. She stated the default RBA for lead may be adjusted with reliable site-specific data (including *in vitro* data). Agnes discussed an area of the Netherlands contaminated by an historical Zn smelter. Contaminated slags and soils from the area were evaluated for bioavailability of Pb and As. RBA information was not applied because for lead it didn't have a major consequence for the site; whereas for As the soil did not pose a human health risk, but an ecological risk.

Q&A

- Might the soils be impacted by lead-based paint, since they are so high?
    - Agnes responded no.

Assessing Contaminant Bioavailability in Soil when *In Vitro* Gastrointestinal Methods are the Only Option

N. T. Basta, Ohio State University, Columbus, OH.
K. G. Scheckel, U. S. Environmental Protection Agency, Cincinnati, OH.
K. D. Bradham; U.S. Environmental Protection Agency, Research Triangle Park, NC.

Nick Basta discussed the utility of *in vitro* methods when they are the only option. Nick reported that the majority of samples to date have been from highly contaminated soils (often >2000 ppm Pb; >1500 ppm As). Nick noted that at highly contaminated soils adjustment of a cleanup level is likely to be influenced by RBA. Development of methods that can be used for

moderately contaminated soils (near 400 ppm) may have greater utility for more sites. Nick noted that most *in vivo* studies require highly contaminated soils (limit of detection issues). *In vitro* methods can be used at moderately contaminates sites, even down to near background levels. He questioned whether we are confident of *in vitro* methods at low levels. Nick suggested that he has greater confidence if it has been validated at high levels for the same contaminant. If the method will be used for a different contaminant, he is hesitant. Also, the source of the arsenic (or metal of interest) and factors that control the bioavailability of the sample (e.g., weathering) need to be better understood to use the method for other samples and concentration ranges. Nick believes that different extraction methods can be used, but first these factors must be understood. Nick provided an overview of some historical data on arsenic and collaboration with other researchers, including data on scorodite, iron oxide, and phosphate. When only *in vitro* methods can be used, As speciation can be used to identify the form of arsenic in the sample and to determine whether the method has been calibrated for those species. Nick suggested that more work is needed in speciation and *in vivo* bioavailability to *in vitro* bioaccessibility correlation and round robin studies. Nick added that collaboration maximizes resources and saves time. Thus, there is a need to share characterized soils and share data.

Q&A

- Which of the speciation methods is essential?
    - Nick responded that advanced spectroscopy will be discussed later. You need to understand the soil type and weathering of the contaminant to select a method.

The Bioaccessibility of Nickel in Contaminated Soils, Can It Be Explained Using Solid Phase Distribution Data?

J. Wragg, British Geological Survey, Nottingham, UNITED KINGDOM.
M. Cave, British Geological Survey, Nottingham, UNITED KINGDOM.
C. Ollson, Jacques Whitford Ltd., Ottawa, ON, CANADA.
K. J. Reimer, Royal Military College of Canada, Kingston, ON, CANADA.

Joanna Wragg discussed Ni bioaccessibility and factors that are known to impact Ni bioaccessibility. Joanna reported on data showing differences in geogenically-influenced soils and anthropogenically influenced soils. Geogenic soils tend to have less organic carbon and lower levels of Ni. Joanna compared Physiologically Based Extraction Technique (Ruby) and *In Vitro* Gastrointestinal (Basta) assays for soil samples that were characterized by chemical speciation. For anthropogenically influenced soils, the two methods compared well: no statistically significant difference in Ni bioaccessibility. Speciation identified 9 distinct soil components. PBET and IVG likely measured a mixture of several of these 9 contaminants. For geogenic-influenced soils, Joanna did not see a significant difference between stomach and intestinal phase of the PBET and IVG assays. She used cluster analysis to understand the speciation data for the anthropogenic soils. In general, anthropogenic soils were more bioaccessible. Joanna reported that aging of the geogenic sources impacted the bioaccessibility of iron complexes. Joanna believes that geochemical data can support the use of bioaccessibility data for risk assessment.

Q&A

- What pH was used for anthropogenic and glycine extractions?
    - Joanna responded glycine was used and pH of 1.8.

Importance of Metal Speciation in Understanding Bioavailability
Kirk G. Scheckel; U.S. Environmental Protection Agency, Cincinnati, OH.

Kirk Scheckel noted that speciation can support remediation and bioavailability. Kirk differentiated between the issues: what do we want to know and what do we need to know. Both chemical and physiology factors influence bioavailability. Kirk noted that research time at the synchrotron facility is free and the only cost is travel associated with getting to the facility. The synchrotron allows for characterization on the atomic scale. For example x-ray absorption near-edge spectroscopy (XANES) can measure down to 10 ppm. Kirk discussed a case study using phosphate amended soil speciation at Joplin, MO. Changes in speciation were observed with different soil treatments. Kirk reported on data showing alteration in bioavailability as a function of time after phosphate treatment where there was a reduction in bioavailability as the time since treatment increased. He noted that requirements are an appropriate measure (methodology and samples) as well as knowledge of the reason for the observed measurement (outliers).

Q&A

- Concerning the figure with the *in vivo* comparison, was what was shown the RBA or the ABA?
    - *In vitro* was RBA others were ABA.
- pH has ranged from 1.5 to 2.5. What should be used?
    - 2.5 was best correlation between PBET and *in vivo* (closer to 1:1 slope).
- Is 250-micron size fraction only correct size fraction?
    - Kirk replied that we need to harmonize our size fraction to allow comparability among assays.

Direct Identification of Metal Compounds in Contaminated Soil Mine Tailings and House Dust Using Synchrotron-based Methods
Heather E. Jamieson, Queen's University, Kingston, ON, CANADA.
S. R. Walker, Queen's University, Kingston, ON, CANADA.
S. E. Fawcett, Queen's University, Kingston, ON, CANADA.
A. Lanzirotti, University of Chicago, Chicago, IL.
P.E. Rasmussen, Health Canada, Ottawa, ON, CANADA.
S. Beauchemin, Natural Resources Canada, Ottawa, ON, CANADA.
M. Parsons, Geological Survey of Canada, Halifax, NS, CANADA.

Heather Jamieson noted that bioavailability is a function of mineral, grain size, and encapsulation. She suggests researchers spend several hours characterizing soils under the microscope for every hour of synchrotron X-ray beam time. Heather discussed two cases for As. The first case was from the Giant mine (an abandoned gold mine in Yellowknife near Slave Lake in Canada), while the second case was a series of sites such as Goldenville and Montague

(abandoned gold mines in Nova Scotia). The Nova Scotia sites are used recreationally today. They are working on paired speciation and bioaccessibility assays for the samples. Heather noted that many As-bearing secondary minerals are nanocrystalline (tens of nanometers), but porosity, cementation, and disaggregation can impact grain size. Heather also discussed the Ottawa house dust samples that Pat Rasmussen discussed earlier. Heather believes that synchrotron methods combined with classical mineralogical studies can provide valuable characterization information on the metal form in the sample.

Q&A

- No time for questions.

Bioaccessibility of Arsenic Adsorbed onto or Incorporated within Freshly Synthesized Iron Oxide Minerals Using the Simulator of the Human Intestinal Microbial Ecosystem (SHIME)

Brian D. Laird, University of Saskatchewan, Saskatoon, SK, CANADA.
T. Van De Wiele, University of Ghent, Ghent, BELGIUM.
D. Peak, University of Saskatchewan, Saskatoon, SK, CANADA.
W. Verstraete, University of Ghent, Ghent, BELGIUM.
S. D. Siciliano, University of Saskatchewan, Saskatoon, SK, CANADA.

Brian Laird suggested that understanding the inverse relationship between bioaccessibility and concentration is a data gap. Does it result from a thermodynamic limitation (liquid:solid ratio) or a kinetic limitation (residence time)? Complex mineralogy in mine tailings makes developing a relationship based on mineralogy challenging. Another hypothesis considered was the impact of colon microbes on As bioavailability. Does microbial activity in the colon also impact other metals? Brian found that for scorodite, stomach phase bioaccessibility was determined by the liquid:solid ratio. By contrast, in the small intestine scorodite bioaccessibility was determined by residence time. Isotherm analysis requires that the sample mineralogy is identical. Other research has shown that scorodite bioaccessibility was reduced in the sterile colon as compared to the microbially active colon. The opposite was seen with ferrihydrite-As(V). This effect was greater at higher concentrations of ferrihydrite-As(V). The effect of GI microbial activity may pose a challenge for validation of *in vitro* models with *in vivo* results. Brian acknowledged that the toxicological implications of the microbial activity are unknown.

Q&A

- Does physical mixing affect bioaccessibility?
    - Brian: Yes. These are kinetic constraints. Contact with solution is important. These models are operationally defined.
- Have you captured the dynamics of the GI tract?
    - Brian: No. We have not. The kinetics of the absorption (pulling arsenic out of the solution) is important, but how do you establish what the rate of absorption is?
- Do we know whether arsenic is absorbed in the colon?

- Brian replied yes. Some data are available concerning rate of arsenic absorption in various stages of the GI tract. For example, Brian found only one study of absorption throughout the GI tract (a study from 50 years ago in Japanese).

- Do the animal models we use have microbial flora in the upper GI?
  - Brian responded: yes. Some species have greater and other species have lesser gut microflora. Brian noted that cecum of the mouse is relatively large, so the microbial alteration may be more important to understand in this species.

Panel Discussion Session II

1 - Is it sufficient for *in vitro* models to have correlation with animal results or is it necessary to make the models accurate physiologic mimics of human gut dissolution?

Mark Maddaloni stated that good correlation across multiple soil types is the objective. Research to develop a physiological model is not necessary (assuming we have validation); however, we would like to have detailed information on physiological mechanisms. Nick Basta agrees.

2 - Is it acceptable to use the terms "bioaccessible", "soluble", "migrateable", and "extractable" interchangeably in the numerator of the % bioaccessibility equation?

In general the terminology has been consistent for the presentations today. Some differences in sample preparation (e.g., pH of digestion and sieving) have come out today. We should work to make these consistent.

Agnes noted that separation method (dialysis vs. centrifugation) also has a large influence on results. This, too, should be standardized.

Nick noted that the methods with more colloidal material may be influenced by separation method to a greater degree than other simpler methods.

Brian noted that we need to consider the role of concentration on kinetics of dissolution.

Kirk noted that we are not trying to establish equilibrium in the *in vitro* tests.

Yvette noted that for other metals (like barium) we may be reaching the solubility limitation.

Marc Stifelman noted that for risk assessment purposes we can probably establish a reasonable upper bound for liquid:solid ratio. Others agreed that would be a start.

3 - Should bioaccessibility values be used on their own without supporting information? If not, what additional information should be included for each soil sample analyzed (e.g., geochemistry)?

Rosalind believes that supporting information is important for better understanding the site. Patricia asked for clarification of the question "be used for what?"

Are we using the bioaccessibility information to make site decisions or for comparing sources? Bioaccessibility is operationally defined.

Beverly Hale agreed. We should collect as much information as possible.

Rosalind followed up that for many of the studies we need to understand the limitations and how we can augment the information.

Kirk noted that electron microprobe studies tell you about chemical composition, it does not provide speciation. The results can be misleading for As. XRD and microscopy can give you some very good information also.

Mark noted that the bioaccessibility and extraction methods are best used in combination.

Nick added that weathering is an important factor. The speciation of soil samples is important and these methods should be used.

What can be done where we have limitations on methods for low concentration and *in vivo* bioavailability methods?

Marc Stifelman noted that he agrees with testing unknowns in as many species as available. We can probably feed monkeys low levels.

Yvette agrees—we should study soils where the metal concentration is of environmental relevance (e.g., 50-100 ppm for As). Moving from there to the risk range is not a scary extrapolation. Mark Maddaloni agrees.

Marc Stifelman noted that with the exception of Florida, most Superfund soil cleanup goals range from 20-245 ppm As.

Nick suggested that if we can characterize the species that is controlling As solubility, then the effect of solubility is likely to be the same at 30 ppm as it is at 3000 ppm.

Kirk and others raised concerns with this related to pH and other factors (as demonstrated with scorodite research).

Particle size distribution issue: Rosalind noted that for oral studies many have sieved to 250-µm particle size (based on adherence of soils to skin and incidental soil ingestion pathway). Recent research on dust is looking at smaller particle size fraction (<150µm). There may or may not be enrichment in the finer fraction. What is the appropriate particle size range cutoff?

Kirk offered that for consistency we should stick with one size fraction.

Rosalind agrees that harmonization is an important issue.

Yvette added that when they examined soils from SERDP, the majority of lead mass was in the <75 µm size fraction.

Patricia noted that house dust has a different particle size distribution. A key issue is to use the same procedure for indoor and outdoor dust. Patricia noted that it is physically challenging to get down below 50 µm. Mark Richardson's paper (Health Canada) encouraged the further characterization of size fractions (look at different cuts).

Agnes added that in the Netherlands they agree that smaller particles tend to stick to the hand, but if even a few large particles adhere to the hand those can dramatically impact the results. RIVM uses the <2 mm size fraction as a result.

Rosalind noted that the particle size fraction to keep in mind is the size fraction commonly used for site characterization, <250 µm.

Patricia noted that the cost of sieving is relatively cheap (a few dollars for full characterization of a sample). A more complete characterization may result in greater consistency across sites. Patricia and Yvette agreed.

Yvette noted that the 3050 method found >100% bioaccessibility for barium (this is a known limitation of the 3050 method). Agreement that consistency in digestion method is important. Heather agrees that results from total digestion may be misleading.

Summary

- The EPA guidance also recommends using a validated methodology and provides recommended evaluation criteria for the development of new methods.

- Establishing a correlation between an *in vitro* method and an *in vivo* method was generally considered an important step. A 1:1 correlation is not necessary, only that the model is predictive for soil types and contaminant concentrations of interest.

- Agreement that mimicking physiology is not necessary, nor is complete characterization and understanding of processes, for an *in vitro* method to be accepted. What is important is that the *in vitro* method is correlated with an *in vivo* model and that it is predictive for soil types and contaminant concentrations of interest.

- While not strictly required, insight into the factors underlying the absorption processes for a given metal and complete characterization of the metal in the media of interest will inform the limits of a test method for a given metal species (e.g., concentration range). This information will also determine confidence in using the method for other forms of the metal or soil phases.

- Any candidate method for evaluation must be presented with bounds of valid use (e.g., metal forms) and methodological constraints (e.g., concentration range, pH, and liquid:solid ratio).

- Agreement that the next logical step is to develop an arsenic assay (*in vivo* and *in vitro*).

- Cost is a factor for an *in vitro* assay. EPA is seeking *in vitro* methods that are cost effective and relatively quick. The *in vivo* model against which it is correlated is not necessarily similarly constrained.

- At this time no decision has been made concerning which animal model is preferred for metals other than soil borne lead from mining, milling, and smelting sites (for which EPA has an SOP).

- For selection of an *in vivo* model for As, comparisons among the available animal models using the same soils are required to make this determination.

- A single animal model may not work for all metals because of differences in pharmacokinetics.

- A more complete characterization of concentration and bioaccessibility for various particle size fractions may result in greater consistency across sites.

- There is a need for harmonization for digestion methods and sieving across sites.

- A combination of laboratory, microscopic, and synchrotron methods may provide useful information for bioaccessibility, bioavailability, and risk assessment.

- Agreement was made to identify, characterize, and share standard reference materials for *in vivo* and *in vitro* assays.

- Agreement to share information and data, as well as soil samples, to identify data gaps and research needs.

- Agreement to collect soil samples from a wide array of relevant sites, preferring weathered soils over spiked soils (because they may differ).

- Agreement to meet again before the 2008 ISEA meeting. This will foster collaboration and further discussion.

Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy.

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Appendix A: Questions for the Panel (submitted in advance)

Questions for Panel Discussion Session I (evaluation/site application and *in vivo* research):

1 - For regulatory applications such as site clean up decisions, is an *in vitro* model that is predictive (i.e., correlates with an *in vivo* model) adequate even if we don't know why it is predictive, or is it important to know why a model is predictive?

2 - Given that many animal models require use of soil concentrations exceeding those of public health concern, what efforts are necessary, if any, to show that bioavailability at these concentrations reflect oral absorption at lower environmental concentrations? In other words, how important is it to evaluate whether bioavailability is concentration dependent?

3 - Should a single animal model be specified, or are studies in a variety of different animal models more likely to improve our understanding of relative bioavailability in humans.

4 - Is there a single animal model that will work for all chemicals considering the widely varying toxicokinetic profiles?

5 - Is relative oral bioavailability of chemicals in soil so routine that it is "ready" for guidance/regulation that will inherently limit science-based research?

Questions for Panel Discussion Session II (*in vitro* and speciation/mineralogy research):

1 - Is it sufficient for *in vitro* models to have correlation with animal results or is it necessary to make the models accurate physiologic mimics of human gut dissolution?

2 - Is it acceptable to use the terms "bioaccessible", "soluble", "migrateable", and "extractable" interchangeably in the numerator of the % bioaccessibility equation?

3 - Should bioaccessibility values be used on their own without supporting information? If not, what additional information should be included for each soil sample analyzed (e.g., geochemistry)?

4 - How can we leverage resources to answer specific research questions to advance the understanding of bioavailability/bioaccessibility?

- Develop Standard Reference Materials or Certified Reference Materials?
- Round robin testing?

5 - In lieu of comparing *in vitro* model results to an *in vivo* model, what criteria should be considered when evaluating whether a particular *in vitro* method is appropriate for providing screening level data versus data to derive quantitative site-specific bioavailability adjustments?

6 - What other metals, or metal species, are of interest for developing additional *in vivo* and *in vitro* bioavailability assays? What criteria are used by regulatory agencies for ranking or prioritizing contaminants?

7 - Is it feasible and cost effective to develop a single standardized *in vitro* method for each metal for simulating oral bioaccessibility? If so, what steps would be needed for that to happen? What criteria would be used to determine the difference between the methods?

- What is available (and in process) and how available/quantities (related to above) how prioritized types of materials needed (ex: soils, tailings, etc.)
- Discussions of desired specifications by type (uses will drive Data Quality Objectives)
- Funding (ex: to National Institute of Standards and Technology for Standard Reference Materials) vs. volunteer analysis via round robins (to develop consensus values) and how determine "reliable" methods? International Atomic Energy Agency could be a model.

Appendix B: Abstracts

Appendix C: Presentations

Abstract 580
Introduction and Overview
K. Bradham; U.S. Environmental Protection Agency, RTP, NC

In human health risk assessments, soil and dust ingestion can be a major route of exposure to many soil contaminants, including metals and metalloids. Site-specific soil physical and chemical characteristics, as well as internal biological factors, determine the oral bioavailability of soil contaminants. Within a single sample, this contamination may be from multiple sources of metals and may exist as different forms and species. Both animal models and *in vitro* bioaccessibility models have been used to estimate relative bioavailability of metals in soil and dust. The bioavailability estimates for soil have a direct impact on current human health risk assessment and risk management practices. This introduction and overview to the symposium will include definitions and specification information necessary for setting the stage for the presentations on recent developments in animal models, new *in vitro* models, the role of mineralogical analyses in assessing relative bioavailability, and the application of physiologically-based models as research tools. Information will also be presented regarding the panel discussions and specific research questions provided to the panelists and presenters for discussion during this symposium.

Abstract 520

Evaluating the Bioavailability of Soil-Borne Contaminants at Waste Sites

M. Beringer[1], A. Yeow[2]; [1]U.S. Environmental Protection Agency, Kansas City, KS, [2]OSRTI, Washington, DC

Site-specific bioavailability is an important consideration in determining potential threats to human health that are posed by metals-contaminated soils at waste sites. It is important to consider bioavailability because metals may be absorbed to a lesser or greater extent following ingestion of contaminated soils as compared to the fraction absorbed in the studies used to establish toxicity values, such as a reference dose or a cancer slope factor. U.S. EPA's Office of Superfund Remediation and Technology Innovation (OSRTI) has led an effort to develop guidance on evaluating and incorporating bioavailability adjustments into human health risk assessments. The guidance outlines a decision framework for deciding when to collect and incorporate site-specific bioavailability information; recommends a process for documenting the data collection, analysis, and site-specific implementation of a validated method; as well as provides recommended method validation and regulatory acceptance criteria for evaluating alternative methodologies. U.S. EPA has used these criteria to evaluate two separate methodologies for predicting the relative bioavailability of lead in soil and soil-like materials. The Agency has determined that both an *in vivo* swine bioavailability bioassay and an *in vitro* bioaccessibility assay have sufficiently satisfied these criteria. Thus, they are considered regulatory methodologies appropriate for determining the relative bioavailability of lead for quantitative use in site-specific risk assessments. This presentation will summarize the bioavailability guidance document and the basis for the Agency's decision regarding the two methodologies for predicting lead relative bioavailability.

Abstract 514

Method Development and the Application of Oral Bioavailability Data in U.S. Risk Assessments

R. A. Schoof; Integral Consulting Inc., Mercer Island, WA

USEPA guidance includes provisions for site-specific adjustments in exposure estimates to account for differing relative bioavailability of chemicals in soil and in the exposure media of toxicity studies. The relative oral bioavailability of lead and arsenic in soil has been assessed in a series of studies that have included both animal models and in vitro test systems. USEPA recommends a default assumption that children will absorb only 60 percent as much lead from soil compared with absorption from water or diet. For arsenic, no such default assumption has been generally accepted. In vivo bioavailability studies should be designed to account for variations in metabolism and excretion of chemicals. The absorption and disposition of lead and arsenic differ substantially. Consequently, bioavailability studies to assess these two chemicals have been designed to reflect these differences. In vivo methods used to assess the bioavailability of soil-borne chemicals are typically modified versions of methods widely used in biomedical research. These methods have been modified to address constraints associated with use of doses relevant to environmental concentrations, the need to reflect weathering behavior in soils over time, and the need to generate data applicable to human health risk assessments. Regulatory acceptance of bioavailability data in a site-specific risk assessment is generally dependent on the use of a validated test method or a careful scientific review of the test method employed. In vivo bioavailability data generated by traditional study designs is usually considered to be acceptable, but in vitro studies face a greater burden in obtaining acceptance. In the US a process developed by the Interagency Coordinating Committee on the Validation of Alternative Methods for validating newly developed alternative toxicity methods provides relevant guidance for assessing in vitro methods. Efforts to obtain regulatory acceptance of in vitro studies of relative bioavailability will be reviewed.

Abstract 461

Arsenic Bioaccessibility Testing Using Various Extraction Methods: Results and Relation to Relative Oral Bioavailability as Measured in the Cynomolgus Monkey

Y. W. Lowney[1], S. Roberts[2], S. Saikat[3]; [1]Exponent, Boulder, CO, [2]University of Florida, Gainesville, FL, [3]UK Environment Agency, Wallingford, UNITED KINGDOM

Recent research has established that the absorption of arsenic from soils following ingestion exposures is lower than absorption of soluble arsenic from water. Because regulatory toxicity values for arsenic are based on studies of human exposures to arsenic in water, understanding the relative oral bioavailability (RBA) of arsenic from soils is important for accurate assessment of exposure to arsenic associated with soil ingestion. Because of the site-specific nature of the controls on arsenic bioavailability, site-specific information is important in assessing potential risk associated with arsenic in soils. To conduct an animal study of bioavailability for every site affected by arsenic would be time- and cost-prohibitive, and may counter policies regarding the use of animals in research. Therefore, recent efforts have focused on developing practical and economical bench-top (*in vitro*) procedures to measure the fraction of contaminants in soils that, following ingestion, would be available for absorption into systemic circulation.
This presentation will discuss recent *in vivo* testing of arsenic bioavailability in the cynomolgus monkey, and the results of *in vitro* extraction tests that have been designed to predict the *in vivo bioavailability* results. The *in vitro* methods include an extraction protocol that has been validated as predictive of the RBA of lead from soil, other extraction methods that have been reported in the literature, methodologies developed by the Dutch RIVM, and the RIVM method as modified by BARGE. This presentation provides the results of extraction testing of splits of several soils that were evaluated *in vivo*, and discusses the differences in results between the extraction methods, and the correlation between the results from extraction testing and RBA as measured in cynomolgus monkeys. Results indicate that additional work remains to identify an individual *in vitro* method that is able to predict the *in vivo* bioavailability satisfactorily for all soils.

Abstract 519

Assessing Bioavailability Using the Swine Model

S. Casteel[1], G. Fent[1], C. Weis[2], W. Brattin[3]; [1]University of Missouri, Columbia, MO, [2]Environmental Protection Agency, Region VIII, Denver, CO, [3]Syracuse Research Corp, Denver, CO

Bioavailability of site-specific environmental contaminants is critical to exposure assessment. Determining the bioavailability of contaminants in a diverse range of soils, allows scientifically derived data to dictate site-specific remedies to reduce the risk for sensitive human populations. Based on a series of dosing trials in a juvenile swine model, site-specific estimates of relative bioavailability of metals and organic compounds, is highly variable and is matrix and chemical species dependent. Results for lead- arsenic- and cadmium-contaminated soils support the view that soil metals are not always as well absorbed as soluble forms; therefore use of default assumptions for assessing human health risk may overestimate the hazard.

Since the selection of appropriate animal models enhances the science and reduces uncertainty in human risk assessment it is critical to use the best available model with reasonable constraints. Numerous rodent model studies are in the literature. Studies in non-human primate (NHP) models have been fewer due to higher purchase and per diem costs, housing availability, zoonotic concerns, and animal rights attention.
Criteria useful in selecting the appropriate animal model include behavior, age, size, ease of bleeding, anatomical considerations and gastrointestinal physiology. Research management factors such as historical database, costs, model availability, and animal rights group interest in the model were also involved in selection.

The swine model has the versatility to assess the bioavailability of a wide variety of materials, including metals, organic compounds, and biodistribution of gold-, palladium- and silver-nanoparticles. When assessing site-specific contaminants, pigs are dosed 2 hours before each feeding twice daily for 14-to-15 consecutive days, at constant dosing times. Multiple doses are provided as a more likely real-world reflection of exposure**.** This approach has been successfully applied at numerous sites for estimation of relative bioavailability for lead, arsenic, cadmium, vanadium, and chromium.

Abstract 521

Assessing Soil Arsenic Bioavailability in the Laboratory Mouse

D. Thomas, M. Hughes, K. Herbin-Davis, P. Seales; U.S. Environmental Protection Agency, RTP, NC

Variation among soils in the bioavailability of arsenic can be a critical determinant of the risk posed by exposure to these soils. Although *in vitro* techniques can provide vital data on aspects of bioavailability of metals and metalloids from soils, these results must be validated in an animal model. A useful animal model provides a measure of bioavailability and allows comparison of bioavailability for different soil matrices. Inbred strains of laboratory mice are potentially good models for development of a bioavailability assay. Laboratory mice are well characterized physiologically and can be manipulated experimentally (e.g., altered dietary components, altered genotype). There is also a large body of data on the absorption, metabolism, disposition, and excretion of inorganic and methylated arsenicals in the mouse which is germane to evaluating the differences and similarities between mouse and human. Initial studies are comparing arsenic bioavailabilities in soils with known arsenic contents with the bioavailability of sodium arsenate. Here, soils (e.g. NIST SRM 2710) or sodium arsenate are added to a standard powdered mouse chow (AIN-93G purified rodent diet) at the one percent (weight/weight) level. Adult female C57BL/6 mice have had free access to this amended chow and tap water for nine days. Urine and feces are collected on a daily basis and food intake is monitored throughout this period. At the end of the nine-day exposure period, mice are euthanized and tissues collected. Data on food consumption and arsenic contents of excreta and selected tissues are used to calculate the bioavailability of arsenic in each soil matrix. Development and refinement of this animal model should provide a convenient and rapid means to assess the absolute and relative bioavailability of arsenic in soils. These data may be of great value in risk assessment. (This abstract does not reflect US EPA policy.)

Abstract 518

In-vitro Bioaccessibility of Soil-borne Contaminants: An Environment Agency Perspective

S. Saikat; Environment Agency, Oxfordshire, UNITED KINGDOM

In the UK, interest in the use of *in-vitro* bioaccessibility data in risk assessment, has been stemmed mainly from the problem associated with elevated arsenic in mineralised and mining effected areas. There is an expectation that *in-vitro* bioaccessibility data can be a 'quick fit solution' to dealing with land contamination where contaminant level exceeds corresponding generic assessment criteria (*e.g*. Soil Guideline Values).

Studies undertaken by the Environment Agency, however, indicate that a number of *in-vitro* methods (*e.g*. physiologically based, semi-physiologically based and simple chemical leaching) are currently available to measure *in-vitro* bioaccessibility but no information to indicate that they are being validated with *in-vivo* data for UK soils. The study also indicated that reproducibility of different *in-vitro* methods, operating procedures and reporting of results could contribute to a large variation in *in-vitro* bioaccessibility data. Laboratories use same *in-vitro* method irrespective of chemicals, concentrations, mineralogy and soil types. Moreover, no reference material containing *in-vivo* data is available to measure the accuracy of *in-vitro* methods.

In order to appreciate and make the best of research progress achieved, a review of outstanding issues is required to consolidate efforts and develop appropriate partnerships. Scientists, risk assessors and regulators need to balance their expectations of the *in-vitro* approach in terms of its capabilities and weaknesses in order to make it more useful in risk assessment.

Abstract 524
Measurement of Metal Bioaccessibility in Urban Household Dust and Corresponding Garden Soils
P. E. Rasmussen; Health Canada, Ottawa, ON, CANADA

Large uncertainties are associated with the measurement of gastric bioaccessibility of metals in household dust, caused in part by the heterogeneous nature of settled dust samples, and in part by variations in analytical parameters. A modified version of European Standard EN 71-3 Toy Safety Protocol was used as a rapid screening method for estimating gastric bioaccessibility of metals in urban geochemical surveys of household dust and corresponding garden soil samples in Ottawa, Canada. In this study, gastric bioaccessibility is defined as the concentration of metal leached from the test sample into 0.07 M HCl (2 h at 37^{o}C; pH 1.5), expressed as a percentage of the total metal concentration. To improve measurements of the total metal concentration (the denominator in the bioaccessibility equation) several modifications were made to the US-EPA 3051 microwave digestion protocol. Increasing the microwave digestion time to 30 min ramp followed by 30 min hold (compared to 5.5 min total digestion time specified by EPA3051) increased total metal recoveries by 15-20%. Increasing the acid volume to sample mass ratio to 1000 (compared to ratios of 20 to 100 specified by EPA3051) increased total metal recoveries by 30-60%. Similarly, for the simulated gastric extraction (the numerator), increasing the acid volume to sample mass ratio to 2000 (compared to ratios of 50 to 500 specified by the Toy Safety protocol) typically increased the extraction efficiency by 20 to 50%. Analytical reproducibility is improved using smaller sieve fractions (<60 micron is best); however, settled dust samples collected in this study were typically very small (1-2 g) necessitating the use of a larger size fraction (<150 micron). In light of the inherent variability associated with settled dust measurements, estimates of gastric bioaccessibility are grouped into simple categories: low (19% and less), medium (20 to 59%) and high (over 60%).

Abstract 391

Assessment of the Use of Dynamic Human Stomach Models for In-vitro Measurement of the Bioaccessibility of Arsenics and Chromium in Soils - Can They Replace Animal Testing?

Mark Cave[1], Helen Taylor[1], Joanna Wragg[1], Andrew Broadway[2: 1]British Geological Survey, Keyworth, Nottingham; University of Edinburgh, School of GeoSciences, Edinburgh, UK

The development of methods for estimating the oral bioavailability of soil contaminants may reduce costs of site remediation and soil cleaning, while still maintaining the required protection level. Currently, simple batch in vitro extraction methods, which broadly mimic the physico-chemical conditions in the human gastro intestinal tract, have been developed as screening methods for bioaccessibility measurement. Regulatory authorities, however, require that the in-vitro methods should produce data that is demonstrated to be comparable to the in-vivo situation. It has been shown, however, that the GI tract of young pigs is similar to humans and that they can be used to validate the results of in-vitro tests. Animal studies are, however, time consuming, costly, have ethical considerations and there are concerns regarding their relevance to the human. The food and drug industry has worked to produce dynamic in-vitro systems specifically designed to mimic the human gastrointestinal system (Wickham, 2007, Minekus, 1995). Such systems may be as relevant as animal models for soil bioavailability studies and have a part to play in either estimating bioaccessibility or in validating the simpler batch tests. This paper will discuss the results obtained for the bioaccessibility of arsenic and chromium from soils with both in-vivo bioavailability and batch in-vitro bioaccessibility data.

Wickham, M. (2007): *The Model Gut*, **2007** (27April), http://www.ifr.ac.uk/science/platform/MG/default.html.
Minekus, M, Marteau, P, Havenaar, R and Huisintveld, JHJ. (1995): "A Multicompartmental Dynamic Computer-Controlled Model Simulating the Stomach and Small-Intestine", *Atla-Alternatives to Laboratory Animals*, **23**(2), 197-209.

Abstract 517

The Use of In vitro Bioavailability Studies in Human Health Risk Assessment: Scientific Research and Application by Policy Makers

A. G. Oomen[1], W. I. Hagens[1], J. P. A. Lijzen[1], E. B. P. Kessels[2], A. J. A. M. Sips[1];
[1]National Institute for Public Health and the Environment, Bilthoven, THE NETHERLANDS, [2]Actief Bodembeheer de Kempen, Eindhoven, THE NETHERLANDS

Today, a relative bioavailability factor of "1" is used for human health risk assessment of contaminated soils. This implicates the assumption that there is no difference in the bioavailability of a contaminant from soil compared to the bioavailability from the matrix used in the studies underlying the Intervention Value for remediation, which is typically a food or water matrix. However, there is ample evidence demonstrating that the bioavailability of a contaminant from soil can be considerably lower than from food or water. Integrating oral bioavailability of contaminants from soil in human health risk assessment will increase the realistic outcome of risk assessment through soil ingestion. The research in this presentation focuses on the contaminant lead, since lead is frequently encountered at human toxicologically high concentrations in soil in the Netherlands. Furthermore, soil ingestion is an important pathway of exposure for lead, especially for children, leading to potential adverse effects. Therefore, the need for a realistic but still protective risk assessment for human health is high.

The RIVM has developed a simple experimental tool, an *in vitro* digestion model, to supply information on the bioavailability of a contaminant in the human body after ingestion of contaminated soil. This model has been used to estimate the bioaccessibility of lead in specific soils. With CSOIL, site specific risk assessment of human health can be modeled to answer specific policy issues.

In this presentation, the experimental setup and outcome of such a project is given. Furthermore, the implementation of the results and the scientific advice towards policy makers is addressed. Actions taken by policy makers following the recommendations are discussed.

Taken together, this presentation gives an overview on the involvement of the RIVM *in vitro* digestion model in site specific risk assessment in the Netherlands.

Abstract 516

Assessing Contaminant Bioavailability in Soil when *In Vitro* Gastrointestinal Methods are the Only Option

N. T. Basta[1], K. G. Scheckel[2], K. D. Bradham[3]; [1]Ohio State University, Columbus, OH, [2]U. S. Environmental Protection Agency, Cincinnati, OH, [3]U.S. Environmental Protection Agency, Research Triangle Park, NC

Incidental soil ingestion is an important exposure pathway for assessing public health risks associated with contaminated soils. The bioavailability of Pb, As, and possibly other contaminants in soils can be determined by conducting dosing trials using acceptable surrogate animal models. To overcome the difficulty and expense associated with *in vivo* trials, *in vitro* gastrointestinal (IVG) methods, that simulate human gastrointestinal conditions, have been developed. Bioaccessible Pb and As determined by several IVG methods has been shown to be correlated with *in vivo* bioavailability data.

Soils must have a very high contaminant concentration, often > 500 or 1000 mg/kg, to accurately measure bioavailability from animal dosing trials. Most contaminated soils are not *highly* contaminated. These moderately contaminated soils require risk assessment but are below the "detection limits" of animal models. IVG methods will be the only methods that can be used for exposure assessment of moderately contaminated soils. Soil chemistry, mineralogy, and other geomedia properties are likely to have more influence on contaminant bioavailability in moderately contaminated soils than highly contaminated soils. Can we rely on IVG methods to assess contaminant bioavailability in moderately contaminated soil without method validation based on *in vivo* bioavailability data? Soil and contaminant chemistry requirements necessary for accurate application of IVG methods to access contaminant (bio)availability will be presented.

Abstract 406

The Bioaccessibility of Nickel in Contaminated Soils, Can It Be Explained Using Solid Phase Distribution Data?

J. Wragg[1], M. Cave[1], C. Ollson[2], K. J. Reimer[3]; [1]British Geological Survey, Nottingham, UNITED KINGDOM, [2]Jacques Whitford Ltd., Ottawa, ON, CANADA, [3]Royal Military College of Canada, Kingston, ON, CANADA

In recent years there has been increased use of bioaccessibility testing to determine the fraction of potentially harmful elements (PHEs) available for uptake in the human gastrointestinal tract, and which therefore may pose a risk to human health. The data produced by such tests may be incorporated into human health risk assessments to determine the risk posed to the critical receptor, by a given land use, for the ingestion pathway.

In tandem, research has focussed on identifying the physico-chemical sources of bioaccessible PHEs (and in some cases non-bioaccessible PHEs) in soils. Identification of the physico-chemical hosts of PHEs can be achieved by the use sequential extractions. Application of these techniques in conjunction with bioaccessibility methods can aid the understanding of soil-contaminant relationships and how contaminant bioaccessibility and mobility may impact on human health risk assessment and future land use. To date, most interest has centred on arsenic but more recently focus has shifted to nickel (Ni). The physico-chemical sources of Ni in soils collected from Sudbury, Ontario will be described, after indentification by the use of the CISED (Chemometric Identification of Substrates and Element Distribution) extraction technique with confirmatory X-ray diffraction information. The information surrounding the solid phase distribution of Ni in the soils will then be used to provide an understanding of measured bioaccessibility data.

Abstract 407

Importance of Metal Speciation in Understanding Bioavailability

K. G. Scheckel; U.S. Environmental Protection Agency, Cincinnati, OH

The speciation or chemical form of metals governs their fate, toxicity, mobility, and bioavailability in contaminated soils, sediments and water. To assess these chemical properties and to accurately gauge their impact on human health and the environment we need to characterize metals at the atomic level. One can employ an array of techniques to address speciation including XRD, DRS, TEM, TGA, and XPS. In addition to these tools, researchers have used synchrotron radiation methods to elucidate metal speciation. The complexity of metal contaminated sites has and continues to be simplified to a measure of the total metal content. While total metal content is a critical measure in assessing risk of a contaminated site, total metal content alone does not provide predictive insights on the bioavailability, mobility, and fate of the metal contaminants. Our ability to determine metal speciation in soils enhances efforts to understand the mobility, bioavailability, and fate of contaminant metals in environmental systems, to assess health risks posed by them, and to develop methods to remediate metal contaminated sites. To attain in situ atomic level information on the speciation of metals we utilize high-energy synchrotron X-rays to probe chemical structure. At the Advanced Photon Source (APS) of Argonne National Laboratory (Argonne, IL), we incorporate X-ray absorption (XAS), X-ray fluorescence (XRF), and micro-tomography spectroscopies to analyze environmental samples to determine the true, in situ speciation of metal contaminants. These innovative research tools are expanding our ability to directly identify the role of metal speciation on many dynamic processes that influence risk.

Abstract 587

Direct Identification of Metal Compounds in Contaminated Soil, Mine Tailings and House Dust Using Synchrotron-based Methods

H. E. Jamieson[1], S. R. Walker[1], S. E. Fawcett[1], A. Lanzirotti[2], P. Rasmussen[3], S. Beauchemin[4], M. Parsons[5]; [1]Queen's University, Kingston, ON, CANADA, [2]University of Chicago, Chicago, IL, [3]Health Canada, Ottawa, ON, CANADA, [4]Natural Resources Canada, Ottawa, ON, CANADA, [5]Geological Survey of Canada, Halifax, NS, CANADA

Contaminated soils can be expected to contain multiple hosts of the metal or metalloid of concern, especially in the case of mine-impacted soils or tailings, where the concentrations are orders of magnitude above soil quality guidelines. For example, we have determined that a single sample of arsenic-rich gold mine tailings contains, in addition to the primary arsenopyrite (FeAsS), five secondary oxidation products namely scorodite ($FeAsO_4 \cdot 2H_2O$) , amorphous Fe arsenate, kankite ($FeAsO_4 \cdot 3.5H_2O$), yukonite (Ca-Fe arsenate), and arsenic bound to iron oxyhydroxides. At sites where ore roasting was used, tailings and soils contain AsIII-bearing roaster-generated iron oxides, as well as AsV-bearing iron oxyhydroxides generated by sulfide weathering. We have also determined that antimony is present in multiple mineral forms and oxidation states in mine waste impacted sediments. The detailed and direct identification of these As- and Sb-bearing phases was achieved using a combination of synchrotron-based techniques microanalytical including microXRF (X-ray fluorescence), microXANES (X-ray near edge spectroscopy) and microXRD (X-ray diffraction) on target grains in polished thin sections with a <10 micron spatial resolution.

Synchrotron-based techniques have also been applied household dust and shown that for a sample from a background urban environment Cu and Zn are associated with distinct matrices. Copper is dominantly hosted in an organic phase while Zn is associated with inorganic minerals.

Each solid host of a metal or metalloid may exhibit different response to bioaccessibility tests, as these phases are known to vary in solubility. The multiplicity of mineral hosts has significant implications for the design of sampling programs that aim to obtain representative ingestable material. Where applicable, synchrotron-based microanalysis provides a tool to unambiguously characterize contaminants in complex samples.

Abstract 525

Bioaccessibility of Arsenic Adsorbed onto or Incorporated within Freshly Synthesized Iron Oxide Minerals Using the Simulator of the Human Intestinal Microbial Ecosystem (SHIME)

B. D. Laird[1], T. Van De Wiele[2], D. Peak[1], W. Verstraete[2], S. D. Siciliano[1]; [1]University of Saskatchewan, Saskatoon, SK, CANADA, [2]University of Ghent, Ghent, BELGIUM

The bioaccessibility of arsenic adsorbed to amorphous ferrihydrite or incorporated within amorphous scorodite was measured in the stomach, small intestine, and colon stages of the Simulator of the Human Intestinal Microbial Ecosystem (SHIME), an in vitro gastrointestinal model that incorporates the microbial community found in the human colon. Arsenic concentrations adsorbed to ferrihydrite ranged between 500 and 9500 ppm while the arsenic concentrations of amorphous scorodite mixed with freeze-dried iron oxide ranged between 4500 and 450,000 ppm. The SHIME digests of these arsenic-bearing minerals were used to construct arsenic dissolution isotherms for the stomach, small intestine, and colon SHIME. Subsequently, the Kd of arsenic in gastrointestinal fluids and the mechanism of concentration-dependent constraints on arsenic bioaccessibility was evaluated. Additionally, the colon digest was repeated with sterilized colon SHIME suspension to investigate the role gastrointestinal microorganisms on the bioaccessibility of arsenic adsorbed onto or incorporated into iron oxide minerals. These experiments investigated the mechanisms by which concentration and gastrointestinal microbes affect arsenic bioaccessibility.

Introduction and Overview for Symposium "Use of *In Vitro* Bioaccessibility/Relative Bioavailability Estimates in Regulatory Settings: What is Needed?"

**Karen Bradham, Mike Beringer,
Aaron Yeow (EPA)
Pat Rasmussen (Health Canada)
Rosalind Schoof (Integral Consulting, Inc.)
Mark Cave (British Geological Survey)**

Symposium Roadmap

- Welcome
- Setting the stage
- Definitions of bioavailability
- ISEA 2006
- ISEA 2007 overview and logistics
- Panel discussion questions
- Closing remarks

Oral bioavailability of metals

- Site-specific human health risk assessments
- Risk assessments used to determine whether a contaminated site poses a current or future threat to human health that warrants remedial action
- Oral ingestion of soil and dust – "risk driver" for human exposure to metal contaminants

Ryan, Scheckel, et al. ES&T, 2004

Exposed to contaminated soil - oral ingestion

- Toxicity of an ingested chemical depends, on the degree to which it is absorbed from the gastrointestinal tract into the body
- Metals can exist in a variety of chemical and physical forms
- Not all forms of a given metal are absorbed to the same extent
  - Physical, chemical, biological
  - Matrix: metal from a contaminated soil absorbed vs. ingestion from dietary exposure

25 March 2005 issue, *Science* Magazine, Simpson et al. The Gut: Inside out. Physiology and biology of the gastrointestinal system

Definitions of bioavailability and related terms

- Many different meanings across various disciplines
- *Guidance for Evaluating the Oral Bioavailability of Metals in Soils for Use in Human Health Risk Assessment* (OSWER 9285.7-80)
- Bioavailability: "*The fraction of an ingested dose that crosses the gastrointestinal epithelium and becomes available for distribution to internal target tissues and organs"*

http://www.epa.gov/superfund/bioavailability/guidance.htm

Definitions of bioavailability and related terms continued

- Bioavailability of metals in a particular matrix may be expressed as absolute bioavailability or relative bioavailability
- "Bioavailability expressed as a fraction (or percentage) of a dose is commonly referred to as *absolute bioavailability (ABA)"*
- ABA – "ratio of the amount of metal absorbed compared to the amount ingested"

http://www.epa.gov/superfund/bioavailability/guidance.htm

Definitions of bioavailability terms continued

- Relative bioavailability (RBA): "*The ratio of the bioavailability of a metal in one exposure context (i.e., physical chemical matrix or physical chemical form of the metal) to that in another exposure context*"
- RBA - ratio of the absolute bioavailability of metal present in a test material compared to the absolute bioavailability of metal in some appropriate reference material
- RBA is usually the most important for risk assessment
  - extent to which the absolute bioavailability of a metal increases or decreases in context with the exposure matrix (*e.g.*, water vs. soil)
- "A related term, pertaining to bioavailability assessment, is *bioaccessibility*. This usually refers to a measure of the *physiological solubility* of the metal at the portal of entry into the body" (IVBA)

http://www.epa.gov/superfund/bioavailability/guidance.htm

Use and application – oral bioavailability

- If the oral dose used for risk assessment is based on studies using metal administered in food or water, then the risk from ingesting metal contaminated soil might be over or underestimated
- Risk assessments that adjust for metal bioavailability may reduce the burden of unnecessary and costly remedial action
  - Small adjustment in oral bioavailability - significant impacts on estimated risks and cleanup goals
  - Bioavailability data used to improve the accuracy of exposure and risk calculations at a site

Methods for Assessing Bioavailability in Soil

- *In vivo* methodologies
  - Quantification of metal present in various tissues and excrement
  - Used to develop quantitative bioavailability adjustments
- *In vitro* methodologies
  - Physiologically-based extraction tests
  - Measures bioaccessibility (e.g. solubility)
  - Used for screening purposes and reducing uncertainty
- Mineralogical/speciation studies
  - Importance of solid phase distribution, speciation and particle size
  - Provides supporting bioavailability/bioaccessibility information

Childhood Exposures to Bioavailable Metals in Soil and Household Dust in Residential Environments

Co Chairs: Pat Rasmussen (Ottawa, Canada), Joanna Wragg (Keyworth, United Kingdom), Mark Cave (Keyworth, United Kingdom)

2006 Symposium

"Childhood exposures to bioavailable and bioaccessible metals in soil and household dust in residential environments"

Joint ISEE/ISEA Conference on Environmental Epidemiology and Exposure, September 2-6, 2006, in Paris

Organized by Health Canada (Pat Rasmussen) and British Geological Survey (Joanna Wragg and Mark Cave)

Abstracts published in Epidemiology 17 (6) pp S39-S42 (2006)

Some observations from the 2006 symposium

- Participants found the International Society of Exposure Assessment (ISEA) to be perfect "home" for the cross-disciplinary nature of bioaccessibility/bioavailability research
- Many countries were represented, and in some areas similar views were expressed:
  - Everyone agreed that a set of criteria is needed, to assess both *in vivo* and *in vitro* methods
  - In addition to Canada, some countries (UK, USA, and EU) were allowing some limited site-specific adjustments
  - At that time, few would be comfortable allowing existing site-specific methods to be universally applied
  - Everyone expressed support for the annual ISEA BA session in 2007

ISEA 2007
The 17th Annual Conference of the International Society of Exposure Analysis

2007 ISEA Symposium "Use of *In Vitro* Bioaccessibility/Relative Bioavailability Estimates in Regulatory Settings: What is Needed?"

- Morning session 9:00 am – 12:30 pm:
  - Site evaluation and application
  - *In vivo* research
  - Panel discussion session I
- Afternoon session 1:30 pm - 6:00 pm:
  - *In vitro* research
  - Mineralogy and speciation
  - Panel discussion session II
  - Closing remarks

Panel discussion and post ISEA

- Two panel discussion sessions
- Questions provided to all presenters/panelists prior to symposium
- EPA plans to develop symposium proceedings to capture current state of the science and research needs
  - Proceedings will be posted on EPA website **http://epa.gov/superfund/bioavailability/links.htm**
  - Presentations included in symposium proceedings
- Plan to publish a series of papers from symposium as a special issue
  - Some recommendations have been made for several journals
  - Symposium organizers will contact presenters following ISEA conference

Questions for Panel Discussion Session I: Evaluation/site application and *in vivo* research

- For regulatory applications such as site clean up decisions, is an *in vitro* model that is predictive (i.e., correlates with an *in vivo* model) adequate even if we don't know why it is predictive, or is it important to know why a model is predictive?
- Given that many animal models require use of soil concentrations exceeding those of public health concern, what efforts are necessary, if any, to show that bioavailability at these concentrations reflect oral absorption at lower environmental concentrations? In other words, how important is it to evaluate whether bioavailability is concentration dependent?
- Should a single animal model be specified, or are studies in a variety of different animal models more likely to improve our understanding of relative bioavailability in humans?
- Is there a single animal model that will work for all chemicals considering the widely varying toxicokinetic profiles?
- Is relative oral bioavailability of chemicals in soil so routine that it is "ready" for guidance/regulation?

Questions for Panel Discussion Session II: *In vitro* and speciation/mineralogy research

- Is it sufficient for *in vitro* models to have correlation with animal results or is it necessary to make the models accurate physiologic mimics of human gut dissolution?
- Is it acceptable to use the terms "bioaccessible", "soluble", "migratable", and "extractable" interchangeably in the numerator of the % bioaccessibility equation?
- Should bioaccessibility values be used on their own without supporting information? If not, what additional information should be included for each soil sample analyzed (e.g., geochemistry)?
- How can we leverage resources to answer specific research questions to advance the understanding of bioavailability/bioaccessibility?
  - Develop Standard Reference Materials or Certified Reference Materials?
  - Round robin testing?

Questions for Panel Discussion Session II: *In vitro* and speciation/mineralogy research continued

- In lieu of comparing *in vitro* model results to an *in vivo* model, what criteria should be considered when evaluating whether a particular *in vitro* method is appropriate for providing screening level data versus data to derive quantitative site-specific bioavailability adjustments?
- What other metals, or metal species, are of interest for developing additional *in vivo* and *in vitro* bioavailability assays? What criteria are used by regulatory agencies for ranking or prioritizing contaminants?
- Is it feasible and cost effective to develop a single standardized *in vitro* method for each metal for simulating oral bioaccessibility? If so, what steps would be needed for that to happen? What criteria would be used to determine the difference between the methods?

For additional information, visit the following websites:

http://epa.gov/superfund/bioavailability/links.htm

http://www.epa.gov/superfund/bioavailability/guidance.htm

http://www.epa.gov/heasd/mdab/mdab.htm

www.bgs.ac.uk/barge

To obtain Proceedings from Health Canada Sponsored Workshops on Bioaccessibility/Bioavailability in Contaminated Site Assessment (2005, 2006, and 2007) go to: http://www.cntc.ca

Evaluating the Bioavailability of Soil-Borne Contaminants at Waste Sites

ISEA Symposium
Durham, N.C.
October 15, 2007

Mike Beringer & Aaron Yeow
U.S. Environmental Protection Agency

Need for Additional Bioavailability Guidance

- Default assumption likely overestimates health risks
  - Bioavailability is equal in soil, diet and water
  - Relative bioavailability or RBA is 1.0
  - Lead is the exception where default RBA is 0.60
- Existing guidance supports bioavailability adjustments
  - Does not address when data collection should be pursued
  - Does not address how to evaluate site-specific bioavailability
- Limited use of site-specific bioavailability information
  - Absence of rapid and inexpensive tools
  - Lack of criteria for evaluating alternative test methods

Guidance for Evaluating the Oral Bioavailability of Metals in Soils for Use in Human Health Risk Assessment

- Limited in scope
- Outlines a decision framework – series of questions
  - Is a validated method available?
  - Does the added value exceed the costs?
- Addresses site-specific documentation
  - Basis for relying on the selected method
  - Data translation
  - Sample collection
- Recommends criteria for evaluating alternative methods

Validation of Bioavailability Test Methods

- Relying on ICCVAM criteria (Interagency Coordinating Committee for Validation of Alternative Methods)
  - http://iccvam.niehs.nih.gov/
- Method validation criteria
  - Demonstrate method is reliable and relevant for its proposed use
- Regulatory acceptance criteria
  - Method fulfills a specific regulatory need
- Regulatory methodologies
  - Must satisfy both sets of criteria
  - Appropriate for making quantitative site-specific adjustments

Method Validation Criteria (ICCVAM, 1997)

- Scientific and Regulatory Rationale
- Relationship Between Test Method Endpoint and Biological Effect
- Detailed Protocol and Known Limitations
- Within-Test Variability and Reproducibility Among Labs
- Test Method Performance with Representative Agents
- Comparison to Existing Test Method
- Data in Accordance with Good Laboratory Practices (GLP)
- Validity Assessment Data Available for Review
- Independent Scientific Review

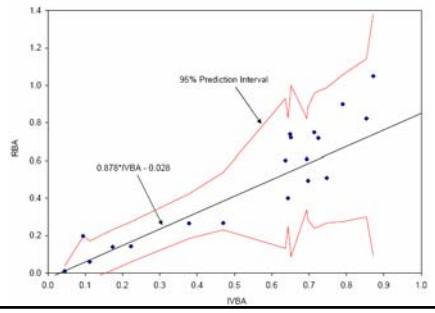
Regulatory Acceptance Criteria (ICCVAM, 1997)

- Independent Scientific Peer Review
- Detailed Protocol with SOPs
- Adequately Predicts Bioavailability and Demonstrates a Linkage
- Representative Chemicals Tested
- Generates Data Useful for Risk Assessment Purposes
- Documentation of Strengths and Limitations
- Robust and Transferable
- Time and Cost Effective
- Can Be Harmonized
- Suitable for International Use
- Reduction of Animal Use

Estimation of Relative Bioavailability of Lead in Soil and Soil-Like Materials Using *In Vivo* and *In Vitro* Methods (Lead TSD)

- Describes *in vivo* and *in vitro* methodologies
  - Juvenile swine model
  - Simplified bioaccessibility method
- Characterizes 19 soil and soil-like test materials
  - Mineral phase
  - Particle size distribution
  - Matrix association
  - Clear differences in RBA between materials
  - Data not sufficient for predictions based on mineral content alone
- Evaluates the correlation between both methods

Lead – Correlation Between *In Vivo* RBA and *In Vitro* Bioaccessibility (IVBA)



Lead TSD Transmittal Memo

- Evaluated both methods using ICCVAM criteria
  - Broad range of relative bioavailability
  - Variety of mineralogical forms
  - Pairwise comparison shows a good fit (r^2=0.92)
- Both methods considered regulatory methodologies
  - Weight-of-evidence determination
  - Method validation and regulatory acceptance criteria achieved
  - Appropriate for use in site-specific risk assessment
- Outlines limitations and considerations for use
  - Quality assurance
  - Sample lead concentration limits
  - Particle size and soil mineralogy
  - Extrapolation to adults
  - Valid for soil samples from mining and milling sites

Future Activities

- Formation of Bioavailability Committee
  - Information archive
  - Provide technical support to the USEPA Regions
  - Develop additional guidance
  - Review new bioavailability methods
- Evaluation of other metals
  - Formal consideration of arsenic bioavailability data
  - Possible derivation of default values for other metals

Method Development and the Application of Oral Bioavailability Data in Risk Assessments

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ISEA 2007

New EPA bioavailability guidance calls for use of validated methods

Validation - Definitions

Validation requirements

- **Relevance**
- **Reliability**

Who oversees validation efforts?

- http://iccvam.niehs.nih.gov

ICCVAM validation criteria

http://iccvam.niehs.nih.gov/docs/guidelines/validate.pdf

ICCVAM regulatory acceptance criteria

http://iccvam.niehs.nih.gov/docs/guidelines/validate.pdf

- Peer review
- Protocol with SOPs
- Measures endpoint of interest, and linkage with existing test
- Representative chemicals tested
- Useful for risk assessment
- Strengths and limitations identified
- Robust and transferable
- Time and cost effective
- Can be harmonized
- International acceptance possible
- Minimizes animal use

What is the process and when is a method validated and implemented?

- **Process: Research > development > pre-validation > validation > review > agency consideration > implementation**
- **Validated when its performance characteristics, advantages, and limitations have been adequately documented for a specific purpose.**

Types and uses of test methods

- **Definitive tests – Used to measure toxic effects**
- **Screening methods – Support preliminary hazard decisions**
- **Adjunct tests – Used to increase the information base and/or aid in the interpretation of results from definitive methods**

Evolution of test methods

- **Development of study design**
- **Refinement of test protocol**
- **Assurance of transferability**
- **Determination of performance characteristics**

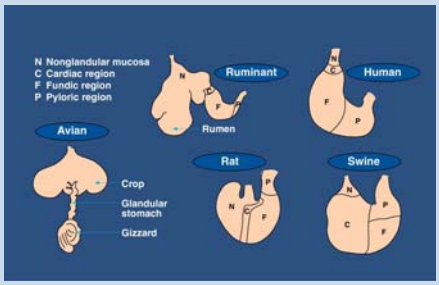
Currently accepted methods

- **Considered validated based on history of use**
- **Applies to many approaches to measuring oral bioavailability of chemicals**

Currently accepted *in vivo* methods of measuring bioavailability

- **Blood concentration over time (area under the curve, or AUC**
- **Absorbed fraction in urine and/or tissues**
- **Comparison of tissue concentrations**
- **Unabsorbed fraction in feces**

Variations in gastric anatomy



Validation for toxicity tests vs. bioavailability methods

- **Are validation criteria for development of alternative toxicological methods appropriate for methods of testing relative bioavailability of chemicals in soil?**

Validation/acceptance issues for oral bioavailability studies of metals in soil

- **Endpoint of interest is relative bioavailability for oral exposures**
- **For some metals there is no standard method available for comparison**
- **SBRC in vitro method meets validation and regulatory acceptance criteria**
- **Should animal studies continue to be used?**

Does the in vitro method meet validation criteria?

- **Rationale and relationship of endpoint to effect of interest are documented**
- **Detailed protocol available**
- **Reproducibility established in interlaboratory study**
- **Performance demonstrated for representative chemicals**
- **Comparison of performance with existing test established**
- **Limitations described**
- **Data quality documented**
- **Data reviewed both in peer-reviewed publications and in independent peer review process**

Bioavailability method development questions for metals

- **How reliable are oral in vivo study methods?**
- **Should validation of in vitro protocols be required on a metal-specific basis?**
- **Should results of unvalidated methods be considered in risk assessments?**
- **If yes, how?**

Exponent®

Relative Oral Bioavailability of Arsenic From Soils:

in vivo and beyond

Yvette Lowney
Presentation to ISEA
October 2007

Presentation Topics

- *in vivo* research
  - cynomolgus monkeys
- *in vitro* extraction testing
  - SBRC method
  - Other chemical extractions
  - RIVM/Unified Barge
- Geochemistry and soil characterization
- Future efforts

Research Funding

- SBRC
- SERDP
- Industry sponsors
- UK Environment

Relative Oral Bioavailability of Arsenic from Soils:

Cynomolgus Monkey Research Model

Relevance of Monkey as a Research Model *(Ohiou and Buehler, 2000)*

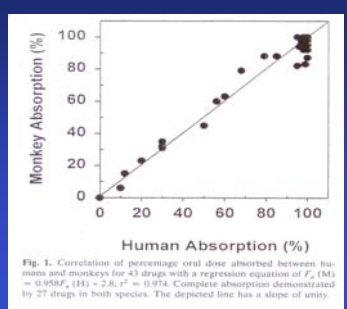
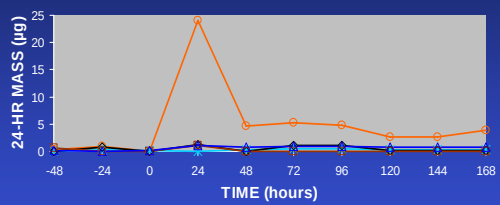


Fig. 1. Correlation of percentage oral dose absorbed between humans and monkeys for 43 drugs with a regression equation of F_a (M) = 0.958F_a (H) + 2.8; r^2 = 0.974. Complete absorption demonstrated by 27 drugs in both species. The depicted line has a slope of unity.

Urinary Arsenic Excretion: Timeline Following Exposure



Study Design

- cynomolgus monkey
- Low arsenic diet prior to dosing
- Dosed with slurry of soil in water
  - Soil dose </= 1 g/kg bw
  - Arsenic dose </= 1 mg/kg bw
- Collection of urine and feces
- n=5

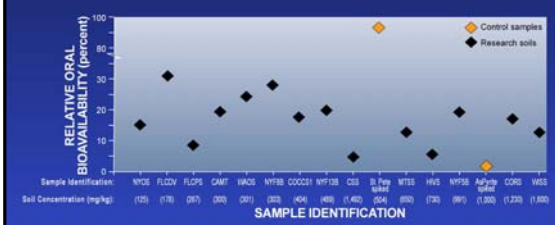
- RBA = (% of soil dose in urine) – (background)
  (% of NaAs dose in urine) – (background)

- Corrections made on animal-specific basis

Results: RBA of arsenic in cynomolgus monkeys

Soil Sample		RBA[a]	Total Recovery (% of dose)
MTSS	0.65	0.18 ± 0.05	95.1 ± 11.1
WISS	1.33	0.18 ± 0.07	86.3 ± 3.0
FLCDV	0.18	0.31 ± 0.04	77.0 ± 15.5
CAMT	0.30	0.19 ± 0.02	92.7 ± 11.5
WAOS	0.30	0.24 ± 0.09	86.4 ± 9.5
NYOS	0.12	0.25 ± 0.08	82.6 ± 13.4
COSCS	0.40	0.28 ± 0.06	79.0 ± 9.2
CORS	1.0	0.17 ± 0.08	78.1 ± 11.1
COSS	1.0	0.05 ± 0.04	87.7 ± 3.7
FLCPS	0.27	0.09 ± 0.04	120 ± 5.7
NYF-5B	0.99	0.19 ± 0.05	88.5 ± 5.0
NYF-8B	0.30	0.28 ± 0.10	93.2 ± 8.7
NYF-13B	0.49	0.20 ± 0.10	92.3 ± 6.7
HIVS	0.73	0.05 ± 0.01	75.7 ± 5.1
AsPyrite spike	1.00	0.002 ± 0.003	101.1 ± 32.8
Arsenate spike	0.50	0.94 ± 0.05	85.1 ± 15.4

Arsenic RBA from 16 Soils



Results from *in vivo* Research Using cynomolgus Monkey

- RBA measured in 14 soil samples from 12 sites
  - smelter soils
  - slag
  - mine tailings.
  - –pesticide facilities,
  - –agricultural soils
  - –cattle dip vat site
  - –wood treatment site
- RBA for arsenic in environmental soils ranged from 5% to 31%
- Indicate that site- or soil-specific factors control the absorption of arsenic from soil
- Roberts *et al*., 2007 ES&T

Development of *in vitro* Methods for Estimating Arsenic RBA, as Measured in the Cynomolgus Monkey

- Goals for in vitro method
  - Simple
  - Repeatable and reproducible
  - Captures rate-limiting step controlling RBA
  - Predictive of animal data

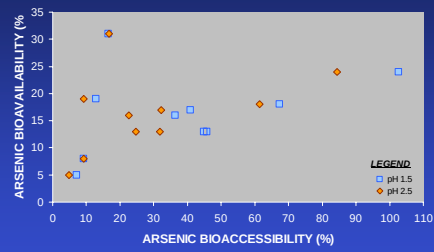
Development of *in vitro* method

- SBRC was used as the starting point
- Phosphate extractions
- Hydroxylamine HCl
- RIVM
- Unified BARGE methods

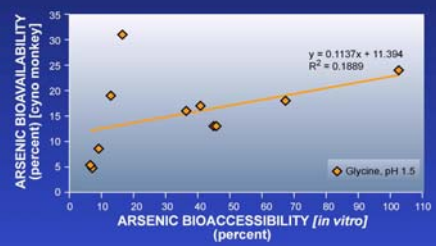
in vitro Extraction Method

- SBRC, phosphate, hydroxlamine buffered "gastric" solution
- <250 um particle size
- 1 g soil: 100 mL fluid
- End-over-end rotation at 37°C
- 1 hr
- Filter (0.45 µm)
- ICP-MS for As

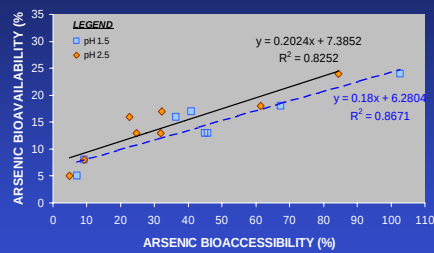
SBRC Method at pH 1.5 and 2.5



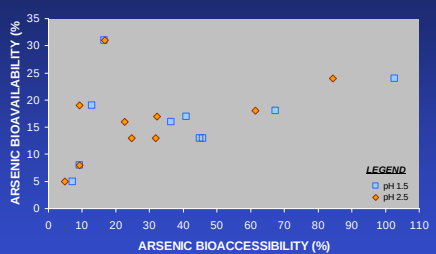
Correlation: *in vivo* and *in vitro*



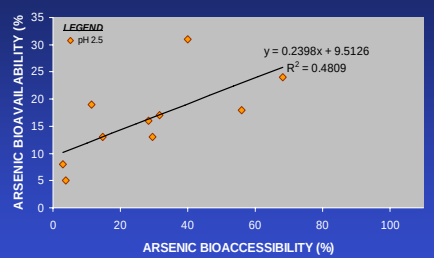
Correlation Without FLCDV or CAMT Soils



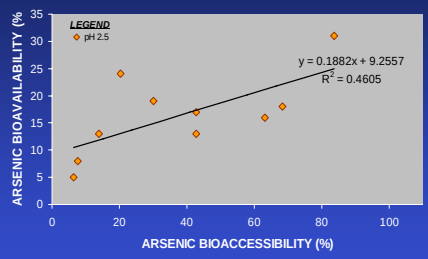
SBRC Method at pH 1.5 and 2.5



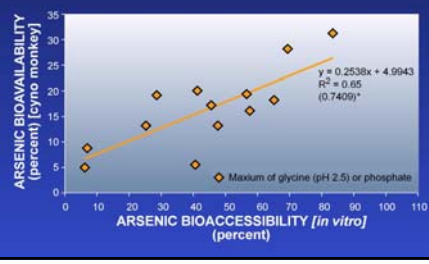
SBRC (pH 2.5) + 500 mg/L PO_4



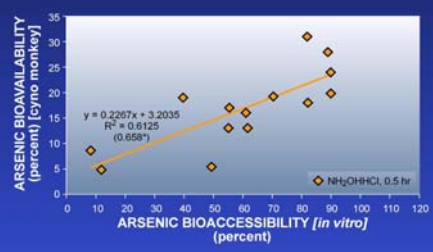
0.4 M KH_2PO_4 in Place of Glycine Buffer



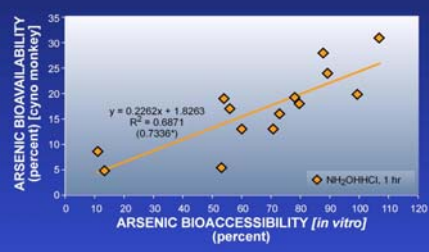
Dual Extraction: Maximum of Glycine or Phosphate "SERDP" Method



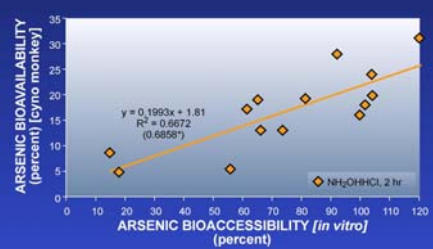
Hydroxylamine HCl: 0.5 Hours



Hydroxylamine HCl: 1 Hour

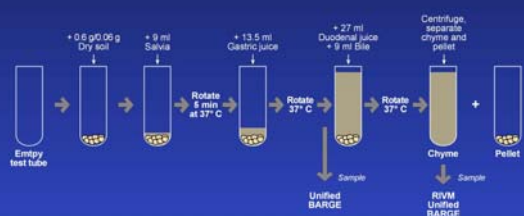


Hydroxylamine HCl: 2 Hours

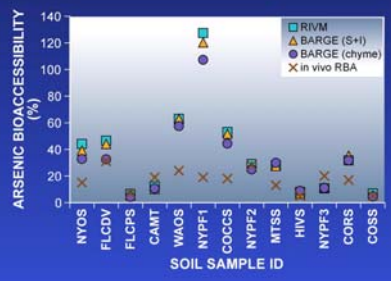


RIVM and Unified Barge Methods:

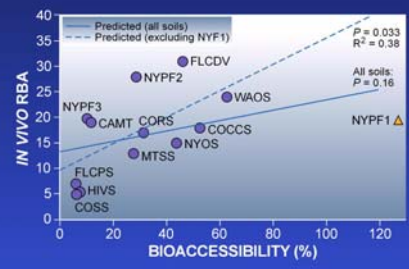
in vitro digestion procedure simulating fasted conditions



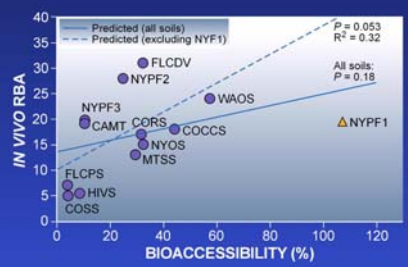
Arsenic Bioaccessbility: RIVM and Unified BARGE Methods



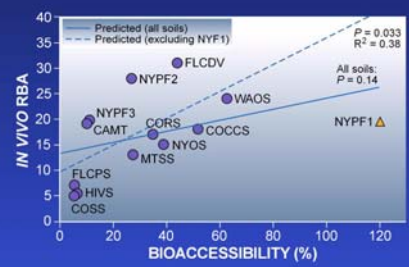
Bioaccessibility and RBA: RIVM Model



Bioaccessibility and RBA: Unified BARGE Method (gastric)



Bioaccessibility and RBA: Unified BARGE Method (s+i)



Status of *in vitro* Method

- Indications that *in vitro* methods may be predictive of *in vivo* results for RBA of arsenic
- Method validated for lead correlates well with arsenic for most soils
- Need method that is predictive for all soils across a diversity of soil types
- RIVM and UBM correlate with each other, poorer relation with *in vivo* data
- Available approaches don't provide 1:1 relation between *in vitro* and *in vivo*, but could find good correlation

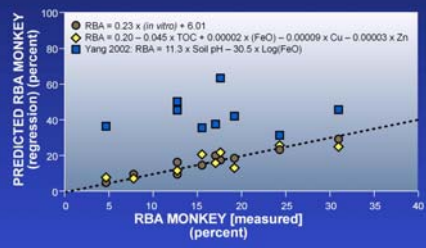
Soil Characterization Data

- Conventional parameters
  - Arsenic concentration
  - TOC
  - Metals
  - Soil pH
- Arsenic source
- Particle size distribution
- Arsenic mineralogy (18 phases)
- Extractable iron oxide

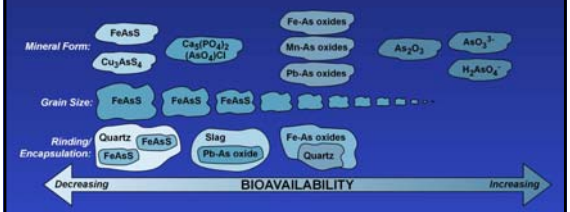
Soil Arsenic Mineralogy Data

	MTSS	WISS	FLCDV	CAMT	WAOS	NYOS	COCCS1	CSS	FLCPS	NYF-8B	NYF-13B	NYF-5B
As bromate							35.8					
Arsenopyrite				70.4								
As(metals) oxide	6.4	7.5					30.0					
AS (metals) sulfate												
Calcite												
Calcium arsenate ($CaAsO_4$)												1.7
CrCuAs												
Clay			85.5									
Fe As oxides (AsFeOOH)	12.3	10.6					3.0					54.5
Iron oxides (FeOOH)	55.9	3.5	14.4	27.2	1.3	6.9	1.5	22.2	35.2	100.0	99.9	32.1
Iron sulfate ($FeSO_4$)	23.1	9.3		2.3			1.4	76.7	64.8			0.5
Lead arsenate ($PbAsO_4$)		66.4			98.6	37.2	24.7					8.1
Lead (metal) oxide		2.5				1.4	3.3					
Manganese oxides (MnOOH)	0.4				0.04	54.5					0.1	3.0
Phosphate		0.02										0.1
Pyrite		0.3										
Slag	1.9											
Zinc (metal) oxide		0.1										
No. particles counted	130	130	147	109	215	112	105	183	153	88	104	118
Arsenic concentration (mg/kg)	650	1,412	189	300	301	125	394	1,492	268	339	549	1,000

Predictions Using Multiple Variables or Extractable Iron Oxide



Factors Affecting Arsenic Bioavailability



Research Status

- ***in vivo***
  - Robust database suggests RBA <30%
  - More data?
- ***in vitro***
  - Method validated for lead correlates well with arsenic for most soils
  - Need method that is predictive for all soil types
  - Available approach doesn't provide 1:1 relation between *in vitro* and *in vivo*, but could find good correlation
  - Progress is potentially rapid
- **Predictive models**
  - Currently no model that is robust across all soil types
  - Theoretically possible
  - Likely to be 'informed' by *in vitro* method development

End

Absolute Bioavailability

Fraction of intake reaching the central compartment; i.e., blood

Relative Bioavailability

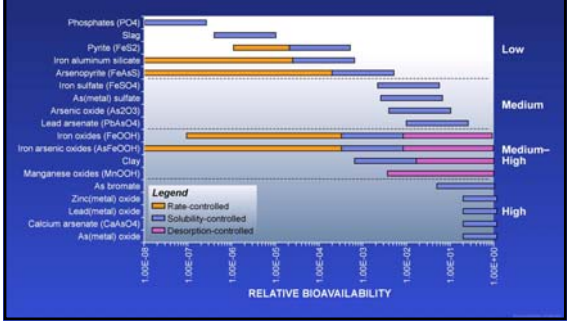
$$\text{RBA} = \frac{\text{Absorption for exposure medium of concern}}{\text{Absorption for medium used in toxicity study}}$$

Bioaccessibility

Bioaccessibility = fraction of the ingested dose that becomes available for absorption (dissolution in surrogate media)

- Bench-top method for evaluating dissolution
- *May* be physiologically based
- Simple
- Reproducible
- Predictive of RBA

Predicted Relative Bioavailability of Minerals at pH=1.5 and 25° C



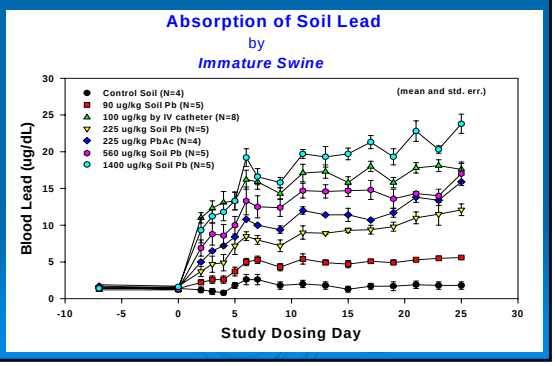
Assessing Bioavailability Using the Swine Model

Stan W. Casteel
College of Veterinary Medicine
University of Missouri

Swine Model Utility

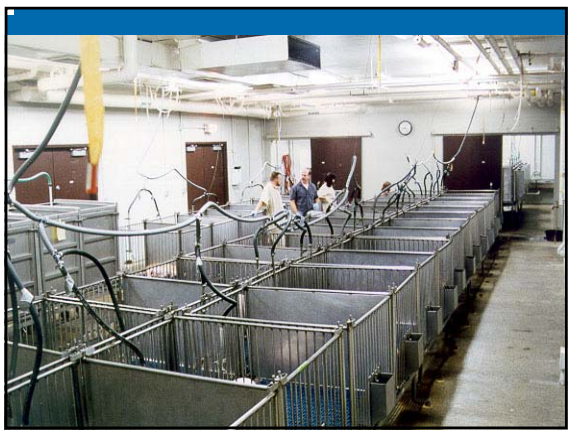
- Versatility--assess metals (As, Cd, Cr, Pb, V), organic compounds (dioxins, DDT) and Au-, Pd- and Ag-nanoparticles.
- Juvenile swine surrogate for children.
- Naïve juvenile pigs used in all EPA and NCI sponsored studies.
- Oral exposure for 12-14 consecutive days at 3 dose levels.
- Statistical Power of the Study > 90%.
- Doses selected to reflect low-dose human exposure (25-160 ug/kg BW).
- Multiple responses to assess RBA—blood, urine, liver, kidney, and bone.

Dose and Time Responses for Absorption of Lead Acetate and a Test Soil-Lead

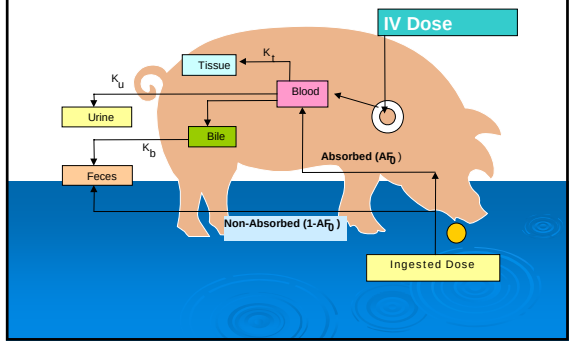


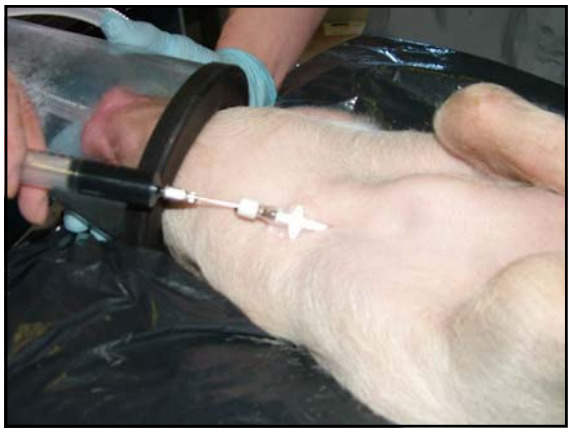
RBA ESTIMATES:Soil-Lead at 20 Sites

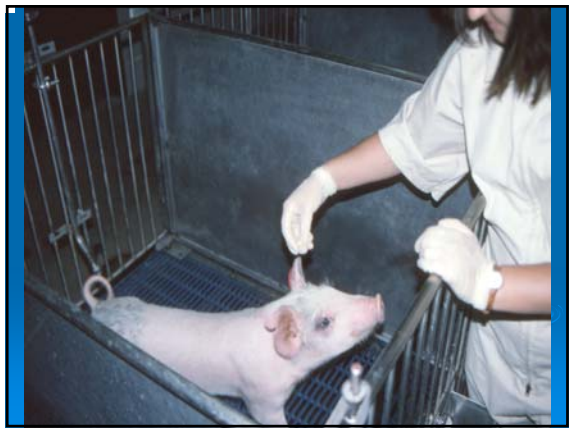
- Studies repeated on two soils: RBAs were **reproducible**: 73 vs 75%
- **Results** for 20 soil-leads, with respect to EPA's 60% default RBA, are:
  - **higher** RBAs (>75%) are associated with $PbCO_3$ and PbMn(M)O
  - **average** RBAs (25% - 75%) are associated with PbO, PbFe(M)O, $PbPO_4$, and Pb-Slags
  - **lower** RBAs (<25%) are associated with PbS, $PbSO_4$, Pb(M)O, $PbFe(M)SO_4$, and metallic Pb



Conceptual Model







General Subchronic As Study Design

Group	Number of Animals	Dose Material Administered	Arsenic Dose (µg/kg-day)
1	4	Sodium Arsenate	25
2	4	Sodium Arsenate	50
3	4	Sodium Arsenate	100
4	4	Test Material 1	40
5	4	Test Material 1	80
6	4	Test Material 1	160
7	4	Test Material 2	40
8	4	Test Material 2	80
9	4	Test Material 2	160
10	3	Control	0

Dosing Regimen

- Animals dosed 2 hours before each feeding, twice (split doses) daily, 12-14 days, constant times
- Doses—3 levels of soil and 3 levels of reference standard + 1 negative control group

Arsenic in Soil

- **Background worldwide**
  - **Range: 0.1 to 40 mg kg -1**
  - **Mean: 6 mg kg -1**
- **Redistribution Sources**
  - **mining, milling, smelting of ores**
  - **raw and spent oil shale**
  - **coal fly ash**
  - **agricultural/orchard pesticides**
  - **wood preservation**

Arsenic Biokinetics Model

- Absorbed As primarily excreted in urine
- Urinary Excretion Fraction (**UEF**) is an approximation of the oral AF or ABA.
- **UEF** does not account for As excreted in bile or As distributed to tissue compartments.
- RBA of 2 orally dosed materials (test and reference material) can be calculated from ratio of $\mathbf{UEF}_{(As\text{- test})}$ / $\mathbf{UEF}_{(As\text{- ref})}$. Keep in mind this is really a ratio of slopes of As excreted as a function of As dosed.

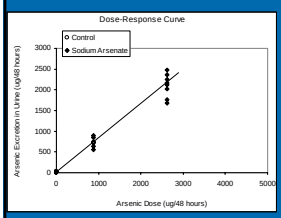
Materials and Methods

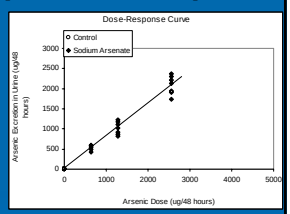
- **7-10 Groups of 4-5 pigs dosed for 12-14 consecutive days**
- **Absorbed As estimated by As excreted in urine (24 or 48 hr)—UEF—urinary excretion fraction**
- **Urinary As excretion--a linear function of dose and independent of time after day 5**

Data Reduction

- **As excreted in urine = C X V (L/48 hrs)**
- **Plot As excreted vs As dosed**
  - **UEF is slope of this line**
- **$RBA_{(x)}$=$UEF_{(x)}$/$UEF_{(Na_2As0_4)}$**
- **Note: Each RBA is a ratio of slopes**

Ref Material (Na Arsenate)





Parameter	Estimate	SE
a	9.8	1.0
b_r	0.83	0.02
b_{t1}	0.60	0.01
b_{t2}	0.42	0.01
Covariance (b_r,b_{t1})	0.0010	--
Covariance (b_r,b_{t2})	0.0013	--
Degrees of Freedom	101	--

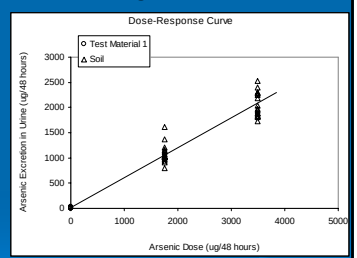
$y = a + b_r{}^*x_r + b_{t1}{}^*x_{t1} + b_{t2}{}^*x_{t2}$

where r = Reference Material, t1 = Test Material 1, and t2 = Test Material 2

Statistic	Estimate
F	1685.429
p	< 0.001
Adjusted R^2	0.9800

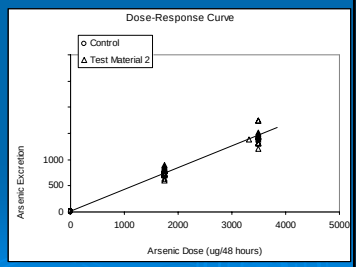
Coal Combustion By-Products

- As concentration in test material 1 is 217 ppm



Coal Combustion By-Products

- As concentration in test material 2 is 80 ppm



Results Coal Combusion By-Products

- Using sodium arsenate as a relative frame of reference, the arsenic RBA estimates are
- approximately 72% for Test Material 1 and 50% for Test Material 2.

Parameter	Estimate	SE
a	9.8	1.0
b_r	0.83	0.02
b_{t1}	0.60	0.01
b_{t2}	0.42	0.01
Covariance (b_r,b_{t1})	0.0010	--
Covariance (b_r,b_{t2})	0.0013	--
Degrees of Freedom	101	--

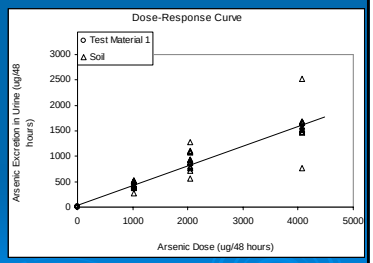
$y = a + b_r * x_r + b_{t1} * x_{t1} + b_{t2} * x_{t2}$

where r = Reference Material, t1 = Test Material 1, and t2 = Test Material 2

Measurement Endpoint	Estimated RBA (90% Confidence Interval)	
	Test Material 1 (TP026)	Test Material 2 (TP041)
Days 6/7	0.74 (0.66 - 0.83)	0.51 (0.46 - 0.58)
Days 9/10	0.69 (0.63 - 0.75)	0.51 (0.46 - 0.56)
Days 12/13	0.73 (0.67 - 0.78)	0.49 (0.45 - 0.53)
All Days	0.72 (0.68 - 0.76)	0.50 (0.48 - 0.53)

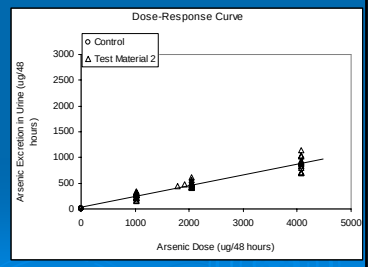
Ottawa Township Flat Glass Site Soil

- As concentration in on-site test soil is 4345 ppm.



Ottawa Township Flat Glass Residential Excavation Soil

- Residential Excavation soil As concentration 4201 ppm.



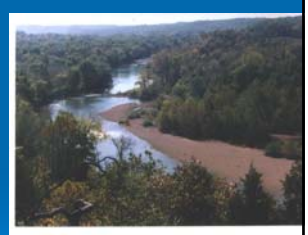
Results

- Using sodium arsenate as a relative frame of reference, the arsenic RBA estimates are
- approximately 48% for Test Material 1 and 26% for Test Material 2.

Measurement Endpoint	Estimated RBA (90% Confidence Interval)	
	Test Material 1 (On-site soil)	Test Material 2 (Residential excavation soil)
Days 6/7	0.49 (0.45 - 0.54)	0.26 (0.23 - 0.28)
Days 9/10	0.50 (0.47 - 0.54)	0.28 (0.26 - 0.30)
Days 12/13	0.55 (0.49 - 0.62)	0.28 (0.24 - 0.32)
All Days	0.48 (0.45 - 0.51)	0.26 (0.24 - 0.28)

Conclusions

- Remedial decisions should consider site-specific data
- In some cases, significant costs are incurred based on default or poorly supported assumptions



Assessing soil arsenic bioavailability in the laboratory mouse

David J. Thomas
PKB, ETD, NHEERL, ORD
U.S. Environmental Protection Agency

International Society of Exposure Analysis Meeting
Durham, NC
October 15, 2007

1

Goals of animal studies

- To develop a mouse model for measurement of metal and metalloid bioavailability
- To determine if the mouse model can be used to compare bioavailability of metals or metalloids in different soil matrices

2

Mouse as animal model

- Well characterized physiologically
- Can be manipulated experimentally (vary dietary components, alter genotype)
- Large body of data on the absorption, metabolism, disposition, and excretion of inorganic and methylated arsenicals in this species

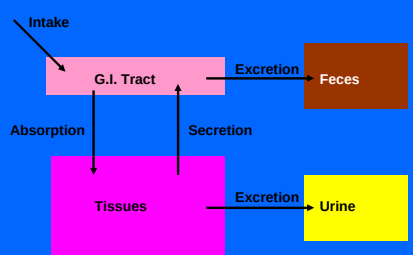
3

Overview of Proposed Studies

- Use soils with known As contents which have been physically and chemically characterized
- Add these soils to diets
- Monitor intake and excretion for mice ingesting these diets
- Collect tissues and excreta to examine distribution and retention

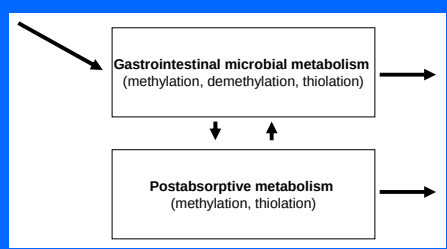
4

Conceptual pharmacokinetic model



5

Conceptual pharmacodynamic model



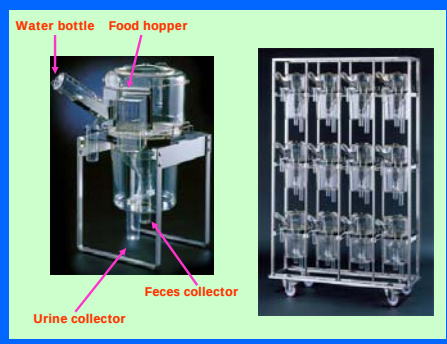
- Pharmacodynamics are a significant issue for arsenic because methylated metabolites are more reactive and toxic than inorganic arsenic

6

Experimental procedure

- Using 3 female C57BL/6 mice (5 to 8 weeks old) per cage in metabolism cages
- Allow free access to tap water and AIN-93G Purified Rodent Diet which may be amended with 1% (w/w) soil
- Monitor food intake and collect urine and feces
- At sacrifice, collect tissues (liver, g.i. tract, carcass)

7



8

Dietary composition

110700 AIN-93G Purified Rodent Diet

AIN-93G Purified Rodent Diet

Ingredient	grams/kilogram
Casein	200.00
Cornstarch	397.486
Dyetrose	132.00
Sucrose	100.00
Cellulose	50.00
Soybean Oil	70.00
t-Butylhydroquinone	0.014
Salt Mix #210025	35.00
Vitamin Mix #310025	10.00
L-Cystine	3.00
Choline Bitartrate	2.50

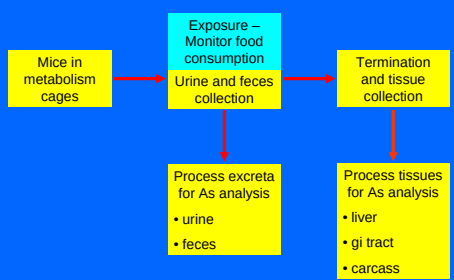
9

Arsenic in test soils and diets

	Soils		Diet
Sample	As (ppm) reference	As (ppm) by NAA	As (ppm) by NAA
NIST-2710	626	657	5.7
VB170/2	983	990	10.8
VB170/4	813	829	8.5
VB170/5	368	379	3.3
Midvale 8	591	837	6.9
Na Arsenate			3.2
AIN-93G	N.D.		<0.12

10

General design



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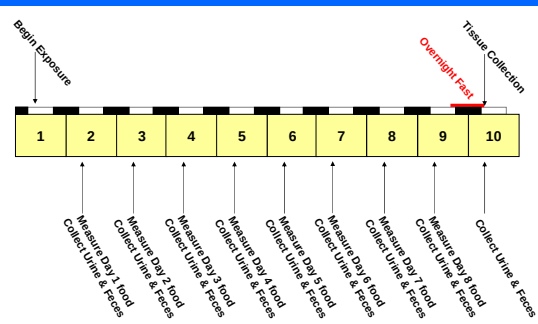

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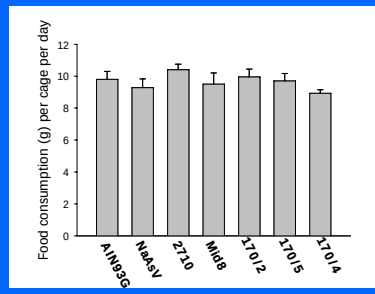
Collecting data from study

- Determine cumulative food intake per cage
- Determine cumulative urine and feces output per cage
- Process tissues, urine, and feces for arsenic analysis by neutron activation analysis or by hydride generation-atomic absorption spectrometry

13

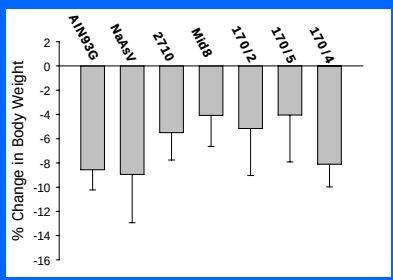
Effects of exposure



- Addition of soil to diet does not affect food consumption

14

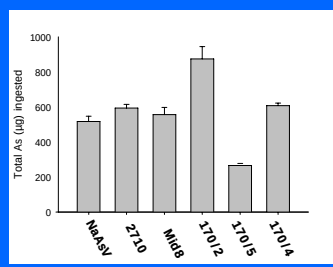
Effects of exposure



- Addition of soil to diet does not increase weight change in metabolism cages

15

Intake of arsenic during exposure



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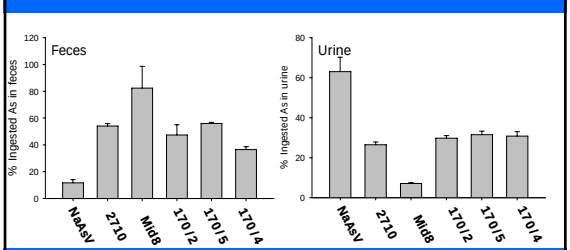
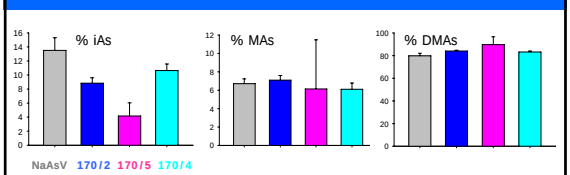
Output of arsenic is affected by arsenic source



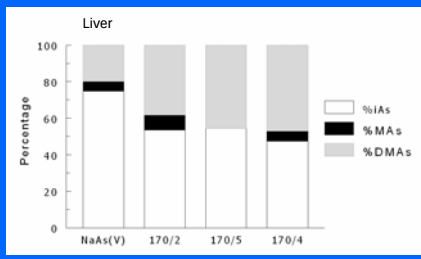
17

Is metabolite profile in urine affected by source of arsenic?



18

Is metabolite profile in tissues affected by source of arsenic?



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Findings to date

- *Mice tolerate a diet containing 1% of mass as soil. No overt toxicity has been noted in mice receiving soil in diet.*
- *The protocol has proven easy to execute.*
- *The patterns of output of arsenic in urine and feces differ among mice receiving different diets.*
- *There may be differences in the patterns of metabolites of inorganic arsenic in excreta and tissues of mice that receive diets amended with different soils.*

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Future directions

- *Replicate results using same soils*
- *Examine the bioavailability of other soils (particularly soils with higher organic matter)*
- *Refine methods for arsenic analysis and speciation*
- *Examine relation between soil source and patterns of arsenic metabolism*
- *Look at effects of changes in basal diet composition on the bioavailability of arsenic (dietary fat, micronutrients – Fe and Cu*

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Contributors

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In-vitro Bioaccessibility of Soil-borne Contaminants: An Environment Agency Perspective

Sohel Saikat

Outline

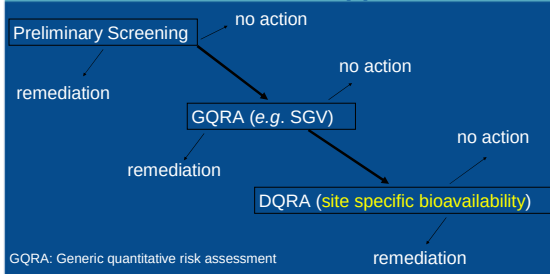
- Risk assessments and land contamination
- Review of currently available *in-vitro* methods
- Conclusion to date
- Forward Look: what is required?

Land contamination

Environment Agency 2006

- 325, 000 ha land potentially contaminated
- Regeneration of contaminated sites
- Socio-economic implications
- Defaults in current risk assessment modelling

Risk assessment approach



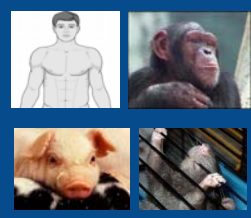
GQRA: Generic quantitative risk assessment

DQRA: Detailed quantitative risk assessment

Definitions

- Absolute bioavailability: Is a measure of the uptake or the fraction of an administered dose absorbed by the body.
  - Needs *in-vivo* studies
- Relative bioavailability: Comparative bioavailabilities of different forms of a chemical or for different exposure media containing the chemical
  - Needs *in-vivo* studies
- Oral bioaccessibility: Is a measure of dissolution, or the fraction of contaminant released into solution from the soil during digestion
  - Attempt to measure in *in-vitro* model

Why *in-vitro*?



- Costs
- Time
- Technical expertise
- Reproducibility
- **Ethical reservation**
- **Absorption is likely to be dependent on solubility**

Environment Agency's work programme

- 2002 Collaborative projects with BGS:
  - Review of *in-vitro* methods
  - Use of PBET for selected soils
- 2005 Onwards, key initiatives:
  - Science Update (s)
  - International workshop
  - Local Authority questionnaire survey
  - Ring test project with UK and overseas labs
  - Translation of Danish EPA report
  - In-vitro method evaluation study with US Exponent
  - Literature review
  - Collaboration initiatives

Questions

1) What are the different *in-vitro* methods in the UK?

2) Can they produce comparable results?

3) Can they adequately predict bioavailability?

4) Can one method be suitable for different chemicals and different soils?

5) Do we have sufficient awareness of these issues and their importance?

Q 1: Methods available

Method		PBET (Ruby *et al.* 1996)	SBRC or Drexler 1998	Chemical leaching	RIVM method	Unified BARGE method
Principle		Physiologically based (PBET)	Simple buffered acid solution	EDTA & CH_3COOH solution.	Physiologically based	Physiologically based
Temperature		37 ºC	37 ºC	Room temp.	37 ºC	37 ºC
Phase	Stomach	✓	✓	None	✓	✓
	Intestine	✓	-	None	✓	✓
pH (Stomach)		2.5	1.5	i) pH 7 (EDTA) ii) pH of (CH_3COOH)	1.1	1.2-1.4
pH (Intestine)		7	-		5.5	6.3±0.5
L:S ratio		100:1	100:1	(i) 10:1 (EDTA extraction) ii) 40:1 (CH_3COOH extraction)	[2]Stomach – 37.5:1 (0.6g) 375:1 (0.06g) [1]Intestine – 97.5:1 (0.6g) 975:1 (0.06g)	Stomach – 37.5:1 (0.6g) Intestine – 97.5:1 (0.6g)

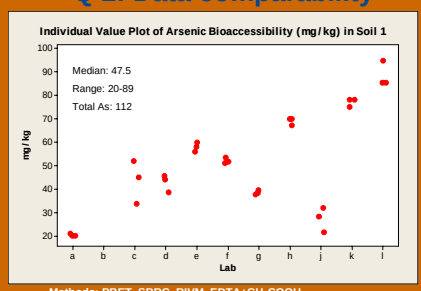
Used for various chemicals

Environment Agency (2007a,b)

Q 2: Inter-lab data comparability

- Study undertaken with UK labs producing bioaccessibility data - available at www.environment-agency.gov.uk/landcontamination
- Eleven labs participated (two from overseas)
- Three prepared samples from UK supplied in triplicate (and one human tested lead contaminated sample from Maddaloni *et al*. 1998)
- Labs were asked to analyse for As, Pb and Ni using their normal protocol.
- 'CONTEST soil proficiency-testing scheme' followed
- *In-vitro* data evaluated as 'consistent' or 'inconsistent'

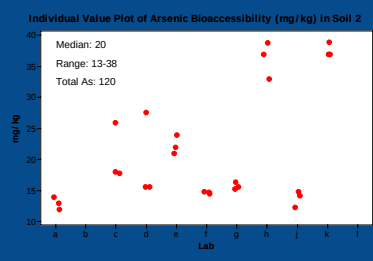
Q 2: Data comparability



Methods: PBET, SBRC, RIVM, EDTA+CH_3COOH

Environment Agency

Q 2: Data comparability



Methods: PBET, SBRC, RIVM, EDTA+CH_3COOH

Q 2: Data comparability (summary)

(excludes overseas and one UK lab)

Test soils	Arsenic (mg/kg)	Lead (mg/kg)	Nickel (mg/kg)
Soil 1 (n = 8)	R: 20-77 Med: 43	R: 1-39 Med: 10	R: 4-23 Med: 8
Soil 2 (n = 8)	R: 13-88 Med: 18	R: 1462-8219 Med: 1911	R: 1-5 Med: 2
Soil 3 (n = 8)	R: 121-7011 Med: 194	R: 2920-84979 Med: 10480	R: 5-25 Med: 9
US Soil (n = 3)	R: 5-9 Med: 5	R: 348-542 Med: 477	R: 1.35-2 Med: 2

Can UK labs produce comparable results?

No, largely due to variability in the types of *in-vitro* methods used

(But labs using the same method and same operating procedure produced comparable results)

Q 3: Predicting bioavailability

- Study undertaken with Exponent USA (report in prep.)
  - Aim: Evaluate selected *in-vitro* methods for their ability to predict bioavailability
  - Used 13 *in-vivo* (Cynomolgus monkey) tested US soils with arsenic bioavailability data obtained from a previous study (Roberts *et al.* 2007)
  - *In-vitro* data produced was studied against *in-vivo* bioavailability data

Environment Agency

Q 3: Predicting bioavailability for arsenic

In-vitro methods		R^2 (range 0-1) (n = 13)
RIVM		0.17 (0.37 excl. outlier)
UBM	Gastric phase	0.18 (0.37 excl. outlier)
	Intestine phase	0.15 (0.32 excl. outlier)
PBET (Ruby *et al.* 1996)[a]		0.18
SBRC/SBET (Drexler 1998)[a]		0.27

[a]Lowney *et al.* 2006

Can *in-vitro* data adequately predict bioavailability?

Not adequately for the soils tested. For UK soils it is unknown as none have gone through *in-vivo* studies

Environment Agency

Q 4: Applicability of one method to various chemicals

In-vitro methods	R^2 (range 0-1)		
	As	Pb	Cd
RIVM	0.17[a] (0.37 excl. outlier)	0.75[b]	0.57[b]
SBRC/SBET (Drexler 1998)	_	0.63[b]	0.23[b]
SBRC/SBET (Drexler 1998)	0.18[c]	0.83[c]	_

[a] Environment Agency 2007b; [b] Danish EPA 2005; [c]US EPA 2005

Can one method be suitable for different chemicals and different soils?

Questionable based on evidence currently available

Q 5: Do we have sufficient awareness in the UK?

- Perception that bioaccessibility and bioavailability are the same thing
- There are reports of extrapolation of bioaccessibility data from literature or different sites
- Inconsistency in the practice (e.g. lab procedure) and use of data in risk assessment

(Environment Agency 2006)

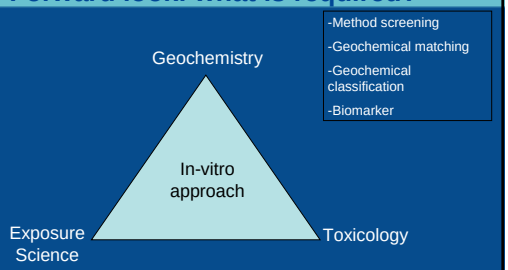
Conclusions to date

- Ability to predict bioavailability by *in-vitro* methods used in the UK is uncertain for UK soils
- Considerable inter-laboratory variability of *in-vitro* data
- Laboratories use same method irrespective of
  - chemical
  - chemical form
  - matrix
- Contaminants of concern differ from country to country
- *In-vitro* bioaccessibility testing is an ongoing research area

Forward look: what is required?

- More needs to be done to develop *in-vitro* methods including validation with appropriate *in vivo* data
- What can be done to increase confidence in *in-vitro* data?
  - Multiple lines of evidence to compliment *in-vitro* bioaccessibility methods/data

Forward look: what is required?



Forward look: what is required?

- Focussed discussion to identify and define what are the essential data gaps
- International harmonisation through a framework of partnership (repeat and working in part would delay achieving the common shared objectives)
- More linkage between research scientist and regulatory scientist (and with Policy makers)

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Measurement of Metal Bioaccessibility in Urban Household Dust and Corresponding Garden Soil

Canada

Outline of Presentation

- Differences between house dust and soil
- High variability of bioaccessible metals in dust
- Solution - simple categories of bioaccessibility: low, medium and high
- What is needed? significance for risk assessment

The "tracking-in" models to estimate indoor exposures

- Use metal concentration of soil to predict metal concentrations in house dust, in the absence of indoor data.
- Assume that main source of indoor metals is dirt tracked from outside.

Coarse fraction of soil

leaves, twigs

stones

agglomerates natural and/or manufactured

Coarse Fraction of House Dust

textiles

"I am Canadian" beer cap

plastics

metals

organics

Sample preparation technique used for soils ….

Size-fractionated sample of garden soil

….are applied to house dust

Vacuum sample of house dust air-drying

To compare indoor dust with outdoor soil, analytical approaches must be consistent

Size-fractionated house dust sample

Same size fraction

- To calculate indoor/outdoor metal ratios (dust/soil)

Same analytical approach

- Aggressive digestion for "total metal" determination, to ensure equally efficient recoveries in different media
- Weak extraction to estimate "bioaccessible metal" fraction

Metals in Dust - References

1. Rasmussen et al. (2001). A multi-element profile of house dust in relation to exterior dust and soils in the city of Ottawa, Canada. *Sci. Tot. Environ.* 267(1-3) 125-140
2. Rasmussen, P.E. (2004). Elements and Their Compounds in Indoor Environments. Elements and Their Compounds in the Environment, 2nd Ed. Editors E. Merian, M. Anke, M Ihnat and M. Stoeppler. V.I(1).Chap. 11; Wiley-VCH, Weinheim. 20p.
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4. Rasmussen, P.E., R. Dugandzic, N. Hassan, J. Murimboh, C. Grégoire (2006). Challenges in Analysing Airborne Metal Concentrations in Residential Environments. *Canadian Journal of Analytical Science and Spectroscopy.* 51: 1-8.
5. Rasmussen, P.E., Wheeler, A.J., Hassan, N.M., Filiatreault, A., and Lanouette, M. (2007). Monitoring personal, indoor, and outdoor exposures to metals in airborne particulate matter: risk of contamination during sampling, handling and analysis. *Atmospheric Environment,* 41: 5897-5907.
6. Hassan, Rasmussen, Dabek-Zlotorzynska, Celo, and Chen (2007). Analysis of environmental samples using microwave-assisted acid digestion and inductively coupled plasma mass spectrometry: maximizing total element recoveries. *Water Air Soil Pollut* 178:323-334
7. Rasmussen, P.E., S. Beauchemin, M. Nugent, R. Dugandzic, M. Lanouette and M . Chénier. 2008. Influence of matrix composition on bioaccessible copper, zinc and nickel in urban residential dust and soil. *Journal of Human and Ecological Risk Assessment* Taylor & Francis Publication (in press).

Detailed Ottawa studies: the unique geochemical profile of house dust

After drying and sieving to fine fraction, settled dust may look the same as soil.

But…

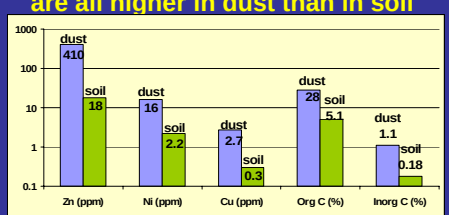
- **Key metals have higher total concentrations.**
- **Bioaccessibility of key metals higher in dust.**
- **Organic content higher**

Dust is very heterogeneous.

- **Particle size**
- **Speciation / mineralogy**

Ottawa: an example of "urban background"

Bioaccessible Zn, Ni, Cu, organic C and inorganic C are all higher in dust than in soil

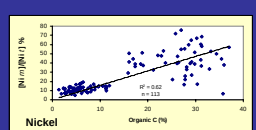


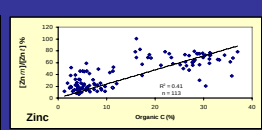
Median values for bioaccessible metals

n = 63 dust and 66 soil samples; < 150 micron size fraction

From Rasmussen et al. (2008) HERA in press

High variability of bioaccessible metal in dust compared to soil

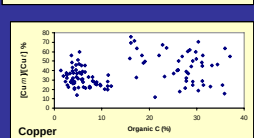




Two clusters in each graph:

Soil samples with low organic carbon contents (median 5%)

Dust samples with higher organic carbon contents (median 28%)

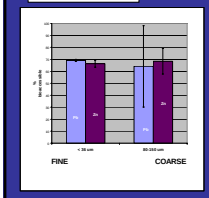


From Rasmussen et al. (2008) HERA in press

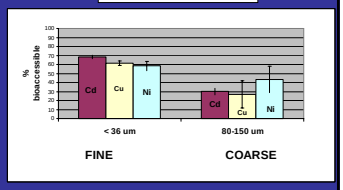
Influence of particle size

fine sieve fraction <36 µm
coarse sieve fraction 80-150 µm

Pb Zn



Cd Cu Ni



Similar bioaccessibility of Pb and Zn in both size fractions

Greater bioaccessibility of Cd, Cu and Ni in fine fraction compared to coarse fraction (same samples).

From Rasmussen et al. (2008) HERA in press

Bioaccessibility as a function of mineralogy

Solid Sample Speciation of House Dust using Synchrotron XAS

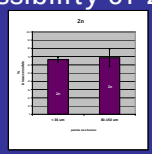
Zinc species	*< 36 µm*	*80-150 µm*
	% of total zinc in house dust	
Zn hydroxyl carbonate	52	65
Zn/Fe-oxides	22	16
ZnS	26	19

- Zn hydroxyl carbonate dominates (>50%) in both samples
- More Zn hydroxyl carbonate in coarse (65%) than fine fraction (52%)
- More ZnS in fine fraction (26%) than in coarse fraction (19%)
- The remainder of the Zn is associated with Fe oxides

From Rasmussen et al. (2008) HERA in press

Influence of Speciation (XAS)

Bioaccessibility of Zn in dust

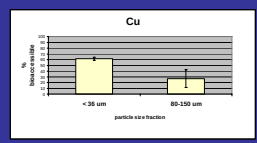


- Speciation (mineralogy) overrides the influence of particle size
- Fine fraction has a higher proportion of less soluble minerals (Zn sulphide)
- Coarse fraction has a higher proportion of more soluble minerals (Zn hydroxyl carbonate)

From Rasmussen et al. (2008) HERA in press

Influence of Speciation (XAS)

Bioaccessibility of Cu in dust



- About one-third of the total Cu is associated with organo-sulphides, in both fractions
- Speciation is similar in fine and coarse fraction
- The difference in bioaccessibility between the two fractions appears to be caused mainly by particle size

From Rasmussen et al. (2008) HERA in press

Summary of histograms

- Particle size is an important control on metal concentration.
- Metal concentration commonly increases as particle size decreases.
- Metal bioaccessibility commonly increases as particle size decreases.
- However, particle size is not the only control on concentration & bioaccessibility: the opposite trends may occur depending on metal speciation.
- Analytical reproducibility is improved using smaller size fractions.

From Rasmussen et al. (2008) HERA in press

Sources of variability
- Bioaccessible metals in house dust-

- **Heterogeneity of dust**
  - Particle size distribution
  - Speciation – inorganic and organic metal compounds
- **Representative ness of the sample**
  - Sampling method
  - Sample size – sieve fraction
- **Analytical method**
  - "bioaccessible metal"
  - "total metal"

Selection of Sampling Method – Depends on Purpose of Study

Ottawa Pilot Study

- High Volume Small Surface Sampler" (HVS3) ASTM method D 5438-00
- Wide room – to – room variability
- 100s ppm in one room, 1000s ppm in another
- Yields small samples
- Coarse size fraction (150 µm) to obtain enough sample mass for analysis

National Baseline Study

- German VDI protocol
- Composite sample integrates all living areas of house
- Larger sample permits sieving to finer size fraction (80 µm)

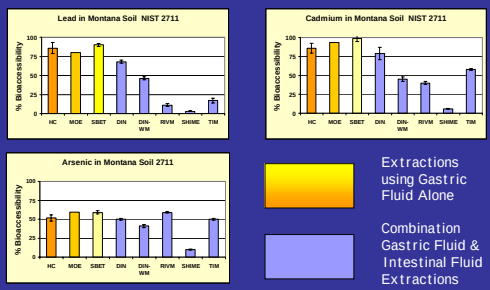
Adaptation of Toy Safety Protocol for House Dust

European Standard EN 71-3: for the migration of certain elements from toys

- **Used in Product Safety lab at HC**
- **Children as target population**
- **Extraction uses only dilute HCl (pH 1.5) to simulate stomach acid**
- **No added ingredients (complexing agents)**
- **Omits mouthing/mastication - assumes toy is small enough to be swallowed**
- **Omits passage through intestine**

From Rasmussen et al. (2008) HERA in press

Modified Toy Safety Protocol Yields Similar Results to Other Gastric Simulations



HC and MOE gastric methods plus multiple laboratory results from Oomen et al (2002)

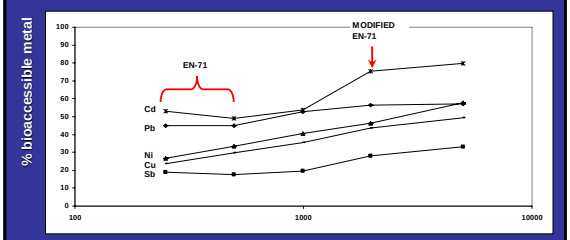
% bioaccessible = bioaccessible metal* / total metal X 100

*Variability in the Numerator

- Mass to volume ratio
- pH
- Other constituents: buffers, complexing agents
- Means of physical mixing
- Filtration *vs* centrifugation

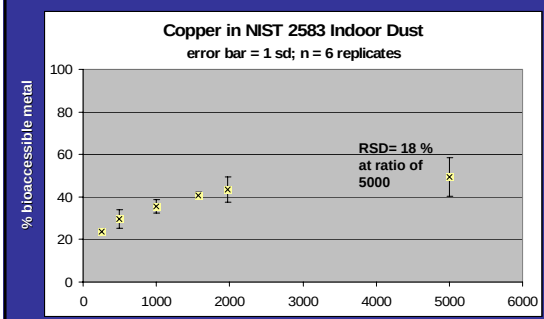
Influence of acid volume: sample mass ratio

Certified Reference Material NIST 2583 – Indoor Dust



stomach acid volume : sample mass ratio (mL/g)

Influence of acid volume: sample mass ratio



stomach acid volume : sample mass ratio (mL/g)

$$\% \text{ bioaccessible} = \frac{\text{bioaccessible metal}}{\text{total metal*}} \times 100$$

*Variability in the Denominator

We had to modify US-EPA 3051 in order to obtain acceptable recoveries for total metals

Only partially successful: HNO_3- HF needed for difficult compounds, difficult matrices

From: Hassan, Rasmussen, Dabek-Zlotorzynska, Celo, and Chen, 2007 Water Air Soil Pollut 178:323-334

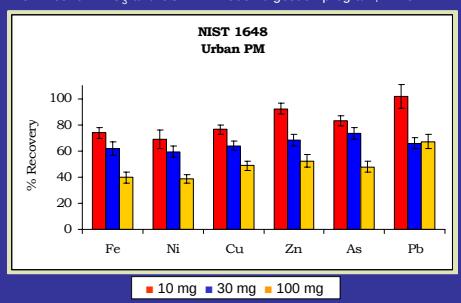
Modifications to US-EPA 3051 microwave digestion method for total metals in dust and soil samples

- Increase microwave digestion time to 30 minutes ramp time & 30 min hold time (EPA3051 specifies 5.5 min total)
  - *recovery improved by 15-20%*
- Increase acid volume to sample mass ratio to at least 1000 (EPA 3051 specifies ratios from 20 to 100)
  - *recovery improved by 30-60%*

From: Hassan, Rasmussen, Dabek-Zlotorzynska, Celo, and Chen, 2007 Water Air Soil Pollut 178:323-334

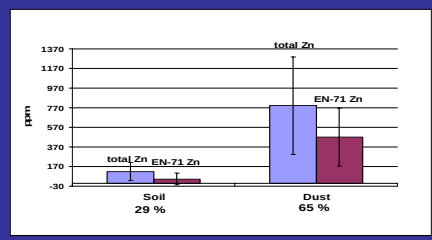
Sample mass to acid volume ratio

Microwave digestion conditions to obtain "total" metal values:
3mL conc. HNO_3 and US – EPA 3051 digestion program; n = 3



From: Hassan, Rasmussen, Dabek-Zlotorzynska, Celo, and Chen, 2007 Water Air Soil Pollut 178:323-334

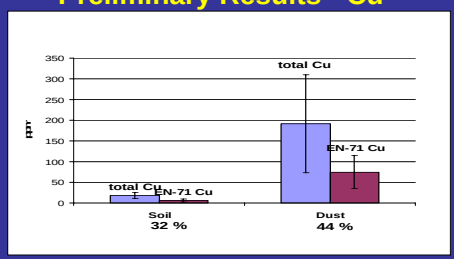
Preliminary Results - Zn



Archived samples (2002 Ottawa Pilot Study); 150 micron size fraction

Error bars represent SD about mean of 63 dust samples (HVS3 vacuum) and 66 garden soil samples

Preliminary Results - Cu

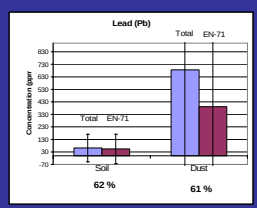


Archived samples (2002 Ottawa Pilot Study); 150 micron size fraction

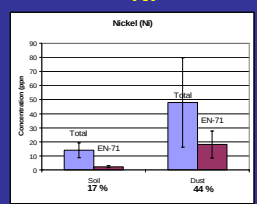
Error bars represent SD about mean of 63 dust samples (HVS3 vacuum) and 66 garden soil samples

Preliminary Results

Pb



Ni

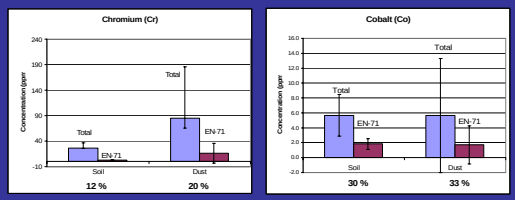


Archived samples (2002 Ottawa Pilot Study); 150 micron size fraction

Error bars represent SD about mean of 63 dust samples (HVS3 vacuum) and 66 garden soil samples

Preliminary Results

Cr **Co**

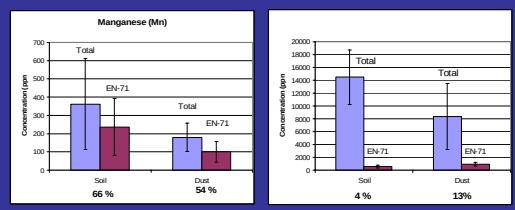


Archived samples (2002 Ottawa Pilot Study); 150 micron size fraction

Error bars represent SD about mean of 63 dust samples (HVS3 vacuum) and 66 garden soil samples

Preliminary Results

Mn **Fe**



Archived samples (2002 Ottawa Pilot Study); 150 micron size fraction

Error bars represent SD about mean of 63 dust samples (HVS3 vacuum) and 66 garden soil samples

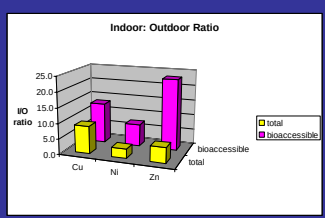
Simple Categories High, Medium or Low?

Bioaccessibility	Soil	Dust
LOW 19% or less	Ni, Fe, Cr	Fe
MED 20% to 59%	Cu, Co, Zn	Ni, Cr, Cu, Co, Mn
HIGH 60% and higher	Pb, Mn	Pb, Zn

Simple Categories High, Medium or Low?

Bioaccessibility	Soil	Dust
LOW 19% or less	Ni, Fe, Cr	Fe
MED 20% to 59%	Cu, Co, Zn	Ni, Cr, Cu, Co, Mn
HIGH 60% and higher	Pb, Mn	Pb, Zn

Indoor/Outdoor ratios are enhanced using bioaccessible extraction rather than total digestion.



Indoor/Outdoor Ratio	Cu	Ni	Zn
Total digestion	9	3	7
Bioaccessible extraction	13	7	23

Arguments for measuring metal bioaccessibility in indoor dust for risk assessments

Exterior soil concentrations do not accurately predict indoor conditions

Yield for Ottawa – an underestimate of indoor exposures

- Concentrations of several key metals (lead, copper, zinc, cadmium, nickel) significantly higher in house dust compared to exterior dust and soil (urban background setting)
- Bioaccessibility greater in dust compared to soil for key metals

Rasmussen, P.E. (2004) CJAAS volume 29, no.3, pp 166-174; Rasmussen et al. 2001. *Sci. Tot. Environ.* 267(1-3) 125-140

What is needed?

Consistent, analytically robust methods
for estimating oral bioaccessibility
of a wide range of metals in dust and soil

- **Numerator**
- **Denominator**

Rasmussen, P.E. (2004) CJAAS volume 29, no.3, pp 166-174.
Rasmussen, P.E., (2005) Proceedings of the Health Canada Bioaccessibility Workshop Aug. 30-31, 2005.

Relevance to Risk Assessment

- **Health Canada's Federal Contaminated Sites Program has identified that house dust is an information gap**
- **Risk assessors need baseline indoor dust data as "background" to compare with indoor dust data from contaminated sites.**
- **Health Canada has launched "The Canadian House Dust Study" to obtain a statistically robust estimate of background levels of metals in urban household dust across Canada.**
  - **13 cities in 4 years**
  - **Sample collection started in January 2007**
  - **To be completed in 2010**
  - **Total and bioaccessible metals, selected metal species, and selected organic compounds**

Thanks to our great lab team

Michelle Nugent, Christine Levesque, Marc Chénier, Jianjun Niu, Monique Lanouette

Acknowledgments & Funding

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Special Thanks to
Federal Contaminated Sites Program
(Mark Richardson and cross-Canada team)

Bev Hale and Ken Reimer Co-chairs of BARC
Canadian Network of Toxicology Centres (Len Ritter) &
NSERC Metals in the Human Environment Network

http://www.cntc.ca

Thank you!

The use of in vitro bioavailability in human health risk assessment

Scientific research and application by policy makers

Human risk assessment of contaminated soils in the Netherlands

- Intervention Value for substance in soil derived from MPR
- MPR: Maximum Permissible Risk (mg/kg/day)
  - Exposure level above which there is a potential health risk
  - Based on toxicological and epidemiological studies
- For most substances it is implicitly assumed that:
  - Oral bioavailability in all exposure matrices = oral bioavailability in the studies underlying the MPR
  - Relative bioavailability =1

Human risk assessment of lead-contaminated soils in the Netherlands

- Most substances: relative bioavailability = 1
- **Lead**
  - Recently changed
  - Intervention value for lead in soil 530 mg/kg for scenario "living with garden" in the Netherlands
  - Relative oral bioavailability = 0.74 for soil ingestion
    - P80 for relative bioavailability of lead in tested soils (soils low in organic matter)
    - P80 for difference between fasted and average state in gastrointestinal tract

Status application relative oral bioavailability in risk assessment

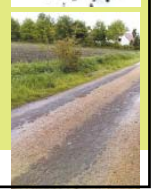
- In the assessment of the risk of contaminated soils a default value for the relative bioavailability of a contaminant from soil is applied (0.74)
- The default value can be changed if reliable site-specific information is available
- Recommendation by RIVM to government to accept the use of in vitro determined bioaccessibility for estimation of relative bioavailability factor (2006)
- Government will probably seek advise from other institutes (Health Council, Technical Soil Committee)

Dutch Kempen area

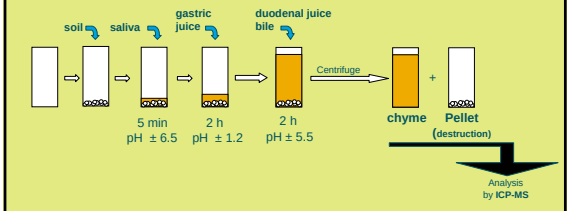
- Zinc smelter for over a century
- Area contaminated with cadmium, lead, arsenic, etc

Experimental set-up

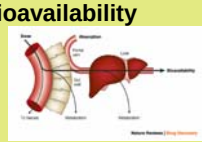
- **Intention:** relative bioavailability as input for an area specific policy
- What is bioavailability of lead and arsenic in soils and slags?
- 20 zinc slags, 16 soils
- In vitro determination of bioaccessibility
- Estimation of relative oral bioavailability



In vitro determination of bioaccessibility



Estimation relative oral bioavailability



External exposure — Exposure to contaminant in a matrix

⇩ mouth — Ingestion of matrix + contaminant

⇩ oesophagus, stomach, small intestine — F_b = Fraction released from matrix = **bioaccessible fraction**

In vitro digestion model

⇩ small intestine portal vein — F_a = Fraction of F_b absorbed by small intestine

⇩ liver — F_h = Fraction of F_a passing liver without being metabolised

⇩ systemic circulation — **F** = Fraction reaching systemic circulation = bioavailable fraction

Internal exposure — $F = F_b \times F_a \times F_h$

Estimation relative oral bioavailability of lead

Described in Oomen et al. (2006):

$$\text{Relative F} = \frac{F_{soil}}{F_{MPR}} = \frac{F_{b,soil} \times F_{a,soil} \times F_{h,soil}}{F_{b,MPR} \times F_{a,MPR} \times F_{h,MPR}}$$

$$\text{Relative F} = \frac{F_{b,soil}}{0.5}$$

Oomen AG, Brandon EFA, Swartjes FA, Sips AJAM (2006) How can information on oral bioavailability improve human health risk assessment for lead-contaminated soils? Implementation and scientific basis. Report no. 711701042, available at http://www.rivm.nl/en/, National Institute for Public Health and the Environment, Bilthoven, The Netherlands.

Research on oral bioavailability lead and arsenic from zinc slags

Relative bioavailability lead and arsenic from soils and slags from the Dutch Kempen area

	Lead	Arsenic
Soils	0.83 ± 0.11 (n=13)	0.15 ± 0.13 (n=12)
Slags	0.35 ± 0.15 (n=17)	0.02 ± 0.01 (n=11)

Application in risk assessment

- Intention: relative bioavailability as input for an area specific policy
- In this case not applied

- **Lead**
  - Relative bioavailability of lead from Kempen soils (0.83) and slags (0.35) was high
  - Minor consequences for area specific policy

- **Arsenic**
  - Soil concentrations in the Dutch Kempen may lead to ecological risks, but in almost all cases not to human health risks

Team

- RIVM
  - Marco Blokland
  - Esther Brandon
  - Menno Duits
  - Werner Hagens
  - Johannes Lijzen
  - Agnes Oomen
  - Adrienne Sips
  - Frank Swartjes
- ABdK
  - Eric Kessels

Assessment of the use of dynamic human stomach models for in-vitro measurement of the bioaccessibility of Arsenic and Chromium in soils – Can they replace animal testing?

Mark Cave[1], Helen Taylor[1], Joanna Wragg[1], Andrew Broadway[2]

1 British Geological Survey, Keyworth, Nottingham, NG12 5GG UK
2 University of Edinburgh, School of GeoSciences, Crew Building, West Mains Road, Edinburgh, EH9 3JN, Scotland, UK.

Kingsley Dunham Centre
Keyworth
Nottingham NG12 5GG
Tel 0115 936 3100

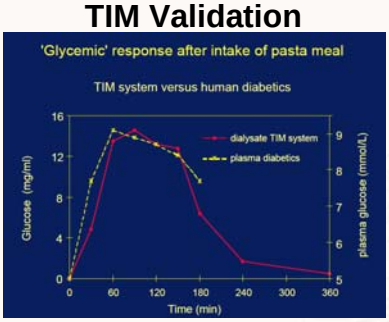
Investigating human digestion

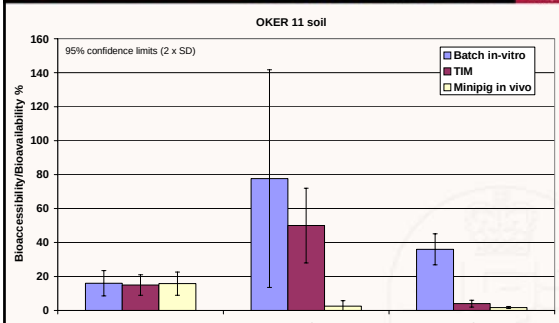
- **Human clinical studies – EXPENSIVE and time consuming (not to mention gross intervention effects and ethics)**
- **Animal studies - may provide an alternative - ethical considerations and concerns on relevance to the human**
- ***In vitro* models - a requirement for accurate *in-vitro* screening tools – currently oversimplified**

Dynamic in Vitro models

- TIM-1 and Tiny TIM -TNO Quality of Life, Zeist, The Netherlands
- Model Gut – Institute of Food Research, Norwich, UK

TIM Validation





Oomen, A. G., A. Hack, et al. (2002). "Comparison of five in vitro digestion models to study the bioaccessibility of soil contaminants." Environmental Science & Technology **36**(15): 3326-3334.

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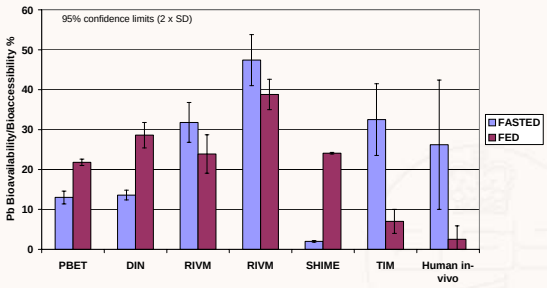
Maddaloni soil



Van de Wiele, T. R., A. G. Oomen, et al. (2007). "Comparison of five in vitro digestion models to in vivo experimental results: Lead bioaccessibility in the human gastrointestinal tract." Journal of Environmental Science and Health, Part A **42**(9): 1203 - 1211.

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BGS reference Material

Bulk soil sample collected

Crushed and dried sample is loaded into the mixing drum

Sample is homogenised in the mixing drum for 10 days

Homogenised sample is split into 50 g batches prior to homogeneity testing and certification

Total As 104±3 mg kg^{-1}

Total Pb 79 ±3 mg kg^{-1}

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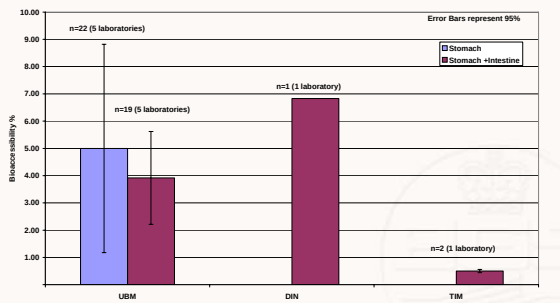
BGS 102



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Chromium In Glasgow:

- Between 1830 and 1968 the Rutherglen area of Glasgow was home to the world's largest producer of chromium chemicals.
- Chromite ore processing residue (COPR) and waste from the chemical works was used as landfill in south-east Glasgow during the 19th and 20th Centuries.
- An estimated 2.5 million tons dry weight of COPR waste was sent to landfill around Southern Glasgow and South Lanarkshire.
- In Glasgow, 15 contaminated sites have been identified. The average Cr(VI) concentration found was 700 mg/kg, with the highest being 15,600 mg/kg.

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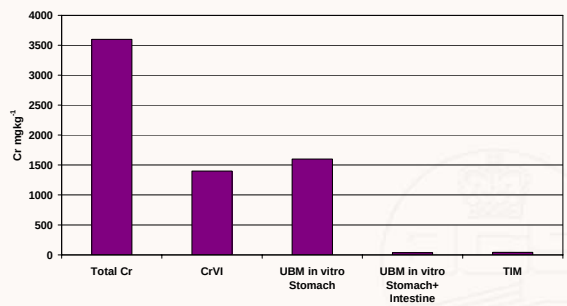
Cr Bioaccessibility in COPR Contaminated Soil



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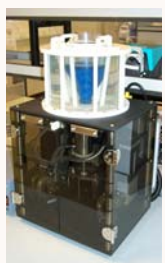
Institute for Food Research (UK)



- **First model to combine emerging knowledge of the physical/ mechanical aspects of digestion with the biochemistry in a single predictive system**
- **It is the only simulation available that can handle real food and pharmaceutical preparations**
- **Also the only model that allows access at any stage of 'digestion' permitting sample collection and analysis at any time point**

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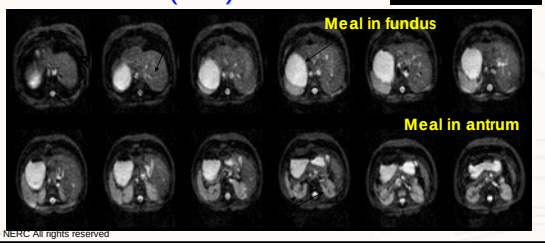
Echo-Planar Magnetic Resonance Imaging (EPI)



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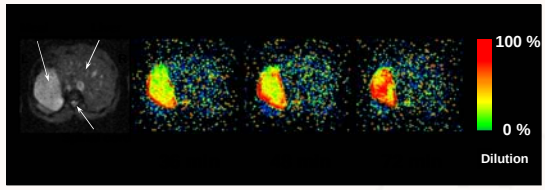
Dilution Maps and Gastric Mixing



Marciani *et al*. Journal of Nutrition,130. 2000

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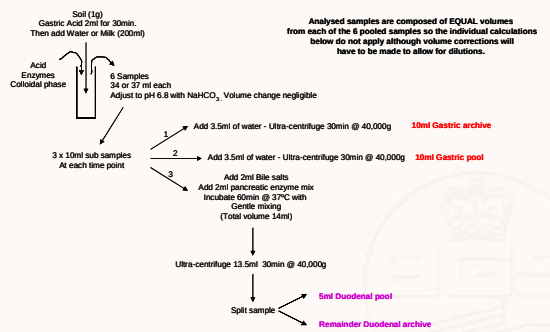

Analysed samples are composed of EQUAL volumes from each of the 6 pooled samples so the individual calculations below do not apply although volume corrections will have to be made to allow for dilutions.

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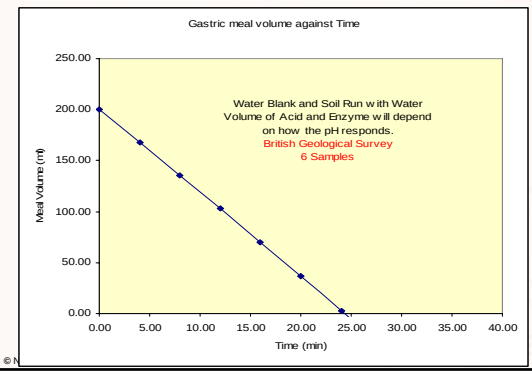

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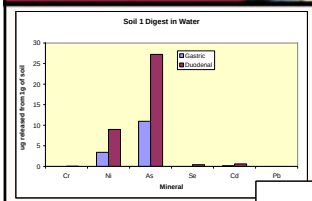

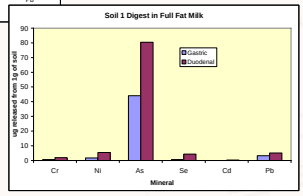

IFR Model Gut

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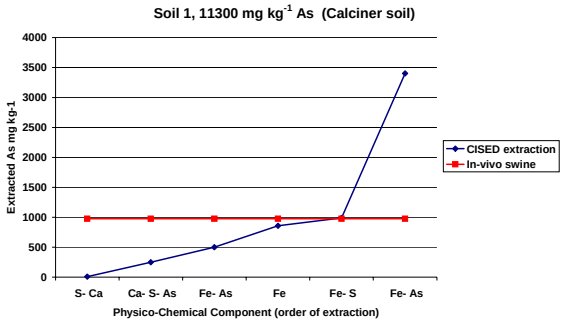
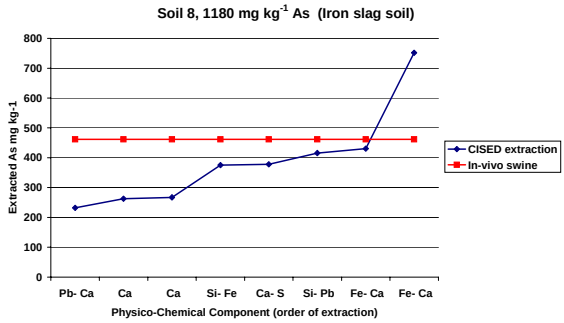
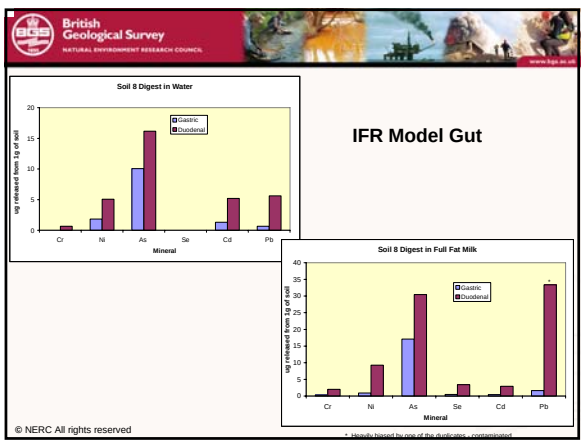
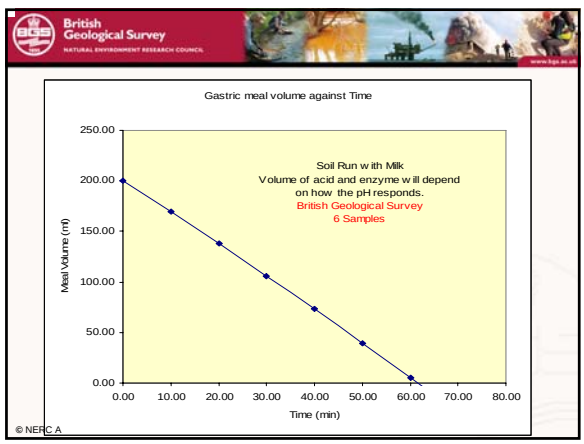
Conclusions

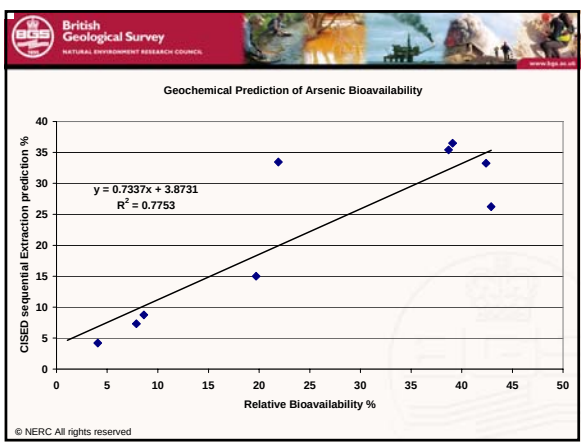
- Dynamic in-vivo models show promising results.
- As yet we do not have a definitive study for soils.
- At present they probably would not replace batch in-vitro methods but they could be used as a ‘reference methods’.
- Commercial units are likely to be available in the near future.
- Could provide a more cost effective, ethical, and scientifically valid alternative to animal testing.

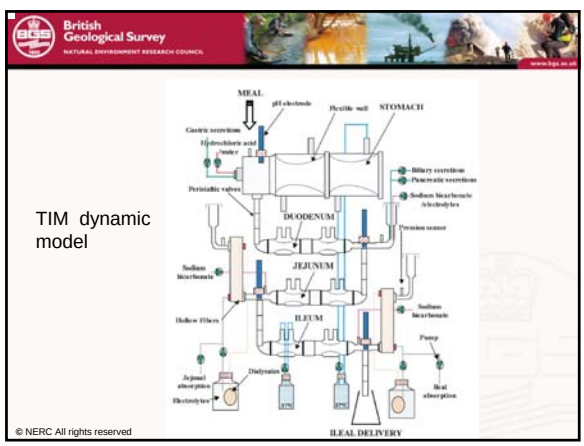






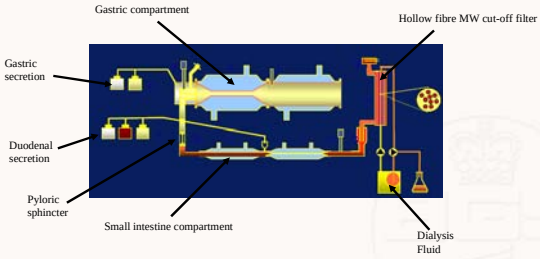






British Geological Survey
NATURAL ENVIRONMENT RESEARCH COUNCIL

Tiny - TIM



British Geological Survey
NATURAL ENVIRONMENT RESEARCH COUNCIL

Bioaccessibility of Chemicals

Not New

- **Jacob Helm (1797-1802) Investigations of the human digestive system and the physiologic manifestation of digestion using gastric and enterocutaneous fistula [Kisch B (1954) Jacob Helm's Observations and Experiments on Human Digestion: A One Hundred and Fiftieth Anniversary, J Hist Med Allied Sci, IX, 311-328]**
- **Carson and Woelfel (1913) The solubility of white lead in human gastric juice and its bearing on the hygiene of the lead. Am J Public Health. 3, 755–769.**
- **Reiman C.K & Minot A.S. (1920) Absorption and Elimination of manganese ingested as oxides and silicates. Journal of Biological Chemistry, 45,133-143**

British Geological Survey
NATURAL ENVIRONMENT RESEARCH COUNCIL

Simulated Peristalsis in the TIM model

Assessing Contaminant (Bio)availability in Soil when *In vitro* Gastrointestinal Methods are the Only Option

Nick Basta
Professor of Soil and Environmental Chemistry
School of Environment and Natural Resources
Ohio State University

Dr. Kirk Scheckel
National Risk Management Research Laboratory
U.S. EPA, Cincinnati, OH

Dr. Karen Bradham
National Exposure Research Laboratory
U.S. EPA, Research Triangle Park, NC

U.S. EPA Guidance for Evaluating the Oral Bioavailability of Metals in Soils for Use in Human Health Risk Assessment

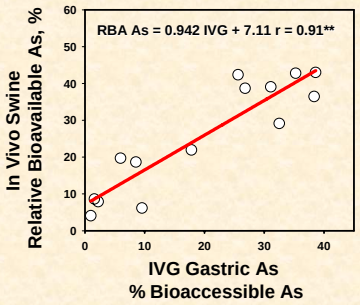
OSWER 9285.7-80, May 2007

Recommended Criteria for Validation of Test Methods adapted from ICCVAM

"Data generated adequately measure or predict the toxic endpoint of interest and demonstrate a linkage between either the new test and effects in the target species."

***In vitro* gastrointestinal (IVG) method must be correlated with an acceptable *in vivo* model**
IVG must be *predictive*

Correlation of IVG method with an *in vivo* model



Basta et al. 2003.
Grant R825410 Final Report.
submitted to U.S. EPA ORD

IVG Method Correlation Studies

most on highly contaminated soils

Most correlation studies conducted on highly contaminated wastes

often > 2,000 mg/kg contaminant of concern

Estimating RBA of Pb in Soil and Soil-like materials
(OSWER 9285.7-77, May 2007)
Most of 19 solid waste materials from smelter origin
Pb content: 1,590 to 14,200 mg/kg, median 7,225 mg/kg

Estimating RBA of Arsenic in Contaminated Soils and Solid Media
(Rodriguez et al., 1999)
As content: 233 to 17,500 mg/kg, median 1,460 mg/kg

Contaminant Concentration in Soil / Solid Waste

when will bioavailability adjustments be made?

$$\text{Risk} = [\text{Soil}] \frac{(\text{EF})(\text{ED})(\text{IR})\ \textbf{(BIO)}}{(\text{BW})(\text{AT})}$$

Highly Contaminated
unreasonable adjustment

Moderately Contaminated
reasonable adjustment

Background

example: 100 mg/kg As target

High level: 7,000 mg/kg total As
bioavailability < 2% to be < target
unreasonable adjustment

Moderate level: 300 mg/kg As
bioavailability < 33% to be < target
reasonable adjustment

Assessing Bioavailability of Moderately Contaminated Soil

The greatest utility of IVG or *in vivo* methods may be to assess risk for soils with mod. level contamination
Pb paint, pesticides, coal ash, CCA, cattle dips, etc.

Moderately contaminated urban and/or old industri

Bioavailable (*in vivo*) vs. Bioaccessible (*in vitro*)
Method Detection Limits and Contaminant Levels

most *in vivo* dosing studies require highly contaminated soil
> 500 to 5,000 mg/kg contaminant

Moderately contaminated soil levels
could be < 1000 mg/kg Pb; < 100 mg/kg As
Below *in vivo* detection limits

Below *in vivo* working range but
easily measured by IVG methods

A Strong Advantage of IVG methods
the ability to estimate (bio)availability
at moderate contaminant levels

Highly Contaminated *in vivo* and *in vitro*
Moderately Contaminated only *in vitro*
Background

Are we confident to use IVG methods to Estimate Contaminant (Bio)availability in Soil for Moderately Contaminated Soils?

More confident to use IVG methods validated for highly contaminated soil if the contaminant source is the same

IVG method validated for scorodite (As contaminant)
in highly contaminated smelter soil;
OK to use IVG method on soil with moderate levels of scorodite

Confident to use IVG method on soil with moderate levels of CCA contamination? other sources of As contamination (pesticides?)

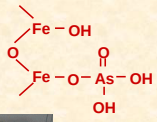
What do we need to establish confidence in using the IVG method for CCA / other arsenic contaminated soils?

1. Evidence the IVG method is correlated with *in vivo* for the CCA (other) arsenic sources
   study conducted with highly contaminated CCA soil(s)
2. Contaminant (i.e., arsenic) speciation in soil
   identify the source term for arsenic (CCA or other?)

How do we do it?
Contaminant Source and Speciation

contaminant source
mineral source: mineral speciation method
i.e., galena in smelter-contaminated soil

Soil-sequestered contaminant
i.e., As sequestered by reactive soil Fe oxides

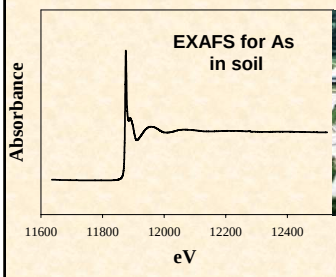


Soil chemical speciation
methods should be used
when soil is the sink

soil sequestered arsenic

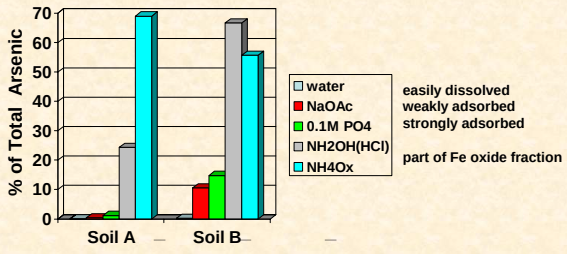
Contaminant Speciation in Soil / Solid Waste using Advanced Spectroscopic Investigation

more from Kirk Scheckel and H. Jamieson at 4 p.m.



Advanced Photon Source
Argonne National Laboratory

Contaminant Speciation in Soil using Extraction
Extract Different Contaminant Pools



Based on "selective" extraction of soil contaminants
must be very carefully applied and interpreted
must have knowledge of extractant and soil / solid waste system

Contaminant Speciation is Essential

Knowledge of chemical form of the soil / solid waste contaminant controlling bioaccessibility

contaminant species in old orchard soil same as contaminant species in smelter soil (*in vivo* correlation study)?

Yes: then we are more confident to use the IVG (*in vitro*) method for the orchard soil

A Case Study

OSU In Vitro Gastrointestinal Method

Simulated GI extraction at 37°C

Gastric bioaccessibility and Intestinal bioaccessibility

Development of Chemical Methods to Assess the Availability of Arsenic in Contaminated Media, R825410

U.S. EPA, Office of Research and Development National Center for Environmental Research

N.T. Basta, R.R. Rodriguez, and S.W. Casteel $431,677; Nov 1996 to October 2000.

Development and Evaluation of IVG Methods

Correlation Studies with Immature Swine

***In vitro* bioaccessible vs. *in vivo* bioavailable**

15 As-Contaminated Soils

Swine Dosing Trial
Measure Urinary As

In Vitro
Gastrointestinal Methods

Bioavailable Arsenic

Compare

Bioaccessible Arsenic

Stan Casteel
Univ. of Missouri

Nick Basta, Robin Rodriguez
Oklahoma State Univ.

Approach to Development of an IVG Method

Occam's Razor

single extraction | **batch *in vitro*** | **dynamic *in vitro***

Simple ⟷ **Complex**

RBA Method complexity

"Pluralitas non est ponenda sine neccesitate" - William of Occam (1280-1349)

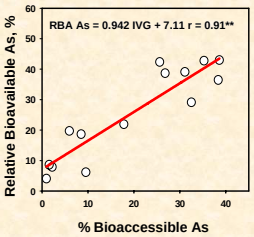
Plurality should not be assumed without necessity

If you have two equally likely solutions to a problem - pick the simplest

"Everything should be made as simple as possible, but not simpler" -A. Einstein

Bioaccessible vs. Bioavailable

OSU IVG correlated with immature swine



Basta et al. 2003.
Grant R825410 Final Report.
submitted to U.S. EPA ORD

OSU IVG correlation with in vivo As with dosing vehicle
Rodriguez et al. 1999.
ES&T 33:642-649

As without dosing vehicle
Basta et al., 2007. J. Environ. Health Sci. Part A 42:1275-1181. Special Publication (BARGE): Bioaccessibility of Soil Contaminants C. Grøn and J. Wragg (eds.)

Pb with/out dosing vehicle
Schroder et al., 2004
J. Environ. Qual., 33:513-521.

Cd with/out dosing vehicle
Schroder et al., 2003.
ES&T 37:1365-1370.

Research on OSU IVG USEPA Project still continuing after 10 yr

1997 **2007**

the soil isn't contaminated

OSU IVG USEPA Project Productivity

Publications (Basta): 39

- 10 refereed publications
- 27 proceedings / abstracts (11 at international conferences)
- 2 (book chapter, technical report)

5 Conference Symposia (3 international)
4 Graduate Student Ph.D. dissertation and M.S. Theses

Collaborative research
Soil samples, reports, data (including bioavailability) sent to 13 research groups
many joint publications / proceedings / symposium
U.S. EPA ORD (NERL, NRMRL)

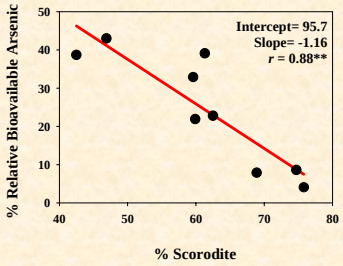
Round robin validation studies
Bioavailability Research Group of Europe (BARGE)

Arsenic Solid Phase Speciation of OSU IVG Soils

Four solid phase species identified by EXAFS

Scorodite ($FeAsO_4 \cdot H_2O$)	43 to 76% As; mean 61% As
Sorbed As	7.3 to 28% As; mean 17% As
"Elemental" As	6.3 to 43%; mean 16%
Löllingite	0 to 8%; mean 4.6%

Bioavailable Arsenic and Solid Phase Speciation (EXAFS)

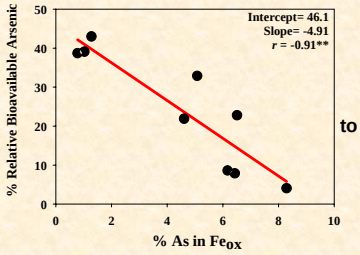


Arsenic identified as Scorodite inversely related to Relative Bioavailable Arsenic

Koch et al. (2005): Soils containing scorodite had lowest bioaccessible As of 6 soils from a military base in gastric solution

Bioavailable As and Soil Reactive Fe Oxide

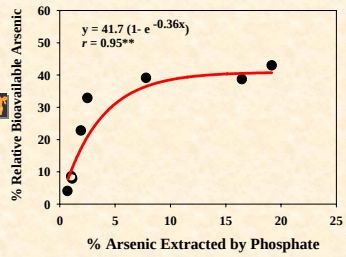
Soil Chemical Extraction Methods



Bioavailable As inversely related to As in Soil Fe_{ox} pool

Arsenic Fractionation

Phosphate extractable arsenic



Phosphate will increase bioavailable arsenic and bioaccessible arsenic

Basta et al. 2007. JEHS Part A, 42:1275-1281

Assessing Contaminant Bioaccessibility in Soil when *In vitro* Gastrointestinal Methods are the Only Option

We could extrapolate the OSU IVG methods for highly contaminated smelter waste soils to soils/solid waste where scorodite or As sequestered by Fe oxide was the source term to moderately contaminated soils

As-sorbed to Fe oxide: A likely source term for many As-contaminated soils and solid wastes

Arsenic speciation by chemical extraction / EXAFS could be performed to verify that the form of As is sorbed to Fe-oxides

U.S. EPA
Guidance for Evaluating the Oral Bioavailability of Metals in Soils for Use in Human Health Risk Assessment
OSWER 9285.7-80, May 2007

"A detailed protocol for the test method....., and a description of the known limitations of the test including a description of the classes of materials that the test can and cannot accurately assess."

- **Specify the contaminant chemical speciation and**
- **whether the IVG method has been correlated with *in vivo* for the contaminant species in the test material**

What may be down the track?
Future Research Needs

Emphasis on a range of contaminant sources and moderately contaminated soils

- **Contaminant Speciation studies (soil chemical speciation)**
- **IVG / *in vivo* correlation and round robin studies**

Collaborative Efforts are Essential
Maximize Use of Resources ($$), Time

BARGE

BARC

Research is expensive
Especially Bioavailability Data

- **Share soils, bioavailability data**
- **Cooperative round robin studies**
- **etc**

Thank you for your attention
More information? Please contact:
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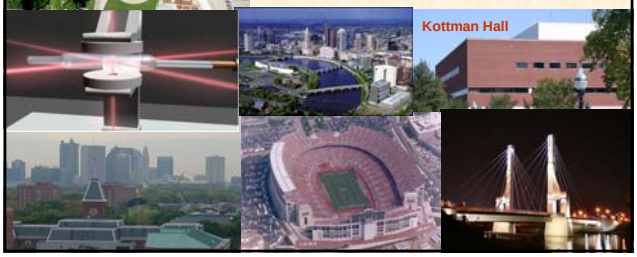

The bioaccessibility of soil Nickel, can it be explained using solid phase distribution data?

Joanna Wragg

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Keyworth
Nottingham NG12 5GG
Tel 0115 936 3100

Health Effects

ACUTE

- Death
  - 90 min exposure to c.400 mg m^{-3} metallic Ni
  - Ingestion (by a child) of 570 mg kg^{-1} bw $NiSO_4$
- Headache, nausea, vomiting etc
  - Inhalation of Ni carbonyl
- GI distress, muscular pain, exacerbation of dermatitis
  - After ingestion of Ni compounds

CHRONIC

- Respiratory effects (bronchitis, emphysema, asthma etc)
  - Repeated inhalation toxicity
- Effects on organ weight and nervous system, reproductive toxicity etc (animal studies)
  - Repeated oral toxicity
- Skin sensitivity from Ni and its compounds
  - Dermal studies /hypersensitivity tests

Nickel in Soil

- Geogenic and Anthropogenic Sources
  - Mined predominantly as Ni-Fe sulphide
  - Dominates in ultramafic and igneous rocks
  - Many industrial uses
    - Ni-Steel alloys, electroplating, jewellery, catalyst etc…
- Soil chemistry is based on the most stable, divalent ion
- Ni (II) is stable over a range of Eh and pH conditions
- Solubility increases with decreasing pH
- Associations with numerous soil components/minerals
  - Mn oxides
  - Fe oxides
  - Sulphides
  - Carbonates
  - Organic matter
  - Etc……

Soils Under Investigation

Anthropogenically Influenced

Sample	Ni %	pH	Organic C
Soil 1	c. 1	c. 6.25	c. 28%
Soil 2	c. 1.4	c. 6.4	c. 6.5%

Speciation

Soil 1	c. 90% Oxidic Ni*	c. 10% Fe oxide & trace Ni
Soil 2	c. 99.6% Oxidic Ni*	c. 0.4% Fe oxide & trace Ni

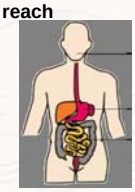
***Oxidic Ni includes all forms of Ni oxide/hydroxide, Ni-Fe oxide/hydroxide and complex multi-metal oxide/hydroxides**

Geogenically Influenced

Sample	Ni mg kg^{-1}	pH	Organic C
1	60.1	7.2	3.52
2	19.4	6.5	1.69
3	53.4	7.2	4.70
4	65.9	6.0	3.02
5	45.9	7.2	2.15
6	78.9	5.8	2.62

Bioaccessibility Methods

- **In vitro gastric and small intestinal simulations**
  - **<250um size fraction**
  - **37°C**
  - **PBET modified from Ruby et al.,**
    - **Direct titration of Na_2CO_3 or $NaHCO_3$ to reach intestinal pH**
    - **Agitation via shaking, not Ar bubbling**
  - **IVG**
    - **Fluids and residence times as per Basta et al., 2007**



Differences in Bioaccessibility Method Parameters for anthropogenic Ni

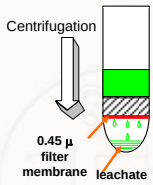
Parameter	PBET	IVG
Soil:Solution Ratio	1:100	1:150
Stomach Fluid Composition	1.25 g l^{-1} pepsin	10 g l^{-1} pepsin, 0.15M NaCl, no citrate, malate or acetic acid
Intestine pH	7.0	5.5
Intestine Fluid Composition	pH adjusted with Na_2CO_3	pH adjusted with $NaHCO_3$
Intestine Residence Time	4 hours	1 hour

Are the results likely to differ significantly?

Solid Phase Distribution of Nickel

CISED Test
Chemometric Identification of Substrates and Element Distributions

- Separate aliquots of aqua regia of increasing concentration
- Passed through the sample under centrifugal force
- Determination by ICP-AES
- Chemometric data processing
- Identification of physico-chemical hosts and the metal distributions within the sample under test



Anthropogenic Contamination

Differences

Between Method

Not significant

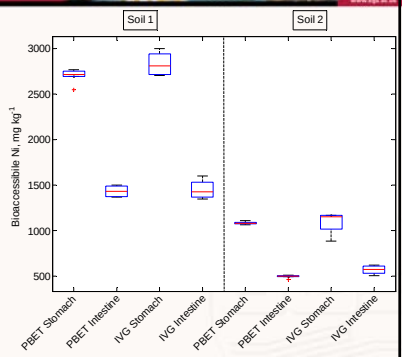
(Stomach or Intestine, for both soils)

Within method

Significant differences between stomach and Intestine data

Between Soils

Significant difference between stomach or intestine data



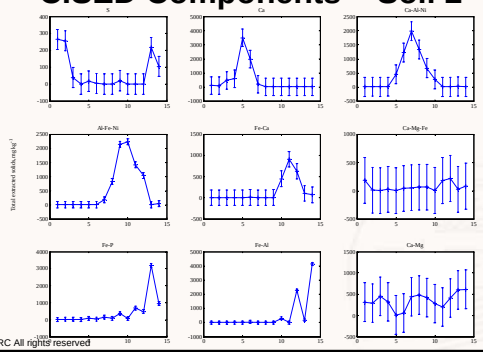
Although there are methodological differences

Soil:Solution ratio

Fluid Chemistry etc…

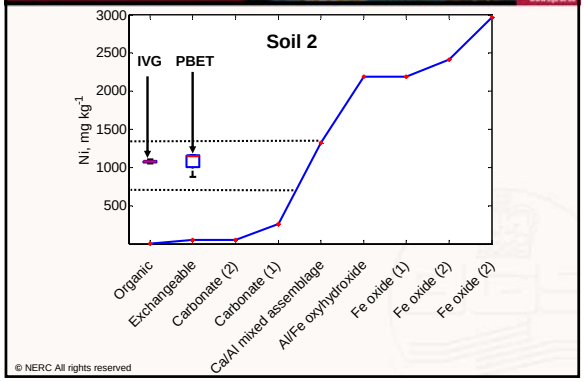
There is no appreciable difference in the measured Ni bioaccessibility

CISED Components – Soil 2

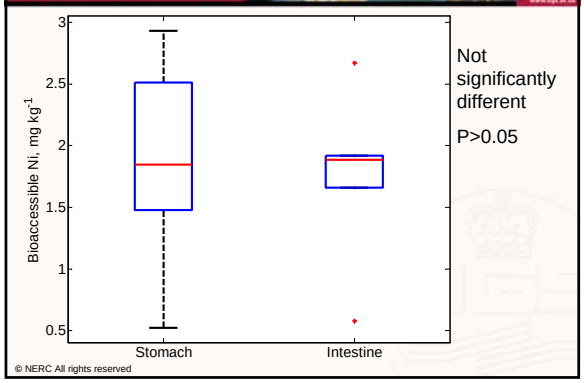


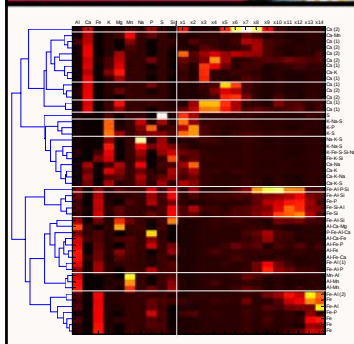
Soil 2 – Ni distribution

Component	Component Composition	Ni mg kg^{-1}
Organic	S	10.7
Carbonate (1)	Ca	208
Ca/Al mixed assemblage	Ca-Al-Ni	1070
Al/Fe oxyhydroxide	Al-Fe-Ni	865
Fe oxide (1)	Fe-Ca	0
Exchangeable	Ca-Mg-Fe	45.6
Fe oxide (2)	Fe-P	220
Fe oxide (3)	Fe-Al	553
Carbonate (2)	Ca-Mg	0

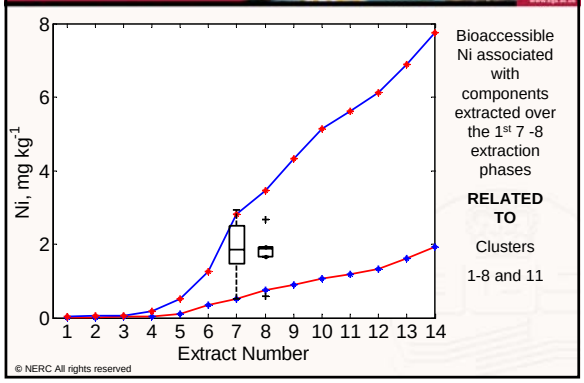


Natural Contamination





Cluster	Name	Ni
1	High Carbonate	1.67
2	Carbonate	0-2.8
3	High Carbonate (2)	0.6-0.9
4	Carbonate (2)	0
5	Organic	0
5	Fertilizer	0.03-0.1
7	Exchangeable	0-0.1
8	Fe/Al Phosphate	3.72
9	Fe/Al oxyhydroxide	0.5-1.3
10	Fe/Al silicate	0.04-1.4
11	Mn oxide	0.06-2.6
12	Fe oxide	0.01-1.9



Summary

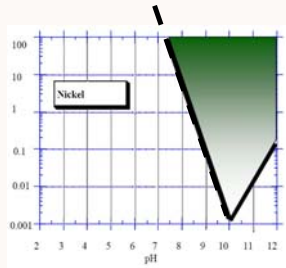
- Higher total and bioaccessibility data observed for the anthropogenic soils
- No appreciable differences between the PBET and the IVG data
- Soil components acting as sources of Ni bioaccessibility were similar for both contaminant types
  - Non Fe dominated components
    - Organic, Fertilizer/Exchangeable/Carbonates/Mn oxides
  - Naturally contaminated soils
    - **Additional contribution from Fe oxyhydroxide components**
    - Thought to be an effect of the different ageing of the Ni within the soil matrix

Summary (2)

- Extraction Efficiencies of the CISED
  - Similar for the anthropogenically contaminated soils, c. 32%
  - > extraction efficiency for the soil with a higher total Ni content
  - 5-15% for the geogenically influenced soils
    - Higher extraction rates are not observed in soils that have the highest total Ni concentrations or absolute Ni bioaccessibilities

Summary (3)

- Anthropogenic soil bioaccessibility data
  - Significant decrease in Ni concentration in the intestine phase
  - Not observed for geogenic Ni soils
  - Function of Ni solubility with pH???



Final Thoughts…..

- **Can solid phase distribution help explain measured bioaccessibility data?**
  - **YES**, Inclusion of geochemical information and testing provides a wealth of additional data
    - Help explain the bioaccessibility data
    - Support the Risk Assessment process
  - Previously adopted method for As and Pb studies in the UK
  - Being applied in to Cr issues in the UK and Canada

Acknowledgements

- Mark Cave, Chemical & Biological Hazards Programme, British Geological Survey, UK
- Ken Reimer, Kim House, Ashley Campbell, Megan Lord-Hoyle, Environmental Sciences Group, Royal Military College, Canada
- Bruce Conard, BRConard Consulting, Canada
- Chris Ollson, Jacques Whitford Ltd, Canada

Importance of Metal Speciation in Understanding Bioavailability

Kirk G. Scheckel
US EPA, Cincinnati, OH

…to protect human health & the natural environment…

SPECIATION

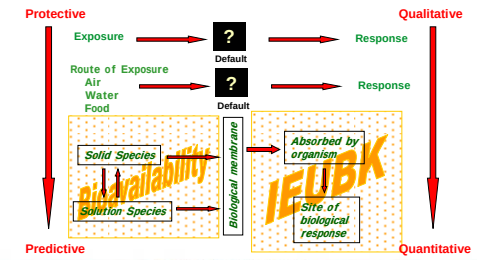
Remediation

- Indicative of metal fate and transport
- Helps decide remediation strategy, i.e. dig-and-haul, in-situ amendment, monitored natural attenuation
- Evaluate effectiveness of remediation

Bioavailability

- Understand the variability of biologically available metal uptake
- Ability to manipulate system to reduce bioavailability
- Develop comprehensive predictive models based on speciation

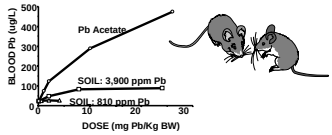
Systematic Characterization of Exposure-Dose-Response Continuum and the Evolution of Protective to Predictive Dose-Response Estimates



What do we need and what do we want to know?

BIOAVAILABILITY

EFFECT OF SOURCE OF Pb ON BIOAVAILABILITY TO RATS
FREEMAN et al. (1991)



EFFECT OF SOURCE OF Pb ON BIOAVAILABILITY TO HUMAN
Steele et al. 1990

SOURCE	BLOOD Pb (µg/dL) / SOIL Pb (1000mg/kg)
SMELTER/URBAN	1.1 - 7.6
MINING	0 - 4.8

WHY?

Components of Bioavailability

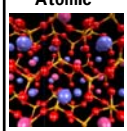
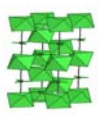
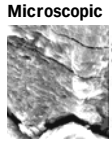
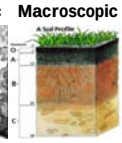
PHYSIOLOGICAL

- ***Pharmacokinetics – Nutritional needs (Ca, P, Fe, etc)***

CHEMICAL

- ***Form of metal***
- ***Geochemical matrix***
- ***Particle size***

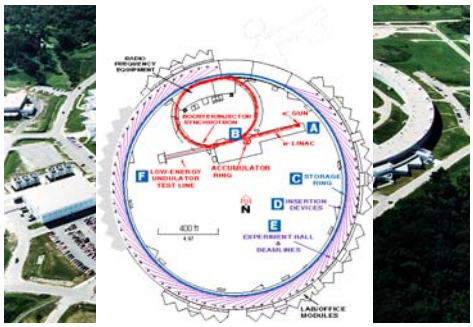
Levels of Detail in Research

Atomic	Molecular	Microscopic	Macroscopic	Field
				
• XRF • XPS • XAS *Requires synchrotron radiation.*	• XRD • TGA • FTIR • DRS	• Enhanced Visual Analysis: 1. SEM 2. TEM 3. AFM	• Field Plots • Equilibrium Studies • Kinetic Studies • Extractions	• Visual/ Intuitive Insight • Field Plots

Adaptation of Bertsch and Hunter, 1996.

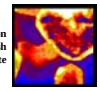
Advanced Photon Source

(Argonne National Laboratory, Argonne, IL)

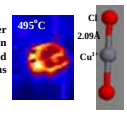


Principal Synchrotron Techniques Used in Environmental Science

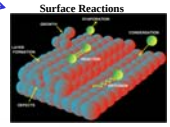
- **X-ray Fluorescence (XRF):** chemical composition (quantification, mapping)
- **X-ray Absorption Fine Structure (XAFS) Spectroscopy:** chemical speciation (oxidation state, coordination, nearest neighbors)
- **Surface Scattering and Diffraction:** surface structure, sorption processes
- **Microtomography:** 3D imaging of internal microstructure (porosity, fluid flow, composition)



Arsenic on Bangladesh Biotite



Copper Speciation in Fluid Inclusions



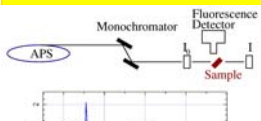
Surface Reactions

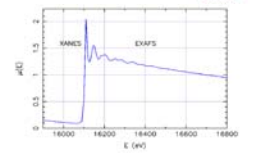
Arsenic in Cattail Root Plaque

Courtesy of Steve Sutton

X-ray Absorption Spectroscopy

X-ray Absorption Spectroscopy: Measure energy-dependence of the x-ray absorption coefficient μ(E) [either $\log(I_0/I)$ or (I_f/I_0)] of a core-level of a selected element





XANES = X-ray Absorption Near-Edge Spectroscopy

EXAFS = Extended X-ray Absorption Fine-Structure

Element Specific: Elements with Z>20 can be examined.

Valence Probe: XANES gives chemical state and formal valence of selected element.

Local Structure Probe: EXAFS gives atomic species, distance, and number of near-neighbor atoms around a selected element..

Low Concentration: concentrations down to 10 ppm for XANES, 100 ppm for EXAFS.

Natural Samples: samples can be in solution, liquids, amorphous solids, soils, aggregates, plant roots, surfaces, etc.

Small Spot Size: XANES and EXAFS measurements can be made on samples down to ~5 microns in size.

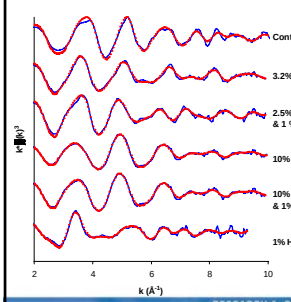
Courtesy of Matt Newville

Pb Immobilization & Bioavailability

- Smelter contaminated site in Joplin, MO
- Various P amendments
- In-vitro and in-vivo bioavailability
- Synchrotron speciation

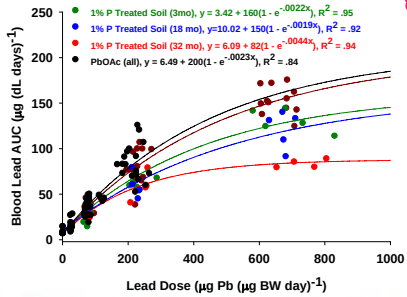
X-ray Absorption Spectroscopy & PCA/LCF

Pb Speciation in a Smelter Contaminated Soil: Results of in-situ Remediation with Phosphorus



	Lead (Pb) Speciation (%)			
	Adsorbed-Pb	Pb-Carbonate	Pb-Sulfur	Pyromorphite
Control	41	3	59	0
3.2% TSP	32	11	17	41
2.5% IRR & 1 % TSP	35	4	20	41
10% Biosolids	63	24	12	1
10% Biosolids & 1% TSP	69	12	19	16
1% H_3PO_4	36	4	16	45

Effect of Time and 1% P Treatment on Soil Lead Bioavailability Joplin Swine Ryan et al 2004



Soil Lead Bioavailability
Joplin 18 mo Sample

	Rat	Swine	In vitro	Human
Control	21.7	34.8	58 pH 2.5 60 pH 2.0 63 pH 1.5	42.2
Treated	7.2	21.6	21 pH 2.5 39 pH 2.0 51 pH 1.5	13.1

Conclusion: Joplin Field Experiment

- Bioavailability of soil lead is not a simple function of total soil lead.
- Soil lead bioavailability can be measured by
  - Swine
  - Rat
  - Human
  - In vitro
- Soil lead bioavailability can be changed by addition of materials to soil.
- The addition of materials to the soil altered the geochemistry of soil lead.

What's next?

Do we have enough information from expensive in-vivo animal bioavailability studies to justify acceptance of affordable in-vitro extraction bioaccessibility?

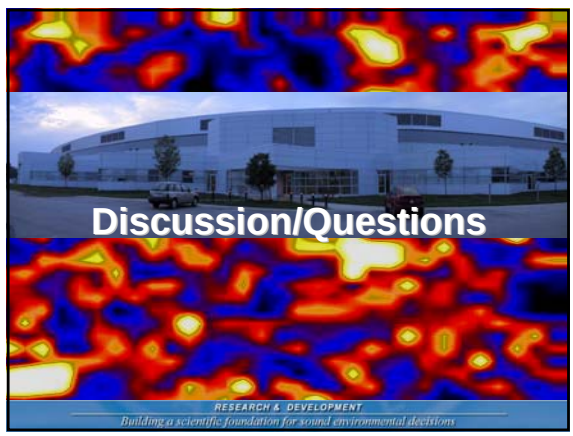
What role does spectroscopic speciation play in support of in-vivo and in-vitro research?

Requirements for using bioavailability in risk management decisions

1) An appropriate measure (methodology and samples)
2) Knowledge of the reason for the observed measurement
3) Knowledge of the long-term stability of the measurement

Lessons learned

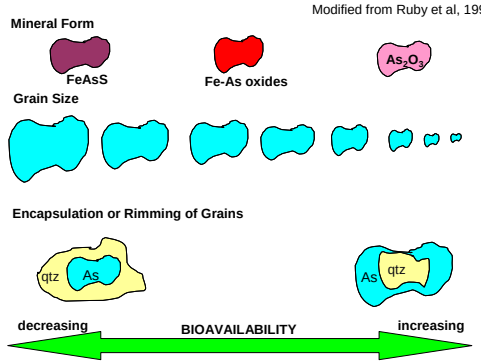
- Total metal content is not a good indicator of exposure or risk
- Soil chemistry important in determination of bioavailability/phytoavailability
  - Form is important
  - Particle size is important
  - Adsorption is important
    - Fe/Mn are important adsorptive surfaces
    - Organic matter is important adsorptive surface
- Cannot always assume an increase concentration in the foodchain equates to increase transfer through the foodchain (plant uptake)
- Predicting the potential transfer of soil metals requires a holistic evaluation of soil, plant, animal, and human processes which may increase or reduce the transfer (bioavailability)



Direct Identification of Metal Compounds in Contaminated Soil, Mine Tailings, and House Dust Using Synchrotron-based Methods

H. E. Jamieson, S.R. Walker, S.E. Fawcett, Queen's University
A. Lanzirotti, University of Chicago
P. Rasmussen, Health Canada
S. Beauchemin, Natural Resources Canada
M.B. Parsons, Geological Survey of Canada

Modified from Ruby et al, 1999



Analytical Techniques

Synchrotron-based

- Micro X-ray Fluorescence (μXRF): Element mapping
- Micro X-ray Diffraction (μXRD): Identify microcrystalline phases
- Micro X-ray Absorption Near Edge Structure (μXANES): oxidation state of As & other elements
- Macro X-ray Absorption (XANES): local molecular environment

Classical

- Electron Microprobe (EMPA): quantitative chemical analysis
- Petrography: visual characterization

Analytical Techniques

Synchrotron-based

- Micro X-ray Fluorescence (μXRF): Element mapping **10 micron**
- Micro X-ray Diffraction (μXRD): Identify microcrystalline phases **10 micron**
- Micro X-ray Absorption Near Edge Structure (μXANES): oxidation state of As & other elements **10 micron**
- Macro X-ray Absorption (XANES): local molecular environment **10 mm**

Classical

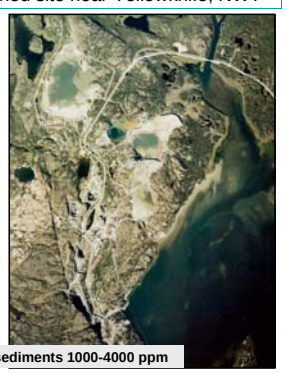
- Electron Microprobe (EMPA): quantitative chemical analysis **10 micron**
- Petrography: visual characterization **1-2 micron**

Results in grain-scale characterization

Giant Mine: Large abandoned site near Yellowknife, NWT

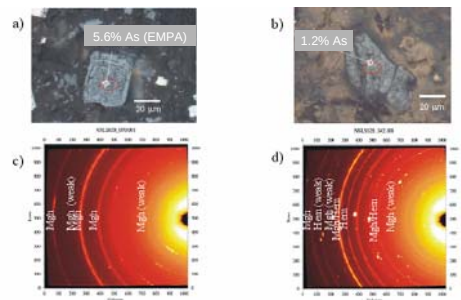
ca. 1955

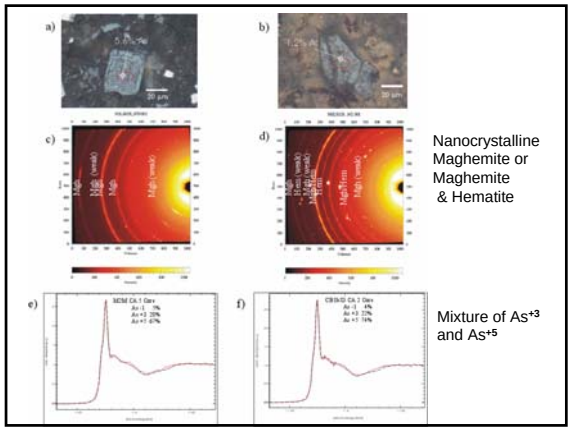
Arsenopyrite-bearing ore was roasted to liberate Au

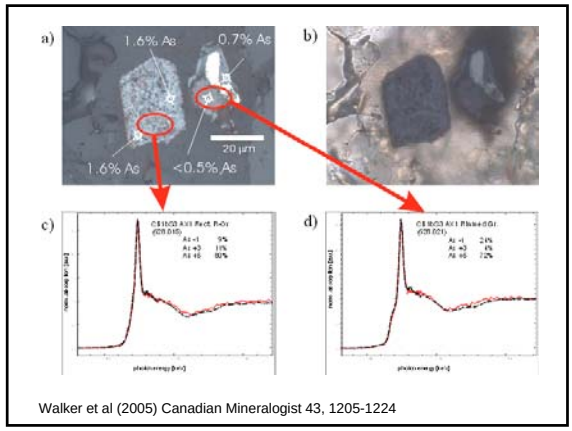


Total arsenic in tailings, soils, sediments 1000-4000 ppm

Roaster-generated Fe oxides: nanocrystalline maghemite γFe_2O_3 and hematite αFe_2O_3

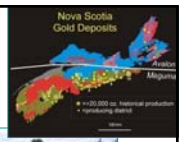






Walker et al (2005) Canadian Mineralogist 43, 1205-1224

Abandoned gold mines (more than 65 sites) in Nova Scotia



Montague

Goldenville

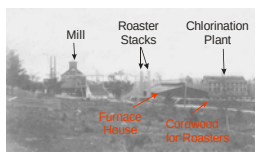
Arsenic concentrations

Total As content in <150 um fraction of tailings, soil, and mill residue samples (ppm)

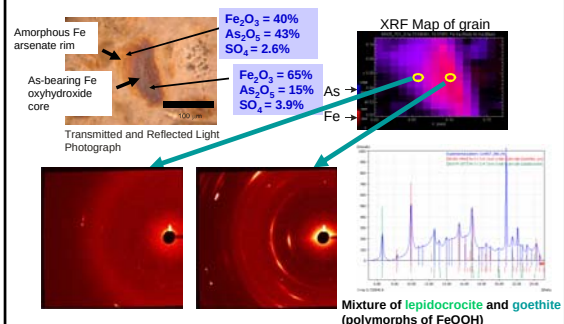
Site	Max	Min	Median
Caribou	313,000	15,200	72,600
Goldenville	210,000	7,200	38,900
Montague	62,100	318 (soil)	10,600
N Brookfield	9,170	195 (soil)	1,590

At North Brookfield sulfide ore was roasted to liberate gold

ca. 1896

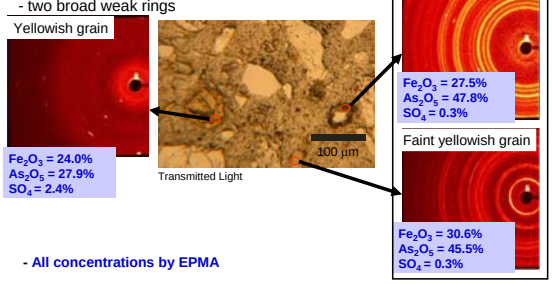


Fe-arsenate rimmed Fe-oxyhydroxide



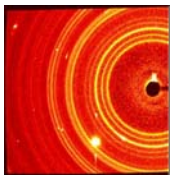
Three different Fe-arsenates

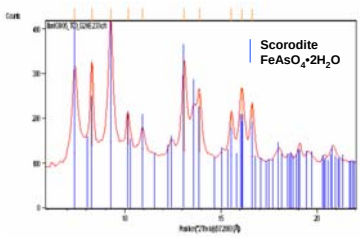
Almost amorphous
- two broad weak rings



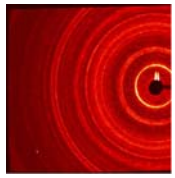
- **All concentrations by EPMA**

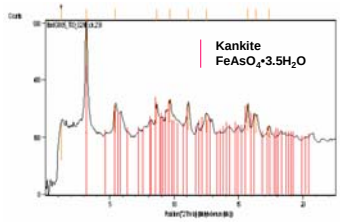
Rimmed Grain - Scorodite





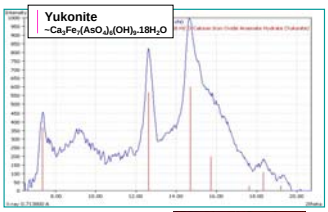
Yellowish grain - Kankite

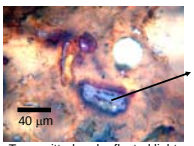




Yukonite


Reflected light

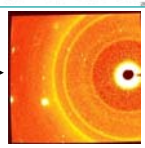




Transmitted and reflected light

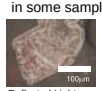
Fe_2O_3 = 32.7%
As_2O_5 = 35.6%
SO_4 = 0.3%
CaO = 7.4%

Ca/As (molar) = 0.3



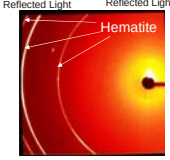
Tailings from gold-ore roasting
North Brookfield

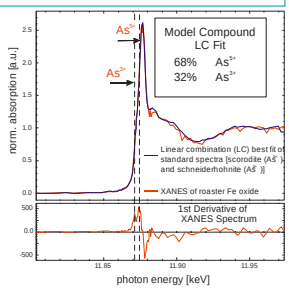
Abundant roaster derived As-bearing Fe-oxide (hematite αFe_2O_3 or maghemite γFe_2O_3) in some samples



Reflected Light

Reflected Light

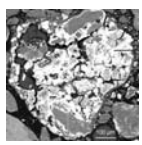




Arsenic-bearing minerals identified in NS gold mine tailings (to date)

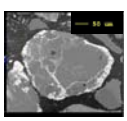

Arsenopyrite

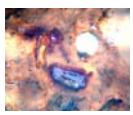

Crystalline scorodite


Nanocrystalline scorodite


Roaster-generated As-bearing hematite


Kankite
$FeAsO_4$•3.5H_2O

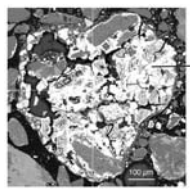

Amorphous Fe arsenate

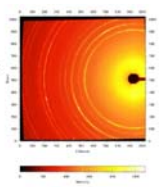

As-Ca bearing Fe oxyhydroxide = Yukonite


As-bearing Fe oxyhydroxide

Information on Grain Size from µXRD

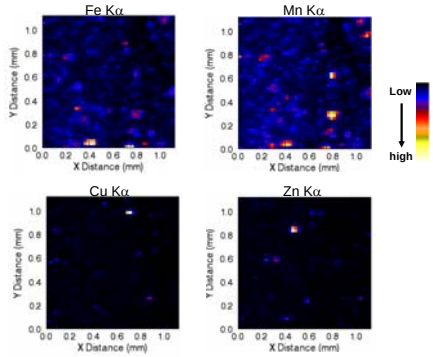

As-bearing Hematite



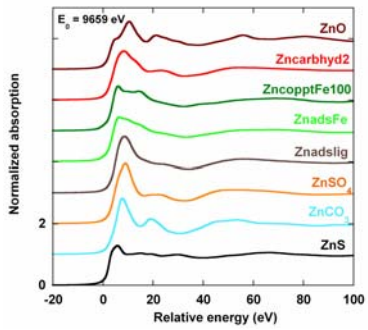

Scorodite

Many As-bearing secondary minerals are nanocrystalline, but porosity, cementation and disaggregation affect grain size

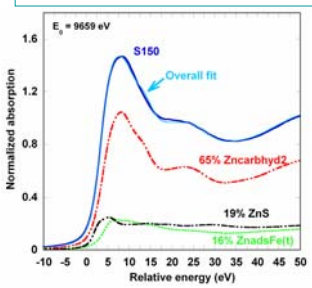
Metal Speciation of House Dust: μXRF mapping



Zn macro XANES spectra of known species



Example of fitting results for Zn



In contrast, Cu in house dust is largely bound to organic species

General Conclusions

- A combination of synchrotron and classical mineralogical analysis can provide direct identification of metal-bearing grains
- Information on grain size and intergrowth textures may also be provided

- Most samples contain at least three mineral forms of a particular metal
- Many particles are aggregates or mixtures of more than one mineral

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Bioaccessibility of arsenic adsorbed onto or incorporated within freshly synthesized iron oxide minerals using the SHIME

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[1]Interdisciplinary Graduate Program of Toxicology
[2]University of Saskatchewan, Saskatoon, Canada
[3]University of Ghent, Belgium

Bioaccessibility and Risk Assesment

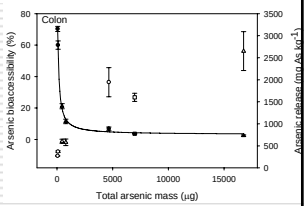
- Total contaminant concentration in a mineral may overestimate exposure
- Bioaccessibility measured using *in vitro* GI models as a surrogate for bioavailability
- Not yet widely incorporated into risk assessment
  - How do we go about validating *in vitro* models?

Bioaccessibility vs Concentration: Inverse Relationships

Two Possible Mechanisms:

1. Thermodynamic limitation of As dissolution
   - Bioaccessibility a function of LS Ratio
2. Kinetic limitation of arsenic dissolution
   - Bioaccessibility a function of residence time

A Cause for Concern?



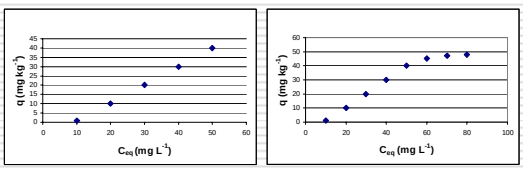
Laird et al., Environ. Sci. Technol., 2007.

Bioaccessibility vs Concentration

- How does mineralogy impact the relationship between bioaccessibility and concentration?
  - Amorphous Scorodite
    - Arsenic incorporated into iron oxide mineral
    - Readily soluble at low pH
  - As(V) - Ferrihydrite
    - Arsenic adsorbed onto surface
    - Ferrihydrite sparingly soluble at low pH

Isotherms in Soil Chemistry

Shape of isotherm is a function of the chemical processes controlling dissolution



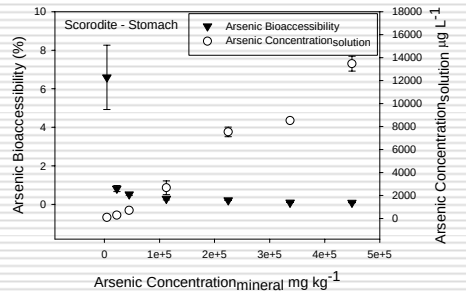
GI Microbes and As Toxicokinetics

- Gastrointestinal microbes increase arsenic bioaccessibility of mine tailings in colon stage of SHIME
- Gastrointestinal microbial community may contain both arsenate reducing bacteria and iron reducing bacteria
- Small intestine is the primary site of absorption
  - Duodenum & Jejunum: Lower microbial densities
  - Ileum: Higher microbial densities
- Arsenic is potentially absorbed in the proximal colon

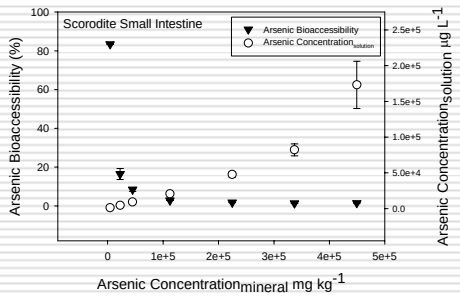
Hypotheses

1. Arsenic dissolution in the SHIME is at equilibrium
   - H_A: Kinetics limit dissolution of arsenic in the SHIME
2. Gastrointestinal microbes impact the bioaccessibility of arsenic absorbed to ferrihydrite and incorporated within scorodite

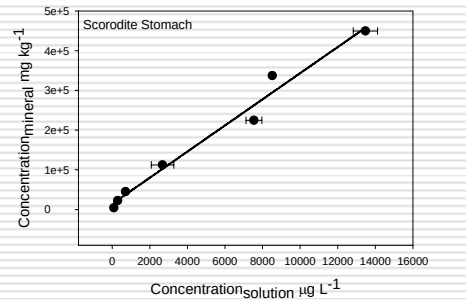
Scorodite Bioaccessibility in the Stomach



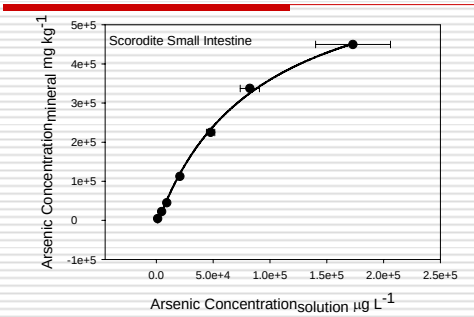
Scorodite Bioaccessibility in the Small Intestine



Scorodite Isotherm: Stomach



Scorodite Isotherm: Small Intestine



Conclusions: Arsenic Bioaccessibility versus Concentration

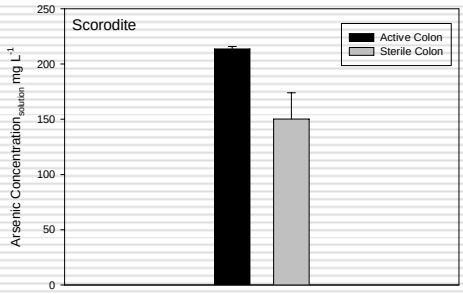
When an inverse relationship between arsenic concentration and bioaccessibility is observed:

- As bioaccessibility from scorodite in the stomach likely determined by LS Ratio
- As bioaccessibility from scorodite in the small intestine may be determined by residence time

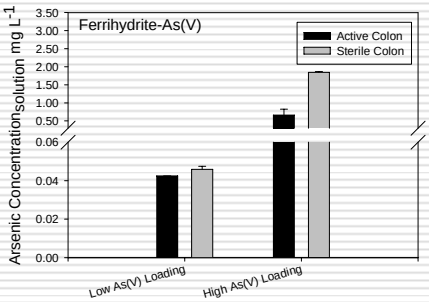
Conclusions: Use of Isotherms in Bioaccessibility Research

- Use of isotherms may not be applicable for use with real-world samples
  - Mineralogy MUST be constant between samples for interpretation of isotherm results
- Should test impact of LS ratio and residence time when inverse relationship observed
  - Current recommendations focus on LS ratio
  - May result in underestimation of bioaccessibility and exposure in risk assessments

GI Microbe Impact on Arsenic Bioaccessibility



GI Microbe Impact on Arsenic Bioaccessibility



Conclusions: Impact of GI Microbes on arsenic bioaccessibility

- GI microbes capable of increasing or decreasing arsenic bioaccessibility from arsenic-bearing minerals
  - GI microbial activity associated with iron reduction
  - Effect of GI microbes likely mineralogy-dependent
- Unknown toxicological implications of this microbial impact on bioaccessibility
  - Mechanisms potentially also active in ileum
  - May pose challenging for the validation of *in vitro* models using *in vivo* animal dosing experiments

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- University of Saskatchewan Department of Toxicology