



*Providing a scientific basis
for evaluating and predicting inorganic
and organic contaminant bioaccessibility
in soils found at contaminated sites
in Canada.*



BioAccessibility Research Canada (BARC)

Webinar Proceedings

October 5, 2010



**Health Santé
Canada Canada**



Proceedings from the BARC Webinar

Tuesday, October 5, 2010

Background

BioAccessibility Research Canada (BARC) was formed in 2006 following a bioaccessibility workshop sponsored by Health Canada in 2005. The workshop was held to discuss the status of bioaccessibility/bioavailability research in Canada and internationally and to determine if there was interest in forming a Canadian working group to further the scientific rationale for making bioaccessibility a component of human health risk assessments at contaminated sites in Canada. There was consensus that a group should be formed and Dr. Ken Reimer and Dr. Beverly Hale were nominated to be the Co-Chairs. Today, the group includes representatives from government, academia, the mining industry and the risk assessment community. It also has a strong international component, and includes members from Europe, the United States and most recently Australia. One of BARC's ultimate goals is to achieve international consensus on protocols for bioaccessibility and bioavailability testing for a range of contaminants, as well as guidance on when and how to incorporate bioaccessibility data in human health risk assessments.

Since 2006, there has been an annual BARC workshop each fall. The workshops are an opportunity for BARC members to report on the progress of BARC research activities, obtain input and feedback on research outcomes, and propose and achieve consensus on research activities going forward.

The annual workshop for FY 2010/2011 has been moved to February, 2011, to allow integration of results from the second international BARC round robin experiment that will be held in November/December, 2010. In lieu of the fall workshop, the Environmental Sciences Group (ESG) at the Royal Military College of Canada (RMC) hosted a BARC webinar on Tuesday, Oct. 5, 2010, from 12 pm until 2 pm EDT. This document summarizes the content of and discussions from the webinar. A list of webinar participants and their contact information can be found in Appendix A and copies of the presentations in Appendix B. The webinar was sponsored by Health Canada.

Summary of Discussion

The webinar was opened by Viviane Paquin, secretary for BARC, with round table introductions of participants on the call. Dr. Ken Reimer, Co-Chair for BARC, welcomed all of the participants and this included a special welcome to the newest international member of BARC Albert Juhasz from the University of South Australia. Next, Dr. Reimer reviewed the agenda for the meeting. The primary objectives of the webinar were to:

1. Update BARC members on recent BARC activities and on new BioAccessibility Research Group Europe (BARGE) initiatives
2. Receive input on the state of science and regulatory status of bioaccessibility testing in the United Kingdom (UK), Australia and the United States (U.S.).
3. Provide an update on results from the organic round robin; and
4. Present and get final consensus on the framework for the second international inorganic round robin experiment.

A summary of the presentations and the ensuing discussions are provided below.

Dr. Reimer opened the presentations with the BARC update. Of special note is that BARC will be chairing a workshop in Toronto in February, 2011 (exact date TBD) with the goal of achieving international consensus on bioaccessibility and bioavailability protocols as well as creating a guidance document for ensuring the correct application of bioaccessibility/bioavailability data in human health risk assessment (HHRA). To this end, two documents will be posted on the BARC web site (www.bioavailabilityresearch.ca) for review and comment: a discussion paper on considerations for bioavailability experiments developed by Dr. Stan Casteel, a professor and research toxicologist at the University of Missouri, Columbia with input from ESG; and a proposal for a step-by-step decision-making framework on when and how to incorporate bioaccessibility data in HHRAs of contaminated sites. These will be discussed at the February workshop. Also of note is that three soils with *in vivo* data have been obtained for use in the 2nd BARC international inorganic round robin experiment. As well, a brownfield soil containing Cr and As, and a soil with naturally elevated As and Co, have been collected and homogenized and will be available to the BARC community for research purposes. Sufficient quantities have been collected to carry out *in vivo* studies with these soils should funds become available.

Dr. Joanna Wragg provided the BARGE update. Dr. Wragg reported that the University of Lille, France, has provided some results with significant implications for the Unified BARGE Method (UBM) relating to the storage temperature of extracts and fixing of the stomach pH at 1.2. As well, a paper summarizing the results of the first international trial using the BARGE method, which included seven laboratories and 34 contaminated soils, has been submitted to the scientific journal *Science in the Total Environment*. A second trial is being planned that will include nine soils that are representative of soils frequently encountered in Europe during contaminated site risk assessment. BARGE welcomes any laboratories that are interested in participating in the second trial.

Methodologies for testing the bioaccessibility of organic contaminants in soil are continually being developed, with a priority focus on polycyclic aromatic hydrocarbons (PAHs).

In terms of regulatory status in the UK, the environment agency does not recommend one specific bioaccessibility method but bioaccessibility data may be incorporated into risk assessment as part of a lines-of-evidence approach.

Questions:

1. Were other organic contaminants included in the organic bioaccessibility testing, or was it PAHs only?
 - The soils included PAHs only because they are a priority contaminant for the UK. Individual analyses were performed for the top 16 U.S. EPA priority PAHs.
2. Which elements are being examined in the next round of testing with the nine soils, and do you have relative bioaccessibility (RBA) values for them?
 - There was no need to have RBA values associated with the soils at this time because BARGE is using a method (the UBM) that has been validated for arsenic (As), lead (Pb) and cadmium (Cd), which are the main contaminants in the soils. The soils include a back garden soil, soils from Portugal, Belgium and Sweden and include a range of concentrations of the elements for which the UBM has been validated.

Albert Juhasz gave the next presentation, providing information on the incorporation of bioaccessibility testing in Australia and some testing that has occurred at the University of South Australia.

A document developed by the Australian National Environmental Protection Measure for the Assessment of Site Contamination (NEPM-ASC) addresses bioaccessibility and bioavailability. The 2010 version of this document includes a detailed discussion of how to accept bioaccessibility in risk assessment; this is outlined in Schedule B4. This document is still under review and comments are currently being accepted.

At the University of South Australia, bioaccessibility testing has focused on arsenic-contaminated soils; two to three of these will be included in the upcoming BARC round robin. These soils are also available to other interested parties. *In-vivo* testing has focused on As, Pb, Cd, dichlorodiphenyltrichloroethane (DDT) and benzo(a)pyrene (B(a)P) using swine or mouse models to determine the absolute bioavailability (ABA) and relative bioavailability (RBA) of these contaminants. *In-vitro* testing has focused on the same contaminants using a variety of methods.

Two conferences are being held in Adelaide, South Australia in the fall of 2011 and all are invited to submit abstracts to these conferences. The **6th International Workshop on Chemical Bioavailability in the Terrestrial Environment** is taking place 7 - 9 September 2011 and the **4th International Contaminated Site Remediation**

Conference is taking place 11 - 15 September 2011. For more information please go to www.cleanupconference.com

Questions:

1. What is the status of acceptance for validated methods by element?
 - State Environmental Protection Agencies (EPAs) will use gastric SBRC for As, RBALP for Pb.
2. Is there anything for organics?
 - No, but EPAs are interested in this information, especially for PAHs as these are a priority contaminant.

Nick Basta followed with a presentation on his participation in a multi-partner project initiated recently in the U.S by the U.S. Department of Defence (DoD) Strategic Environmental Research and Development Program (SERDP), in partnership with the U.S. Department of Environment (DoE) and U.S. Environmental Protection Agency (EPA). The project focuses on the soil binding mechanisms that affect the bioaccessibility of As and Pb species. A range of soils with representative As and Pb species will be researched to determine if correlations between *in-vivo* and *in-vitro* data still hold. The project is looking for collaborators and also for soils that exhibit different binding mechanisms preferably with associated RBA data.

Questions:

1. How much soil are you looking for?
 - A minimum of 500 g of <250 µm sieved soil. (Albert Juhasz has about 5kg of some As contaminated soils of <250 µm size and some bulk soils in greater amounts).
2. What is the timeframe for this project?
 - It is a three-year project and is now in the second month.
3. Why does the new standard operating procedure (SOP) for the IVG method have an increased pH to 6.5 and why is the argon bubbling removed?
 - Argon was not making any difference. The pH of the bulk solution in the intestines is 6.5-7, whereas the pH near the membrane is 5.5. Correlations with *in-vivo* data were not affected over a range of pH from 5.5-7, so the bulk solution pH was chosen to correspond with available literature.
4. Are you working with anyone from the United States Geological Survey (USGS)?
 - Not specifically on this project. Discussion to continue off-line.

Iris Koch presented the results of the initial organic round robin experiment. Dr. Koch reported that a preliminary analysis of the data revealed that results using the single method selected, the Fed Organic Extraction Human Simulation Test (FORE(h)ST), were variable for both spike recovery (which was less than 100 percent) and soil bioaccessibility, and that alternate methods tested gave results comparable to those obtained with FORE(h)ST. The results will be compiled in a report and likely be

prepared for publication in a peer-reviewed journal. Future steps should include studies with soils for which *in-vivo* data are available.

Questions:

1. Was the analysis for PAH totals or B(a)P?
 - The contaminant was polychlorinated biphenyls (PCBs) and the analyses were for total PCBs. PAHs are future work.
2. When calculating bioaccessibility, did you use the recovery from each lab, or did you calculate bioaccessibility based on average recovery of spikes?
 - No spike recovery was included in the bioaccessibility results.

Dr. Koch continued her presentation with a recap of the first round robin experiment and a discussion of the goals and proposed framework for the second. The primary aim of the second round robin experiment is to establish bioaccessibility control ranges for a limited number of methods and test materials to aid in bioaccessibility testing. The experiment will include four methods (RBALP, UBM, PBET and IVG), for which detailed SOPs will be provided, and four or five soils. Three of the soils will have *in vivo* data.

Questions:

1. How prescriptive is the IVG method to include paddle stirrers?
 - Nick Basta has used end-over-end mixing and results have been similar but he is hesitant to suggest the use of end-over-end because nothing has been published yet. It was decided that if people wish to use the IVG method they could choose to use either or both mixing methods.
2. Why is a chromium (Cr^{6+}) spike being used when the species in soil is likely Cr^{3+} ?
 - Research with the compound used in the TRV for Cr^{3+} is showing that it is insoluble and the goal is to use the most soluble compound.
3. Will the analysis be for Cr species or total Cr?
 - The analysis will be for total Cr.
4. Albert Juhasz offered to do all four methods and asked if any forms were needed for the transportation of soils.
 - Dr. Koch will be working on them.
5. Will the results be published?
 - Yes, ESG are working with INERIS to publish results.

The webinar was concluded at this point with Dr. Reimer providing closing remarks and a reminder of the upcoming BARC workshop to be held in February, 2011.

Please contact Viviane Paquin (viviane.paquin@rmc.ca) if you want your contact information and/or publications related to the broad topic of bioaccessibility/bioavailability listed on the BARC website. The URL for the website is www.bioavailabilityresearch.ca

Annex A

Participation List

Name	Contact
Albert Juhasz	Albert.Juhasz@unisa.edu.au
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Annex B

Presentations



Bioaccessibility Research Canada

**Webinar hosted by Environmental Sciences
Group, Royal Military College of Canada**

Tuesday , October 5th , 2010



Webinar Agenda



1. Welcome and introductions
2. Updates on the progress of scientific research and related activities for advancing the use of bioaccessibility testing in contaminated sites risk assessment
 - a. Canada - BARC
 - b. Europe - Barge
 - c. Australia – CRC Care
 - d. US EPA
3. Presentation of results of the initial BARC organic round robin experiment
4. Presentation of the framework for inorganic round robin experiment #2
 - a. Goals and objectives
 - b. Participating labs
 - c. Methods and protocols
 - d. Soils
 - e. Time lines
 - i. Soil ship date
 - ii. Date Extracts due at central lab
5. Other Items



BARC Activities Past and Present



- 2005:** BARC is born to address the need for a coordinated effort to advance the science of contaminant bioaccessibility in Canada
- 2006:** BARC chairs workshop to get industry perspective on bioaccessibility issues
- 2007:** BARC chairs workshop to plan the initial inorganic RR experiment
- 2008:** BARC chairs workshop to report on inorganic RR #1 results and to solicit input on results and next steps from BARC members
- 2009:** BARC chairs workshop on bioaccessibility of organic contaminants to identify state of science and develop framework for initial organic RR experiment
- 2009:** BARC chairs workshop to address issues surrounding the reliability, accuracy, and correct incorporation of site specific bioaccessibility/bioavailability data into HHRA
- 2010:** BARC hosts international webinar to share progress on bioaccessibility advances internationally and present plan for 2nd inorganic Round Robin
- 2011:** BARC to chair workshop in Toronto, February 2011, to achieve international consensus on when and how to incorporate bioaccessibility data in HHRA



BARC Updates

November 2009 BARC Workshop



- A primary aim of the November 2009 workshop was to consolidate existing technical and professional knowledge with respect to:
 - Fundamentals
 - Methodological considerations; and
 - Validation using *in vitro/in vivo comparisons*.
- This knowledge will inform the development of a decision-making framework for the correct application of bioaccessibility data in HHRA

Next Step: a proposed framework is being developed and will be posted on the BARC website for review and comment and discussion at the February workshop



BARC Updates

Inorganic Round Robin Experiment #1



- Objective was to determine the variability in bioaccessibility results among different laboratories using different methods for NIST 2710
- Following completion of the RR INERIS contributed *in vivo* data for NIST 2710 and *In vitro/in vivo* comparisons were carried out
- Final report draft including results of *in vitro/in vivo* comparisons has been submitted to Health Canada for review
- Report will be available on BARC website

Next Step: Publication in a peer reviewed journal



BARC Updates

In vivo Protocols



- A discussion document on considerations for bioavailability experiments has been prepared by Stan Casteel with input from ESG. Topics addressed are:
 - Appropriate animal models
 - Dosing of animals
 - Organs or tissues measured
 - Control experiments
 - Calculation of relative bioavailability
 - Replication and uncertainty
 - Information gaps
- Consensus around these topics is needed to develop a framework for conducting bioavailability experiments

Next Step : Paper will be posted on the BARC website for review and comment and discussion at the February workshop



BARC Updates Canadian Reference Materials



- Proposal for a suite of reference materials submitted to Health Canada but funding not available at this time
- As an interim measure two candidate soils for *in vivo* testing have been collected and homogenized for research purposes
 - A brownfield soil containing Pb and Cr
 - A soil with naturally high As and Co



BARC Updates 2010/11 Activities



1. 2nd international inorganic round robin experiment
2. February 2011 BARC workshop in Toronto, Canada
 - Information on the current status of bioaccessibility testing in Canada will be solicited from industry and regulators prior to the workshop.The workshop will aim to:
 - Consolidate results from RR#1 and RR#2
 - Achieve international consensus on *in vitro* and *in vivo* protocols and decision-making framework for when and how to incorporate bioaccessibility data in risk assessment
 - Identify way forward for bioaccessibility of organic contaminants



BARGE 2010 Update



BARGE Activities



- University of Lille Investigations – significant data implications
 - The affect of storage temperature on enzyme activity and resulting bioaccessibility - 4, 20 and 37°C
 - The affect of not fixing the stomach pH at 1.2
- Preparation and submission of the paper for the 1st inter-laboratory trial
 - 7 labs, 3 elements, 34 contaminated soils (complicated matrices)
 - Taken time to un-pick the story and get agreement between the participants
 - Now submitted to STOTEN



The UBM – where are we?



- We have the French SOP
 - In the process of finalising technical/practical details
 - Distribute to all BARGE members
- Awaiting a peer reviewed publication of the validation of the UBM from INERIS prior to posting the SOP on the BARGE website
- INERIS are in the process of making a video to show how to carry out the UBM
 - Will be posted on the BARGE website with French, English, Belgian, Portuguese and Spanish translations
- A second inter-laboratory trial is being organised
 - Within & between laboratory uncertainty

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Bits and Pieces



- RIVM
 - Pb study
 - To provide a RBA factor for Pb in made ground in Dutch cities
 - Used the RIVM 'Original method' and TNO 'Tiny TIM' method
 - Significant differences in bioaccessibility data
 - Reviewed by an International expert panel
 - Recommendations for the differences and a way forward – *in vivo* trial
- Consoil 2010 – Salzburg
 - Dutch protocol
 - RBA factor of 0.7 for children as the receptor
 - 0.4 (the average between the RIVM and TNO data) for made ground
 - Funding not currently available for an *in vivo* study

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2nd Inter-laboratory Trial



- Validated BARGE UBM method
 - Within and between uncertainty
- 9 contaminated soils to date, not slags or mine wastes
- 5 participating labs to date
 - Please contact us if you wish to participate
- All samples are about to be characterised by XRFS at the BGS
- BGS will sub-sample, send out materials, collate and statistically analyse the data
- As yet we have not decided on the analysis
 - Financial constraints

- Peer reviewed publication

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Organics



- Continuing development of organic methodologies
 - Possible *in vivo* trial carried out by University of Nancy (supported by the French Agency ADEME)
- FORE(h)ST is published ES&T
 - FORE(h)ST not yet scaled up as a commercial method
 - Some R&D required to identify sources and extent of interferences
- Research is continuing at Reading and Ghent to include the colon
 - CePBET and SHIME
- Collaborative efforts between individual BARGE members continues
 - Look at differences in methods and PAH sources

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Regulatory and Public Acceptance



- UK
 - EA - Do not recommend any specific test
 - But, the results may be used for As as part of a “lines of evidence approach” for site-specific risk assessment
 - DEFRA announced in February 2010 to review the Statutory Guidance which underpins the contaminated land regime under Part 2A of the Environmental Protection Act 1990
 - c. 70% of UK and Welsh local authorities see bioaccessibility as a useful tool
 - Want further statutory guidance on its use
 - CIEH documentation on how to apply the UBM in a HHRA, how many samples per SSRA to analyse etc

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Regulatory and Public Acceptance



- Europe
 - For Ni compounds an assessment strategy has been devised to comply the REACH regulations
 - Bioaccessibility and transformation/dissolution data provide specific information on Ni release for evaluating toxicity
 - Netherlands – as previously mentioned
- Awaiting votes on the Soil Framework Directive
 - Belgium currently hold the presidency
 - UK, France, Germany are blocking the acceptance of the policy

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Join BARGE research activities

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Regulatory Status of Bioaccessibility Testing and State of the Science in Australia

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Regulatory Status of Bioaccessibility Testing in Australia

National Environmental Protection Measure for the Assessment of Site Contamination (NEPM-ASC)

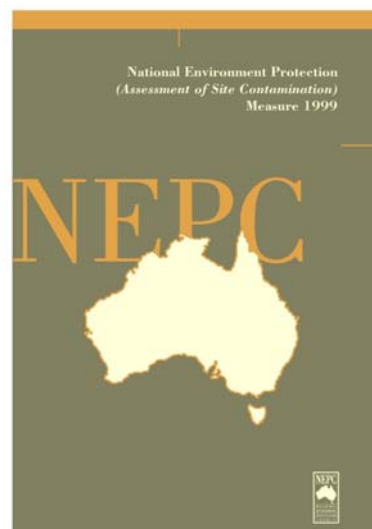
The NEPM establishes a nationally-consistent approach to the assessment of site contamination to ensure sound environmental management practices by the community which includes regulators, site assessors, contaminated land auditors, land owners, developers and industry.

NEPM-ASC contains two schedules:

Schedule A – identifies the recommended process for the assessment of site contamination.

Schedule B – comprises 10 general guidelines for the assessment of site contamination.

The implementation of the NEPM-ASC is the responsibility of each participating jurisdiction

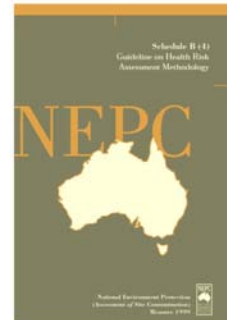




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Regulatory Status of Bioaccessibility Testing in Australia

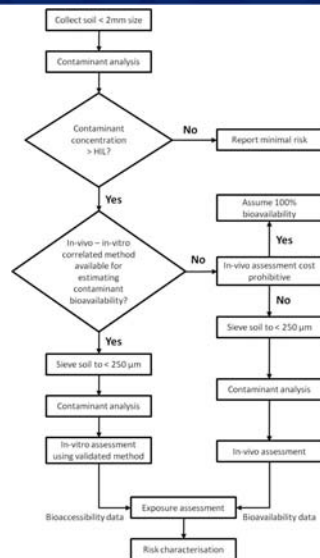
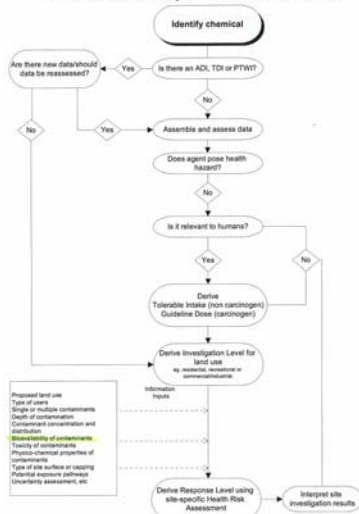
- Schedule B1** Guideline on investigation levels for soil and groundwater
- Schedule B2** Guideline on data collection, sample design and reporting
- Schedule B3** Guideline on laboratory analysis of potentially contaminated soil
- Schedule B4** Guideline on health risk assessment methodology → Bioavailability / bioaccessibility
- Schedule B5** Guideline on ecological risk assessment
- Schedule B6** Guideline on risk based assessment of groundwater contamination
- Schedule B7a** Guideline on health-based investigation levels
- Schedule B7b** Guideline on exposure scenarios and exposure settings
- Schedule B8** Guideline on community consultation and risk communication
- Schedule B9** Guideline on protection of health and the environment during the assessment of site contamination
- Schedule B10** Guideline on competencies and acceptance of environmental auditors and related professionals



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NEPM-ASC Bioavailability and Bioaccessibility

Decision Tree for the development of health-based soil criteria





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NEPM-ASC Bioavailability and Bioaccessibility

ASC NEPM Variation 2010 - Public Consultation Documents

Background

The Assessment of Site Contamination NEPM is the premier guidance document in Australia for the assessment of site contamination. It addresses a complex area that is particularly subject to new developments in scientific knowledge and technology, as recognised during the original development of the NEPM.

With the high cost of assessment and remediation, it was recognised by NEPC that new scientific and technical information needed to be incorporated into the NEPM. This would serve to minimise overly conservative investigation levels and provide clarification on the site investigation process, and ultimately minimise unnecessary remediation.

In June 2007, NEPC initiated the variation to the Assessment of Site Contamination NEPM. Since that time, the project team has developed a draft varied Assessment of Site Contamination NEPM (including revised schedules) that address the review recommendations endorsed by NEPC in 2006 (a PDF of the review report can be downloaded from here).

The major changes included in the draft varied Assessment of Site Contamination NEPM are:

- new and improved methodologies for deriving Ecological Investigation Levels (Schedule 3a) and Health Investigation Levels (Schedule 3b)
- incorporation of Health Screening Levels and Ecological Screening Levels for petroleum hydrocarbons (Schedule 3c)
- incorporation of guidance for assessing asbestos impacts (Schedule 3d and 3e)
- incorporation of guidance on stockpile sampling (Schedule 3f), contaminant bioavailability (Schedule 3g) and bioaccessibility (Schedule 3h)
- incorporation of guidance for assessment of volatile substances (Schedules 3i, 3j and 3k).

<http://www.ephc.gov.au/contam/pdocs>



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State of the Science in Australia *In Vivo* Bioavailability

Contaminant	Animal Model	Endpoint	Comments
Arsenic	Swine	Blood	ABA determined for As ^{III} , As ^V , MMA, DMA RBA determined for 12 contaminated soils RBA determined for 8 spiked soils (2 soils – ageing effects)
Cadmium	Mouse	Liver Kidney	ABA determined for Cd-acetate RBA determined for 5 contaminated soils RBA determined for 2 spiked soils
Lead	Swine Mouse	Blood	ABA determined for Pb-acetate RBA determined for 5 contaminated soils (swine) RBA determined for 12 contaminated soils (mouse)
DDT	Mouse	Adipose tissue	ABA determined for DDT RBA determined for 8 contaminated soils
BaP	Mouse	Organs Urine faeces	Preliminary studies to determine BaP pharmaco-kinetics



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State of the Science in Australia *In Vitro* Bioaccessibility

Contaminant	<i>In Vitro</i> Assay	Comments
Arsenic	SBRC	Comparison of in vitro methodologies
Cadmium	IVG	Correlation of in vitro assays with RBA
Lead	PBET	Effect of gastric phase pH on As bioaccessibility (and RBA correlation)
	DIN	Effect of ageing on bioaccessibility
	UBM	Effect of soil particle size on bioaccessibility
		Contaminant distribution using synchrotron analysis
DDT	PBET	Comparison of in vitro methodologies
	DIN	Correlation of in vitro assays with RBA
	FOREhST	
PAHs	FOREhST	Preliminary studies to determine PAH bioaccessibility



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2011 CleanUp Conference



6th International Workshop on Chemical Bioavailability in the Terrestrial Environment – 7 - 9 September 2011

4th International Contaminated Site Remediation Conference – 11 - 15 September 2011

Adelaide, South Australia: www.cleanupconference.com





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Further Information

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**Mechanisms and Permanence of Sequestered Pb
and As in Soils: Impact on Human Bioavailability
Project Number (ER-1742)**

**Dr. Nicholas T. Basta
The Ohio State University**



Performers



Dr. Nicholas Basta, The Ohio State University
Project PI, Soil Chemistry, In Vitro Gastrointestinal (Bio)availability

Dr. Mark Failla, The Ohio State University
Bioavailability (Pb, As) using Caco-2 Human Intestinal Cell Lines

Dr. Kirk Scheckel, USEPA NRMRL, Cincinnati, OH
Pb and As Speciation in Soils by Advanced Spectroscopic Methods

Dr. Karen Bradham, USEPA, NERL, RTP, NC
Environmental Soil Toxicology, Bioavailability Technology Transfer

Dr. David Thomas, USEPA NHEEL, RTP, NC
Toxicology, In Vivo Bioavailability of Pb and As

Dr. Christopher Schadt, Oak Ridge National Laboratory
Microbial Ecology and Permanence of Remediation

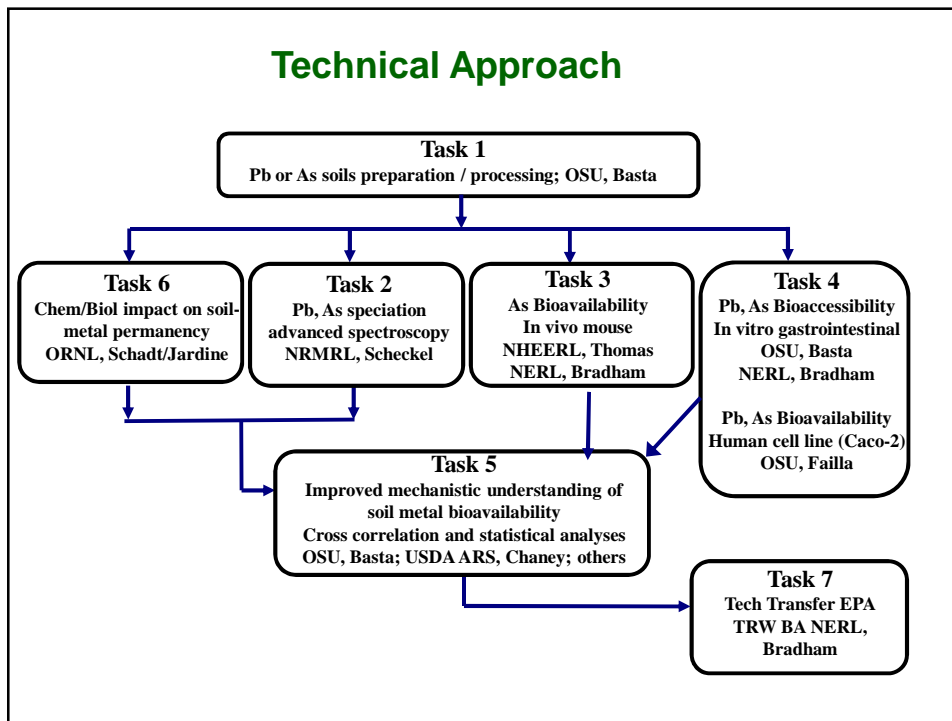
Dr. Philip Jardine, Oak Ridge National Laboratory
Soil Chemical Processes and Permanence of Remediation

Dr. Rufus Chaney, Beltsville, MD, USDA Agricultural Research Service, Beltsville, MD
Soil Amendments, Cross Correlation of (Bio)Availability Methods

Technical Objective

- Conduct a comprehensive study to link the binding mechanism of Pb and As in soil (i.e., speciation) with in vitro and in vivo methods used to predict current and potential future bioavailability of soil Pb and As to humans.
- Evaluate the effect of key biological and chemical processes on the permanence of binding and bioavailability of Pb and As in untreated and treated (i.e., remediated) soils.

Technical Approach



Study Soils

It is critical to use study soils that have representative Pb/As species and a range of metal binding mechanisms

Study soils contaminated with Pb or As are selected from USEPA, DoD sites with representative metal species and binding mechanisms.

Three soils remediated by soil amendments which bind Pb/As and reduce their bioavailability.

Cross Correlation and Statistical Analysis

- Metal speciation and IVG bioaccessibility results are useful if soil element's *in vitro* bioaccessibility is well correlated with *in vivo* bioavailability.
- Identify methods/frameworks that permit inclusion of metal speciation, bioaccessibility, and/or bioavailability measures into site-specific management decisions.

USEPA Bioavailability and Technology Transfer

**USEPA Technical Review Workgroup Metal Bioavailability Committee
Dr. Bradham, co-Chair; Drs. Thomas, Scheckel, members**

- TRW members will assist with bioavailability method development as well as guidance development
- Implementation and technology transfer
Workshops and webinars designed to increase confidence of site managers to use bioavailability measures in site management decisions.



PCB Round Robin Preliminary Results



Participating Laboratories



- British Geological Survey
 - Mark Cave and Joanna Wragg



- Environmental Sciences Group, RMC
 - Ken Reimer

-  Simon Fraser University
 - Margo Moore

-  Exponent
 - Yvette Lowney



- Umeå University
 - Staffan Lundstedt



- Ghent University
 - Tom Van de Wiele

-  University of Reading
 - Chris Collins

-  Golder Associates
 - Tammie Morgan-Gray



Objectives

- To determine the variability in results obtained from different laboratories using a single method for a PCB contaminated soil

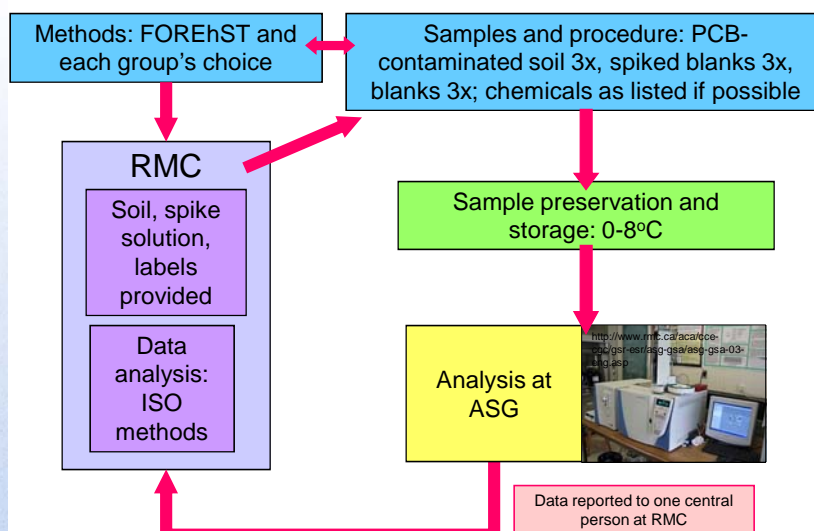


- FOREhST = Fed ORganic Extraction Human Simulation Test: resembles UBM/RIVM methods

- To compare these results to those obtained using different methods



Study Design





Preliminary Points

- Six laboratories carried out the FOREhST test:
 - U Ghent
 - BGS
 - RMC
 - Exponent
 - Golder
 - SFU
- Three additional methods were tested:
 - Exponent – BMOC (from Finley et al 2009)
 - Golder
 - Sweden (butanol extraction)
- University of Reading carried out FOREhST and CEPBET (small intestine and colon), but were excluded from data analyses because no results were detectable
- RMC carried out PBET on soils only



QA/QC

- Blanks were all $<25 \mu\text{g/L}$ in the extracts (corresponding to 2.5 mg/kg in soil) with a single exception:
 - A single blank for Exponent BMOC method had $39 \mu\text{g/L}$ in extract (= 4 mg/kg in soil)
- Duplicate analyses of individual extracts resulted in RPDs of 0-16%
- Blanks accompanying extract analyses were all $<25 \mu\text{g/L}$
- Control recoveries accompanying extract analyses were 74-122%

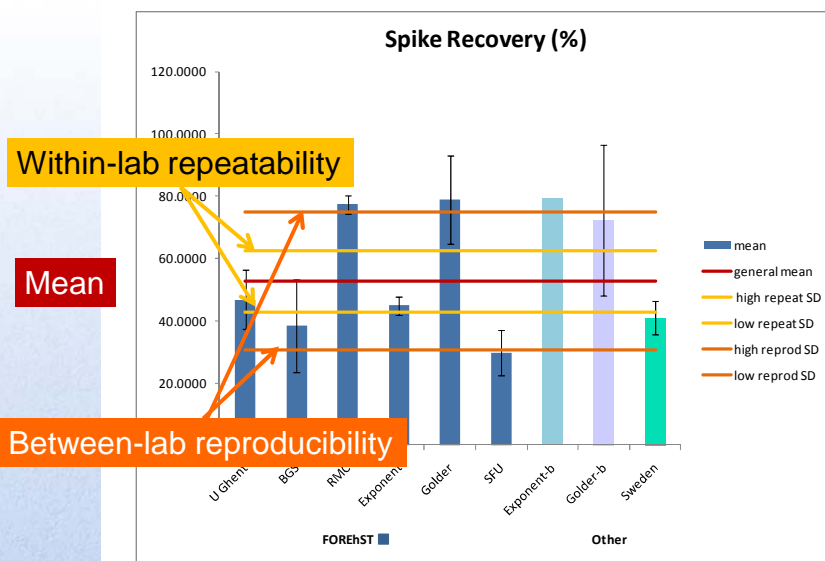


Statistics for FOREhST method

FOREhST method Parameter	Total PCBs	
	Soil	Spike
# Laboratories	6	6
General Mean	13	53
Repeatability variance	5.7	97
Between laboratory variance	77	393
Reproducibility variance	83	490
Repeatability SD	2.4	9.9
Between laboratory SD	8.8	20
Reproducibility SD	9.1	22
% RSD (repeatability)	18	19
% RSD (reproducibility)	70	42
Median	11	46
Minimum	3.3	24
Maximum	30	90

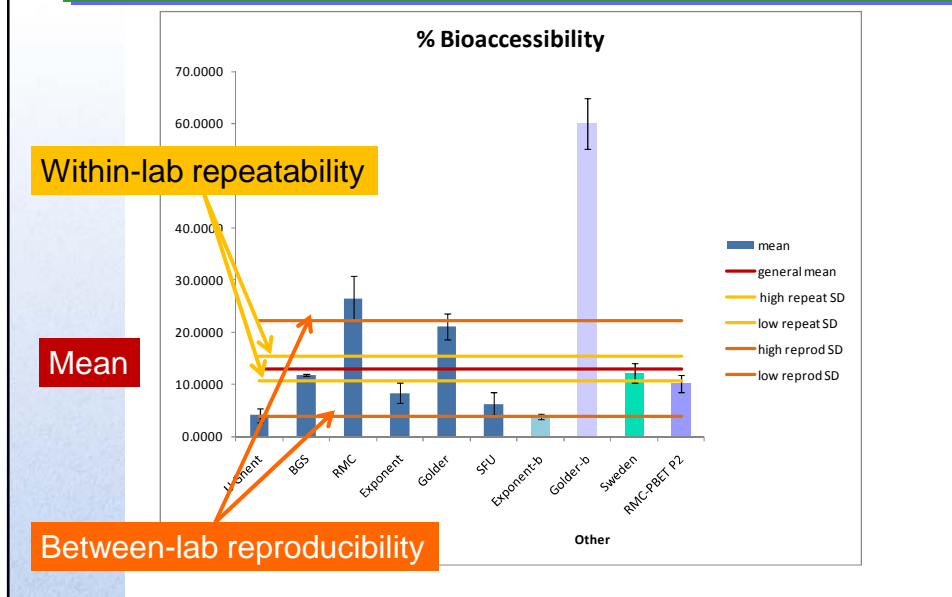


Spiked Blanks





Soil Results



Summary

- Source of reagents for FOREhST method should be updated (most laboratories had problems sourcing the exact suppliers and catalog numbers)
- Variability, even within-laboratory (~ 20%), is substantial
- Most alternate methods are within reproducibility standard deviation of the FOREhST method
- Spike recovery is substantially <100% (general mean of 53%)
- % Bioaccessibility of PCBs in the studied soil is low (3-30%)



Next Steps

- Write report and possible publication
- Literature review of bioavailability/
bioaccessibility results to determine impact of
including in HHRA
 - Past workshops have indicated that PAHs are a
potential concern in Canada
- *In vivo* studies, and bioaccessibility trials with
these soils



Health Santé
Canada Canada

Inorganics Round Robin #2

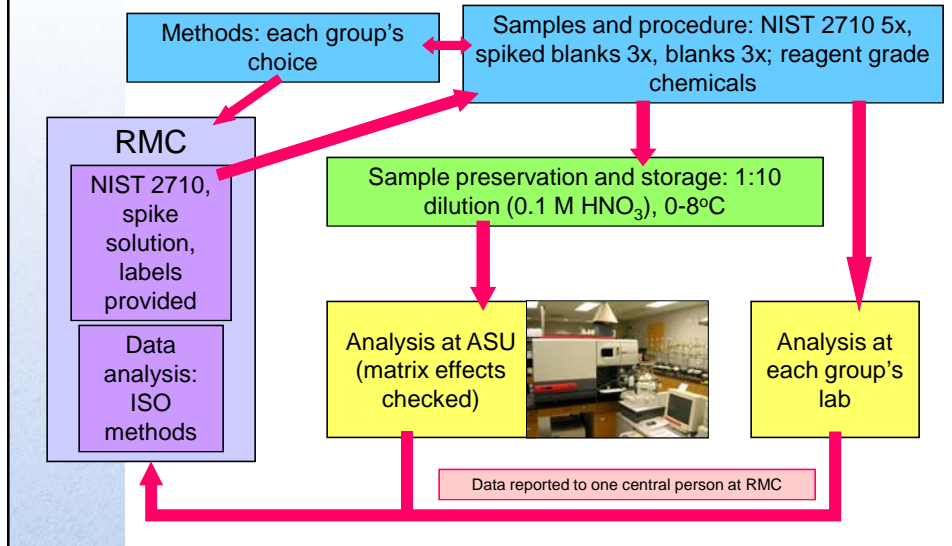


Outline

- Recap of Round Robin #1
- Plans for Round Robin #2
- Discussion



RR1: Study Design



In Vitro Data from RR#1: Participating Laboratories



• British Geological Survey

• Environmental Sciences Group, RMC



• Exponent

• Golder Associates

• Health Santé Canada Canada (Rasmussen)

• OHIO UNIVERSITY (Basta)

• riyman Netherlands

• ROYAL ROADS UNIVERSITY (Dodd)

• SGS Lakefield

• Simon Fraser University (Moore)



• Stantec

• UNIVERSITY OF GUELPH (Hale)

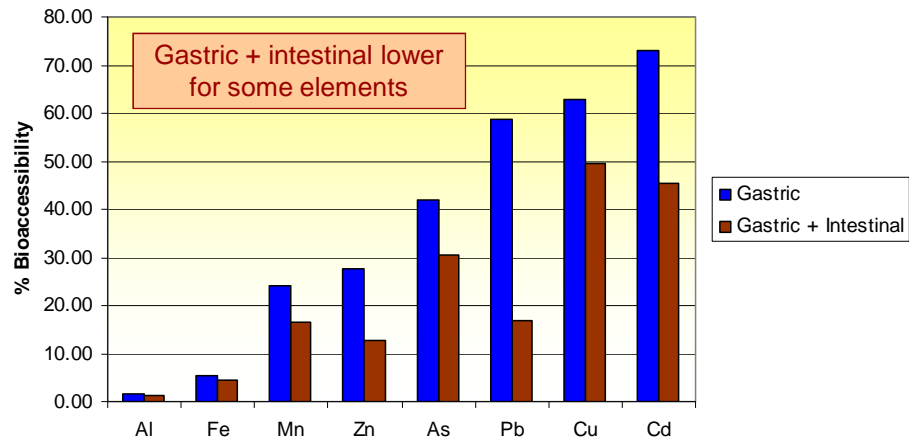
• (Zagury)

• UNIVERSITY OF SASKATCHEWAN (Siciliano)



Results for NIST 2710

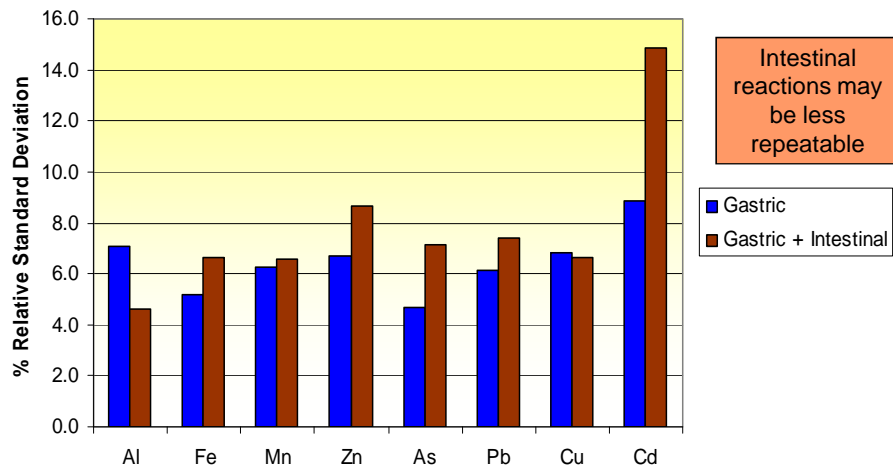
General Mean of % Bioaccessibility



Variability - Within Lab

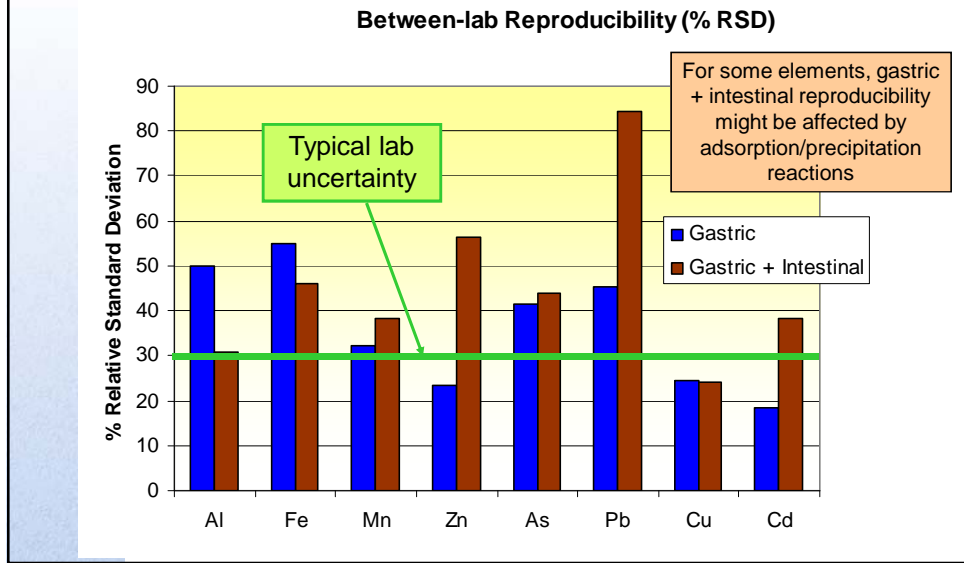
Within-lab Repeatability (% RSD)

Generally around or <10%

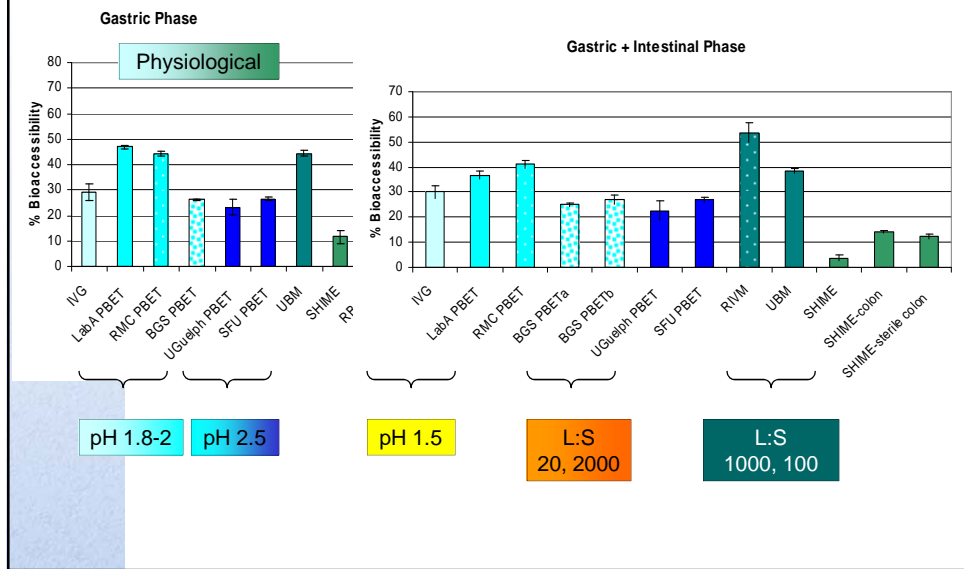




Reproducibility (Repeatability + Between-lab Variability)

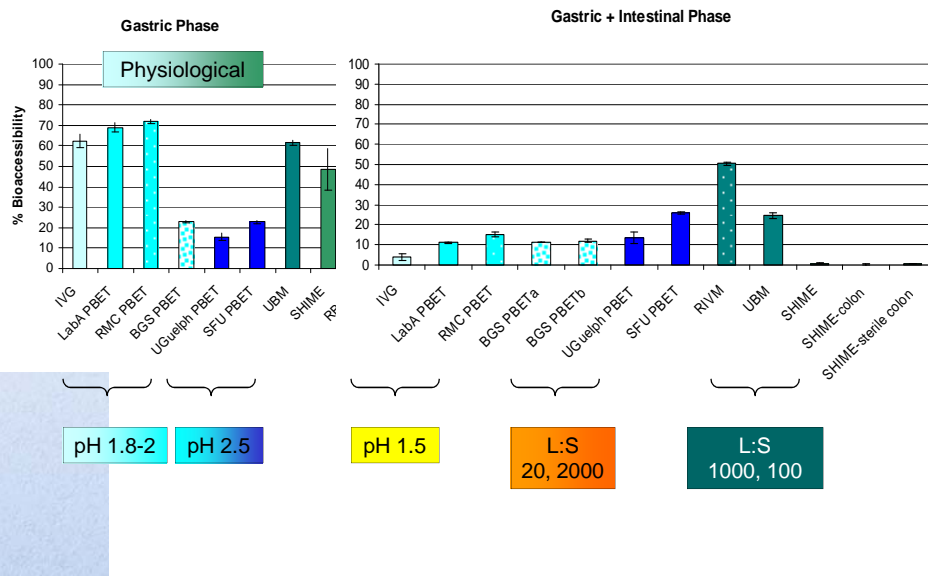


Method Trends - As





Method Trends - Pb



Comparison to In Vivo Data

- INERIS: swine study
 - 3 doses of NIST 2710 and control
 - Control = sodium arsenate, lead acetate in water
 - Tissues analyzed: kidney, urine, bone, liver





INERIS

Arsenic

Laboratory/ method	Smallest difference (%)	Source of data	Spike corr?	Phase
RRU-PBET	3.5	Individual	No	Gastric
Exponent-phosphate				
RIVM				al
Lab A-RBALP				
RMC-PBET				al
SGS-Modified TCLP	5.7	ASU	Yes	Gastric
BGS-UBM	-9.4	ASU	No	Gastric
HC-HCl	14	Individual	No	Gastric
Lab B-RBALP	18	ASU	Yes	Gastric
RRU-RBALP	29	ASU	Yes	Gastric
OSU-IVG	-39	ASU	No	Gastric + intestinal
Exponent-glycine	-42			
BGS-PBET	-43			
SFU-PBET	-45			
UG-PBET	-50	ASU	Yes	Gastric
US-SHIME	-64	ASU	Yes	Colon

Lighter coloured methods indistinguishable when measurement error is considered:
3 simple, 3 physiological

Larger deviations associated with underestimation



INERIS

Lead

Laboratory/ method	Smallest difference (%)	Source of data	Spike corr?	Phase
RMC-PBET	0.22	ASU	Yes	Gastric
HC-HCl				
Lab B-RBALP				
SGS-Modified TCLP				
RRU-PBET				
RRU-RBALP	5.6	ASU	Yes	Gastric
Lab A-RBALP	-8.9	ASU	Yes	Gastric
OSU-IVG	-12	Individual	No	Gastric
RIVM	-13	ASU	Yes	Gastric + intestinal
BGS-UBM	-19	ASU	No	Gastric
Exponent-glycine	-27	Individual	No	Gastric
US-SHIME	-36	ASU	No	Gastric
UG-PBET	-63	Individual	No	Gastric
SFU-PBET	-66	ASU	No	Gastric + intestinal
Exponent-phosphate	-66	ASU	Yes	Gastric
BGS-PBET	-69	Individual	No	Gastric

Lighter coloured methods indistinguishable when measurement error is considered:
5 simple, 2 physiological



Summary

- Variability between methods was substantial but could mostly be explained by gastric pH
 - For some elements, reproducibility RSD was comparable to lab uncertainty
- Swine data have very small error margins
 - For arsenic, 3 simpler and 3 more physiologically based methods were close enough to the *in vivo* results that the difference was within the bioaccessibility measurement error
 - For lead, 5 simple and 2 physiological methods were within the measurement error
- Data generated in individual laboratories did not introduce more variability



Proposed Round Robin # 2



Objective

- To establish bioaccessibility control ranges for a limited number of methods and test materials to aid in Canadian and international bioaccessibility testing



Lessons Learned from RR#1

- Using a soil that has *in vivo* data is valuable
- Need to limit the number of methods, since it is difficult to interpret results when many different methods are used
- Use of spikes to calculate relative bioaccessibility requires careful selection of spike solution
- Personnel and lab conditions must be available so that each lab's INTERNAL QC requirements are met
- Personnel and other resources may become unavailable for analysis by individual laboratories – thus a single lab approach is valuable



Aspects from RR#1

- Central organizing body (ESG)
 - Ship soils, spikes, labels and instructions
 - Receive results
 - Statistical analysis
- Analysis in central commercial laboratory only
- Preservation: dilute to 0.1 M nitric acid, refrigerate
- ISO methods of statistical analysis



Soils and Contaminants

- Soils that have already been subjected to in vivo testing:
 - Australian soil (As)
 - US soil (As)
- Kingston brownfield soil (Cr, Pb)
- Nova Scotia soil (As, Co)
- Limit to 4 (budgetary constraints)



Proposed Methods

RBALP

UBM

PBET

IVG

- All methods have been validated against *in vivo* results, and RBALP and PBET compared favourably with *in vivo* (swine) results in RR1
- RBALP is standard for Pb (USEPA); UBM is being standardized in Europe
- Aiming for 5 laboratories to carry out each method
 - Based on response so far, 8 RBALP, 7 PBET, 4-6 UBM, 2 IVG



Reagents, Equipment

- Reagents:
 - Specified grades or purities
 - Some manufacturers that distribute in Europe do not distribute in North America
- Equipment
 - Mixing as specified by method:
 - End-over-end for RBALP and UBM
 - End-over-end proposed for PBET
 - Paddle stirrers for OSU-IVG
 - Plastic bottles and shipping containers (no glass)



Replicates and Spikes

- 3x soil, 3x spikes and 3x blanks
 - 12 soil extracts in total
 - 3 spiked blank + 3 blank = 6 more extracts
 - Total of 18 extracts/phase/method
- Soluble compounds used to make spikes should reproduce the solutions used in *in vivo* control experiments
 - As as sodium arsenate; Pb as lead acetate; Cr as K_2CrO_4 ;
 - Mixture of elements ok



Timing

- Soil/spikes/labels to be sent out early Nov
- Aim for extracts to be sent to ASU in Dec/early Jan.
- Data analysis in Jan/Feb
- Presentation of preliminary results in Feb.



Anticipated Outcomes

- Control limits = variability between labs for a given method
- Methods will be consistent with *in vivo* data
 - Similar values or predictive
- Some methods may give similar results
- Publication in peer-reviewed literature
- Regulator confidence in use of bioaccessibility

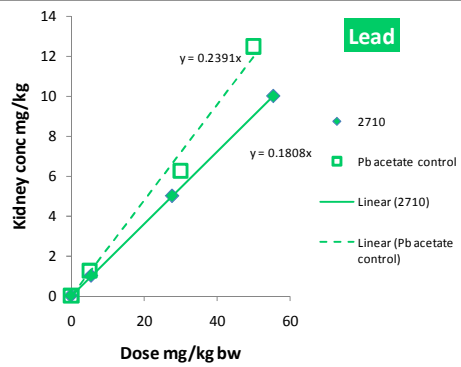
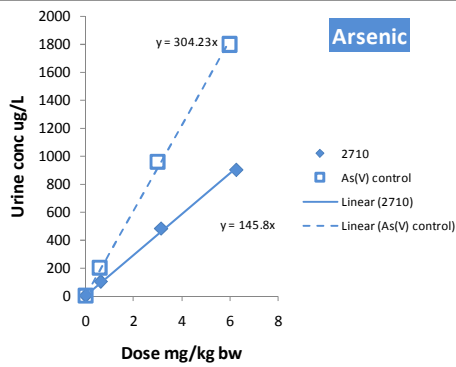


Discussion points

- IVG method – currently 2 labs will perform
 - How prescriptive are paddle stirrers?
- If only shaker is available for other methods, how prescriptive is end-over end?
 - There may be sufficient numbers of labs carrying out other methods
 - Shaker at high speed (e.g., 275 rpm) can be tested, but extracts considered lower priority for analysis
- Replicates, reagents, preserving etc.?



INERIS RBAs



$$RBA_{As} = 146/304 * 100\%$$

$$RBA_{As} = 48\%$$

$$RBA_{Pb} = 0.181/0.239 * 100\%$$

$$RBA_{Pb} = 76\%$$



In Vivo Data cont'd

	Arsenic		Lead
Data Source	INERIS	Ellickson et al.	INERIS
Animal	swine	rat	swine
Control	NaH ₂ AsO ₄	none used, estimated from literature	Pb(CH ₃ COO) ₂
Tissue	urine	all except feces	kidney
RBA (%)	49	44	76
RBA range (%)	46-52	37-52	73-79
range source	95% CI	1 SD (n=19)	95% CI



Relative Bioaccessibility

- When comparing bioaccessibility with relative bioavailability, it is often useful to compare with relative bioaccessibility:

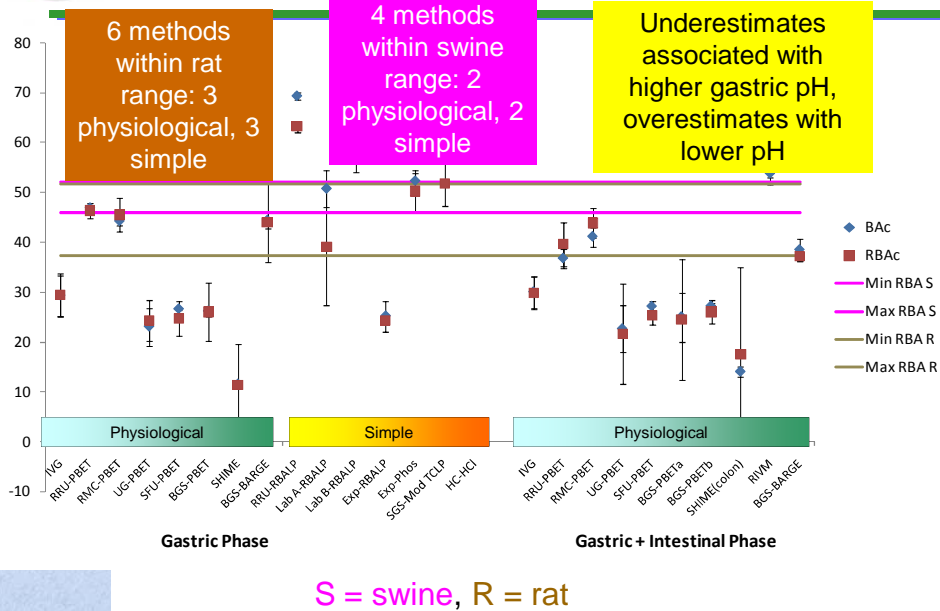
$$RBAc = \text{Bioaccessibility}_{2710} / \text{Bioaccessibility}_{\text{control}}$$

Where the control resembles the control in the bioavailability (in vivo) experiment

- Spike was used as a surrogate



Comparison of % Bioaccessibility to %RBA - As

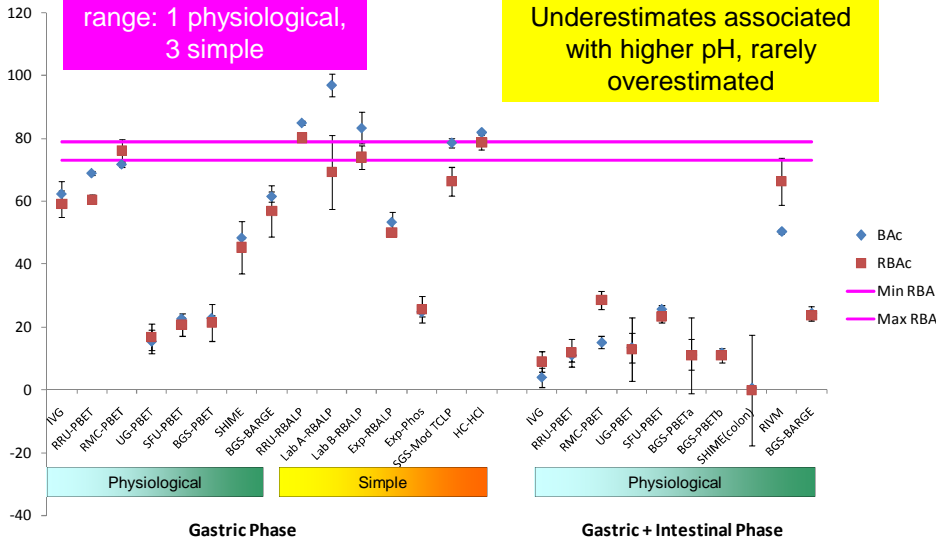




Comparison of % Bioaccessibility to %RBA - Pb

4 methods within swine range: 1 physiological, 3 simple

Underestimates associated with higher pH, rarely overestimated



As control rat experiments				Ellickson results			Calculated results	
As species	Measurement time	Deviations (Ellickson: difference from feces, oral)	Source	ABA	ABA 2710	ABA 2710 corrected	RBA 2710	RBA 2710 corrected
AsIII	24 hr	12 hr, organs+blood, IV	Suzuki 2001	75	34.3	37.5	45.7	50.0
AsIII	24 hr	ip	Hilmy 1991	97	34.3	37.5	35.4	38.6
AsIII	48 hr	whole body	Vahter 1981	79.7	37.9	56.5	47.6	70.9
AsIII	48 hr	ip	Hilmy 1991	94.5	37.9	56.5	40.1	59.8
AsIII	72 hr	RBC	Naranmandura 2007	83	41.2	48.1	49.6	57.9
AsIII	72 hr	ip	Hilmy 1991	93.6	41.2	48.1	44.0	51.4
AsIII	72 hr	96 hr, ip	Hilmy 1991	93.1	41.2	48.1	44.3	51.6
AsV	24 hr	ip	Hilmy 1991	98.3	34.3	37.5	34.9	38.1
AsV	48 hr	whole body	Vahter 1981	65.4	37.9	56.5	58.0	86.4
AsV	48 hr	ip	Hilmy 1991	96.4	37.9	56.5	39.3	58.6
AsV	48 hr	stomach tube, cellulose	Katayama 1994	93	37.9	56.5	40.8	60.7
AsV	72 hr	10 d, "gastrointestinal"	Dutkiewicz 1977	70	41.2	48.1	58.9	68.7
AsV	72 hr	ip	Hilmy 1991	96.1	41.2	48.1	42.9	50.0
AsV	72 hr	96 hr, ip	Hilmy 1991	95.9	41.2	48.1	43.0	50.1
Descriptive Statistics								
AsIII	Minimum						35.4	38.6
AsIII	Maximum						49.6	70.9
AsIII	Mean						43.8	54.3
AsV	Minimum						34.9	38.1
AsV	Maximum						58.9	86.4
AsV	Mean						45.4	59.0
All	Minimum						34.9	38.1
All	Maximum						58.9	86.4
All	Mean						44.6	56.6
All	Mean - 1 SD						37.4	
All	Mean + 1 SD						51.7	

#	SRM NAME	Source	As	Cd	Co	Cr	Cu	Hg	Ni	Pb	Sb	Se	V	Zn
1	2709	NIST	17.7	0.38	13.4	130	34.6	1.4	88	18.9	7.9	1.57	112	106
2	1648	NIST	115	75	(18)	403	609		82	65.5%	(45)	27	140	47.6%
3	2586	NIST	8.7	2.71	(35)	301	(81)	(0.367)	(75)	432		(0.6)		(352)
4	2587	NIST	13.7	1.92	(14)	92	(160)	(0.29)	(36)	3242			(78)	(336)
5	CRM032-050	RTC	369	131	103	15	585	21.4	14.8	4601	48.9	171	9.38	2577
6	CRM033-050	RTC	129	89.2	95.2	105	95.5	6.45	56.04	60.6	79	88.9	112.5	227
7	CRM214-225	RTC	2.92	0.41		3.2		0.06		0.32		5.48		
8	CMI7004	LGC	49.6	1.52	20	82.2	183	0.223	33.3	93.4			126	227
9	CMI7002	LGC	32.4	0.31	12.6	179	29.3	0.09	42	58.9			54.9	69
10	CMI7001	LGC			9.66	89.6	30.8	0.087	31.9	43.8			58.7	120
11	CMI7003	LGC			11.5	79.8	29.1	0.096	31.3	33.5			76.2	81
12	CRM142R			0.34	12.1		69.7	0.067	64.5	40.2				
13	CRM141R			14.6	10.5	195	46.4	0.25	103	57.2				283
14	LGC6135	RTC	70			455	107		291	411			139	345
15	RTC-CRM020	LGC	400	15.4	4.51	13.6	729	1.12	16.9	5111	8.38	6.57	6.47	3011
16	RTC-CRM021	LGC	25	1		11	4792	5	13		2725			546
17	RTC-CRM022	LGC	5	3	6	19	12	5	16	415			23	46
18	RTC-CRM023	LGC	380	0.9	8.9	31.1	4.7	77.8	11	213		116	21.7	93.8
19	RTC-CRM024	LGC	3.42	2.15		25.4	8.7	0.71	15	15.7			20.8	37.3
20	RTC-CRM025	LGC	339	369	4.07	441	7.76	99.8	12.2	1447		518	19.3	51.8
21	RTC-CRM026	LGC	5.64	11.9	6.77	27.2	18.8	2.42	14.4	25.6		116	32	140
22	RTC-CRM027	LGC	12.5	11.9	4.77	27	9.79	3.85	10.7	51.7		13.8	21.8	51.5
23	RTC-CRM028	LGC	3.93	0.5	4.3	19	8.51		11	10.4			19.2	75
24	CRM025-050	RTC	339	369	4.07	441	7.76	100	12.2	1450	(≤ 3.2)	518	19.3	51.8
25	CRM026-030	RTC	5.64	11.7	6.77	27.2	18.8	2.42	14.4	25.6	(≤ 3.2)	1.86	32	140
26	CRM027-050	RTC	12.5	11.9	4.77	27	9.79	3.85	10.7	51.7		13.8	21.8	51.5
27	CRM028-050	RTC	3.83	0.504	4.00	19	8.51		13.4	10.4			19.1	75.0
28	CRM030-050	RTC	13.1	58.4		43.8	5.68	6.55	6.63	7.13	2.32	18.5	29	74.8
29	NCS DC 73319	China NACIS	34	4.3	14.2	62	21	0.032	20.4	98	0.87	0.14	86	680
30	NCS DC 73321	China NACIS	4.4	0.06	5.5	32	11.4	0.06	12	26	0.44	0.09	36	31
31	NCS DC 73322	China NACIS	58	0.35	22	370	40	0.59	64	58	6.3	0.64	247	210
32	NCS DC 73323	China NACIS	412	0.45	12	118	144	0.29	40	552	35	1.6	166	494
33	NCS DC 73324	China NACIS	220	0.13	7.6	75	390	0.072	53	314	60	1.34	130	97
34	NCS DC 73325	China NACIS	4.8	0.08	97	410	97	0.061	276	14	0.42	0.32	245	142
35	NCS DC 73326	China NACIS	12.7	0.13	12.7	68	24.3	0.017	31.5	21	1.0	0.10	81	68
36	SOIL-7	IAEA	13.4		8.9	60	11		60	1.7			66	104
37	TILL-1	CANMET	18		18	65	47		24	22	7.8		99	98
38	TILL-2	CANMET	26		15	74	150		32	31	0.8		77	130
39	TILL-3	CANMET	87		15	123	22		39	26	0		62	56
40	TILL-4	CANMET	111		8	53	237		17	50	1		67	70
41	RTS-1 tailings	CANMET	8.2		16.6		595		22	105				553
42	RTS-2 tailings	CANMET	6.3		72		670		2430	45				117
43	RTS-3 tailings	CANMET	9.1		260		2820		71	146				1850
44	RTS-4 tailings	CANMET	207		186		280		7940	60				158
45	SO-3 soil	CANMET			18	26	17	0.017	16	14			38	52