HEALTH CANADA BIOACCESSIBILITY WORKSHOP

Health Canada

Santé Canada

August 30-31, 2005
Delta Chelsea Hotel, Toronto
1.0 Introduction

Regulations and guidelines governing contaminated site assessment and remediation are based in part on minimizing human health risks based on predicted exposures using the total concentration of the target substance in a particular substrate, e.g. soil, sediment or water. A default assumption for many human health risk assessments is that a contaminant in soil as an exposure medium is 100% bioavailable. Bioavailability is the extent to which a substance can be absorbed by an organism into its systemic circulation. While chemicals that are dissolved in water may be readily available for uptake by various organisms, this may not be the case for soils and sediment where the contaminant may be tightly bound to the soil matrix.

It can be argued that only the bioavailable fraction of a contaminant poses risk. True bioavailability can only be determined by means of in vivo (usually animal) studies. Laboratory (in vitro) tests are increasingly being developed to model exposure; the fraction of the contaminant that dissolves in such tests is referred to as the bioaccessible portion.

A workshop was held on Aug. 30-31, 2005 at the Delta Chelsea Hotel in Toronto, Canada, to discuss bioavailability/bioaccessibility research both in Canada and internationally. The meeting was sponsored by Health Canada in order to determine the status of this research in Canada, to establish linkages with activities in Europe and the United States, and to identify data gaps that could be filled as part of a larger co-ordinated effort. As well, the meeting sought to determine whether there was sufficient interest to form a Canadian Bioaccessibility Working Group.

The workshop was co-chaired by Drs. Beverley Hale of the University of Guelph and Ken Reimer from the Royal Military College of Canada. Logistical support was provided by staff from the Canadian Network of Toxicology Centres. The organizers tried to include key researchers in this area as well as representatives
from regulatory agencies. The goal was to be inclusive and there was general agreement that this was achieved. The agenda for the meeting can be found at the end of this section.

Twenty-nine people (see list at Annex A) attended the workshop and 12 participants gave presentations on their areas of interest. The session concluded by identifying some data gaps and challenges. There was a consensus to form a Canadian working group and Drs. Hale and Reimer were nominated to be the initial co-chairs. Dr. Reimer committed to producing a quarterly newsletter that could be used as a communication tool and to attract other members of the group. More recently, Drs. Hale and Reimer have proposed calling the group Bioaccessibility Research Canada, or BARC.

1.1 Structure of this Report

This report serves as a record of the proceedings of the Workshop. Section 2 provides a background document prepared by Dr. Mark Richardson of Health Canada. It was designed to provide context for the meeting and was distributed to participants in advance. It defines the terms bioavailability and bioaccessibility and identifies Health Canada’s rationale for sponsoring the Workshop.

A summary of the key points provided by the speakers and the ensuing discussion is provided in Section 3. Abstracts and copies of all of the presentations can be found in Annex B. The discussion focusing on research gaps and future directions is summarized in Section 4.
## Agenda - Health Canada Bioaccessibility Workshop
### August 30-31, 2005

**Carlyle Room, Delta Chelsea Hotel, 33 Gerrard St. W., Toronto, ON**

### Day 1: Aug. 30, 2005

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<thead>
<tr>
<th>Time</th>
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<th>Topic</th>
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<tr>
<td>12:30</td>
<td>Cold Lunch</td>
<td>Outside Carlyle Meeting Room</td>
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<tr>
<td>13:00</td>
<td>Hale / Reimer</td>
<td>Chairs' introduction to the Workshop</td>
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<tr>
<td>13:15</td>
<td>Richardson</td>
<td>Health Canada perspective</td>
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<tr>
<td>13:30</td>
<td>Ollson</td>
<td>Critical review of <em>in vivo</em> and <em>in vitro</em> Studies of Arsenic and Lead Bioavailability</td>
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<tr>
<td>13:50</td>
<td>Dodd</td>
<td>Effect of Soil Particle Size and Different Liquid-to-Soil Ratios on the Bioaccessibility of Metals in Contaminated Soils</td>
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<td>14:10</td>
<td>Rasmussen</td>
<td>Determination of Bioaccessible Metals in Household Dust: Modifications to the European Standard Toy Safety Protocol</td>
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<td>14:30</td>
<td>Moody</td>
<td>Dermal Absorption Research Team (DART): Bioavailability of Soil Contaminants in Overview</td>
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<td>15:00</td>
<td>Bright</td>
<td>Pulmonary Bioavailability of Particle-Bound Contaminants: A Review</td>
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<td>15:20</td>
<td>Coffee Break</td>
<td>Outside Carlyle Meeting Room</td>
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<tr>
<td>15:30</td>
<td>Wragg</td>
<td>BARGE Perspective on Oral Bioaccessibility</td>
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<tr>
<td>16:15</td>
<td>Basta</td>
<td>Using <em>in vitro</em> Gastrointestinal Bioaccessibility Methods to Quantify Trace Element Bioavailability and Risk From Soil Ingestion: A Perspective on Research Needs and Activities in the United States</td>
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<tr>
<td>16:50</td>
<td>Reimer/Hale</td>
<td>Synthesis of &quot;research needs&quot; identified by Day 1 presentations</td>
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<td>18:00</td>
<td>Adjournment</td>
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### Day 2: Aug. 31, 2005

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<tr>
<th>Time</th>
<th>Presenter</th>
<th>Topic</th>
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<tr>
<td>7:30</td>
<td>Continental breakfast</td>
<td>Outside Carlyle Meeting Room</td>
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<tr>
<td>8:30</td>
<td>Hale / Reimer</td>
<td>Chairs' introduction to Day 2</td>
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<tr>
<td>8:45</td>
<td>Hale</td>
<td>Cadmium Bioavailability vs. Bioaccessibility</td>
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<tr>
<td>9:15</td>
<td>Moore</td>
<td>Polycyclic aromatic hydrocarbon (PAH) Bioaccessibility Using Caco-2 Cells</td>
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<td>9:45</td>
<td>Coffee</td>
<td>Outside Carlyle Meeting Room</td>
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<tr>
<td>10:15</td>
<td>Siciliano</td>
<td>Microbial Processes in Estimating Food Metal Bioaccessibility</td>
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<td>10:45</td>
<td>Birmingham</td>
<td>Bioaccessibility and Bioavailability in Human Health Risk Assessment</td>
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<tr>
<td>11:15</td>
<td>Reimer</td>
<td>Application of Bioaccessibility for Contaminated Site Risk Assessment</td>
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<tr>
<td>11:45</td>
<td>Cold Lunch</td>
<td>Outside Carlyle Meeting Room</td>
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<tr>
<td>13:00</td>
<td>Reimer/ Hale</td>
<td>Gap Analysis and the Canadian Way Ahead</td>
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<tr>
<td>15:00</td>
<td>Richardson</td>
<td>Closing Remarks</td>
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<tr>
<td>15:10</td>
<td>Adjournment</td>
<td>Coffee available outside Carlyle Room</td>
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Under the Canadian Federal Contaminated Sites Action Plan (FCSAP), Health Canada is an *Expert Support Department*, responsible for the provision of guidance, training and advice on human health risk assessment (and related topics) to federal departments with custodial responsibilities for contaminated sites.

It is broadly recognized that the site-specific bioavailability of soil-borne contaminants is seldom complete (i.e. typically less than 100 percent) and should be considered when undertaking contaminated site risk assessments. A chemical that is ingested, inhaled and/or dermally applied, but is not absorbed would theoretically present no risk, at least in most instances (inhalation of some chemicals, such as asbestos or nickel, are exceptions).

Adjustments for dermal bioavailability are routinely included in Canadian risk assessments, due primarily to the necessary characterization of risks posed by dermally-absorbed doses through comparison to reference doses established on the basis of oral exposures. It is important to adjust dermal doses to reflect the equivalent systemically-absorbed oral dose to make such comparisons and risk characterizations valid.

The application of adjustments for oral bioavailability is also becoming commonplace. The most common surrogate for true ingestion bioavailability is bioaccessibility. It is determined from an *in vitro* assay as the ratio of the mass of contaminant dissolved into a volume of simulated gastric fluid (acidic leachate) to the original mass of contaminant in the soil sample as determined by HF ("total digest") or (reverse) *aqua regia* ("environmentally available"). Although solubility in simulated gastric fluid is not a direct measure of bioavailability, it is considered to be a reasonable surrogate since solubilization in the gastrointestinal tract is
generally a necessary step in the systemic absorption process. However, such adjustments must consider not only the potential for systemic absorption from soil, but also the likely absorption of the toxicant in the key toxicological study upon which the reference dose is based. In many cases, the systemic absorption in that key toxicological study was also less than 100 percent.

Adjustments for inhalation (respiratory) bioavailability are seldom introduced into contaminated site risk assessments in Canada. This is particularly true where inhalation reference doses or concentrations are available from inhalation-specific toxicological studies. However, adjustments are seldom made, even when inhalation exposures are being compared with oral reference doses. It is simply assumed that the inhalation absorption is equivalent to ingestion absorption (i.e., the relative bioavailability factor = 1).

Health Canada Activities with Respect to Bioavailability of Contaminants in Soil

Health Canada has initiated research to quantify the *in vitro* dermal penetration of various soil-borne contaminants using viable human skin. The work initiated to date, which has focused on method development and validation, will be discussed by Dr. Rick Moody of the Environmental Health Science Bureau (EHSB) of Health Canada.

Health Canada has funded some limited research on oral bioavailability, which will be presented. This research includes:

- the influence of soil particle size and the ratio of gastric fluid volume to soil mass on measures of bioaccessibility (Matt Dodd, Royal Roads University);
- bioaccessibility of metals from indoor house dust versus outdoor soils, and the potential applicability of OECD methods designed to assess lead risk from toys to the issue of soil bioaccessibility (Pat Rasmussen, EHSB);
Health Canada was interested in the relevance and applicability of *in vitro* simulated lung fluid solubility assays as a means of quantifying the potential inhalation bioavailability of particle-borne contaminants, such as might arise if contaminated soil particles were aerially entrained and inhaled. To that end, Doug Bright (UMA, Victoria) will present on the status and results of a literature review and evaluation of this issue.

**Health Canada Guidance on Bioavailability Adjustment Factors (BAFs)**

Health Canada’s guidance on preliminary quantitative (screening level) risk assessment for federal contaminated sites acknowledges that BAFs for dermal exposures are appropriate. To that end, Health Canada provides recommended dermal BAF values for a variety of substances. These dermal BAFs are based on a review of literature conducted by the Ontario Ministry of Environment and Energy (OMEE) several years ago. Those BAFs are acknowledged to be dated and inadequate; hence the reason that new research using actual human skin was initiated with EHSB.

International efforts have been made over the past decade to validate *in vitro* measures of bioaccessibility of lead and arsenic using *in vivo* models. However, the incorporation of oral BAFs (based on site-specific bioaccessibility assay results) for other inorganic contaminants are now being applied in Canada, despite not being subjected to similar *in vivo* validation. It is anticipated that the application of *in vitro* bioaccessibility to adjust ingestion exposures for virtually all inorganic elements will continue and increase in the absence of *in vivo* validation. This is certainly evident in Canada.

In order that sufficient data are generated to satisfy Health Canada that the site-specific BAFs (based on bioaccessibility) are reasonably valid for application at federal contaminated sites in Canada, and that the data are interpreted and applied correctly, Health Canada has prepared an initial review of this issue and proposed some recommended data submission requirements. Those
recommendations will be incorporated into our guidance document on detailed site-specific risk assessment (Draft 3 in progress), and are also presented in:


This manuscript will be distributed to workshop participants for information, and discussion as may be appropriate.

**What Further Work or Research Should Health Canada Facilitate With Respect to Soil Bioavailability?**

Relative to US and European agencies, Health Canada’s resources for this issue are very limited. However, there are likely specific areas of interest or investigation that Health Canada might pursue that could have specific relevance to this country (and hence are not being actively pursued elsewhere).

Alternately, or as well, linkages could be made with US and European working groups on this topic and Health Canada could consider filling certain “niche” data gaps as part of a larger coordinated effort.

The overall purpose of this first meeting to establish a Canadian Bioavailability Working Group is to become current with Canadian research in this field, and to identify a direction we (both Health Canada and as a working group) should take.
Section 3.0 Proceedings

Workshop co-chairs, Beverley Hale and Ken Reimer, welcomed the participants and outlined the structure of the Workshop. They encouraged active participation from the audience and stressed that the objective was to exchange ideas, identify research gaps, and explore the potential for forming a Canadian bioaccessibility working group.

Mark Richardson, Senior Health Risk Assessment Specialist with Environmental Health Assessment Services, Contaminated Sites, Health Canada, outlined his objectives for the Workshop (see Section 2.0). He described his department’s role in advising federal government departments as part of the contaminated sites program and the challenges of including bioaccessibility measurements, given that there is no standard laboratory method. Health Canada is developing a bioavailability data package describing the information that has to be included if such measurements are included in risk assessments. Key elements of this are the simulated gastric fluid-to-soil mass ratios and the soil particle grain sizes – both of which can potentially influence the experimental outcome. Such measurements are applied to simulated ingestion scenarios but Health Canada is also interested in oral bioavailability/bioaccessibility from dust and dermal contact. Finally, he described his goals for the Workshop – especially to create linkages among Canadian and international researchers in order to complement, not duplicate, efforts.

The first series of talks described the results of projects conducted on behalf of Health Canada. Christopher Ollson, the director of Environmental Risk Assessment at the Ottawa office of Jacques Whitford, gave a critical review of the most current data on the oral bioavailability of arsenic and lead from soil and raised the question of whether in vitro testing had progressed to the point where it could be used for human risk assessments. His review had found that for both elements the bioavailability was less than 100 percent but that in most cases, the studies had not included sufficient information on several key physical and
chemical properties of soils: soil grain size, chemical fractionation, chemical speciation and organic carbon content. Also, it was apparent that rat models were returning results that seemed uncharacteristic of other animal bioavailability tests. The ensuing discussion emphasized the limited variety of soil types and the need to relate to bioaccessibility results to soil characteristics.

Matt Dodd, a Research Professor at Royal Roads University, continued the discussion on various soil properties – total organic carbon, particle size, pH, cation exchange capacity and mineralogy and especially grain size – that might influence bioavailability and bioaccessibility. He outlined the results of experiments conducted on soils taken from light stations in British Columbia. He noted that the effect of soil particle size and dilution on bioaccessibility was not straightforward, and the other soil characteristics were also important. For some of the elements, such as arsenic in one soil, grain size effects were significant. During the discussion period, the influence of settling times for different particle sizes was raised but it was determined that they did not have a measurable effect. There was general agreement that the source of the material – e.g. oxidized lead from smelter sources – could influence the results.

Pat Rasmussen, a Research Scientist with Health Canada, revealed findings regarding the applicability of the European Standard Toy Safety Protocol for the determination of bioaccessibility of metals in household dust. She particularly noted that the prescribed fifty-fold acid volume to sample mass ratio underestimated bioaccessible metal and that optimum results were obtained using a ratio of 2,000. Her results also demonstrated that particle size is a key factor affecting metal bioaccessibility and is a critical parameter in residential dust and soil surveys. Several members of the audience expressed opinions regarding ratios, and the point was made that the method also has to be practical in terms of laboratory implementation.

Richard Moody, another Research Scientist with Health Canada, discussed findings of the Dermal Absorption Research Team (DART) regarding
bioavailability of soil contaminants that come in contact with the skin. He described experiments with organic contaminants such as benzo[a]pyrene (BaP) and various skin models. This is a complex field and several members of the audience wondered about the effects of sweat and changing permeabilities with age, although it was concluded that there was little information on these points in existing literature.

Doug Bright, Manager, Earth and Environmental, British Columbia, UMA Engineering, presented the results of a review of the pulmonary bioavailability of particle bound contaminants. He pointed out that inhaled particulates are often ignored but that there is no good reason for doing so. He described the various categories of inhalation risks and concluded that the solubility of particle-borne contaminants in lung fluids can have a significant influence on risk. He made the point that it is not currently feasible to include in vitro pulmonary measurements in risk assessments. There was general agreement that current risk models assume 100 percent bioavailability because of the absence of reliable alternative information but that it was possible that the field could move in the same direction as soil ingestion work.

Joanna Wragg from the British Geological Survey provided a European perspective on bioaccessibility. She described the historical development and achievements of the Bioaccessibility Research Group of Europe (BARGE) which was formed to develop more realistic risk factors when dealing with the legacy of contaminated lands in 16 EU countries. She noted that a variety of bioaccessibility models have been used across Europe, and discussed the outcome of the first BARGE publication comparing five in-vitro models used in a round-robin trial to measure the bioaccessibility of arsenic, cadmium, and lead in three soils. The reasons for the resulting variability of bioaccessibility values were discussed, as were the results of a second round robin that included the simulation of fed and fasted conditions. Currently, BARGE is working on the development of a unified bioaccessibility method. She indicated BARGE’s interest in international collaboration and noted that two participants of the
Workshop (Canada’s Ollson and Basta from the US) were members of the BARGÉ committee. There was considerable discussion about the TNO Gastrointestinal Model (TIM) as it incorporates microorganisms but is time consuming; it was thought that it might be useful to put some soils through this method. As well, people considered, without conclusion, the role of other organic matter in the GI tract and the significance of fasted versus fed conditions. It was noted that BARGÉ will emphasize this in future tests.

A United States perspective was provided by Nick Basta, Associate Professor of Soil and Environmental Chemistry with the School of Natural Resources at Ohio State University. He addressed some key points including the reliability of bioaccessibility tests, their proper application and public acceptance. He presented data from animal testing experiments for arsenic, lead, and cadmium and compared these to the results from in vitro measurements using the same soils. He noted that, given the high initial concentrations of these contaminants in the feeding studies, there may not be animal models for moderately contaminated soils. Specifically, most validation studies use doses at 1,000 or 10,000 mg/kg – can these results be extrapolated to 100 mg/kg of a contaminant? Acceptance of bioaccessibility testing by policy makers is important and currently in the United States such acceptance is on a regional, rather than a national basis. Lastly, Dr. Basta indicated that there was a need for more collaboration among U.S. scientists and applauded the efforts of BARGÉ and encouraged the formation of an equivalent organization in Canada. Questions ranged from specifics – are there any differences between fresh and stored soils (answer – no), how important are redox controls (not for arsenic), to the concern noted above regarding the high concentrations used in animal models.

This ended the discussion on the first day and, given the intense discussions following each of the talks, it was decided to adjourn.
DAY TWO

Co-Chair Beverley Hale, of Land Resource Science at the University of Guelph, presented research completed by herself, Debbie Chan, W. Black and M. Waisberg on *in vitro* digestion dialysis of cadmium compared with *in vivo* data obtained from rabbit feeding studies. These studies utilized plant incorporated and soluble salt amended lettuce and grain. They found that dialysis membranes could rank foods for the bioaccessible portion of Cd, but couldn't predict the actual fraction of Cd that would be absorbed by the ingesting animal. Caco-2 cells were used to simulate intestinal absorption and it was determined that if the *in vivo* bioavailable fraction had been determined for the whole body, the *in vivo* and Caco-2 cell values would be similar. A point was raised regarding the ability to differentiate between ab- and ad-sorption in the Caco-2 experiments, but it was noted that it was not possible in this case – although they are working towards this goal.

Margo Moore, Professor of Toxicology/Microbiology with the Department of Biological Sciences at Simon Fraser University, Vancouver, BC, discussed the bioavailability of hydrophobic contaminants. She began her presentation by noting the difficulties in assessing the properties of the thousands of organic industrial chemicals come onto the market each year and speculated that bioaccessibility might be able to play a role. Her work focused on $^{14}$C-benzo[a]pyrene (BaP) bound to digestible (skim milk powder) and non-digestible (soil) matrices. She used an *in vitro* model that incorporated full gastrointestinal digestion plus sorption to Caco-2 cells or to ethylene vinyl acetate (EVA) film. The study found that it was possible to extrapolate uptake into biological lipids (Caco-2 cells) from EVA data (at least for BaP). While changes in the bioaccessible fraction were reflected in the differences in the bioavailable fraction under non-equilibrium conditions, at equilibrium sorbed concentrations were not directly correlated with differences in the bioaccessible concentration. This raised a question: which condition is physiologically relevant? One participant asked if one could expect the lipids to saturate at the same rate as EVA, but it was
concluded that it would have to go to very high concentrations. The thickness of the layer was important — the thinner it is, the more rapid the chemical equilibrium but it handling problems would arise if it was too thin. An interesting possible difference was noted between metals and organic molecules in that there was the potential to form micelles in the gastric fluid.

Steven Siciliano, of Soil Science at the University of Saskatchewan, emphasized his belief that microbes are the most important constituent of the GI tract and that key mechanistic processes included the following factors: biosorbent behavior (the sorption of cationic metals to bacteria cell walls), metals acting as electron acceptors for bacteria as oxygen is depleted and bacterial transformations (e.g. alkylation of arsenic) of metals. He currently believes that the relative bioavailability of metals in ingested food depends on: the concentration of the metal in the food, the mode of metal uptake into the foodstuff, and the activity of the gut microflora. Siciliano felt that mechanical methods for estimating bioaccessibility may be confounded by flocs and do not account for direct uptake and that the dynamic nature of bacterial communities will make measurements challenging. There was some discussion of the importance of microbial transformation since during the reference dose experiments similar transformations could have also occurred. It was clarified that the actual redox potential of the gastrointestinal tract is not easily known but it can be low. There was a question regarding how tightly metals are bound to microbes — namely is it so tight that they are no longer available, but this was not considered to be the case. There was some discussion of the potential risk posed by the greater potential for transformations during the longer residence time in the colon.

Brendan Birmingham, the Senior Research Toxicologist with the Human Toxicology and Air Standards section of the Standards Development Branch of Ontario’s Ministry of the Environment, presented findings of research performed with Dino Manca on using bioavailability and bioaccessibility in human health risk assessments. The Ontario process has allowed for the inclusion of such
measurements in site specific assessments for some time but is currently preparing a guidance document addressing oral bioavailability in human health risk assessments. The contents of this document were discussed and the importance of standardization was stressed. Some case studies were also presented including metal results as well as PAHs.

**Ken Reimer**, a Professor in the Chemistry and Chemical Engineering Department and Director of the Environmental Sciences Group at the Royal Military College of Canada described three case studies that were conducted in collaboration with Iris Koch and Chris Ollson. The first study examined the bioaccessibility of arsenic in soils contaminated from gold mining operations in Yellowknife, NWT. The results clearly indicated the importance of such measurements for both human health and ecological risk assessments. Samples had been collected from a wide range of substrates, yet there was no difference in the bioaccessibility for solid:liquid ratios from 1:100 to 1:5000. As well, there were no differences using grain sizes of <250µm or <63 µm. The percent bioaccessibility was a function of both the organic carbon content and the total arsenic concentration in the original samples. Bioaccessibility measurements were also obtained for ironite® and these had good agreement with the absolute bioavailability from hamster feeding studies conducted at the University of Arizona. Results obtained for nickel indicated the need for a modified method that does not include glycine as this complexes with the nickel and artificially increases the bioaccessibility when compared to the animal model. Collaborations have been established between the RMC group, Basta in the US, and BARGE.
Section 4.0 Research Gaps and Future Directions

The afternoon discussion addressed the current status of public and policy acceptance of bioaccessibility/bioavailability. Mark Richardson reiterated that Health Canada supports the use of such measurements in assessments involving federal contaminated sites but stressed that Health Canada only provides guidance and advice and does not have a regulatory role. The Ontario Ministry of the Environment (MOE) does have regulations and the guidance documents for brownfield remediation will clearly describe the data requirements – adequate QA/QC information, numbers of samples, etc. are important issues. It was noted that British Columbia also has regulations which are currently being reviewed but that the province has always been receptive to adjustments for bioavailability. It was suggested that this was also the case for Alberta. Nick Basta indicated that the situation in the United States is split.

There was considerable discussion about the appropriateness of animal models. While there have been significant advances over the years, there are still challenges in that soil dosing experiments are probably at too high a concentration. Nevertheless, there is a soil versus no soil effect and the requirement for in vivo measurements at lower concentrations is quite important.

There was widespread agreement that there is no one model that works for all inorganics (metals and metalloids); indeed, it was accepted that there may need to be several methods developed. The importance of in vitro tests for organic contaminants was emphasized. It was noted that the MOE is not prescribing a bioaccessibility method in their guidance documents, but instead is defining the scientific requirements that have to be satisfied. It was understood that the field is evolving and improvements will continue to be made and methods updated, perhaps on a regular basis. One suggestion was for this group to propose a method to the Canadian Council of Ministers for the Environment (CCME) but with the caveat that it be reviewed every five years with new science. Such an approach would assist in getting ‘buy-in’ and would accomplish the important
objective of public acceptance. Another suggestion was for Health Canada to propose a method and have it reviewed by the group.

It was agreed that there were many factors that still needed to be examined: the effect of grain size, the appropriate solid: liquid ratio, strategies to ensure representative sampling. The group identified a requirement to develop a standard material to which they could compare and develop methods – similar to the approach being used by BARGE. The possibility of using standard reference materials (e.g. NIST 2710, 2711), especially those with a range of metal/metalloid concentrations and varying organic carbon content was explored. Bev Hale indicated that MITHE-RN could play a role here as it already has a QA/QC protocol in place.

Funding sources to support round robin and other activities were explored. The Mining Association of Canada was identified as one possibility and everyone agreed that it would be mutually beneficial if the funds were managed in an ‘arms-length’ manner to ensure that the work was seen to be independent. It was further noted that the type of developmental activities being discussed were not contract work but rather research. This led to a general discussion of the viability of a working group and the benefits that would come from having a central organization coordinate activities both nationally and internationally. The need for on-going communications and an annual meeting was identified.

There was unanimous agreement that a working group be formed and Beverley Hale and Ken Reimer agreed to be the co-chairs. Some initial action items were identified:

- Creation of a newsletter to be distributed on a quarterly basis (Ken Reimer agreed to facilitate this).
- Identification of appropriate reference materials for round-robin testing (noted that eventually such materials will have to include organic contaminants and food references).
- Contacting appropriate organizations (e.g. Mining Association, BC Environment officials, etc.) to identify collaboration and funding opportunities.
Editor’s Note: Since the meeting was concluded, it has been proposed to call the group Bioaccessibility Research Canada (BARC). As well, BARGE has included members of BARC (Ollson and Reimer) to participate in their imminent round-robin testing. It is anticipated that the inaugural newsletter will be distributed in early 2006.
ANNEX A

Health Canada Workshop on Bioaccessibility/Bioavailability in Contaminated Sites
Aug. 30-31, 2005

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* Speaker
Section 5.0 Presentations
Health Canada Perspective

Mark Richardson, Health Canada
HC’s Science and Research Activities

- Oral bioavailability & bioaccessibility from soil
  - Compilation of data on As, Cd & Pb (Jacques-Whitford)
  - Meta-analysis of bioavailability & bioaccessibility data as a function of soil properties, assay methods, and other factors
  - Review of key factors influencing bioaccessibility (particle size, ratio of volume of acidic leachate to soil mass) – HERA publication pending
  - Define data package (and eventually perhaps standard method) for conducting bioaccessibility assays at federal contaminated sites – discussed in HERA publication
  - Preliminary research on the influence of particle size and liquid:soil ratio on measures of bioaccessibility (Royal Roads U) – publication(s) in prep

HC’s Science and Research Activities (cont’d)

- Oral bioavailability & bioaccessibility from indoor dust
  - Is the bioavailability/bioaccessibility of contaminants in dust different from that in soil?
  - If so, how is it different? And if so, why is it different?
  - Dr. Pat Rasmussen, Environmental Health Sciences Bureau, Health Canada

HC’s Science and Research Activities (cont’d)

- Dermal bioavailability
  - Data are very limited on dermal penetration of soil-borne contaminants through human skin
  - HC is initiating research on the in vitro dermal penetration of soil-borne contaminants through excised viable human skin
  - Dr. Rick Moody, Environmental Health Sciences Bureau, Health Canada
Contaminated Sites Program SEPSEP

HC’s Science and Research Activities (cont’d)

- Respiratory bioavailability
  - Should adjustments be applied for respiratory bioavailability of inhaled particle-borne contaminants?

- Pulmonary Bioavailability of Particle-Borne Contaminants: A Review – Dr. Doug Bright, UMA Consultants/RBU
  - Review of lung fluid solubility assays
    - Why are they used?
    - How are they conducted?
    - What do they mean?
    - Can/should they be applied to contaminated site risk assessment?

HC’s Current Proposals of Bioavailability ‘Data Package’ for Federal Contaminated Sites

- A range of soil particle size fractions (such as ≤ 45 µm; ≤ 125 µm; ≤ 250 µm) should be assayed
  - If bioaccessibility increases (or decreases) as particle size decreases, then use data for smallest particle size fraction.
  - If bioaccessibility unrelated to particle size, then use data for <250 µm size fraction.

- A range of ratios of simulated gastric fluid (mL) to soil mass (g) must be employed, ranging from 100:1 to perhaps 5,000:1 (possibly up to 10,000:1)
  - If bioaccessibility increases as ratio increases, then use data for highest ratio tested (or testable).
  - If bioaccessibility not influenced by this ratio, then use data from standard methods (100:1 or other ratio).
  - Confirm (through statistical analysis or other means) that limited contaminant solubility in simulated gastric fluid is not confounding the measure of bioaccessibility.

HC’s Goals for this 1st Canadian Bioavailability Workshop

- Establish a Canadian Working Group on Bioavailability
  - How does HC link broader industry and consultant interest with researchers?
  - How to link to US and European (and elsewhere?) activities

- Come up to speed on what Canadian researchers are doing in this subject area

- What further work or research should HC facilitate with respect to soil bioavailability?
  - Compliment, don’t duplicate, work elsewhere
  - What niche can Health Canada fill?
Critical Review of As and Pb \textit{in vivo} and \textit{in vitro} Studies

\textit{Christopher Ollson, Jacques Whitford Limited, Ottawa Office}
Critical Review of As and Pb in vivo and in vitro Studies

Christopher Ollson, Ph.D.
Director, Environmental Risk Assessment
Ottawa Office
Jacques Whitford Limited

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

Federal Government - Health Canada

Unless on-site soils have been subjected to tests of bioavailability / bioaccessibility, it should be assumed for purposes of risk assessments that the gastrointestinal absorption for any contaminant is 100%.

(Health Canada, 2003)

At this point Health Canada has not settled on a specific test methodology. Current position is that in vitro tests are here to stay and in vitro test being used must have been validated against in vivo animal models for inorganic elements.

Scope of Work

In keeping with the need to standardize the manner in which risk assessment is conducted, Health Canada retained Jacques Whitford Limited to conduct a critical review of the most current data on the oral bioavailability of arsenic, lead and cadmium from soil and to provide recommendations on how it can be incorporated into site specific risk assessment (SSRA).
Soil Properties

Research has shown that there are several key physical and chemical soil properties that would govern the potential bioavailability or bioaccessibility of inorganic elements from soil.

- soil grain size
- chemical fractionation
- chemical speciation
- organic carbon content

In Vivo Arsenic Bioavailability Studies

Thirteen in vivo studies involving the oral administration of arsenic-bearing soil to laboratory animals were reviewed:

- rats (3),
- rabbits (3),
- dog (1),
- swine (4),
- monkey (2).

In Vivo Arsenic Summary

- 55 soil samples were subjected to bioavailability experiments on an average soil concentration of arsenic of 1950 ± 3760 ppm.
- The average percent bioavailability of arsenic was 23 ± 20%.
- Range <5% to 98%
- Unfortunately, the majority of the bioavailability studies did not report the physiochemical properties of the soils tested, other than the total arsenic concentration.
- The majority of studies appear to have been conducted on soils impacted by mining or smelter operations and don’t reflect a wide variety of contaminated soils.
**In vitro Arsenic Tests**

- 102 soil arsenic bioaccessibility results were reported.
- Average soil concentration of 1204 ± 2775 ppm.
- The average percent of arsenic that was bioaccessible was 25.6 ± 19.0%.
- Ranged from <1% to as high as 95%.

**Arsenic Summary**

- 100% of soluble arsenic in the GI Tract is assumed to be absorbed across intestinal wall, thus arsenic bioaccessibility = bioavailability.
- Examination of the bioaccessibility testing that has been reported in the literature indicates that regardless of the soil type or area of the world it was collected, it is unlikely that the oral bioaccessibility of arsenic would be 100%.
- The range of <1% to 95% in arsenic bioaccessibility indicates that arsenic bioaccessibility varies considerably by site.
- However, the 95th percentile of arsenic bioaccessibility (in vitro experiments) was 59%, and the 95th percentile in vivo bioavailability was 52%.
- Therefore, a reasonable upper limit oral bioavailability / bioaccessibility of arsenic from soils might be 60%.

**Gastric pH and liquid:solid Ratio**

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<thead>
<tr>
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<td>pH1</td>
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</tr>
<tr>
<td>pH2</td>
<td>0.903</td>
<td>1.000</td>
<td>1.000</td>
</tr>
</tbody>
</table>

**Total Soil Arsenic Influence on Bioaccessibility**

- 100% of soluble arsenic in the GI Tract is assumed to be absorbed across intestinal wall, thus arsenic bioaccessibility = bioavailability.

**In Vivo Lead Relative Bioavailability Experiments**

- Studies reviewed involving lead soil administration:
  - Male swine models
  - Rat models
  - Human feeding trial.
- Many of the researchers did not report the physiochemical properties of the soils that may be important determinants of lead bioavailability – pH, TOC, grainsize, etc.

**Relative Bioavailable Fraction**

The RBA is generated using the following equation:

$$RBA_{soil} = \frac{ABA_{soil}}{ABA_{lead\ acetate}} \cdot RBA_{rel}$$

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

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

$$RBA_{rel} = \frac{ABA_{soil}}{ABA_{lead\ acetate}}$$
**In Vivo Lead Relative Bioavailability Studies**

- 27 soil samples were subjected to relative bioavailability experiments.
- Average soil concentration of lead of 6067 ± 3521 ppm.
- The average percent relative bioavailability of lead was 46 ± 27%.
- US EPA traditionally uses a RBA of 60% in risk assessment, both in the IEUBK model and straight risk assessment dose calculations.
- Assumption lead in soil has a constant absolute bioavailability of 30%, while lead as lead acetate (using in TRV development by WHO and IEUBK modeling by US EPA) has a ABA of 50%.
- RBA of soil lead of 60% (0.3/0.5 = 0.6 or 60%).

**In Vitro Lead Experiments**

- A total of 86 discrete soil lead bioaccessibility results.
- Average soil concentration of lead of 3180 ± 3285 ppm.
- The percent of lead that was bioaccessible varied dramatically between the stomach phase and intestinal phase.
- The average bioaccessibility of lead in the stomach phase from all tests was 51 ± 26%, and decreased to only 4.2 ± 5.0% in the intestinal phase.
- The bioaccessible fraction of lead from soil ranged from <1% to as high as 86%.

**Summary of Pb In vivo Studies**

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

**Total Soil Lead Influence on % Bioaccessibility**

- $y = -0.0205x + 120.05$  $R^2 = 0.3413$
Influence of Soil pH on Lead Bioaccessibility

\[ y = 7.2698x - 5.587 \]
\[ R^2 = 0.3585 \]

Pb Summary

- The 95th percentile of relative bioavailable fraction from the stomach phase was 80%
- This suggests that a reasonable maximum oral relative bioavailability of lead from soils of 80% might be appropriate for use in human health risk assessments.

The Questions that Still Remain!

- Do in vivo animal experiments need to be conducted on a site specific basis?
- Has the science of in vitro bioaccessibility experiments advanced to the point where they can be used to provide a valid representation of bioavailability that can be used in risk assessment?
- Can literature default values be used in human health risk assessment in lieu of site specific soil testing?
- How should bioaccessibility numbers be incorporated into site specific risk assessments
  - Dose adjustments
  - TRV adjustments
Effect of Soil Particle Size and Different Liquid-to-Soil Ratios on the Bioaccessibility of Metals in Contaminated Soils

Matt Dodd, School of Environment & Sustainability, Royal Roads University

Apart from the characteristics of the metal contaminant itself, it has been suggested that bioavailability is dependent on various physical and chemical properties of a soil, including total organic carbon (TOC), particle size, pH, cation exchange capacity (CEC) and mineralogy. However, limited literature data demonstrate the effects of these properties, especially soil particle size distribution, on bioavailability.

Bioaccessibility, the most common surrogate for bioavailability, is generally determined from an in vitro extraction using simulated gastric juice and soil sieved to <250 µm. The typical gastric fluid-to-soil ratio is 100:1 (ml:g). Due to the lack of literature data on the effect of soil particle size distribution and gastric fluid-to-soil ratio on bioaccessibility, studies were conducted to evaluate how changes in these two parameters affected bioaccessibility using the simplified stomach phase extraction protocol developed by the Solubility/Bioavailability Research Consortium (SBRC).

Bulk soil samples were collected from three Canadian Coast Guard light stations in British Columbia and one non-light-station location in Southern Ontario to represent different sources of metal contamination and variable concentrations. In addition to the conventional <250 µm fraction, the soil samples were also sieved into the following size fractions: <250 – 125 µm; <125 – 63 µm; <63 – 44 µm and <44 µm. The bioaccessibility of As, Cd, Co, Cr, Cu, Pb, Ni and Zn in these soil particle size fractions were determined using the SBRC protocol. The results obtained – including the effect of other bulk soil properties such as pH, TOC, Fe and Mn content on bioaccessibility – will be presented.

To study the effect of dilution, extractions were conducted using liquid-to-soil ratios of 100:1, 250:1, 500:1, 1,000:1 and 2,000:1. Data obtained for three of the soil samples and NIST 2711 standard reference material will be discussed. The presentation will conclude with our work to date on the collection of PM-10 size fraction for bioaccessibility assays and proposed studies using sequential stomach and intestinal phase extractions.
Effect of Soil Particle Size and Different Liquid to Soil Ratios on Metal Bioaccessibility

Matt Dodd, Doug Bright and Mark Richardson

Factors that Affect Bioavailability of Metals in Soils Include:
- Soil pH
- Soil organic matter content (TOC)
- Redox reactions
- Fe and Mn content
- Metal speciation
- Weathering/aging
- Soil particle size

Summary of Factors that Affect Lead Bioavailability

Test Method
- Bioaccessibility conducted using SRBC simplified stomach phase extraction protocol
- SOP
  - <250 µm soil
  - 1.0 g soil
  - 100 mL glycine/HCl buffer at pH 1.5
  - Liquid to soil ratio 100:1
  - 1-h extraction with end-to-end rotation
- Triplicate analyses

Inter-Lab Comparison

From Ruby et al. 1999, EST, 23(12): 3697 - 3705
Test Soils
- 3 CCG Pacific Lightstations
  - Cape Mudge
  - Portlock Point
  - Discovery Is.
- Southern Ontario
  - Port Colborne

Soil Properties
- Cape Mudge (CPM)
  - Near light tower
  - Paint flakes
  - Sandblasting grits
  - Sand, silt and gravel

Soil Properties (cont’d)
- Portlock Point (PLP)
  - Former refuse burn area
  - Fused metal bits, glass, melted plastic and other burnt residue
  - Dark brown to black sand and silt
- Discovery Island (DIS)
  - Overgrown landfill
  - Organic rich silty sands
- Southern Ontario (SOT)
  - Residential backyard in Port Colborne
  - Nickel refining
  - Sand and silt

Analysis
- Soil pH
- Total metals
- Organic carbon
- Inorganic carbon
- Sulphur
- Major oxides
  - SiO₂, Al₂O₃, Fe₂O₃, CaO, MgO, Na₂O, K₂O, Cr₂O₃, TiO₂, MnO, P₂O₅, SrO and BaO

Some Properties of the Test Soils

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<th>PLP</th>
<th>DIS</th>
<th>SOT</th>
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<td>TIC (%)</td>
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<td>0.52</td>
<td>0.07</td>
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<td>0.55</td>
<td>0.88</td>
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</table>

Particle Size Effect Studies
- Homogenized soil oven dried at <40°C
- Sieved to
  - <250 - 125 µm
  - <125 - 63 µm
  - <63 – 44 µm
  - <44 µm
Soil Particle Size Distribution in Test Soils

TOC and pH Changes by Particle Size
- TOC increased with decreasing size fraction
- No significant changes in pH with size fraction

Cape Mudge Total Metal Conc in Soil Size Fractions

Southern Ontario Metal Bioaccessibility as a Function of (A) Total Fe and (B) TOC
Summary of Soil Particle Size Effects

- Effect of soil particle size distribution on bioaccessibility is not straightforward
- Bioaccessibility also dependent on other soil properties such as TOC, mineralogy and source of metal contamination
- For some of the elements, changes in bioaccessibility values between the different particle size ranges were substantial
  - Bioaccessibility of As in CPM
    - 34% using SOP particle size of <250 µm
    - 30% for <250 - 125 µm
    - 98% for <45 µm

Effect of Varying Liquid to Soil Ratio

- CPM, SOT and PLP sieved to <250 µm
- NIST SRM 2711 Montana soil used as is
- Liquid to soil ratios of
  - 100:1
  - 250:1
  - 500:1
  - 1000:1
  - 2000:1
- Triplicate analyses

Current Studies

- Bioaccessibility of metals in PM-10
- Soil particle size and dilution effects using the sequential stomach and small intestinal phase extraction
- Bioaccessibility of barite using both the simplified stomach phase extraction and the sequential stomach and small intestinal phase extraction
PM-10 Sampler

PM-10 Sampling
Determination of Bioaccessible Metals in Household Dust: Modifications to the European Standard Toy Safety Protocol

Pat Rasmussen, Health Canada

In this study, the European Standard Toy Safety Protocol (EN 71-3:1994) was evaluated for its applicability to dust and soil samples, to provide a simple and reproducible in vitro method of estimating the ingestion bioaccessibility of metals.

The EN-71 protocol is a simulated stomach acid extraction in which the sample is mixed with 50 times its mass of a dilute HCl solution (pH 1.5) for two hours at 37°C. The solution is then centrifuged and analyzed by ICP-MS.

In this study the acid volume to sample mass ratio was varied over two orders of magnitude using certified reference material (NIST 2583). Relative bioaccessibility of metals increased as the acid volume-to-sample mass ratio increased, indicating that the fiftyfold acid volume to sample mass ratio prescribed by the EN-71 Toy Safety protocol is inappropriate for testing household dust, due to the likelihood of underestimating bioaccessible metal.

Optimum results were obtained using a ratio of 2,000. The modified method was then applied to different size fractions of indoor dust. Relative bioaccessibility of Cd, Pb, Ni and Cu in the fine size fraction of indoor dust (<36 micron) ranged from 66 percent to 80 percent (n=6), while bioaccessibility of these metals in the coarse fraction of dust from the same house (80 to 150 microns) ranged from 23 percent to 33 percent (n=6). These results show that particle size is a key factor affecting metal bioaccessibility and is thus a critical parameter in residential dust and soil surveys.
Determination of bioaccessible metals in household dust: modifications to the European Standard Toy Safety Protocol

Pat Rasmussen, Environmental Health Sciences Bureau, Safe Environments Program, Health Canada

Health Canada Bioavailability Workshop Delta Hotel, Toronto
August 30, 2005

Factors affecting bioaccessibility of metals in household dust

- Analytical protocol to determine bioaccessible metal
  - mass to volume ratio
  - particle size fraction
- Mineral form of metal (species) in dust
- Relationship with organic matter in dust matrix
  - Future focus for our research
  - Organic carbon is elevated in house dust compared to soil and appears to be an important factor influencing bioaccessibility
- Considerations in survey design

Residential dust surveys:
50 single family homes in Ottawa


Applicability of Toy Safety Method for Household Dust

- Goal - develop simple, inexpensive, and reliable test for migratable metals in household dust surveys.
- Responsibility to provide information to participants in residential surveys.
- Requirements:
  - fast turn-around time
  - reproducible values

Total Pb in Ottawa dust & soil (geometric mean; n=50)

Test for Migratable Metals in Toys

EN 71-3: European standard for the migration of certain elements from toys (Sb, As, Ba, Cd, Cr, Pb, Hg, Se)

- Extraction uses only dilute HCl (pH 1.5) to simulate stomach acid
- Omits mouthing/mastication - assumes toy is small enough to be swallowed
- Omits passage through intestine
- Product Safety Bureau, Health Canada

Summary of Results

Certified Reference Material NIST 2583 – Indoor Dust

- EN-71 Protocol:
  - Place test sample in 5 mL 0.07 M HCl solution (pH is kept at 1.5); 2 hr in water bath at 37°C; 1 hr shaking followed by 1 hr no shaking; centrifuge & dilute as required for ICP-MS
  - EN-71 prescribes a volume:mass ratio of 50 (but allows ratio up to 500)

- Modification to EN-71 protocol:
  - Test a range of volume:mass ratios from 250 to 5000
  - Incrementally decrease sample mass from 100 mg to 10 mg and incrementally increase acid volume from 25 mL to 50 mL
  - For test sample use NIST indoor dust standard reference material
  - Minimum 6 replicates for every volume:mass ratio
  - Calculate % bioaccessibility using migratable metal value as numerator and certified total metal value as denominator

Migratable metals in indoor dust vs. outdoor soil (EN-71)
Ottawa and Lanark County control samples

Summary of Results

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  - Calculate % bioaccessibility using migratable metal value as numerator and certified total metal value as denominator
Analytical reproducibility at different volume:mass ratios

Pb in NIST 2583 Indoor Dust
error bar = 1 sd; n = 6 replicates

% bioaccessible metal

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

RSD = 10% at ratio of 5000

Nickel in NIST 2583 Indoor Dust
error bar = 1 sd; n = 6 replicates

% bioaccessible metal

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

RSD = 26% at ratio of 5000

Copper in NIST 2583 Indoor Dust
error bar = 1 sd; n = 6 replicates

% bioaccessible metal

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

RSD = 18% at ratio of 5000

Tin in NIST 2583 Indoor Dust
error bar = 1 sd; n = 6 replicates

% bioaccessible metal

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

RSD = 113% at ratio of 5000

Molybdenum in NIST 2583 Indoor Dust
error bar = 1 sd; n = 6 replicates

% bioaccessible metal

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

RSD = 57% at ratio of 5000

Beryllium in NIST 2583 Indoor Dust
error bar = 1 sd; n = 6 replicates

% bioaccessible metal

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

RSD = 23% at ratio of 5000
**Experimental Design:**

**Purpose:**
Quantify changes in bioaccessibility & reproducibility in different particle size fractions of dust.

- Vacuum samples (2 yr) from 50 yr old Ottawa single family home.
- Dry and sieve to 150, 80, 56, 36 micron size fractions.
  - Place 25 mg test sample in 50 mL 0.07 M HCl solution (pH is kept at 1.5); 2 hr in water bath at 37°C; 1 hr shaking followed by 1 hr no shaking; centrifuge & dilute as required for ICP-MS.
  - 6 replicates for every size fraction.
  - Calculate % bioaccessibility using migratable metal value as numerator and total metal value as denominator.

---

**Improvement in reproducibility with decrease in particle size**

*“Faraday” household dust control*

---

**Factors affecting bioaccessibility of metals in household dust**

- Analytical protocol to determine bioaccessible metal
  - mass to volume ratio
  - particle size fraction
- Mineral form of metal (species) in dust
- Relationship with organic matter in dust matrix
  - Future focus for our research
  - Organic carbon is elevated in house dust compared to soil and appears to be an important factor influencing bioaccessibility Rasmussen (2004) CJASS, volume 29, no.3, pp 166-174.
- Considerations in survey design

---

**Particle size vs. metal bioaccessibility**

*“Faraday” household dust control*
• Cadmium in rural soil samples is largely associated with fraction defined as soluble organics, suggesting that it would be readily solubilized in the human digestive system.

*note: Watch the Denominator!*

\[
\% \text{ bioaccessible} = \frac{\text{migratable metal value}}{\text{total metal value}} \times 100
\]

We found that we needed to modify US-EPA 3051 in order to obtain acceptable recoveries for total metals in urban environmental samples.
Dermal Absorption Research Team (DART):
Bioavailability of Soil Contaminants in Overview

Richard Moody, Health Canada

An overview of the Automated In Vitro Dermal Absorption (AIDA) method under development in our laboratory will be given while focusing upon its use for determining the bioavailability of soil contaminants.

Details of the method used for preparing dermatomed thin specimens of fresh (viable) human skin will be given, and the basic AIDA techniques will be used (e.g. robotic fraction collector, Bronaugh flow-through diffusion cells).

Various aspects pertinent to method validation, such as use of fresh versus frozen skin, will be briefly considered. How this relates to the analysis of blood simulant receiver samples and the levels of soil contaminant detected persisting in the skin specimen (i.e. the skin depot) will be discussed.

Finally, new data concerning the lipophilic polycyclic aromatic hydrocarbon (PAH), benzo[a]pyrene (BaP) will be presented with particular emphasis on data obtained for the skin depot for 24 hours versus extended 42-hour exposure tests conducted with 14C-labelled-B[a]P-spiked soil.

The bioavailability of this skin depot and its relevance to Bioavailability Adjustment Factors (BAFs) will be explored. Our contention is that the BAF for B[a]P needs to include at least some of the skin depot as being percutaneously absorbed, hence bioavailable.
Dermal Absorption Research Team (DART): Bioavailability of Soil Contaminants in Overview

Richard P. Moody Ph.D.
Presented August 30, 2005
HC Bioaccessibility Workshop, Toronto, ON

Purpose of today’s talk

• to give an overview of our Automated In vitro Dermal Absorption (AIDA) theory/methods and our latest data re. benzo[a]pyrene, a lipophilic soils contaminant.

• To generally introduce our new project entitled “Dermal absorption of hazardous waste site soil contaminants: Petroleum hydrocarbons and metals”.

AIDA Research/Development Plan

Dermatotoxicokinetics

The Complexity of Human Skin Tissue

Mentionables

- Our standard AIDA method uses the Bronaugh system. All tests use fresh viable human skin from the Ottawa Hospital.
- We have 3 ongoing project areas, CRTI tests with RMC at Kingston, soil exposure (HIB) and cold storage (our E&OT) project. We are presently winding down our CRTI tests.

Methods

- We have now modified our AIDA method to test dermal absorption of soil contaminants.
- We selected a common gardening soil (President’s Choice Magic brand) for our tests sieved through 710 um (composition, soil particle size?).
- We spike soil with radiolabel dissolved in acetone and evaporate the acetone under nitrogen (effect of soil ageing?).
Fridge/Freezer Cold Storage Research

Early AIDA Cell Data

Effect of fridge (red) or freezer (green) storage on skin permeability to tritiated water

More recent AIDA data for Nonyl Phenol (AIDA system used)

Foxy Oxygen Fibre Optic Probe

Soils Project: ^14C-B[a]P data (soap ‘wash-in’ effect?)

Data with ^14C-B[a]P applied in soil

Recovery Data (%)

Absorption Data (%)

Total % absorption into receiver

Total % absorption into membrane (depot)

Total % absorption (receiver + depot)

Recovery Data (%)

Soap & water skin wash % recovery

Apparatus wash % recovery

Accountability (%)

Total mass balance % recovery

n = # of skins/volunteers

24 hour ^8[a]P data

Without Soil (n = 6) With Soil (n = 5)

Mean S.D. Mean S.D.
### Absorption Data (%)

<table>
<thead>
<tr>
<th></th>
<th>Without Soil (n = 5)</th>
<th>With Soil (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean</strong></td>
<td>15.30</td>
<td>3.78</td>
</tr>
<tr>
<td><strong>S.D.</strong></td>
<td>2.74</td>
<td>0.86</td>
</tr>
<tr>
<td>Total % absorption into receiver</td>
<td>10.7</td>
<td>8.6</td>
</tr>
<tr>
<td>Total % absorption into membrane (depot)</td>
<td>9.23</td>
<td>7.4</td>
</tr>
<tr>
<td>Total % absorption (receiver + depot)</td>
<td>15.8</td>
<td>9.3</td>
</tr>
<tr>
<td>Recovery Data (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soap &amp; water skin wash % recovery</td>
<td>30.6</td>
<td>8.01</td>
</tr>
<tr>
<td>Apparatus wash % recovery</td>
<td>5.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Accountability (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total mass balance % recovery</td>
<td>85.2</td>
<td>8.74</td>
</tr>
</tbody>
</table>

*n = 4 due to outlier exclusion. (low % recovery).

### Soil data for skin exposed to 14C-B[a]P for 24 & 42 hrs

(placeholder for actual data)

**In case I forget: Why is stats comparison of depots important?**

- The purpose of conducting tests for the longer 42 hr duration was to test the bioavailability of the skin depot. This is a method advocated by the Bronaugh (US-FDA) research group.
- Very simply, if the B[a]P levels (% recovery) declined following an extended exposure period (from 24 to 42 hrs), this would be reflected in a significant loss of B[a]P from the depot.
- As this was not observed one could assert that the depot is not bioavailable... however →

### Continued jet-lag:

- However, in our dataset the S.D. was high:
  - Depot 24 hr – soil was 45 ± 10.3% (n = 6) at 24 hrs
  - but was 39 ± 9.2% (n = 5) at 42 hrs.
- This leaves some room for an actual difference.
- Hence it could still be argued that at least some of this depot was bioavailable.
- Also it could certainly become available later on.
Other chemicals to be tested

- At present tests are underway for mercury and we are planning to test other metals such as Pb, Ni, Cd, Cu and other PAHs such as benzantracene or possibly lipophiles such as nonyl phenol, dioxin, PCBs
- The first Hg test showed 3% in the receiver and 72% in the skin depot.

Final Points

- The issue of the bioavailability of the skin depot cannot be overly stressed. It is not even considered by most of the early in vitro dermal absorption literature. For example it is not included in the Flynn database skin permeability ‘Kp’ coefficients.
- Our contention is that the depot should be included as bioavailable unless data exists to the contrary.
- Hence Bioavailability Adjustment Factors (BAFs) should ‘err on the side of safety’ by including the skin depot.

Acknowledgements

- thanks especially to Julie Joncas for technical assistance in the conduct of the new soils B[a]P data and Ih Chu for project management.
- and of course to Mark Richardson (HIB) for project funding☺
Pulmonary Bioavailability of Particle-Bound Particulates: A Review

Doug Bright¹, G. Mark Richardson² and Ross Wilson

1) UMA Engineering Ltd.
2) Health Canada,

The dissolution rate of particulates within the extra-thoracic and thoracic regions of human lungs is an important determinant of systemic exposures versus particulate biopersistence, both of which might have negative health implications.

One way to improve human health exposure estimates when assessing the risks of situations involving the inhalation of particulates, therefore, is to apply an increasingly realistic understanding of the fate of particulates in the pulmonary tract and the substances they carry. Whereas there are strong conceptual and mathematical models describing the deposition and clearance of particulate matter within different parts of the pulmonary tract, there is considerably more uncertainty regarding solubility rates of deposited particles.

Several researchers have measured dissolution rates \textit{in vitro} in simulated lung fluids (such as Gamble’s fluid) especially to evaluate the solubility of radionuclides in alveoli or organic contaminants in combustion-derived particulates. In addition, \textit{in vitro} solubility assays have been used to improve delivery of pharmaceuticals to patients via inhalation (e.g. through a puffer), and to examine the relative dissolution rates of glass-like fibres that can cause chronic irritation when lodged in the thoracic regions of the lungs.

For studies on radionuclide risks, the volume of experimental data provides some confidence regarding the similarity between \textit{in vivo} dissolution rates and solubility rates in Gamble’s fluid, but not in more simplistic \textit{in vitro} media. Given the complexity of particulate distribution, clearance, dissolution and interaction with macrophages in the pulmonary tract, there is insufficient experimental evidence for other types of solubility assessments to allow for the confident use of \textit{in vitro} assays as estimates of \textit{in vivo} contaminant bioavailability.
Of particular importance is the concept that dissolution rate, not absolute percentage solubilization potential, is an important aspect of bioavailability within lung fluid. Whereas in vitro tests of gastrointestinal tract bioaccessibility generally rely on estimates of solubility that reach an asymptote with time, in vitro bioavailability tests in simulated lung fluid must also account for the fact that essentially all undissolved material in inhaled particles within one day of introduction are phagocytized by macrophages, after which further dissolution in the phagolysosome is controlled by chemical and biochemical interactions within the intracellular environment that are very different from those in extracellular lung fluid.

Based on the available studies, any arguments for decreased bioavailability of substances derived from inhaled particulates are necessarily accompanied by a very low level of confidence. The preferred order of lines of evidence for bioavailability/solubility estimates from potentially inhaled particulates is: (i) in vivo studies (for example, intratracheal implantation studies using radiolabelled and non-radiolabelled substances sorbed to particulates); (ii) flow-through tests that use either lung tissue cell cultures or perfused isolated lungs and various simulated lung fluids; (iii) static tests in simulated Gamble’s fluid; and (iv) static or other in vitro tests in other simulation media. Use of the ICRP or similar models to estimate pulmonary bioavailability for non-radionuclides and for particulate types for which they were not intended in situation-specific risk assessments is problematic, since no means currently exist to validate the resulting predictions.
Pulmonary Bioavailability of Particle-Bound Contaminants

Doug Bright
(doug.bright@uma.aecom.com)
Mark Richardson
Ross Wilson

Exposure Pathways

Vapour-phase Inhalation
Drinking Water
Particulate Inhalation
• Deposition in Lower Lungs
• Deposition in Upper Lungs
Dermal
Soil/Sediment Ingestion

At least 4 categories of inhalation-related risks:

1) Substances inhaled with PM$_{2.5}$ fraction (settle in lower lungs) for which RfCs exist (e.g. diesel exhaust: USEPA 1993 RfC for non-cancer effects): tox data typically implicitly take into account pulmonary and systemic fate (whole animal inhalation studies).

2) In larger particles trapped in or transported to upper respiratory tract by "muco-ciliary elevator".

3) In mostly smaller particles with no inhalation-specific TRVs developed; assumed systemic mode of action.

4) “Biopersistent” particles.

Questions

• Does bioavailability from particles $>2.5$ $\mu$m in the upper respiratory tract contribute significantly to systemic uptake, when considering subsequent uptake in the GIT following particle clearance?

• Is dissolution from fine particles ($<2.5$ $\mu$m) in the lungs an important contributor to systemic concentration?

• Can the use of solubility assays in simulated lung fluid, for either finer or coarser particles, improve human exposure estimates based on inhalation pathways?

• Are there other promising methods for the estimation of pulmonary bioavailability that might be used routinely as part of contaminated sites assessment and environmental risk assessment?
“5.1.3 Inhalation Exposures
No guidance has been identified related to relative bioavailability of chemicals inhaled from resuspended soil particles.”

“6.1.3 Inhalation Exposures
No case studies considering the site-specific pulmonary bioavailability of metals in soil have been identified for sites where EPA was the lead regulatory agency.”

“6.2.3 Inhalation Exposures
No case studies considering the relative bioavailability of metals inhaled from resuspended soil particles have been identified in which a state was the lead regulatory agency for the site.”

---

**Improving pulmonary biokinetic fate models:**

1. Estimated amount of air and amount and characteristics of inhaled particles (particle size distribution and biases in contaminant distribution important);
2. Particulars of deposition (dependent on particle size);
3. Rates of clearance;
4. Kinetics of compartmental transfers at 1st and 2nd deposition sites (including solubility controls).

---

**Table 1: Pulmonary areas (after ICRP, 2002)**

<table>
<thead>
<tr>
<th>Extrathoracic Area</th>
<th>Thoracic Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbrev.</td>
<td>Abbrev.</td>
</tr>
<tr>
<td>ET1</td>
<td>BB</td>
</tr>
<tr>
<td>Posterior Nasal Region</td>
<td>Bronchial (trachea, generation 0, and bronchi, airway generations 1–8)</td>
</tr>
<tr>
<td>EE2</td>
<td>sb</td>
</tr>
<tr>
<td>Posterior Nasal Region</td>
<td>Bronchiolar (airway generations 9–15)</td>
</tr>
</tbody>
</table>
| LN
| AI                |
| Lymphatic tissue  | Alveolar Interstitial (airway generations ≥ 16) |
| LN
| LN
| Lymphatic tissue  | Lymphatic tissue |

---

**Other important features of the pulmonary system**

- Thoracic extracellular fluid (e.g., lavage fluid) – pH 7.3
- Contains proteins, lipids, and both organic and inorganic salts.
- Within ~ 1 day, virtually all undissolved particles deposited in lower lung phagocytized by macrophages
- Transferred to an intracellular organelle (phagolysosome) with a pH of ~ 4.5 to 5.5.
- In extrathoracic region, mucociliary clearance rate in the upper air passages ~ 1 to 2% per minute (half-life of ≤ 1 to 2 hours)
Health Canada Bioavailability Workshop
Aug 30-31, 2005, Toronto

PM10 chemical and physical characteristics in two urban areas in northern Italy. Measurements and pulmonary deposition estimate.
http://secus.met.fu-berlin.de/veranstaltungen/Abstracts%20PM10/Zanini.htm

Available Methods

In vivo:
- Mammalian (rodent, dog...) inhalation studies (total, nose only, mouth only) – includes biophysical aspects
- Intratracheal implantation – ignores biophysical aspects (Problem with between-species extrapolation either way)

In vitro:
- Tissue culture or perfused isolated lung tissue
- Simulated extracellular lung fluid (e.g. Gamble’s)
- Simulated intracellular fluids (phagolysosomes)
- Simple, non-physiological solutions
  [Batch or Continuous Flow Thru]

Areas of Research Interest:
- Radionuclide risks (e.g. occupational exposures in uranium mills)
- Biopersistence studies (vitreous fibre dissolution, e.g. relative risks of asbestos-like material)
- Pharmacological (enhanced therapeutant delivery, e.g. by puffer)
- Risks from combustion-type emissions (e.g. incinerator fly ash) or urban and/or industrial air pollution (PM2.5, PM10)
- Risks from particulate inhalation from contaminated soils

Common thread – noted by virtually all researchers - is lack of standardized, validated in vitro techniques

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3. Rates of clearance;
4. Kinetics of compartmental transfers at 1st and 2nd deposition sites (including solubility controls).
Simulated Lung Fluid Used by Davies and Feddah, 2003 (meq/L)

- pH: 7.3-7.4
- Total anions: 156 meq/L
- α-Phosphatidylcholine: 1.0 meq/L
- Protein: 1.0 meq/L
- Sulphate, SO$_4^{2-}$: 2.0 meq/L
- Phosphate, HPO$_4^{2-}$: 7.0 meq/L
- Acetate, H$_3$C$_2$O$_2^{-}$: 1.0 meq/L
- Citrate, H$_5$C$_6$O$_7^{3-}$: 1.0 meq/L
- Chloride, Cl$^{-}$: 31.0 meq/L
- Bicarbonate, HCO$_3^{-}$: 156 meq/L
- Total cations: 145.0 meq/L
- Sodium, Na$^+$: 4.0 meq/L
- Potassium, K$^+$: 2.0 meq/L
- Magnesium, Mg$^{2+}$: 5.0 meq/L
- Calcium, Ca$^{2+}$: 2.0 meq/L

Table 4: Composition of Simulated Serum Ultrafiltrate (after Eidson and Mewhinney, 1983)

<table>
<thead>
<tr>
<th>Salt</th>
<th>Molar Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>0.116</td>
</tr>
<tr>
<td>NH$_4$Cl</td>
<td>0.011</td>
</tr>
<tr>
<td>NaH$_2$PO$_4$</td>
<td>0.001</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.005</td>
</tr>
<tr>
<td>Na$_2$ Citrate</td>
<td>0.0003</td>
</tr>
<tr>
<td>CaCl$_2$</td>
<td>0.0002</td>
</tr>
<tr>
<td>L-Cysteine</td>
<td>0.001</td>
</tr>
<tr>
<td>H$_2$SO$_4$</td>
<td>0.0005</td>
</tr>
<tr>
<td>Na$_2$HPO$_4$</td>
<td>0.001</td>
</tr>
<tr>
<td>DTPA</td>
<td>0.0002</td>
</tr>
<tr>
<td>ABAC</td>
<td>50 ppm</td>
</tr>
</tbody>
</table>

*Diethylenetriaminepentaacetic acid, not present in blood serum.
*Alkylbenzyldimethylammonium chloride added as an antibacterial agent.

Biopersistence studies:

- In vivo (intratracheal installation) dissolution rate constants, $K_{dis}$, agree well with in vitro estimates (using modified Gamble’s);
- However, long fibers (> 20 µm) are too long to be enveloped effectively by the alveolar macrophages, thought to be most affected by dissolution because they spend the most time following inhalation in the extracellular environment of the lung.

What Bioavailability Estimate Should We Use For Tin?

- Sn – oral RfD available, but no dermal or inhalation RfD(?)
- Estimated oral bioavailability (ORNL) from ingested soil: ~5-7%
- Systemic availability from inhaled fine particulate fraction of soils?
- If assume 100% absolute bioavailability by pulmonary route, this is ~ 14 x greater than via oral route.
1. oral bioavailability in lab rats by gavage;
2. pulmonary bioavailability in lab rats by intratracheal instillation
3. In vitro solubility studies (static pH2 and pH7.4) and packed column extractions (buffered, pH9, saline)

"Intratracheal installation of flyash resulted in an order of magnitude greater absorption of lead into the kidney after twenty-four hours as compared to G.I. tract absorption."

In vitro solubility studies did not agree with gavage or intratracheal instillation results. "not good predictor"

Batch and column studies did provide information on MSW fly as buffering properties.

Conclusions

- Solubility of particle-borne contaminants in lung fluids can have a significant influence on estimates of risk, ... relatively widespread application of these assays in health physics (radiological risk assessment).
- Not currently feasible to confidently use in vitro pulmonary solubility assays within contaminated site risk assessments for other particle-borne contaminants.
- Lack of unambiguous research and information regarding the interpretation and application of such assays.

Next directions:

Particle size distribution/composition always an important aspect.

Further research needed to -

1. Ascertain the relative contribution of inhaled doses to absolute systemic load in combination with oral and dermal exposures, to refine screening level and detailed RA assumptions;
2. Further characterize lung fluid(s) to ascertain the most appropriate surrogate or simulated lung fluid (both extracellular and intracellular) for use with non-radioactive contaminants and the quantities of both fluid and particulate that should be employed in such in vitro assays;
3. Measure in vitro solubility of PM10 and PM2.5-borne inorganic contaminants in a variety of simulated or surrogate lung fluids to determine likely maximal dissolution rates to refine and compare to results of tasks 1 and 2.
The Bioaccessibility Research Group of Europe (BARGE) was established within the Contaminated Land Rehabilitation Network for Environmental Technologies (CLARINET) to address the urgent need for more realistic measures for bioavailability of soil contaminants from the ingestion pathway than the 100-percent uptake model. The cost of dealing with the legacy of contaminated land faced by the 16 CLARINET member countries was a significant driver for the development of these more realistic bioavailability measures.

Since its inception, BARGE has primarily been funded by its original member institutes, BGS, RIVM, TNO, the University of Ghent, VITO and the University of Bochum. More recently, an international flavour has been added to the group with the inclusion of Canada’s Jacques Whitford, Ohio State University and Oak Ridge National Laboratory from the United States and Denmark’s DHI Water and Environment.

A priority of the BARGE group is to provide robust and defensible data on bioaccessibility that can be used in human health risk assessment and policy making. The elements of concern at present for most member countries are arsenic, lead and cadmium; however there is increased interest in human health issues relating to Ni and organic contaminants such as benzo(a)pyrene (BaP).

Currently, the United Kingdom is the only European country routinely using bioaccessibility data in human health risk assessments. The other European BARGE member countries are participating in bioaccessibility trials to investigate the potential of the data produced, using the data to refine risk assessments or including the data in risk assessments at the local government level on a site-specific basis.

Across Europe, a wide variety of models have been used to measure the bioaccessibility of contaminants ingested with soil. The models include static and dynamic systems, and have been applied to both natural and anthropogenic contaminated soils and geological materials. All of the models were included in the first BARGE publication, ‘Comparison of Five in vitro Digestion Models to Study the Bioaccessibility of Soil-borne Contaminants,’ where the experimental results were published of a round-robin trial measuring the bioaccessibility of arsenic, cadmium and lead in three contaminated soils.
A goal of the round robin was to cross-validate each method employed by the European institutes against the other contributing partners’ methodologies. A wide range of bioaccessibility values were measured for the three trial soils. Although there were differences in the gastrointestinal models used in the round robin, the main differences in the absolute bioaccessibility data are thought to be a result of the differences in the gastrointestinal pHs used and the separation techniques employed. The choice of total digestion method used by each laboratory has a pronounced effect on the reported relative bioaccessibility data.

A second BARGE publication is currently being prepared. This round-robin study measured the bioaccessible lead content of a soil with human bioavailability data. The second round-robin study included the simulation of both fed and fasted conditions and different gastrointestinal models, due to the ever-changing participants of the group. In addition to the effects of the fed and fasted state, the effect of the separation step, identified as a possible critical parameter from the first BARGE paper, was also studied. Some results obtained from this study will be presented.

As a result of the two BARGE round-robin studies carried out to date and a closer working relationship – among both the individual member groups, and between BARGE and the International Organization for Standardization (ISO) working group on bioaccessibility – the development of a unified BARGE bioaccessibility method has been proposed. The unified method is currently under review by member institutes and will be tested using contaminated soils where bioavailability data are available.

All BARGE member countries have raised concerns about the quality control associated with the measurement of bioaccessibility. Two certified reference materials, NIST 2710 and NIST 2711, are commonly used as part of the standard operating procedures within each of the member institutes. As part of research carried out for the UK Environment Agency, the BGS identified the quality control issues of bioaccessibility measurements as an area to be addressed. Although the use of NIST 2710 or 2711 is suitable in terms of the concentrations of contaminants present in the UK, the materials are not similar to those currently being analyzed by laboratories. To this end the BGS has begun to develop reference materials for bioaccessibility testing that are applicable, in the first instance to the situation in the UK.
BARGE Perspective on Oral Bioaccessibility

Joanna Wragg

History of BARGE

1998 CLARINET Meeting
Contaminated Land Rehabilitation Network for Environmental Technologies

Presentation Outline

- Background to BARGE
  - Our History, who we are, our aim etc
- European situations
  - Bioaccessibility status, contaminants, methods and environment agencies
- BARGE round robins
- Unified Method
- Conclusions

Barge Funding

- Initial Funding (1999)
  - VROM (Dutch Ministry of Housing Planning and the Environment)
  - Feasibility study for a group such a BARGE
- BARGE Established
  - Outcome of VROM study
  - Researchers decided to join together
  - Funded by individual research institutes

Who are BARGE

- Bioaccessibility Research Group of Europe
  - www.bgs.ac.uk/barge
  - Email: barge@bgs.ac.uk
  - BGS - UK
    Chair and Secretary
  - RIVM - Netherlands
  - University of Ghent – Belgium
  - DHI – Denmark
  - University of Nottingham - UK
  - Ohio State University – USA
  - Jacques Whitford – Canada
  - ORNL - USA

History of BARGE

- 1998 CLARINET Meeting
  Contaminated Land Rehabilitation Network for Environmental Technologies

- NEED
  Realistic Bioavailability factor – NOT 100%

- DRIVER
  Cost of contaminated Land remediation
BARGE AIM

- Study the human bioaccessibility of priority contaminants in soils via the gastrointestinal tract
  - As, Pb and Cd
  - Ni

- Priority objective
  - provide robust and defensible data on bioaccessibility that can be used in human health risk assessment and policy making

UK situation

- As = Priority contaminant
  - Present at levels > current soil guideline value, 20mg kg⁻¹ (SGV)
- Other contaminants of interest
  - Pb
    - BUT Bioaccessibility measurements not carried out
  - Ni
  - Organics (BaP)

Denmark

- Danish EPA
  - Currently seeking advice from DHI, amongst others
  - Financed several projects investigating contaminated soils, including bioaccessibility issues
  - Evaluating methods for bioaccessibility testing
  - Potential use of bioaccessibility data in the RA of contaminated Sites

Denmark II

- Pb = Priority contaminant
  - Cd, Ni, Organics also assessed
- DHI recommendations
  - Commercial in Confidence
Netherlands

- RIVM
  - Pb, Cd, As, Organics
  - Developing models for specific needs
  - Release of contaminants from soil, food and toys
- TIM gastro-intestinal model (TIM)
  - Dynamic modelling of the stomach and intestine
  - Applied to the food and pharmaceutical industries
  - Used in the first BARGE round robin, As, Cd, Pb
  - A potential alternative to in-vivo testing

Belgium

- Work included the BARGE round Robin with VITO (Belgium)
- Bioaccessibility of As, Pb and Cd for BARGE
- Bioaccessibility of PAHs using the SHIME reactor
  - Soils from a playground area North of Ghent
- Work includes investigating the biotransformation products of PAHs in the colon
  - Potentially more hazardous than bioaccessible PAHs

Netherlands II

- Dutch Department of the Environment
  - not reached a decision on whether to accept bioaccessibility testing in risk assessment
  - The current approach is to assume that the relative bioavailability is 1
  - Pb bioaccessibility data sometimes accepted
    - By local governments
    - On a site specific basis

Who is using bioaccessibility in HHRA?

- UK – Definitely for arsenic, but its status under review by UK Environment Agency
- The Netherlands and Belgium
  - for refining and checking purposes
- Denmark
  - not at present, still under review

Belgium II

- Simulator of Human Intestinal Microbial Ecosystem (SHIME reactor)
  - 5 double jacketed vessels
  - Dynamic system
  - Developed over a decade ago
  - Labmet, University of Ghent
  - Tom van de Wiele

European Environment Agencies

- Danish EPA
  - Supportive, moving bioaccessibility forward
  - Good relationship with DHI
- Dutch DoE
  - Supportive of bioaccessibility research
  - Funded projects in the past
  - Good relationship with research institutes
- Belgium EPA
  - Supportive of research in bioaccessibility
European Environment Agencies II

- UK EA
  - Jointly funded research with BGS
  - Has not included bioaccessibility in CLEA UK (Risk Assessment model)
  - Has a mixed relationship with research institutes
  - Has carried out own ring test – UNSUITABLE materials for the UK

*UK EA STATEMENT ON BIOACCESSIBILITY
*Recognise the potential of bioaccessibility
*Feel the methodology is scientifically sound and robust
*Lack of uncertainty data?
*Bioaccessibility data is limited at this time

BARGE Round Robin

- Comparison of 5 in-vitro methods for As, Pb and Cd
- 3 soils
  - Oker 11 and Flanders soils – historically contaminated
  - Montana 2711 – NIST contaminated soil reference material
- Total Digestions and bioaccessibility by in-house methods

BARGE Round Robin Results

- Total data
  - Showed some variability due to the method of digestion and the method of analysis
  - Recovery data for NIST 2711 was not 100%
- Bioaccessible data
  - Reported as a % of the total
  - Dependant on the total data
  - Failing of the paper
  - Overestimates the uncertainties associated with bioaccessibility measurements if taken at face value

BARGE Round Robin Results

Why are there differences in the BARGE results?

- Model variations
  - Stomach pH
  - 1.2 or 4.0?
  - Soil:solution ratio
    - 1:1000, 1:100 or lower?
  - GI fluid matrix
  - Separation technique
    - Centrifugation, 0.45um or ultrafiltration
  - Total digestion method
    - Low total digestion recovery
    - High bioaccessible content (%)
BARGE Round Robin II

- Maddaloni Bunker Hill Soil
  - In-vivo data for humans
- Fed and fasted trial of the BARGE models
  - Paper in preparation
  - BGS conditions
    - Fed
      - 1 g of soil + 100 ml of stomach simulant
    - Fasted
      - 1g of soil + 0.5g of infant formula + 100 ml of stomach simulant

Problems with Round Robin II

- 1 lab carried out all analysis – GOOD
- FED STATE different each time!!!!
  - Exactly like Madaloni
  - Infant formula
  - Baby food
  - Separation step differs!!!!!!
    - Centrifugation
    - 0.45um filtration
    - Ultrafiltration

Comparison of Bioaccessible and Bioavailable Lead (solution)

Fed State testing

Why the Differences?

- Complexes formed in the fed state
  - Too large to cross cell wall and become bioavailable
  - But not to big to be filtered out of bioaccessible solution
- RESULT
  - Separation step is a critical factor in determining bioaccessibility and estimating bioavailability
- SO
  - Centrifugation >0.45um filtration>ultrafiltration
  - Bioaccessible>Bioavailable
  - Conservative>Realistic value
Copenhagen 2005

- Joint BARGE/ISO Meeting
- Beginning of a new era!
- A unified BARGE Bioaccessibility method
  - Inorganic contaminants
  - Fasted state
  - Validation against animal models
  - Where material available
  - Address issues of uncertainty
  - Provide a physiologically based, simple, robust and reproducible method

Method trials

- Method currently under review by members
- Validation of method
  - Available soils with in-vivo data for As, Pb and Cd
  - Contaminated materials donated by Nick Basta
    - Rodriguez et al 2003, Schroder et al 2004
  - Participating member institutes
    - BGS, RIVM, DHI, Ghent, Ohio State, Jacques Whitford
  - Extraction of materials, reference materials and blanks in duplicate at least
  - BGS to carry out all analysis

The Unified Method

- RIVM method = basis of the unified method
- Method parameters
  - Conservatively set in the 1st instance
  - Inclusion of stomach and intestinal phases
    - For both cationic and anionic contaminants
    - Sampling and measurement of both phases

The Unified Method II

- Simulated fluids
  - Based on RIVM recipe –
    - adjusted for new pH regime and buffering capacity of materials encountered in each member country
- pH
  - Stomach 1.2 ± 0.5
  - Intestine 6.3 ± 0.5
- Residence times
  - Stomach 1 hour
  - Intestine 3 hours
- Separation Step
  - Centrifugation @ 3000G for 5 minutes

Reference materials

- NIST 2710 or 2711
- Newly created BGS reference soils
  - To ensure the quality and reproducibility of Bioaccessibility data (including total element data)
  - Representative of the UK situation
    - Naturally elevated contaminants, As and Ni
  - Consensus ‘bioaccessible’ values will be arrived at by a round robin with UK labs, and BARGE participants

The Material

- 0.5 tonne of soil
  - collected from North Lincolnshire
- Soil type
  - similar to the Banbury series – a ferritic brown earth
  - Total As concentration c.90 mg kg⁻¹
  - Bioaccessible As c.5 mg kg⁻¹
  - Dried, ground, homogenised and characterised
  - Split into sub samples and subjected to homogeneity testing by XRF

A greenish and reduced limestone weathers at the surface to a brown rock
including iron, other weathering products present i.e. include pyrite "hematite" and siderite
BARGE perspective

- Questions still to be answered, it’s not a done deal yet
- The tip of the iceberg
  - Many countries have problems from different contaminants
    - Natural and anthropogenic
    - Inorganic and organic

- Many parameters to consider
- Must keep it physiologically based
- Separation step
- Do we want a realistic test (ultrafiltration) or a conservative test (centrifugation) for HHRA? Can have both and keep it simple and robust for commercial facilities
- Questions about fed and fasted state

Acknowledgements

- Health Canada
- BARGE
- BGS

BARGE perspective

- Developments in bioaccessibility measurement and applications in human health risk assessment
- www.consoil.de or www.bgs.ac.uk/barge
- Application and results from bioaccessibility testing
  - Canada – Chris Olsson
  - UK – Ben Klinck
  - ISO bioaccessibility standard
  - Denmark – Lilli Andersen
  - Use of bioaccessibility data in a UK risk assessment
  - UK – Paul Nathanail

Consoil Special Session on Bioaccessibility

- Application and results from bioaccessibility testing
  - Canada – Chris Olsson
  - UK – Ben Klinck
  - ISO bioaccessibility standard
  - Denmark – Lilli Andersen
  - Use of bioaccessibility data in a UK risk assessment
  - UK – Paul Nathanail

- We need to gain support from our individual EA’s and the European EA
- Improve bioaccessibility communications across the board, Europe, US, Australia
- Funding needs to be found for joint research
  - We all have similar problems and are trying to get to the same end
  - Start addressing other contaminants
  - BaP

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- Improve bioaccessibility communications across the board, Europe, US, Australia
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Using *in vitro* Gastrointestinal Bioaccessibility Methods to Quantify Trace Element Bioavailability and Risk from Soil Ingestion: A perspective on research needs and activities in the United States

Nicholas Basta, School of Natural Resources, Ohio State University

Remediation science has matured and bioavailability-based risk assessment frameworks are now being developed and implemented. Many of these frameworks incorporate adjustments for contaminant solubility and/or bioavailability in remedial investigation or risk-based remediation endpoints. Adjusting risk for contaminant bioavailability often requires modifying exposure assessments to quantify human and ecological intake of soil contaminants. One approach to evaluate exposure from contaminant bioavailability in soil uses *in vitro* models and bioassays based on risk-based exposure pathways.

Animal models have been used to estimate bioavailability of inorganic contaminants to humans via the soil-ingestion exposure pathway. In order to overcome some of the difficulties and expenses associated with animal dosing trials, research efforts have been directed at developing *in vitro* chemical extraction methods that simulate the gastrointestinal environment. Several *in vitro* methods have been shown to correlate with animal models used to determine heavy metal bioavailability including juvenile swine, monkeys, rabbits and dogs. Contaminant bioaccessibility, measured by *in vitro* gastrointestinal (IVG) methods, has been applied to soils contaminated with As, Cr, Cd and Pb. *In vitro* GI methods are being adopted for risk assessment in the United Kingdom and are under consideration in other countries including the United States.

However, several science and policy issues remain before IVG methods measuring trace element contaminant bioaccessibility become an accepted means for assessing contaminant bioavailability via incidental ingestion by humans. A brief overview of several science and policy *in vivo* and *in vitro* issues in the US will be presented. The most cost effective and efficient means to address scientific issues are collaborative efforts among scientists in research groups focused on *in vitro* methods. Current and planned research efforts between groups in the European Union, Canada, Australia, and the US are underway. Research promoting international collaborative efforts will be discussed.
Using \textit{in vitro} Gastrointestinal Bioaccessibility Methods to Quantify Trace Element Bioavailability and Risk from Soil Ingestion

A perspective on research needs and activities in the USA

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

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Bioavailability Use in USEPA CERCLA Risk Framework
Soil Screening Levels (SSL)

\[
\text{Risk} = \left[ \frac{\text{Soil}}{\text{SSL}} \right] \times \frac{(\text{EF})(\text{ED})(\text{IR})(\text{BIO})}{(\text{BW})(\text{AT})}
\]

Risk Assessment for Contaminant
Adjustments for Bioavailability in USEPA CERCLA R.A.G.S.
are possible BUT
its use depends on USEPA regional acceptance
and is on a case-by-case basis (and mostly for Pb)

---

Soil Ingestion Pathway and Human Health
"Gastrointestinal Soil Contaminant Bioavailability"

Soil ingestion often "risk driver" for human exposure to contaminated soil

\[
\text{Risk} = \left[ \frac{\text{Soil}}{\text{SSL}} \right] \times \frac{(\text{EF})(\text{ED})(\text{IR})(\text{BIO})}{(\text{BW})(\text{AT})}
\]

[Soil] = Soil Contaminant Content
(BIO) = "Gastrointestinal Bioavailability"

GI bioavailability drives risk
Bioavailability-based remediation reduces GI bioavailability

---

Using Bioavailability in Risk Assessment Remedial Investigation /Feasibility Study of Highly Contaminated Sites

Tri-State Mining Region
Extensive Pb, Zn Mining
Smelting / Processing

Highest Child Blood Pb in US

---

Risk Assessment / Remedial Action
Contaminant bioavailability has been used to determine remedial action
mostly limited to soil excavation, soil replacement of residential yards
Desire to Use Bioavailability Adjustment for Non-extraction in situ Soil Remediation

Before Remediation

After Remediation

Bunker Hill, ID; Joplin, MO; Leadville, CO; others

http://faculty.washington.edu/slb/


Use of in vivo or in vitro methods to measure reductions in risk from risk-based remediation still in the research stage

Desire to Use Bioavailability Adjustment for Environmental Assessment of Real Estate

- Disclosure of Pb-based paint
- Pb testing of soil
- Residential development of agricultural land, old orchards, cotton fields (arsenical pesticides), CCA - treated wood products
- Use of industrial by-products on lawns / gardens

Proposed Arsenic Soil Screening Standards

Florida 0.7 mg/kg; Delaware 6 mg/kg

New USEPA drinking water standards for As Jan. 23, 2006

Animal Models Used to Determine Gastrointestinal Bioavailability

GI Bioavailability Determined from Soil-dosing Trials

$25-50K/soil

$75-125K/soil

Desire to Use Bioavailability Adjustment for Environmental Assessment of Real Estate

- Disclosure of Pb-based paint
- Pb testing of soil
- Residential development of agricultural land, old orchards, cotton fields (arsenical pesticides), CCA - treated wood products
- Use of industrial by-products on lawns / gardens

Proposed Arsenic Soil Screening Standards

Florida 0.7 mg/kg; Delaware 6 mg/kg

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in vitro Gastrointestinal Availability Methods

Sequential extraction, 37°C

Gastric phase

4 g soil + 600 mL gastric solution

1% pepsin in 0.15 M NaCl, pH 1.8

60 minutes

Intestinal phase

raise pH to 5.5, add bile + pancreatin, 60 min

In vitro “availability” = dissolved contaminant = bioaccessible contaminant

Using Bioavailability to Adjust Risk in the Soil Ingestion Pathway “Gastrointestinal Soil Contaminant Bioavailability”

Soil ingestion often “risk driver” for Human exposure to contaminated soil

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

[Soil] = Soil Contaminant Content

(BIO) = “Gastrointestinal Bioavailability”

GI bioavailability drives risk

Remediation reduces GI availability

Application of in vitro GI Methods to Evaluate Remediation Treatments

Control Alk. NViro PO4 Biosolids

Basta et al., 2001. J. Environ. Qual. 30:1222-1230
Use of *in vitro* or *in vivo* methods to quantify contaminant bioavailability to human (child) receptors

**Serious Issues**

- How reliable are in vivo and in vitro methods in measuring contaminant bioavailability?
- Proper application of method / limitations
- Public acceptance

Bioequivalency of *in vivo* Models

Maddoloni, Bunker Hill Superfund soil, dosed for Pb

- Human bioavailability = 50%; immature swine = 33%

Artifacts
- Dosing methods: food vehicle, gavage, etc
- Method used to calculate bioavailability
- Lack of thorough interspecies comparison

Bioequivalency of *in vivo* Models

- Use of *in vitro* or *in vivo* methods to quantify contaminant bioavailability to human (child) receptors

**Step 1**

- Contaminant dissolution
- Gastrointestinal System
- Intestinal Membrane

**Step 2**

- Contaminant absorption
- Systemic circulation

Contaminant bioavailability is a two-step process

- **Which elements?** extensive Pb, some As, Cd, Ni
- **Is the rate-limiting step contaminant dissolution (Step 1)?**
- Yes – may be able to use an *in vitro* method to measure GI availability
**Pb GI (Bio)availability in vitro vs. in vivo**


\[
\text{Bioacc Pb} = 3.0 + 0.39 \text{ In Vivo } \quad r = 0.93^{**}
\]

**As GI (Bio)availability in vitro vs. in vivo**


\[
\text{Bioacc As} = -1.2 + 0.77 \text{ In Vivo } \quad r = 0.87^{**}
\]

**Cd GI (Bio)availability in vitro vs. in vivo**


\[
y = 0.60x + 25.3, \quad r = 0.86^{**}
\]

---

**In vitro models**

**Method validation issues**

Which media: Soils contaminated with mining wastes (slags, etc), paint, pesticides, battery waste, coal ash, etc.

---

**In vitro models**

**Method validation issues**

Which concentration ranges?

- most validation studies > 1,000 or 10,000 mg/kg contaminant
- can we extrapolate the method to < 100 mg/kg contaminant?

Recommended for swine (in mg/kg):

- > 2500 Pb
- > 500 As

Soil background levels usually:

- < 50 mg/kg for Pb
- < 20 mg/kg for As

---

**Background**

**Moderately Contaminated**

**Highly Contaminated**

in vivo and in vitro

---

**Is fasting worst case? Pb --yes; As --yes?**


- + food: in vitro bioaccessible Pb = 0.39 (in vivo RBA) + 2.97; \( r = 0.93, P < 0.05 \)
- fasting: in vitro bioaccessible Pb = 0.65 (in vivo RBA) - 1.44; \( r = 0.89, P < 0.05 \)

Fasting 40% higher Pb -- more conservative estimate

Comparison of 9 materials from Rodriguez et al (1999)

<table>
<thead>
<tr>
<th>Phase</th>
<th>Pb</th>
<th>As</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric phase</td>
<td>+ food</td>
<td>IVG</td>
</tr>
<tr>
<td></td>
<td>Pb = 0.39 (in vivo RBA) + 2.97; ( r = 0.93, P &lt; 0.05 )</td>
<td>As = -1.2 + 0.77 (in vivo RBA) - 1.61; ( r = 0.91 )</td>
</tr>
<tr>
<td></td>
<td>fasting</td>
<td>IVG</td>
</tr>
<tr>
<td></td>
<td>Pb = 0.65 (in vivo RBA) - 1.44; ( r = 0.89, P &lt; 0.05 )</td>
<td>As = 0.86 (in vivo RBA) - 0.05; ( r = 0.95 )</td>
</tr>
</tbody>
</table>

Intestinal phase:

<table>
<thead>
<tr>
<th>Phase</th>
<th>Pb</th>
<th>As</th>
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<tr>
<td></td>
<td>+ food</td>
<td>IVG</td>
</tr>
<tr>
<td></td>
<td>Pb = 0.64 (in vivo RBA) - 4.8; ( r = 0.89 )</td>
<td>As = 0.24 (in vivo RBA) - 0.9; ( r = 0.77 )</td>
</tr>
</tbody>
</table>

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Page 4
Use of risk-based endpoint criteria bioavailable, soluble forms (not total content) criteria affects site assessment and remediation

Public Policy

Use of risk-based endpoint criteria bioavailable, soluble forms (not total content) criteria affects site assessment and remediation

Public acceptance?
contaminant removal (no risk)
reduced availability (acceptable risk)

Acceptable Risk in Oklahoma?
F5 tornado in Oklahoma 0.5 to 1 mile wide for 100 miles (4.5 hr)
May 3, 1999

Future Research and Using (Bio)Availability

A few more studies or are we just at the “tip of the iceberg”? Policy decision: adoption of bioavailability in risk assessment by federal and state agencies is essential

Good news research funding opportunities for bioavailability / risk assessment research
Momentum towards bioavailability in risk assessment of contaminated soil

Soils -- The Final Frontier (6/11/04)

“Interest in soil is booming” --ecology, biogeochemistry, terrestrial (e.g., soil) physics, critical zone processes, etc

National Academy of Science
Availability, Bioavailability, and Toxicity
Not available Available Bioavailable Toxic

In vitro / in vivo contaminant activities in the USA

Historical
Contractual studies on USEPA Superfund sites
Almost all Pb contaminated soils

Contractual arrangements – data may not be
shared released / in some or all cases

Collaboration / publication of results
illegal or strongly discouraged

Competitive Grant Research
USEPA ORD, SERDP, other agencies
Universities / federal agencies

Federal agencies
USEPA research laboratories with mission

Potential for Collaborative Studies
Little collaboration from groups under contractual Superfund sites

Other disincentives – patents ($$), etc

More collaboration between competitive grant research

Universities: Ohio State, Auburn, Missouri, Stanford, Columbia, others

Federal laboratories: USEPA, DOD, DOE

FREE WORKSHOP

BIOAVAILABILITY OF LEAD AND ARSENIC:
USING in vivo AND in vitro MEASUREMENTS

TUESDAY 13 SEPTEMBER 2005, 8:30 AM – 4:00 PM
Byron Sher Auditorium, Cal/EPA Headquarters
1001 "I" Street, Sacramento, CA

Also WEBCAST at http://www.calepa.ca.gov/broadcast

Sponsored by:
California Department of Toxic Substances Control

Cal/EPA
DTSC

ESTCP Project Overview

Coordination/Workshop

DoD Soil Selection

Contaminant Solid Phase Speciation
Stanford University

Contaminant Bioaccessibility
ORNL
Auburn University

Ecological Bioassays
Plant / Soil Invertebrates
Ohio State University

In Vivo Bioavailability
Swine Dosing Trials
Univ. of Missouri

† Oak Ridge National Laboratory

The Use of In Vitro Soil Metal Bioavailability
Methodologies to Adjust Human and Ecological
Risk Assessment

ESTCP Sponsored Workshop
September 15, 2005
Holiday Inn by the Bay San Diego, CA
Principal Investigators

- Oak Ridge National Laboratories, Oak Ridge, TN
  Dr. Philip Jardine, Distinguished Research Scientist, Soil and Environmental Science

- Naval Facilities Engineering Service Center, Port Hueneme, CA
  Amy L. Hawkins, Ecological Risk Technical Assistance Team

- Auburn University, Auburn, AL
  Dr. Mark O. Barnett, Associate Professor of Environmental Engineering

- The Ohio State University, Columbus, OH
  Dr. Nicholas Basta, Associate Professor, Soil and Environmental Chemistry
  Dr. Roman Lanno, Associate Professor, Soil and Environmental Ecotoxicology
  Dr. Elizabeth Dayton, Research Scientist, Soil and Environmental Chemistry

- University of Missouri at Columbia, Columbia, MO
  Dr. Stan Casteel, Professor of Toxicology and Director of Veterinary Medical Diagnostic Laboratory

- Stanford University, Stanford, CA
  Dr. Scott Fendorf, Associate Professor, Geological and Environmental Science

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USEPA policy on "Good Science"

Regulations should be based on "good science."

"Good Science:" science that is peer-reviewed and published in scientific journals.

Rodriguez et al. (1999), and Oomen (2002) meet the USEPA Good Science definition.

Entire process of method development should be open to review or "transparent"

Highly desirable for the collaborative research to be international

---

Issue Paper on the Bioavailability and Bioaccumulation of Metals

- Eastern Research Group, Inc.

Submitted to USEPA Risk Assessment Forum
August 19, 2004

section (7 pages) on bioavailability methods for human risk assessment — Dr. John Drexler, University of Colorado

In vitro method must a "Bioequivalent Test." The in vitro method must:

1. be correlated with an acceptable *in vivo* model
2. be validated - defined as a inter- and intra-lab round robin
3. QA/QC -- defined as proper lab procedures
4. have a sensitivity analysis (test parameters relative effect)

---

Bioavailability Research Group of Europe

Collaborative Effort on in vitro soil contaminant bioavailability research between research groups in Europe, Canada, USA, Australia (hopefully soon), others?

Canadian Bioavailability Working Group
US Bioavailability Working Group?
North American Bioavailability Group?

---

Issue Paper on the Bioavailability and Bioaccumulation of Metals

Conclusions of issue paper (in bold):


Rodriguez et al. (1999), and Oomen (2002) are not defensible?

These methods have completed or are completing the "bioequivalency" requirements. But more importantly — they have been peer-reviewed and published in Environmental Science and Technology —which ranks 2nd of 131 Environmental Science scientific journals

---

Thank you for your attention
More information?
Please contact:
Nick Basta
School of Natural Resources
410 Kottman Hall
basta.4@osu.edu
www.snr.osu.edu

Kottman Hall
Cadmium Bioavailability and Bioaccessibility as Determined by *in vitro* Digestion Dialysis and Intestinal Epithelial Monolayers, and Compared to *in vivo* Data

Beverley Hale ¹, Debbie Chan¹, William Black² and Michael Waisberg¹

¹Department of Land Resource Science, ²Department of Biomedical Sciences, University of Guelph

The transfer of Cd from food to target organs in the body is influenced by the bioavailability of Cd, which in turn is influenced by the chemical speciation of Cd, likely both before and after digestion of food. Bioavailability is defined as the proportion of total metal ingested in food that is absorbed, while bioaccessibility refers to the proportion of total metal that is absorbed in simulated gastrointestinal processes. These studies examined how Cd bioavailability and bioaccessibility differed between plant-incorporated and soluble salt amended lettuce and grain following an *in vitro* digestion procedure. The results were compared with *in vivo* studies of the same dietary matrices.

Three lettuce diets (control, amended and incorporated) were grown hydroponically and subjected to the *in vitro* digestion procedure which uses various gastric and intestinal enzymes. Dialysis membranes with molecular weight cutoffs (MWCOs) of 1, 10 and 25 kD were filled with supernatant from the *in vitro* digestion procedure (defined to be the bioaccessible fraction of Cd in food) and placed in saline solution for 24 hours. Samples from inside the membrane and the outer solution were analyzed for Cd. To simulate intestinal absorption, confluent Caco-2 cell monolayers were exposed to filtered supernatants from the same *in vitro* digestion procedure of the same lettuce materials as described for the dialysis experiment, for one hour. The Caco-2 cells were grown and exposed in a T-75 cell culture flask. For the *in vivo* testing of the same diets, rabbits were fed with one of three lettuce diets for up to 10 weeks; these diets were supplemented with Cd-free water and alfalfa pellets. Target organs (liver and kidney) were collected and analyzed for Cd; accumulation of Cd was regressed against the cumulative dose separately for each diet, and compared.
More Cd from the amended diet diffused out of the tubing for all MWCOs (Fig 1: higher number = more Cd retained in tubing), indicating that more Cd was present in the free ionic form or bound to molecules less than 1 kD in size (e.g. Cd-glutathione). If greater diffusion from inner to outer solution is due to the presence of more Cd\textsuperscript{2+} in the amended diet, then more Cd-metallothionein (MT) complexes are likely formed since Cd\textsuperscript{2+} induces the formation of MTs. Because Cd-MTs preferentially accumulate in the kidney, our results can help explain the approximately 10 percent increase in Cd accumulation in the kidney from the amended diet in the \textit{in vivo} study (Chan \textit{et al.}, 2004). Layers of Caco-2 (intestinal epithelial) cells did not differentiate between amended and incorporated diets, although this experiment did not separate Cd bound to the surface of the cells, from Cd taken up into the cells (Table 1). Experiments are in progress that use cell culture inserts to examine this difference. In these new experiments, the Cd moving to the basolateral side of the monolayer will also be measured, as has been done by Oomen \textit{et al.} (2003) for soil.

![Figure 1: Ratios of Cd inside the dialysis tube to Cd found in the outer solution for Cd incorporated and amended lettuce relative to MWCO.](image-url)
Table 1: Estimated "bioaccessible" and "bioavailable" fraction of metals in lettuce; values are percent of total in original lettuce material.

<table>
<thead>
<tr>
<th>Method</th>
<th>Incorporated</th>
<th>Amended</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>In vivo</em> (kidney)</td>
<td>0.20</td>
<td>0.27</td>
</tr>
<tr>
<td><em>In vitro</em> (dialysis)</td>
<td>44</td>
<td>58</td>
</tr>
<tr>
<td><em>In vitro</em> (Caco-2 cells)</td>
<td>1.9</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Dialysis membranes have the potential to rank foods for the bioaccessible fraction of Cd, but not to predict the actual fraction of the total Cd in foods that would be absorbed by the animal. The estimates of the bioaccessible fraction using intestinal cells were much more similar to the *in vivo* bioavailable fraction (kidney), but did not distinguish between the diets as did the dialysis method. If the *in vivo* bioavailable fraction had been determined for whole body, rather than kidney only, the *in vivo* and Caco-2 cell values would be more similar.
Cadmium Bioavailability and Bioaccessibility as Determined by *in vitro* Digestion, Dialysis and Intestinal Epithelial Monolayers, and Compared to *in vivo* Data

Hale, B., D. Chan, W. Black and M. Waisberg
University of Guelph, Guelph, ON, Canada

**Objective**
- compare estimates of the bioavailability of Cd in same diet material (lettuce) from *in vivo* study, with bioaccessibility estimates from two *in vitro* studies

**Question**
Can bioavailability (proportion of total metal ingested in food that is absorbed by the animal) be estimated by bioaccessibility (the proportion of total metal that is solubilized by simulated gastrointestinal fluid)?

**Introduction**
- Cd known carcinogen and toxicant
- Cd elevated in some soils and in plant and animal tissues consumed by humans
- Cd in plants complexed with organic molecules (phytchelatins, proteins, phytates) which may affect bioavailability

**Experimental Plan**
- *in vivo* Feeding Study
- *in vitro* Simulated Digestion (Estimate 1)
- Dialysis (Estimate 2)
- Caco-2 Cells (Estimate 3)

**Diet Material**
- hydroponically grown lettuce (*Latuca sativa* L. cv. Ostinata):
  - **control** (no Cd in nutrient solution),
  - **incorporated** (grown in nutrient solution containing Cd)
  - **amended** (surfactually sprayed with CdNO₃ to match the Cd concentration in the incorporated diet)
**In vivo Feeding Study**

- rabbits were fed with one of three lettuce diets (control, incorporated or amended) for up to 10 weeks
  - diet supplemented with Cd free water and alfalfa pellets
- target organs (liver and kidney) collected and analyzed for Cd using GF-AAS
- Chan et al., 2004 J Tox Environ Health A 67:397-411

**In vivo Results**

![Graph showing cumulative Cd in diet vs total Cd in kidney](image)

**Simulated Digestion Results**

<table>
<thead>
<tr>
<th></th>
<th>Incorporated</th>
<th>Amended</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lettuce</td>
<td>407 µg Cd</td>
<td>795 µg Cd</td>
</tr>
<tr>
<td>Chyme</td>
<td>11.4 µg Cd</td>
<td>21.3 µg Cd</td>
</tr>
<tr>
<td>Chyme (% of lettuce)</td>
<td>2.8 %</td>
<td>2.7 %</td>
</tr>
</tbody>
</table>

**In vitro Simulated Digestion**

gastric: pepsin, pH 1.8, 1 h
intestinal: pancreatin, bile extract, pH 5.5, 1 h
shaking water bath at 37°C

solid fraction removed, tubes centrifuged; supernatant passed through 0.22 µm filter

**Dialysis**

- Supernatant placed in dialysis membrane (MWCOs 1, 10 and 25 kD)
- Dialysis membranes placed in saline solution (osmolality matched) on shaking table for 24 h

**Dialysis Results**

![Graph showing inner Cd / outer Cd](image)
Dialysis Results

<table>
<thead>
<tr>
<th>1 kD</th>
<th>10 kD</th>
<th>25 kD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inc.</td>
<td>1.40%</td>
<td>1.23%</td>
</tr>
<tr>
<td>Ammd</td>
<td>1.43%</td>
<td>1.57%</td>
</tr>
</tbody>
</table>

(% of Cd originally in lettuce)

Caco-2 Results

<table>
<thead>
<tr>
<th>% of Cd in chyme</th>
<th>% of Cd in lettuce</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inc.</td>
<td>2.06%</td>
</tr>
<tr>
<td>Ammd</td>
<td>1.80%</td>
</tr>
</tbody>
</table>

How do they compare?

(as % of Cd originally in lettuce)

<table>
<thead>
<tr>
<th>Incorporation</th>
<th>Amended</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vivo</td>
<td>0.39%</td>
</tr>
<tr>
<td>Simulated Digestion</td>
<td>2.8%</td>
</tr>
<tr>
<td>Dialysis (10 kD)</td>
<td>1.23%</td>
</tr>
<tr>
<td>Caco-2 Cells</td>
<td>0.058%</td>
</tr>
</tbody>
</table>

Caco-2 Cell Results

![Caco-2 Cell Results Diagram]

Y = 85.7 + 19.3pH - 9.4pH² + 0.71pH³

(aqueous simulated digestion)
Acknowledgements

• Peter Smith
• Anna Gasior
• Nick Fry
• Anne Marie Landis

• NSERC and CIHR
• Canadian Network of Toxicology Centres

Funding

• Canadian Network of Toxicology Centres,
• Natural Sciences and Engineering Research Council of Canada
• Metals in the Environment Research Network (www.mite-rn.org)
• Ontario Ministry of Agriculture and Food
PAH Bioaccessibility Using Caco-2 Cells

Margo Moore, Department of Biological Sciences, Simon Fraser University

Accurate, reproducible and inexpensive in vitro screening models are needed to estimate the oral bioavailability of hydrophobic contaminants. The aim of this research was to determine the bioavailability of $^{14}$C-benzo[a]pyrene (BaP) bound to digestible and non-digestible matrices – skim milk powder (SM) and soil, respectively – using an in vitro model that incorporated full gastrointestinal (GI) digestion plus sorption to cultured human enterocytes (Caco-2) or to ethylene vinyl acetate (EVA) film.

We first compared BaP mobilization from soil containing high or low percentage of organic matter. The plateau concentrations of BaP in fixed Caco-2 cells or EVA were not significantly different between the two soil types. The fugacity capacity of Caco-2 lipids for BaP was found to be 2.44 times higher than EVA; this relationship was linear over a range of concentrations.

We compared the uptake of $^{14}$C-BaP from the two soils into live Caco-2 cells. BaP achieved a rapid equilibrium in the aqueous compartment for both soils and, as observed with fixed cells, a significantly higher amount was mobilized from soil with low organic matter. However, the BaP concentration in live Caco-2 cells was significantly higher from low organic matter versus high organic matter soil. Because the concentration in Caco-2 cells was not at equilibrium, sorbed BaP levels correlated with those in the aqueous compartment.

We then measured the mobilization of BaP from a digestible matrix (SM). BaP was aged onto SM for either seven days or six months. The extent of uptake and mobilization of BaP from aged and non-aged SM was about fourfold higher than from soils.

Despite significant differences in mobilization into GI fluids between the aged and non-aged SM, the extent of uptake into cells was the same. Thus, uptake into sorptive epithelia may not correlate with aqueous concentrations when the contaminant has reached equilibrium.
Determining Bioaccessibility/Bioavailability of PAH using Caco-2 Cells

Margo Moore
Department of Biological Sciences
Simon Fraser University
August 31st, 2005

Contributors to this work

Graduate students: Luba Vasiluk, F. Gobas, SFU
Jas Minhas, C. Eickhoff, Vizon SciTec

Undergraduate students: Zahra Walji, Sandy Tsang, Sheila Smith

Research Technician: Linda Pinto

Financial support from NSERC (Strategic) and Vizon SciTec Inc. is gratefully acknowledged.

Overall aim of our research

To develop a rapid in vitro screening method to estimate relative oral bioavailability of hydrophobic organic contaminants (HOC) bound to a solid matrix (e.g., food, soil) by using synthetic gastrointestinal fluids and cultured human enterocytes, Caco-2

The model includes exposure to digestive fluids as well as exposure to sorptive epithelium or its surrogate.

Objectives

Measure the mobilization into gastrointestinal fluid and binding to epithelial cells or their surrogate, of two PAH, benzo[a]pyrene and chrysene, bound to soil

Effect of:
- percent soil organic matter
- sorptive surfaces: Caco-2 vs. EVA thin film
- components of the gastrointestinal fluid

Background

- Ingestion of contaminated food and soil is the major route of exposure to many hydrophobic organic contaminants
- Thousands of chemicals awaiting categorization by Canadian government for which there is little data on oral bioavailability beyond $K_{ow}$
- Animal testing is expensive and unethical for such large scale screening
- In vitro methods are needed for screening compounds with the potential for intestinal absorption

Experimental Approach

STEP 1. Gastric digestion

STEP 2. Intestinal digestion

STEP 3. Exposure to intestinal epithelium

NaHCO$_3$ + intestinal components

Apical

Caco-2 cells

Membrane filter ($\varnothing$ = 1 cm)
Rationale for using Caco-2 in this model

- Closely resemble mature intestinal enterocytes, both morphologically and biochemically
- Fully differentiated cells express various transport systems and many brush border membrane enzymes
- Widely used to measure trans-epithelial transport and cytotoxicity of various nutrients and pharmaceuticals, more recently xenobiotics

The fugacity (f) approach in chemical partitioning

- The fugacity gradient provides driving force for net passive chemical transport
- Advantages of this approach:
  - Normalized for the sorptive capacity of each medium, rather than concentrations
  - Predicts the static and dynamic behavior of compound within organism
- Mathematically:
  \[
  f = \frac{C}{Z}
  \]
  where:
  - \(C\) is the concentration
  - \(Z\) is the fugacity capacity

Different sorptive surfaces: comparison of EVA and Caco-2 models

Ethylene vinyl acetate (EVA) thin film was used to mimic absorptive epithelium

- Advantages
  - Inexpensive and doesn’t require special training
  - Durable, can withstand longer incubation periods
  - Quick absorption kinetics resulting in rapid chemical equilibrium
- Disadvantages
  - No protein-mediated transport
  - May not have the same properties as biological lipids

Study compounds: Polyaromatic hydrocarbons

- [7,10-14C]Benzo[a]pyrene (BaP)
  - Very hydrophobic, log \(K_{ow}\) = 5.97
  - By-product of incomplete combustion, found in coal oil and crude oil
  - Pro-carcinogen of great concern as it is widely dispersed in the environment
- [5,6,11,12-14C]Chrysene (Chr)
  - Very hydrophobic, log \(K_{ow}\) = 5.8
  - By-product of incomplete combustion, found along with BaP in the environment, usually in higher concentrations
  - Probable human carcinogen

Different sorptive surfaces: comparison of EVA and Caco-2 models

Soil characterization

<table>
<thead>
<tr>
<th>Depth</th>
<th>Topsoil</th>
<th>Subsoil</th>
</tr>
</thead>
<tbody>
<tr>
<td>cm</td>
<td>0 to 15</td>
<td>15 to 20</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Organic content</th>
<th>Topsoil (high)</th>
<th>Subsoil (low)</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>38.1%</td>
<td>15.2%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>pH in water</th>
<th>Topsoil (3.4)</th>
<th>Subsoil (3.8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Particle size</th>
<th>Topsoil (125-250µm)</th>
<th>Subsoil (125-250µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>µm</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Particle size most likely to adhere to skin

Soil to Fluid Ratio:

10 mg of soil per 1ml of gastrointestinal digestion per cell insert = 1:100

Physiological values are 1:100-1:5000 g/ml
The effect of varying % soil organic matter on partitioning of 14C-BaP from soil: Caco-2 cells

After 5 h, BaP levels sorbed into Caco-2 cells (bioavailable fraction) paralleled BaP concentrations in gastrointestinal fluid (bioaccessible fraction). Sorbed concentrations are 100-fold higher.

What happens at equilibrium?

Although the mobilized fraction was higher in the soil containing low organic matter, the cell culture system cannot reach equilibrium because cells cannot withstand incubation periods in GI fluid of > 5-6 hours.

...we therefore measured uptake of 14C-BaP into EVA thin films and into fixed Caco-2 monolayers.

Is EVA a reasonable surrogate to measure PAH uptake?

BaP uptake into lipid normalized Caco-2 cells was ~ 2.5 fold greater than into EVA, regardless of soil organic matter content.

Determining fugacity capacity (Z) of Caco-2 cell lipids for BaP

Using linear bivariant fit equation with 95% confidence:

\[ C_{\text{Lipid}} = 2.44 \times C_{\text{EVA}} \]

\[ f_{\text{Lipid}} = f_{\text{EVA}} \]

Yes, EVA is a good surrogate, at least for BaP uptake, but EVA cannot account for metabolism.
The effect of altering the composition of gastrointestinal fluids

Measuring the desorption of hydrophobic contaminants into water may not provide an accurate measure of desorption into fluids of the gastrointestinal tract.

But how do the individual components of gastrointestinal fluids affect desorption?

In particular:
- Bile acids
- Proteases
- Proteins
- pH shift

The bioavailable fraction remained the same despite significant differences in the bioaccessible fraction

Conclusions

1. The bioavailable fraction (sorbed) depends on the fugacity capacity ($Z$) of the matrix as well as the $Z$ of the epithelium. Changes in the bio accessible (mobilized) fraction do not always translate into changes in the bio available (sorbed) fraction.
2. Biological lipids have higher fugacity capacity than EVA thin film for BaP; however, the relationship is linear. We can extrapolate uptake into biological lipids (Caco-2 cells) from EVA data, at least for BaP.

However, EVA does not account for metabolism or transport.

Conclusions

3. Changes in the bioaccessible fraction were reflected in the differences in the bioavailable fraction under non-equilibrium conditions. However, at equilibrium, sorbed concentrations were not directly correlated with differences in the bioaccessible concentration.

Which condition is physiologically relevant?

Method for quantitative analysis of $^{14}$C-BaP metabolites
Thin layer chromatography analysis of $^{14}$C-BaP/metabolites from gastrointestinal fluids

The rate of $^{14}$C-BaP metabolism to Phase II metabolites in Caco-2 system

The effect of metabolic induction on $^{14}$C-BaP/metabolites uptake by Caco-2 monolayers
Microbial Transformations of Ingested Contaminants

Stephen Siciliano
Microbial Transformations of Ingested Contaminants

Steven Siciliano

Mechanistic Processes

- **Biosorbent**
  - Bacteria’s cell wall is negatively charged and typically sorbs cationic metals.

- **Electron Acceptor**
  - Bacteria are using the metals as a terminal electron acceptor as oxygen is depleted.

- **Transformations**
  - Bacteria will often add alkyl groups to metalloids, transforming inorganics to organo-metalloids.

Predictive Modeling of Bioaccessible Contaminant

\[
\begin{align*}
\text{predicted} &= K_d \times \text{observed} \\
K_d &= \frac{K_{d,\text{in}} \times K_{d,\text{free}}}{K_{d,\text{complex}} + K_{d,\text{bound}}} \\
K_{d,\text{in}} &= K_{d,\text{in}} \times \text{in} \\
K_{d,\text{free}} &= K_{d,\text{free}} \times \text{free} \\
K_{d,\text{complex}} &= K_{d,\text{complex}} \times \text{complex} \\
K_{d,\text{bound}} &= K_{d,\text{bound}} \times \text{bound}
\end{align*}
\]

The slope of the predicted versus observed plot was 1.08, while the intercept, 0.61 µg PAH/L, indicated a slight underestimation of release. The majority of the error was accounted for by the model (\(r^2=0.7937\)), with only 20% of the variance in observed PAH release being unexplained.

Bacteria sorb Metals


Kinetics or Equilibrium?

The first time I studied thermodynamics I thought I understood it but for a few points.
The second time I studied thermodynamics I realized that I understood less than I thought.
The third time I studied thermodynamics I realized I still knew nothing but it had ceased to bother me.

Transformations

Relative concentrations of PAHs and estrogen response upon stomach, small intestine and large intestine digests on PAH contaminated soil samples. Data show that with lower released PAH concentrations in the digests, estrogenicity of the digests increases. Error bars represent standard deviation values of 4 replicates.


Current Hypothesis

Relative bioavailability of metals in ingested food depends on:
(1) concentrations of metals in food
(2) mode of metal uptake into food
(3) activity of gut microflora

Challenges

- Estimating bioaccessibility
  - Mechanical methods may be confounded by "flocs" and do not account for direct uptake
  - Biological methods are susceptible to microbial cytotoxicity
- Reproducibility
  - Bacterial communities are highly dynamic. The current approach has not been validated for batch SHIMEs
- Accuracy
  - What would be a good animal model?
The Use of Bioavailability and Bioaccessibility in Human Health Risk Assessment

Brendan Birmingham, Dino Manca, Ontario Ministry of the Environment
This talk discusses current issues related to the use of bioavailability and bioaccessibility in HHRA including:

1. Nomenclature and definitions of terms used
2. Current and proposed in vitro methodologies
3. Validation of in vitro data

Some examples of in vitro soil data for metals and PAHs from some recent HHRA.
3. INFORMATION NEEDS FOR BIOAVAILABILITY ADJUSTMENTS

4. DESIGNING AND CONDUCTING BIOAVAILABILITY STUDIES
   - In Vivo Methods
   - In Vitro Methods

4. CHEMICAL-SPECIFIC BIOAVAILABILITY INFORMATION
   - Nonpolar Organic Chemicals, Dioxins and Furans, PCBs, PAHs, Chlorinated Pesticides
   - Metals and Metalloids (As, Pb, Cd, Cr, Hg, Ni)

Use of Bioavailability and Bioaccessibility in HHRA

NOMENCLATURE AND DEFINITIONS

absolute bioavailability: the fraction or percentage of a compound which is ingested, inhaled, or applied on the skin surface that is absorbed and reaches the systemic circulation

bioaccessibility: a term for the fractional dissolution of a chemical from an environmental medium (generally measured in vitro)

bioavailability: the extent to which a substance can be absorbed by a living organism into the systemic circulation

relative bioavailability: a measure of the difference in extent of absorption among two or more forms of the same chemical (e.g., lead carbonate vs. lead acetate), different vehicles (e.g., food, soil, water), or different doses. In the context of environmental risk assessment, relative bioavailability is the ratio of the absorbed fraction from the exposure medium in the risk assessment (e.g., soil) to the absorbed fraction from the dosing medium used in the critical toxicity study.

relative bioavailability adjustment (RBA): the fraction obtained by dividing the absolute bioavailability of a chemical present in the environmental media by the absolute bioavailability of that same chemical present in the dosing medium used in the toxicity study from which the reference dose for human health risk assessment was determined.

Use of Bioavailability and Bioaccessibility in HHRA

Issues with current and proposed in vitro methodologies for bioavailability testing
- Soil particle size, e.g., 250 microns
- Extraction at acid and neutral pH
- Sample number
- Concentration range in medium tested
- Ratio of extraction fluid volume to sample weight
- Use of additives in the extraction fluid
- Metal speciation
- Role of soil ageing

Currently, in vitro extraction is not a routine or standardized methodology. MOE would like to see consensus on acceptable in vitro methodologies.

Similarly, metal speciation is not a routine or standardized methodology.

Use of Bioavailability and Bioaccessibility in HHRA

Validation of in vitro data
- Choice of laboratory animal (primate, swine or rodent) or humans
- Nature of the test (excreta, blood, tissue)
- Nature of the chemical of interest
- Resources available and timing issues
- Detailed protocol (GLP)
- Species, age, number of animals per dose, dose range, dosing frequency
- Tissue/sample collection
- Analytical methods, QA/QC
- Mass balance approach or measurement of chemical in blood, excreta or tissue

Some examples of in vitro soil data for metals and PAHs from some recent MOE HHRA.

Port Colborne HHRA
The extensive soil sampling program identified eight metals of potential concern in the soil at levels that exceed the 1997 MOE residential soil guidelines (Antimony, Arsenic, Beryllium, Cadmium, Cobalt, Copper, Lead, Nickel, Selenium, Zinc).

Details of the bioaccessibility testing can be found in the report available at:
http://www.ene.gov.on.ca/envision/portcolborne/4255e.htm
Simulated Stomach Acid Leachate Tests - Soil samples were extracted at MOE’s laboratory with 0.17 N HCl (pH 1) only (10 samples)

Bioaccessibility Testing - Soil bioaccessibility extractions were performed at Exponent’s laboratory (Boulder, CO) using the method developed by the Solubility/Bioavailability Research Consortium (Ruby et al, 1999, Exponent, 2001). Samples were extracted in glycine buffer (pH 1.5) for 1 hr, then at pH 7 for an additional 4 hours (20 samples)

Bioaccessibility Value (%) selected for Nickel and other metals

This result, expressed as a percentage of the total metal level in the original soil sample was used to correct the estimates of metal intake from soil ingestion.

As noted, metal bioaccessibility testing is not a routine or standardized methodology.

Use of Bioavailability and Bioaccessibility in HHRA

Use of Bioavailability and Bioaccessibility in HHRA

<table>
<thead>
<tr>
<th>Metal</th>
<th>MOE Mean (range)</th>
<th>Exponent* Mean (range) (Ground &amp; Sieved)</th>
<th>Exponent* Mean (range) (Sieved Only)</th>
<th>Bioaccessibility Value used for Exposure Estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antimony</td>
<td>2.8 (2.1 - 3.9)</td>
<td>38 (7 - 66)**</td>
<td>32 (5-64)</td>
<td>32</td>
</tr>
<tr>
<td>Arsenic</td>
<td>22.8 (12.6 - 28.4)</td>
<td>31 (17 - 53)</td>
<td>35 (23-58)</td>
<td>-</td>
</tr>
<tr>
<td>Beryllium</td>
<td>N/A</td>
<td>92 (9 - 95)</td>
<td>99 (2-81)</td>
<td>59</td>
</tr>
<tr>
<td>Cadmium</td>
<td>N/A</td>
<td>70 (9 - 83)</td>
<td>74 (4-86)</td>
<td>76</td>
</tr>
<tr>
<td>Cobalt</td>
<td>18.2 (12.5 - 24.7)</td>
<td>22 (10 - 34)</td>
<td>28 (8-35)</td>
<td>29</td>
</tr>
<tr>
<td>Copper</td>
<td>28.1 (13.8 - 44.6)</td>
<td>30 (11 - 49)</td>
<td>46 (1-68)</td>
<td>45</td>
</tr>
<tr>
<td>Lead</td>
<td>75.7 (61-90.3)</td>
<td>87 (2-81)</td>
<td>74 (10-84)</td>
<td>-</td>
</tr>
<tr>
<td>Nickel</td>
<td>16.5 (11.8 - 23.3)</td>
<td>35 (9 - 22)</td>
<td>19 (11-20)</td>
<td>19</td>
</tr>
</tbody>
</table>

* = highest bioaccessibility value from either acid or neutral pH extractions
** = based on only 21% recovery from NIST soil standard
N/A = Not Available

PAH-Contaminated Neighbourhood in Toronto

Bioaccessibility Testing - Soil bioaccessibility extractions were performed at the Centre for Toxicology Laboratory, Guelph University using a method developed for soil dioxins (Ruby, 2001, 2002). Samples were extracted in glycine buffer (pH 1.5) containing NaCl, pepsin, BSA, mucine and oleic acid for 1 hr, then at pH 7.2 following addition of NaOH, porcine pancreatin and bovine bile for an additional 4 hours.

The mean BAF value for the non-carcinogenic PAH (acenaphthene, anthracene, phenanthrene, benzo[a]pyrene, fluoranthene, pyrene, fluorene, naphthalene) of 8.6% was higher than the mean BAF for the carcinogenic PAH (benzo[a]anthracene, benz[a]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, chrysene, indeno[1,2,3-cd]pyrene, dibenzo[a,h]anthracene) of 7.2%.
Application of Bioaccessibility for Contaminated Site Risk Assessment

Kenneth J. Reimer¹, Christopher Ollson²

1) Royal Military College of Canada
2) Jacques Whitford Limited, Royal Military College of Canada
Application of Bioaccessibility for Contaminated Site Risk Assessment

Iris Koch, Ken Reimer
Royal Military College of Canada

Christopher Olsson
Jacques Whitford Limited
Royal Military College of Canada

Bioaccessibility Test (BAT)

- Typically used a two stage method that simulates chemical and physical environment of GI tract.

Research Partners

Jacques Whitford Limited
Dr. Chris Ollson
Dr. Eric Veska
Cecile Willert

Royal Military College of Canada
Dr. Iris Koch
Numerous M.Sc., Ph.D. and 4th year students

University of Guelph
Dr. Beverly Hale and her lab

Memorial University of Newfoundland
Dr. Naill Gogan
Kelly Johnson

Case Study 1: Bioaccessibility of Arsenic from Gold Mine Contaminated Soils

Yellowknife, NWT Canada
- 2 Gold Mines since 1938
- Gold ore associated with FeAsS
- Sole contaminant of concern (CoC)
- Public concern has increased as mining comes to a close
- Risk assessment required

Exposure Dose from Inadvertent Soil Ingestion

Exposure Dose = Contaminant Concentration x Exposure Factors x Bioavailability

Body Weight
Bioaccessibility of Arsenic from Yellowknife Soils Using BAT

<table>
<thead>
<tr>
<th>Sample Category</th>
<th>Organic Carbon Content (%)</th>
<th>Arsenic (ppm)</th>
<th>GFE Arsenic (ppm)</th>
<th>% Bioaccessible</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rock</td>
<td>0.88 ± 0.79</td>
<td>3170 ± 5220</td>
<td>110 ± 127</td>
<td>5.0 ± 3.6</td>
</tr>
<tr>
<td>Tailings</td>
<td>1.6 ± 1.6</td>
<td>116 ± 4220</td>
<td>116 ± 112</td>
<td>2.9 ± 1.7</td>
</tr>
<tr>
<td>Mine Organic</td>
<td>38 ± 17</td>
<td>850 ± 1020</td>
<td>164 ± 211</td>
<td>20 ± 11</td>
</tr>
<tr>
<td>Residential</td>
<td>47 ± 21</td>
<td>142 ± 128</td>
<td>72 ± 112</td>
<td>31 ± 28</td>
</tr>
</tbody>
</table>

Sequential Selective Extraction

Dilution Factors

- No significant difference across ratios
- Does work need to be done on other soil types or on a site specific basis?

Table 4.1: Bioaccessible arsenic concentration (%) across varying dilution factors.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Soil Type</th>
<th>% Bioaccessible</th>
<th>% MBE</th>
<th>% MBE</th>
<th>% MBE</th>
<th>% MBE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mine Organic</td>
<td>Residental</td>
<td>Rock</td>
<td>Tailings</td>
<td>Rock</td>
<td>Tailings</td>
<td>Rock</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(%)</td>
<td>(ppm)</td>
<td>(%)</td>
<td>(ppm)</td>
<td>(%)</td>
</tr>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>
Grain Size

- No difference between ESG standard lab prep (<250um) and <63 um

First time GFE extracts have been speciated
- Dominant species As(V), with minor amounts of As(III)
- Important as toxicity values used in human health risk assessment based on As(V)

<table>
<thead>
<tr>
<th>Soil Type</th>
<th>Grain Size</th>
<th>% Soil As</th>
<th>GFE As</th>
<th>% Bioaccessible</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rock</td>
<td>Standard preparation</td>
<td>7</td>
<td>1750 ± 150</td>
<td>41.44</td>
</tr>
<tr>
<td>Rock</td>
<td>Fancy filter</td>
<td>5</td>
<td>2570 ± 200</td>
<td>61.12</td>
</tr>
<tr>
<td>Tailings</td>
<td>Standard preparation</td>
<td>3</td>
<td>2780 ± 150</td>
<td>107 ± 17</td>
</tr>
<tr>
<td>Tailings</td>
<td>Fancy filter</td>
<td>8</td>
<td>2790 ± 150</td>
<td>70.5 ± 6</td>
</tr>
<tr>
<td>Mine Organic</td>
<td>Standard preparation</td>
<td>4</td>
<td>303 ± 150</td>
<td>14.5 ± 1.4</td>
</tr>
<tr>
<td>Mine Organic</td>
<td>Fancy filter</td>
<td>4</td>
<td>303 ± 150</td>
<td>14.5 ± 1.4</td>
</tr>
</tbody>
</table>

Speciation of GFE Extracts

- First time GFE extracts have been speciated
- Dominant species As(V), with minor amounts of As(III)
- Important as toxicity values used in human health risk assessment based on As(V)

Hazard Quotient = EDI / RfD

<table>
<thead>
<tr>
<th>Soil Type</th>
<th>Total Arsenic (ppm)</th>
<th>% Bioaccessible Arsenic</th>
<th>Traditional Hazard Quotient</th>
<th>Bioaccessible Hazard Quotient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rock</td>
<td>3180</td>
<td>5.6</td>
<td>13.9</td>
<td>0.008</td>
</tr>
<tr>
<td>Tailings</td>
<td>4500</td>
<td>2.9</td>
<td>2.1</td>
<td>0.73</td>
</tr>
<tr>
<td>Mine Organic</td>
<td>850</td>
<td>20.0</td>
<td>47.4</td>
<td>0.094</td>
</tr>
<tr>
<td>Residential</td>
<td>142</td>
<td>31.2</td>
<td>0.078</td>
<td>0.024</td>
</tr>
</tbody>
</table>

Implications of Arsenic Bioaccessibility on Risk Assessment

HQ>0.2 → potential risk to human health

Case Study 2:
Bioavailability of Nickel from Contaminated Soils

A Comparison of in vivo and in vitro Methodologies

Study Goals

- Evaluate the bioavailability / bioaccessibility of nickel from impacted soils and its influence on risk assessment
- Conduct in vivo rat soil feeding study to determine the relative oral bioavailability and the absolute oral bioavailability of nickel in impacted soil
- Validate an in vitro bioaccessibility extraction to estimate the oral bioaccessibility of nickel impacted soil

Design of in vivo Rat Study

- Male Sprague Dawley rats, 8 to 10 weeks old
- Single dose via oral gavage
- Three Ni contaminated soils
- Five dosing groups – 18 rats/group
Absolute Oral Bioavailability of Nickel

• Less than 0.40% of the total concentration of nickel in soils was found to be absorbed across the gastrointestinal tract (absolute bioavailability)

### Sample Group

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood (µg)</td>
<td>0.37</td>
<td>1.7</td>
<td>0.22</td>
<td>0.23</td>
<td>0.35</td>
</tr>
<tr>
<td>Urine (µg)</td>
<td>2.0</td>
<td>880</td>
<td>19</td>
<td>35</td>
<td>26</td>
</tr>
<tr>
<td>Tissues (µg)</td>
<td>11</td>
<td>17</td>
<td>9.9</td>
<td>14</td>
<td>6.0</td>
</tr>
<tr>
<td>Total (µg)</td>
<td>13</td>
<td>900</td>
<td>29</td>
<td>49</td>
<td>32</td>
</tr>
</tbody>
</table>

### Absolute Bioavailability (%)

| Sample Type     | NA | 8.0 | 0.39 | 0.35 | 0.20 |

**in vitro** BAT Methodology

• Soil: Liquid ratio 1:100 used in a two stage extraction

  - **Stomach Phase:**
    - HCl solution at pH 1.5 or glycine / HCl solution pH 1.5
      - extracted on temperature controlled shaker table @ 150 rpm, 1 hr
  - **Intestinal Phase:** pH raised to 7 with 10% NaOH
    - addition of bile extract and pancreatin returned to shaker for 4 hrs

• End of Extraction: samples filtered, acid digested and analyzed for Ni, Co, Cu, As by ICP-AES

### Comparison of In Vitro and In Vivo Results (Ni)

<table>
<thead>
<tr>
<th>Soil Type</th>
<th>Absolute Calculated Bioavailability (%)</th>
<th>Absolute Bioavailability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stomach Phase</td>
<td>Intestinal Phase</td>
</tr>
<tr>
<td></td>
<td>With Glycine</td>
<td>Without Glycine</td>
</tr>
<tr>
<td>Welland Clay</td>
<td>9.8</td>
<td>8.1</td>
</tr>
<tr>
<td>Organic Soil</td>
<td>12</td>
<td>11.3</td>
</tr>
<tr>
<td>Fill Soil</td>
<td>6.8</td>
<td>6.5</td>
</tr>
</tbody>
</table>

**Ni at pH 7 (no glycine)**

\[
\text{Ni}^{2+} + 2\text{OH}^- \rightleftharpoons \text{Ni(OH)}_2
\]

**Glycine at stomach pH 1.5**

\[
\text{Ni}^{2+} + \text{H}_3\text{N}^+ - \text{CH}_2 - \text{C} \equiv \text{O} \rightleftharpoons \text{Ni}^{2+} + \text{H}_3\text{O}^+
\]

**Glycine at intestinal pH 7**

\[
\text{Ni}^{2+} + \text{H}_2\text{N} - \text{CH}_2 - \text{C} \equiv \text{O} \rightleftharpoons \text{Ni}^{2+} + \text{H}_2\text{N} - \text{CH}_2 - \text{C} \equiv \text{O}
\]
Implications of Nickel Relative Oral Bioavailability on Risk Assessment

Hazard Quotient = \[\text{exposure dose (ED)} \times \text{oral reference dose (RfD)}\]

where RfD = 20 ug/kg-d (US EPA IRIS)
HQ>0.2 → potential risk to human health

<table>
<thead>
<tr>
<th>Soil Type</th>
<th>Total Soil Nickel (ppm)</th>
<th>100% Bioavailable Hazard Quotient</th>
<th>Relative Oral Bioavailable Hazard Quotient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Welland Clay</td>
<td>10 400</td>
<td>0.15</td>
<td>0.0958</td>
</tr>
<tr>
<td>Organic Soil</td>
<td>14 300</td>
<td>0.2</td>
<td>0.0065</td>
</tr>
<tr>
<td>Fill Soil</td>
<td>14 600</td>
<td>0.21</td>
<td>0.0044</td>
</tr>
</tbody>
</table>

Case Study 3: Bioavailability of Arsenic in Ironite®

A Comparison of \textit{in vivo} and \textit{in vitro} Results
Ironite®

- Website claims 95% of arsenic is as arsenopyrite

#### Arsenic Speciation/Toxicity

- Inorganic Arsenic
- Arsenobetaine
- Scorbite (S)
- Arsenopyrite (AP)

#### Bioaccessibility of Ironite® Mineral Phases

- I = Ironite
- AP = arsenopyrite
- S = scorbite

#### Effect of Organic C

- Comparison to Bioavailability
  - Experiments carried out with hamsters by Vas Aposhian et al. (same Ironite®)
    - Absolute bioavailability = 26%
    - Bioaccessibility = 8-18%

#### Food Bioaccessibility Measurements

- Asian medicine
- Rice
Hijiki Bioaccessibility and Bioavailability

- Foods analyzed by ICPMS and NAA
- Gastric extraction:
  - 1.25g/L pepsin, 0.15 M NaCl, pH 1.8
  - 3% (m/m) hijiki in liquid
  - 30% (m/m) total food with representative proportions
  - ICPMS analysis of extracts
- ICPMS analysis of urine samples (2 volunteers)

Other food appears to decrease % extraction

<table>
<thead>
<tr>
<th>Conditions</th>
<th>% Extraction ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>GE hijiki</td>
<td>90 ± 6</td>
</tr>
<tr>
<td>GE hijiki + food</td>
<td>14 ± 4</td>
</tr>
<tr>
<td>Bioavailability (%) (2 volunteers)</td>
<td>37, 48</td>
</tr>
</tbody>
</table>

Method Development

- Need to evaluate the effects of buffering agents on “other” elements
  - As and Pb studied most frequently
- Simple method
  - Including a buffer ideally
  - Readily available laboratory equipment
- Effects of filtration/digestion of filtrate
- Comparison to in vivo results

International Collaborations

- University of Arizona
  - Dr. Vas Appochain
    - Ironite - As
- Ohio State University
  - Dr. Nick Basta
    - Nine soils: As, Cd
- BARGE
  - Round robin validation

Questions?