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Accounting for Bioavailability in Contaminated Land Site-Specific Health Risk Assessment

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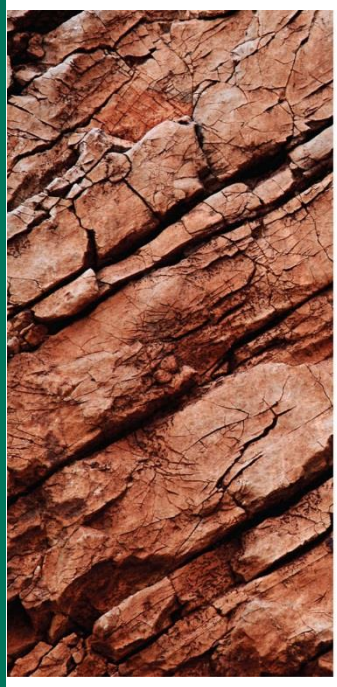

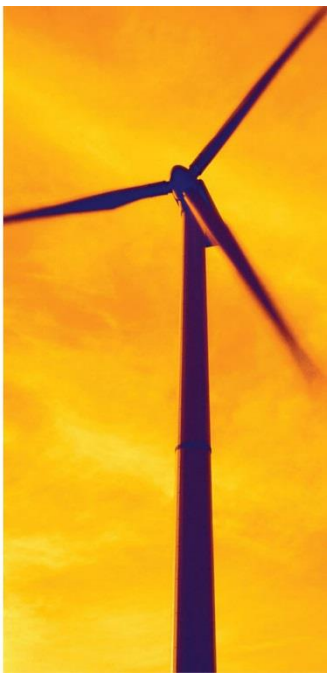

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REPORT

Golder Associates

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Executive Summary

Economic and industrial development since the mid-1800s has left New Zealand with a legacy of contaminated land. This land ranges from highly contaminated gasworks and chemical storage sites to residential properties and farmland subject to diffuse low level contamination.

On face value, large areas of agricultural land pose a potential risk to human health when developed for residential use. For example, spraying pipfruit orchards with lead arsenate has left tens of thousands of hectares exceeding residential SCS for arsenic. Consequently, such land poses current and future management issues.

Soil Contaminant Standards (SCS) were developed as generic criteria to assess contaminated land. One key assumption in the derivation of the SCS is that contaminants are 100 % bioavailable. There is increasing evidence that this is not the case for arsenic (and lead) in soils impacted by a range of historical land use activities. Management issues with former agricultural land could be alleviated if arsenic bioavailability could actually be measured for specific sites or groups of sites.

This Review aimed to determine whether there is now an appropriate methodology for taking bioavailability into account during site-specific health risk assessment. One possible measurement tool is a validated 'bioaccessibility' test that extracts a similar proportion of arsenic and lead from soils as would be absorbed by people ingesting the soil. Five years ago, the Ministry for the Environment (MfE) took the position that there was insufficient scientific evidence to support bioaccessibility testing in New Zealand.

Golder Associates (NZ) Limited (Golder) screened ten published bioaccessibility test methods to see which were most likely to be appropriate, and analysed the two leading tests in detail. In Golder's view the gastric phase tested developed by the Solubility/Bioavailability Research Consortium (SBRC-G) meets relevant scientific, economic and social assessment criteria. It has been validated for arsenic in a wide range of conditions, and performed well in those validation studies. The SBRC-G test has already been used in New Zealand (Golder 2012, Gaw et al. 2008) and has USEPA approval for lead in soils (USEPA 2009).

Contaminated land regulators and practitioners are likely to need national guidance on bioavailability testing for site specific health risk assessment. In addition, a policy position is needed as to whether risk assessment calculations should use the mean bioavailability or some upper bound statistic. Golder suggests that this would best be done within a wider guidance document for site-specific health risk assessment.

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List of Abbreviations and Units

Term	Definition
As	Arsenic
BARC	Bioaccessibility Research Canada
BARGE	Bioaccessibility Research Group of Europe
CSM	Conceptual Site Model
Golder	Golder Associates (NZ) Limited
HCl	Hydrochloric acid
Hills	RJ Hill Laboratories Limited
HRA	Health risk assessment
IVBA	*In Vitro* Bioaccessibility Assay
IVIVC	In-vitro in-vivo correlation
IVG	In Vitro Gastrointestinal extraction method
GI	Gastrointestinal
µm	Microns (micrometres)
NES:CS	Resource Management (National Environmental Standard for Assessing and Managing Contaminants in Soil to Protect Human Health) Regulations 2011
Pb	Lead
PBET	Physiologically Based Extraction Test
pH	Negative of the logarithm of the hydrogen ion concentration
RAF	Relative Absorption Factor
RBAPL	Relative Bioaccessibility Leaching Protocol
SBET	Simplified Bioaccessibility Extraction Test
SBRC	Solubility/Bioavailability Research Consortium
SCS	Soil Contaminant Standard
SGV	Soil Guideline Value
SHIME	Simulator of the Human Intestinal Microbial Ecosystem
TIM	TNO Intestinal Method
UBM	Unified BARGE Method
USEPA	United States Environmental Protection Agency

Glossary

Term	Definition
Bioavailability	Bioavailability is defined as the proportion of a substance that is absorbed from soil in the digestive system into the body.
Bioaccessibility	The bioaccessibility of a contaminant is the proportion that can be extracted under simulated digestive conditions. The oral bioaccessibility of a substance is the fraction that is soluble in the gastrointestinal environment and is available for absorption.
IVIVC	In-vitro in-vivo correlation is defined as "a predictive mathematical model describing the relationship between an in-vitro property of a dosage form and an in-vivo response".
In vivo	Latin for “within the living” and comprising studies in which the effects of various biological entities are tested on whole, living organisms usually animals including humans, and plants.
In vitro	Latin for “within the glass” and comprising studies that are performed with microorganisms, cells or biological molecules outside their normal biological context.
Relative Bioavailability	Relative bioavailability refers to comparative bioavailabilities of different forms of a substance or for different exposure media containing the substance (e.g., bioavailability of a metal from soil relative to its bioavailability from water).

1.0 INTRODUCTION

1.1 Purpose

The Ministry for the Environment (MfE, the Ministry) commissioned Golder Associates (NZ) Limited (Golder) to undertake a review (the Review) of methods for assessing bioavailability of key soil contaminants as part of site specific health risk assessments.

The international understanding of bioavailability, and thus the need for national guidance on bioavailability, both appear to have advanced substantially since MfE last issued guidance on contaminant risk assessment (MfE 2011a, 2011b). Accordingly, the purpose of this Review is to evaluate whether there is now sufficient weight of evidence to adopt an appropriate methodology to assess contaminant bioavailability during site specific health risk assessment. Definitions and the relationship between bioavailability and bioaccessibility are presented in Section 2.0 of this Review.

This Review was commissioned by the Ministry under Statement of Work 0559-09-RFQ and the scope presented in Golder's proposal dated 18 November 2015.

1.2 The Issue

Economic and industrial development since the mid-1800s has left New Zealand with a legacy of contaminated land. This land ranges from highly contaminated gasworks and chemical storage sites, to residential properties and farmland subject to diffuse low level contamination. Given this legacy, New Zealand contaminated sites have generally arisen through single source contaminating activities impacted by a limited range of contaminants, key trace metal impacts include arsenic, lead, copper, and chromium.

Until the 1960s, New Zealand's pipfruit orchards were routinely sprayed with lead arsenate to kill codling moth and other chewing insects. Sheep were dipped in arsenic solutions to kill insect parasites. Market gardens and sports greens were sprayed with organoarsenical herbicides. Timber was, and still is, treated with chromated copper arsenate (CCA). Since arsenic and lead are toxic trace elements and persist in surface soils, these practices have created tens of thousands of hectares of contaminated land that will remain with us for the foreseeable future.

On the face of it, this land could present a potential risk to human health. Many pipfruit orchards and market gardens were located close to town and city boundaries, and consequently much of the affected land has since been developed for residential use. Arsenic concentrations in former New Zealand pipfruit orchard soils have frequently been found to exceed the current Soil Contaminant Standards (SCS) for rural residential use (17 mg/kg) and ordinary residential use (20 mg/kg) (PDP 2004, Gaw 2003, PDP 2007).

On this basis, this pipfruit orchard land poses current and future land management issues for many territorial and regional councils. Such land has to be placed on contaminated land registers, and in accordance with regulations made under the Resource Management Act 1991 (RMA), land use consents are typically required for subsequent redevelopment, subdivision or soil disturbance. Arguably, landowners may be committing offences under the Health Act 1956 by allowing affected residential properties to be occupied, and could have to remediate at their own expense, or face enforcement by their territorial or local authority. These factors have the potential to lead to reductions in affected land value, create a reluctance to developed affected land or increase land costs given the remediation required before land development occurs.

These potential adverse consequences rest on the premise that the generic SCS accurately predicts health risk on contaminated land. SCS are necessarily conservative because they must be applicable to a range of different situations, and by definition cannot respond to site-specific factors.

One key assumption in the SCS is that contaminants are 100 % bioavailable. That is, if we happen to swallow contaminated soil and dust, the contaminants will be completely absorbed into the body. This assumption is likely to be significantly conservative for arsenic and lead in former agricultural soils. Consequently, land management issues could be alleviated if arsenic and lead bioavailability could be estimated more accurately.

Some animals are confidently expected to take up contaminants in much the same way as people do. Consequently, other international jurisdictions have used animal testing to assess bioavailability. However, toxicologists are always looking for ways to reduce the need for animal testing, for humane reasons and because animal testing is necessarily time-consuming and expensive.

The alternative is 'bioaccessibility' testing using non-living *in vitro* ('in glass') test systems. In recent years, researchers in the United States, Europe and Australia have developed laboratory tests that extract a similar proportion of arsenic and lead from soils, as is absorbed by animals eating the soil. If there are bioaccessibility tests that are scientifically valid, practical, and acceptable to regulators and the public, they would offer a way forward for New Zealand's arsenic-and lead-contaminated sites to be assessed. Bioaccessibility testing has been readily adopted to various degrees by international environmental regulators as part of site-specific health risk assessment (Appendix A).

1.3 Scope of Review

The scope of the Review comprises the following:

- Providing the background to bioavailability and bioaccessibility and the application of these concepts to site specific health risk assessment for contaminated soil.
- Assessing suitability and appropriateness of bioaccessibility analysis in New Zealand.
- Identifying contaminants that can be assessed by the preferred analysis.
- Identifying gaps in knowledge and guidance that will need to be addressed to allow implementation of the proposed assessment methodology.

In developing the scope of the Review, the Ministry specified a number of key constraints/controls, including:

1) Bioaccessibility testing should be undertaken using an in vitro method.
2) Consideration should only be given to methods that have been validated against in vivo test data.
3) The adopted method must be applicable to the assessment of arsenic, for the reasons set out above.

2.0 BIOAVAILABILITY AND BIOACCESSIBILITY

2.1 Definition of Bioavailability and Bioaccessibility

There are two terms that are central to this review – bioavailability and bioaccessibility. These are properties of contaminants in soils, ideally equivalent in value but determined in different ways. This section introduces these concepts.

The chemical relationships between trace elements and soils are complex. Most trace elements can take many different chemical forms in the soil environment. Depending on the element, these forms can include oxides and sulfides, carbonates and phosphates, complexes with iron or calcium, coatings on particles of iron or manganese oxides, associations with humic (organic) materials or clay minerals. The actual mix of chemical forms present in any specific soil depends on a host of factors. Key factors include soil acidity, degree of oxidation, the source of the trace elements, and ongoing biological and geological processes.

When water comes into contact with soil, some forms of the trace elements that are present dissolve and leach out, while other forms stay put. If soils are exposed to stronger leaching agents such as acids and chelators, more forms dissolve and greater proportions of the trace elements are leached. Soil particle size is also a factor; large particles are slow to dissolve and small particles have a greater surface area on which contaminants can be bound.

Different leaching agents have been shown to extract specific chemical forms of trace elements from soils. The scientific literature on the subject is extensive. Tessier et al. (1979) proposed a sequential extraction method to operationally distinguish between five increasingly intractable forms of trace elements – those that were readily exchangeable; those that were bound as carbonates; those bound to iron or manganese oxides; those bound to organic matter; and the residual trace elements in mineral form, which would not dissolve in a reasonable time frame under common environmental conditions.

From a contaminated land health risk assessment perspective, the important question is: Which chemical forms are extracted when soils containing trace elements are exposed to the leaching conditions in the human digestive system? Those forms of a trace element that dissolve in digestive fluids can be absorbed through the linings of stomach and intestines. This fraction enters the bloodstream and is transported to different parts of the body. What happens next depends on the biochemistry of the particular trace element, for example it may be excreted in urine, or stored in bone, brain, liver, fatty tissue, etc. The fraction that is not dissolved and absorbed is eventually expelled from the digestive system along with other solid waste.

> Definition 1. *The bioavailability of a trace element is the proportion of that element that is absorbed from soil in the digestive system into the body.*

For some trace elements, such as lead, the digestive system is never very efficient at absorbing them, and it is more useful to talk about *relative* bioavailability compared to some readily absorbed form. Note that the bioavailability of trace elements from food or water, to other animals, to plants, or through skin or lung tissue may be quite different. Some literature sources may use different definitions.

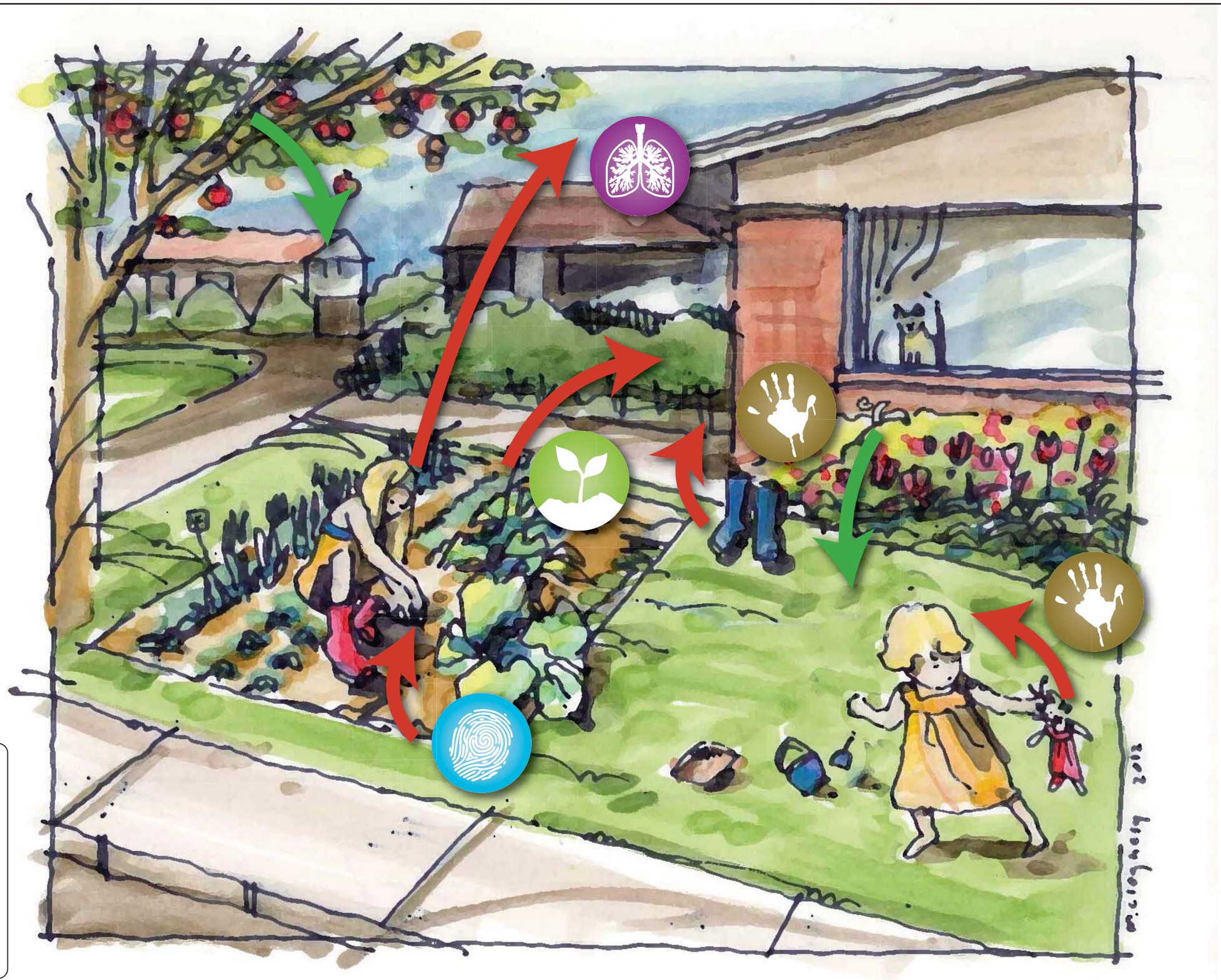
Bioavailability of trace elements is not generally determined directly from measurements in human body tissues. There are practical collection difficulties, it is hard to attribute findings to a specific source, and there can be ethical issues. Bioavailability is typically measured using (*in vivo*) tests with animals that are physiologically similar to people, for example piglets (“juvenile swine”) are considered a particularly good surrogate for small children. However, conducting live animal bioavailability tests on a site-by-site basis is time-intensive and costly, even for a single test per site, and still poses ethical issues.

Accordingly, laboratory-based (*in vitro*) extraction procedures have been developed by researchers to mimic biological ‘extraction’ using simulated digestive fluids. Dissolved trace elements in the simulated biological fluid are then measured by standard analytical techniques. The result is called the ‘bioaccessible’ fraction – the fraction that is ‘accessible’ for absorption into the bloodstream if ingested.

> Definition 2. *The bioaccessibility of a trace element is the proportion of that element that can be extracted under simulated digestive conditions.*

2.2 Rationale for Bioaccessibility Testing

Soil ingestion is thought to be the dominant health risk exposure pathway for many trace elements in soils, including arsenic and lead (Figure 1). Small amounts of contaminated soil and soil-derived dust can adhere to children’s hands and to toys, which are then intentionally or accidentally put in the mouth. For both children and adults, some contribution may come from soil attached to vegetables, and from coarse dust that is inhaled and then swallowed. That there are exceptions to this general rule, notably cadmium, for which the dominant exposure pathway is thought to be consuming produce grown on contaminated soil.



Legend

- Produce consumption
- Soil ingestion
- Dermal absorption
- Dust inhalation
- Exposure pathway
- No exposure pathway in this assessment

1. Schematic only, not to be interpreted as an engineering design or construction drawing
2. DRAWN BY: Michele Cloghesy (Lanarc) / VG REVIEWED BY: HG

Golder Associates

TITLE | SITE CONCEPTUAL MODEL FOR HEALTH RISK ASSESSMENT

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In generic health risk assessments, such as those underlying the development of the SCS, models of soil ingestion necessarily assume that contaminants have 100 % relative bioavailability (RBA). This assumption cannot be tested on people. However, the consistent theme emerging from bioavailability studies is that the actual value may be much less than 100 %. As of 2011, out of 64 animal in vivo studies of arsenic bioavailability in United States soils, arsenic RBA ranged from 4.1 % to 78 %; even taking uncertainty into account, and it was estimated that less than 5 % of values exceeded 60 % (USEPA 2012).

Health risk assessment is an imprecise science and some conservatism is always advisable. However, there may be compelling reasons to collect more information, reduce uncertainty and remove conservatism for specific sites. Unlike petroleum hydrocarbons or organochlorine compounds, trace elements do not degrade. Remediation is often expensive and involves excavation of contaminated soil to a less sensitive area (i.e., disposal at Class A landfills distant from the subject property). If a site exceeds SCS by a moderate margin – say no more than 5- to 10-fold – it may be a better use of time and resources to refine the health risk assessment by taking bioavailability into account. This may have benefits in comparison to pursuing remediation or abandoning the land. This is more likely to be the case for arsenic, which was widely used in New Zealand agriculture and has relatively low SCS compared to natural background, than for other potentially toxic trace elements such as lead.

Animal testing is one option for assessing bioavailability. However, as already outlined, animal testing is necessarily time-consuming, expensive, difficult and arguably unethical. If a laboratory test could be found that would consistently give a similar result, that would be highly preferable.

2.3 Test Development

In the mid-1990s, Ruby et al. (1996) in the United States initiated work on oral bioaccessibility studies of arsenic and lead from soils and household dust collected in the vicinity of a historical copper smelter. The in vivo oral bioavailability of arsenic and lead from these samples had been previously determined in rabbits (Freeman et al. 1993) and cynomolgus monkeys (Freeman et al. 1995), and shown to be significantly less than 100 %.

Ruby et al. (1996) termed its in vitro chemical extraction method the “physiologically based extraction test” (PBET) because conditions were based on a child’s gastric system – two sequential extractions representing stomach and small intestine, using what were believed to be realistic parameters for gastric and small intestinal pH, soil mass, fluid volume, stomach mixing and emptying rate, and small intestinal transit time. These studies found a reasonable correlation existed between bioavailability of arsenic and lead in animal feeding studies and the bioaccessibility of those elements in the PBET’s gastric phase. The PBET tended to overestimate bioavailability, but not excessively so.

Other groups soon published their own results. Some used the same PBET methodology or variations thereon, others developed their own simulations of the gastrointestinal tract. Meanwhile, bioavailability studies moved to laboratory animals that were better analogues of human digestion (pig) or easier to work with (mouse). Researchers extended the method to soils contaminated by mining or pesticide manufacture, soils with naturally high levels of contaminants, and then soils contaminated by agricultural activities at lower levels.

These studies identified several key parameters for in vitro experiments (Health Canada 2006). The pH of the simulated gastric fluid has proved to be the single most influential variable. Gastric pH also varies considerably between methodologies – as low as 1.2 and as high as 4.0 (a 600-fold difference in acidity). Soil:solution ratio is another parameter that has varied considerably, causing problems for some studies with low ratios (5:1, 25:1, even 37.5:1), where the extractant became saturated by high concentrations of contaminant, leading to artificially low results.

Other experimental parameters seemed to have less effect on results, such as residence time, temperature, whether the leaching solution is shaken or stirred, and how it is analysed at the end of the experiment.

Some researchers added a salivary / mouth phase, and more complex reagents such as salts, stomach and intestinal enzymes, bile and pancreatic juice. The question of whether to mimic a fasted state, or a fed one, and in the latter whether to add food material, has been actively debated. Some European methods are 'flow-through' systems intended to better represent movement of food through the digestive system. Other researchers actively looked to keep the method simple – and developed the Solubility / Bioavailability Research Consortium (SBRC)'s method which did away with the intestinal phase altogether. The SBRC's gastric phase simply involves shaking for one hour in a glycine hydrochloride buffer at 37 °C.

Appendix B presents and summarises the 20 arsenic bioaccessibility studies incorporating validation against animal models that Golder has identified in the literature.

2.4 Current New Zealand Position

In 2011, New Zealand established a process for generating and applying SCS for 'priority contaminants' including arsenic and lead (MfE 2011a, 2011b, 2011c). The process is articulated in the 'Methodology for Deriving Standards for Contaminants in Soil to Protect Human Health' (the Methodology (MfE (2011a)). Under the relevant National Environmental Standard, SCS are default 'applicable standards' when removing underground fuel storage systems, sampling soil, disturbing soil, subdividing land, and changing land use (NES:CS).

Regulation 7 of the NES:CS, and section 9 of the Methodology, allow for site-specific risk assessment. In site-specific risk assessment, parameters are varied from the generic values, and exposure pathways are added or removed, as dictated by a conceptual site model, to generate a site-specific soil guideline value (SGV). Mathematically, it is straightforward to account for bioavailability within the SCS methodology, by multiplying the soil ingestion rate and the soil loading on vegetables by the percentage bioavailability. Nonetheless, the Methodology strongly discouraged this, on the basis that:

> "*Until the science is better developed for New Zealand soils and conditions bioavailability considerations in site-specific assessments are not appropriate. Any adoption of site-specific assessment using reduced bioavailability in New Zealand should use a multiple lines of evidence approach. At present, the science does not support in vitro testing for other than lead and perhaps arsenic, despite wider use overseas. The present knowledge within the contaminated land community in New Zealand, both practitioners and regulators, is insufficient to give confidence that bioavailability test results would be applied correctly.*"

Moreover, it is unclear precisely how the percentage bioavailability ought to be calculated. Most parameters within the Methodology are central tendencies (averages, or upper confidence limits to the mean) but a few are upper bounds.

The Ministry of Health's (MoH 2012) guidance on managing environmental lead exposure confirms that lead exposure is dependent on bioavailability, but does not provide any further guidance on how bioavailability is to be assessed or taken into account.

We are aware of one precedent for incorporating bioaccessibility test results into a contaminated land assessment. Golder included arsenic and lead bioavailability, among other considerations, within a site-specific health risk assessment for the Moanataiari subdivision, Thames (Golder 2012). The assessment was accepted by the regulator, Thames-Coromandel District Council. Golder was able to address the issues raised in the SCS methodology by collecting supporting physico-chemical data, by locating its methodology within USEPA practice, and by drawing on staff with relevant international experience. Moreover, the approach was demonstrably suited to Moanataiari as arsenic concentrations were only moderately above SCS (at least in the west of the subdivision), the cost of remediating or relocating an existing subdivision was estimated at as much as $80M, and mining-impacted soils at Moanataiari appeared similar to mining-impacted soils studied overseas.

2.5 Future Application

With the above considerations in mind, it appears that there may be a place within the New Zealand contaminated land regulatory framework for bioavailability assessment through bioaccessibility testing, provided that:

- Bioavailability remains a matter for site-specific risk assessment, since it is likely to vary from soil to soil.
- There is already a well-developed conceptual site model.
- The principal contaminant of concern is arsenic or lead.
- Taking bioavailability into account could affect the outcome of the assessment (i.e., the site currently exceeds SCS, but not by a large margin) and the site management decision.
- There is a validated bioaccessibility test appropriate to the contaminant(s), concentration range(s), source(s) and soil(s) in question.
- There is supporting information on physico-chemical characteristics of the soil(s) and the contaminant binding phase(s).
- There is national guidance on how to incorporate bioaccessibility test results into risk assessments, including a policy position on whether a central estimate or high-end parameter is to be used.

The following section of this report examines which bioaccessibility tests might be fit for this purpose.

3.0 SELECTING A SUITABLE BIOACCESSIBILITY TEST

3.1 Preliminary Screening Assessment

USEPA (2007) recommends twelve criteria be met before a bioaccessibility test method is considered suitable for regulator use. Some of these criteria are difficult to adapt to the New Zealand context, such as a requirement that validation data meet a particular quality assurance standard, Good Laboratory Practice (GLP). Few academic laboratories are GLP participants, so insisting on this requirement would mean discarding most published studies on the subject. In other respects, the USEPA guidance is not broad enough, for example, it does not explicitly address social acceptability.

Accordingly, Golder has developed a preliminary screening approach based on the USEPA (2007) criteria and expanded the evaluation criteria to incorporate a broader assessment of scientific / technical, economic / practicable, and social / regulatory attributes (Appendix C). Bioaccessibility test methods are rated on eleven scientific / technical aspects, three economic / practical aspects, and five regulatory / social aspects, where:

- '1' indicates that the method has been rigorously assessed as satisfactory.
- '2' indicates that there is some information to indicate that the method is satisfactory.
- '3' indicates little or no information.

None of the methods were proven unsatisfactory for arsenic, or they would not have been considered. The ratings were summed within each category, then the three category sums were multiplied out, to give an overall score between 165 (proven ready for use) and 4,455 (manifestly unsuitable). The aspects, ratings and overall scores are presented in Table 1.

Table 1: Preliminary screening assessment of bioaccessibility test methods.

	SBET / SBRC / RBALP	UBM	DIN	A/NZ ISO8124-3	PBET	IVG	Hydroxylamine hydrochloride	'Dutch' method	TIM	SHIME
Scientific / Technical Attributes										
In vitro method validated by in vivo	1	1	2	3	2	2	2	3	3	3
Suitable for key contaminant - arsenic	1	1	1	1	1	1	1	1	2	2
Validated for a range of contaminant sources and chemical forms	1	1	1	2	1	1	2	3	3	3
Validated for concentrations relevant to residential SCS	1	1	1	3	1	1	3	3	3	3
Detailed laboratory method	1	1	1	1	2	2	2	2	2	3
Previous experience with method in NZ	1	2	2	1	2	2	2	3	3	3
Validated for a range of soil types and uses	1	1	1	3	1	1	2	3	3	3
Accuracy in reproducing in vivo	1	1	2	3	2	2	2	2	2	2
Consistency of method	1	1	2	1	2	2	2	2	1	1
Are results reproducible between laboratories?	1	1	2	1	2	2	2	2	3	3
Data from reference materials?	1	1	1	1	1	1	3	3	3	3
Scientific / Technical Score	**11**	**12**	**16**	**20**	**17**	**17**	**23**	**27**	**28**	**29**
Economic / Practical Attributes										
Is there a Standard Operating Procedure?	1	1	1	1	2	2	2	1	2	3
Does the test require significant laboratory investment?	1	1	1	1	1	1	1	3	3	3
Licensing fees for test method?	1	1	1	1	1	1	1	1	3	3
Economic / Practical Score	**3**	**3**	**3**	**3**	**4**	**4**	**4**	**5**	**8**	**9**
Regulatory / Social Attributes										
Was the method developed by a reputable authority?	1	1	1	1	1	2	2	1	2	2
Has validation been published in a peer-reviewed journal?	1	1	1	1	1	1	1	1	1	1
Does performing test raise ethical issues?	1	1	1	1	1	1	1	1	1	1
Ethical issues with development/validation of test?	2	2	2	1	2	2	2	1	1	1
Potential Te Tiriti o Waitangi / Treaty of Waitangi issues?	1	1	1	1	1	1	1	1	1	1
Regulatory / Social Score	**6**	**6**	**6**	**5**	**6**	**7**	**7**	**5**	**6**	**6**
Three Factor Overall Score	**198**	**216**	**288**	**300**	**408**	**476**	**644**	**675**	**1344**	**1566**

3.2 'Long List' of Bioaccessibility Test Methods

Golder identified ten bioaccessibility tests potentially applicable to arsenic primarily as well as other contaminants. They are:

- The Physiologically Based Extraction Test (PBET: Ruby et al. 1996, Rodriguez et al. 1999, Bruce et al. 2007, Juhasz et al. 2009, Juhasz et al. 2014a, Diacomanolis et al. 2015, Li et al. 2015).
- The In Vitro Gastrointestinal test (IVG: Rodriguez et al. 1999, Basta et al. 2007, Juhasz et al. 2009, Nagar et al. 2009, Hawkins et al. 2013, Li et al. 2015).
- Hydroxylamine hydrochloride extraction (Rodriguez et al. 2003).
- The Simplified Bioaccessibility Extraction Test (SBET) or Solubility and Bioaccessibility Research Consortium test (SBRC) or Relative Bioaccessibility Leaching Protocol (RBALP: SBRC 1999, Juhasz et al. 2007a,b, Juhasz et al. 2009, Bradham et al. 2011, Juhasz et al. 2014a, Griffin and Lowney 2013, Hawkins et al. 2013, Juhasz et al. 2014b, Bradham et al. 2015, Li et al. 2015: see also Diamond et al. 2016).
- The Standardised German In Vitro Assay (Deutsche Institut für Normung standard method DIN 19738: Juhasz et al. 2009, Juhasz et al. 2014a, Li et al. 2015).
- The RIVM or 'Dutch' in vitro digestion method (Oomen et al. 2003).
- The Unified Bioavailability Research Group of Europe Method (UBM: Wragg et al. 2011, Denys et al. 2012, Juhasz et al. 2014a, Li et al. 2015).
- The Simulator of the Human Intestinal Microbial Ecosystem reactor (SHIME: www.prodigest.eu).
- The TNO intestinal model (TIM: www.tno.nl).
- The Australian / New Zealand (A/NZ) Standard for the Safety of Toys, ISO8124-3:2010.

Summary details of the methods used to assess arsenic that have been subject to peer review, the soils they were applied to, and the animal bioavailability assessments they were validated against, are provided in Appendix B. The aspects, ratings and overall scores are presented in Table 1.

Briefly, the hydroxylamine hydrochloride extraction is a simple, mild chemical extraction targeting freely exchangeable and oxide-bound trace elements, reported in a single academic paper.

The SBRC (SBET, RBALP) method is just as simple, often with only a gastric phase (SBRC-G), but conducted at physiologically relevant pH and temperature, and extensively validated with USEPA support. The SBRC-G method was used by Golder at Moanataiari, and in an earlier academic study of arsenic, lead and cadmium bioavailability in New Zealand orchard soils (Gaw et al. 2008).

PBET, IVG and DIN are two-phase methods with more physiologically representative additives in the extractants, such as pepsin, pancreatin and bile salts. They have been studied in some depth and the DIN method has been developed to regulatory standard level.

The UBM and its precursor the 'Dutch' method are three-phase methods, incorporating a short salivary phase. The extractants are prepared to complex formulae, to achieve a high degree of chemical and biochemical representativeness. The UBM has been extensively validated for arsenic including inter-laboratory studies, with the support of European regulators. The 'Dutch' method is principally intended for lead and is approved for that purpose in the Netherlands.

SHIME and TIM are multiphase models designed to be as physiologically accurate as possible, down to realistic bacterial communities, and are principally used for nutritional studies, drug development and the like. Both are proprietary models and could not be implemented in New Zealand without the consent and assistance of their respective developers, universities in Belgium and the Netherlands. As far as Golder can determine, neither has been applied to the specific problem of arsenic bioavailability let alone validated, but the high degree of physiological accuracy should give a reasonable degree of confidence in results obtained using these tests; and there has been some application to lead.

The A/NZ standard is intended for assessing bioavailability of contaminants in paint, rubber, cardboard and other toy components for child safety purposes. To Golder's knowledge it has not been applied to soil, but as against that, it is a well-defined method already in use in New Zealand with regulator approval.

3.3 Summary of Preliminary Assessment

The weighted scoring assessment shown in Table 1 showed that the SBRC-G and UBM tests scored well. These two methods have been extensively applied to arsenic, validated against in vivo studies, at contaminant concentrations relevant to residential SCS and above. They have been evaluated for accuracy, consistency and reproducibility, and data are available for performance on standard reference materials. Both have weaknesses in that they were developed with reference to animal testing while the UBM drops another point in that it has not to Golder's knowledge been used in New Zealand.

The DIN and A/NZ Standards follow close behind. DIN scores very much like the leading tests, but falls behind because less work has gone into validation, and when validated it has been less accurate and less consistent. The A/NZ Standard seems likely to outperform the soil-specific methods on laboratory and ethical measures, but the apparent lack of applications to soil counts against it. Neither of these methods is quite as promising as SBRC and UBM.

The remaining tests are progressively less attractive. The most complex / physiologically accurate systems, TIM and SHIME, are not appropriate due to a lack of information about potential application to soil that results in a very poor technical score, and their proprietary nature which results in a very poor practical score. Still, all methods scored well on social / regulatory aspects, in part because of the inherent ethical advantages of bioaccessibility tests over animal bioavailability studies discussed in Section 2.2.

3.4 Detailed Assessment

The brief screening assessment set out above showed the SBRC-G and UBM bioaccessibility tests to be strong candidates for adoption in New Zealand. However, in a matter of such technical complexity, where results will be used to make decisions bearing on human health, a screening assessment is far from sufficient. It is necessary to understand test performance in detail.

Accordingly, Golder has undertaken a more rigorous evaluation and comparison of the SBRC-G and UBM tests when applied to arsenic, using an expanded version of the preliminary screening framework. The full evaluation is attached as Appendix D. In summary:

- The tests were developed by reputable authorities – research consortia backed by the USEPA (SBRC-G) and by government agencies of the United Kingdom, the Netherlands, France, Denmark and Canada (UBM). Validation studies have been published in peer-reviewed journals (10 papers for SBRC, three for UBM)
- The SBRC-G test has been validated for different contaminant sources, including herbicide / pesticide application, mining and smelting waste, and natural sources. **However the UBM may be limited to mining and smelting sources.** The tests have been validated for soils containing arsenic in a wide range of different binding phases. Neither test appears to have been validated for organoarsenic

herbicides as they should probably not be used for that purpose. Although soils used for validation have varied widely, no formal descriptions of soils that the tests can or cannot accurately assess have been developed. Both tests have been validated for soils in residential and some agricultural uses, in the case of SBRC-G specifically including orchards. The tests have been validated for a wide range of soil arsenic concentrations, and no factors have been identified that might lead to different performance at concentrations above the 20 mg/kg residential SCS. The tests have been validated for a wide range of bioavailability values.

- The SBRC-G test for lead has been formally approved for use by USEPA (2009) (Appendix E). The UBM can also be applied to lead, as well as cadmium and perhaps antimony (though this is of little relevance in New Zealand since the dominant exposure pathway for cadmium is uptake via produce, and antimony is not considered to be a priority contaminant.)
- The UBM mimics the human ingestion and (fasted) digestion process quite closely. **The SBRC-G test was not intended to be physiologically accurate, though it is explicitly carried out at pH, temperature and duration approximating stomach conditions.**
- SBRC-G results closely reflect oral bioavailability, **although raw UBM results tend to overestimate bioavailability.** Results of both tests are consistent with animal testing and broadly consistent with soil chemical and physical characteristics where these have been determined.
- Both SBRC-G and UBM are repeatable within-laboratory, but have never been subjected to an inter-laboratory study with five or more participants, so **have not had the opportunity to meet standards for inter-laboratory reproducibility**.
- Laboratory quality assurance checks, such as blanks and spikes, have been undertaken throughout validation of both tests, and were reported to be satisfactory throughout. Data quality objectives have been achieved for a wide range of samples, though **one UBM study reported saturation or matrix effects in highly contaminated materials**. Results have been obtained for standard reference materials.
- Test stability has been explored for both SBRC-G and UBM.
- The cost of the SBRC-G should be comparable to common commercial laboratory procedures. **However, the UBM is expected to be relatively expensive**, because it would take a full day to run, comprises three extraction phases using complex solutions including unusual reagents. Neither test requires special equipment or precautions. It seems likely either could gain certified method status if required.
- No ethical, social, or Te Tiriti o Waitangi / Treaty of Waitangi issues have been identified.

3.5 Preferred Option

Considering the detailed assessment set out in Section 3.4, Golder considers the SBRC-G test to be the better option for assessing bioavailability in New Zealand. It has been validated for arsenic in a wide range of conditions, and performed well in those validation studies. Given that good performance, the fact that it is only weakly physiologically based, seems less important. It has already been used in New Zealand (Golder 2012, Gaw et al. 2008) and it already has USEPA approval for lead in soils (USEPA 2009).

One residual weakness of the SBRC-G test is that it has never been subjected to an inter-laboratory study with five or more participants, so it has not had the opportunity to meet standards for inter-laboratory reproducibility. If the test were introduced to New Zealand, it might be advisable for the laboratory(ies) offering the test to be members of SBRC, so they have opportunities to benchmark themselves.

The UBM also appears satisfactory in many respects. Again, there is a question mark over reproducibility, and there must be some concern that it has not been validated for arsenic from pesticide / herbicide application. The UBM also tends to overestimate bioavailability. Possible poor performance in highly contaminated materials is of little relevance since it would not be applied to such materials in New Zealand. In practice, perhaps the most significant barrier to adopting the UBM is that it would be more expensive.

4.0 SUMMARY

Economic and industrial development since the mid-1800s has left New Zealand with a legacy of contaminated land. This land ranges from highly contaminated gasworks and chemical storage sites to residential properties and farmland subject to diffuse low level contamination.

On face value, large areas of agricultural land pose a potential risk to human health when developed for residential use. For example, spraying pipfruit orchards with lead arsenate has left tens of thousands of hectares exceeding residential SCS for arsenic. Consequently, such land poses current and future management issues. But SCS are generic as they must be applicable to almost any site. For this reason, they reflect conservative assumptions. One key assumption is that contaminants are 100 % bioavailable. There is increasing evidence that this is not the case for arsenic (and lead) in soils impacted by a range of historical land use activities. Management issues with former agricultural land could be alleviated if arsenic bioavailability could actually be measured for specific sites or groups of sites.

This Review aimed to determine whether there is now an appropriate methodology for taking bioavailability into account during site-specific health risk assessment. One possible measurement tool is a validated 'bioaccessibility' test that extracts a similar proportion of arsenic and lead from soils as would be absorbed by people ingesting the soil. Five years ago, MfE took the position that there was insufficient scientific evidence to support bioaccessibility testing in New Zealand.

Golder screened ten published bioaccessibility test methods to see which were most likely to be appropriate and analysed the two leading tests in detail. In Golder's view the SBRC-G test meets relevant scientific, economic and social assessment criteria. It has been validated for arsenic in a wide range of conditions and performed well in those validation studies. The SBRC-G test has already been used in New Zealand (Golder 2012, Gaw et al. 2008) and has USEPA approval for lead in soils (USEPA 2009). The UBM may also be acceptable, but has rarely been applied to agricultural soils. Moreover, it tends to overestimate bioavailability, and would be more expensive.

One residual weakness of the SBRC-G test is that it has never been subjected to an inter-laboratory study with five or more participants, so it has not had the opportunity to meet standards for inter-laboratory reproducibility. If the test were introduced to New Zealand, it might be advisable for the laboratory(ies) offering the test to be members of SBRC, so they have opportunities to benchmark themselves.

Contaminated land regulators and practitioners are likely to need national guidance on bioavailability testing for site specific health risk assessment. The guidance would need to cover how to collect bioavailability data, and how to collect supporting information on physico-chemical characteristics of the soil(s) and the contaminant binding phase(s). It would also advise on how to incorporate bioavailability values into health risk assessments. In addition a policy position is needed as to whether risk assessment calculations should use the mean bioavailability or some upper bound statistic. Golder suggests that this would best be done within a wider guidance document for site-specific health risk assessment.

5.0 LIMITATIONS

Your attention is drawn to the document, “Report Limitations”, as attached (Appendix F). The statements presented in that document are intended to advise you of what your realistic expectations of this report should be, and to present you with recommendations on how to minimise the risks to which this report relates which are associated with this project. The “Report Limitations” is not intended to exclude or otherwise limit the obligations necessarily imposed by law on Golder Associates (NZ) Limited, but rather to ensure that all parties who may rely on this report are aware of the responsibilities each assumes in so doing.

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APPENDIX A

Bioaccessibility Adoption by International Environmental Regulators

Similar to the approach adopted in the Methodology, the majority of environmental regulatory agencies have established SGVs set using a default of 100% bioavailability (e.g., NEPC 2013). Typically, bioavailability is used as part of a Tier 2 risk assessment and in determining remediation goals; it is seldom used in setting Tier 1 health risk-based criteria (Ng et al 2010).

Ng et al (2010a) noted that although there is recognition of the importance of bioavailability, there is reticence to include this into regulatory guidelines. Typically this is a function of the following:

- The test results can depend on the assessment method used, the soil type and contaminant
- The suitability and applicability of validated methods between different contaminants
- The suitability and applicability of validated methods between different soil types
- No reference standards to validate the results and evaluate reproducibility
- No advocacy for the incorporation of in vitro data into risk assessment without supporting evidence from in vivo testing

Internationally, there is variability in the acceptance and adoption of bioavailability for the assessment of human health risk, as summarised below:

Australia

In Australia environmental human health risk assessment (HHRA) is carried out in a number of circumstances. These include:

- Contaminated sites regulated by the Environmental Protection Act requirements in each state (and thus the audit schemes in most states).
- Adverse health investigations for communities where chemical contamination is suspected.
- Approvals for industrial facilities that are regulated by approval authorities (e.g., Department of Mines).

Schedule B7 of the NEPM (NEPC 2013) details Health Investigation Levels (HILs) for application to contaminated sites. With respect to lead and arsenic, the HILs include assumptions regarding bioavailability that can be tested to check whether the HIL can be altered for a particular site. When bioaccessibility testing is done for this purpose, then it is done according to guidance produced by CRC Care (Ng et al 2010). While the CRC Care guidance has no regulatory weighting, it is typically used by practitioners and auditors as the reference guide for conducting standardised bioaccessibility assessments. No prior approval from an authority is required to proceed to this testing.

Health risk assessment is undertaken with respect to the methodology presented in Schedule B4 of the NEPM (NEPC 2013). Under Schedule B4, bioaccessibility testing can be used for compounds other than lead and arsenic, but there is a greater standard of scientific justification necessary. HHRA's are typically subject to independent third party review (formal or informal audit) and as such it is the role of the auditors expert support team in HHRA to validate the scientific justifications for the use of bioaccessibility testing. This inserts a less standardised approach to the application of bioaccessibility testing for metals other than lead and arsenic. No prior approval from an authority is required to proceed to this testing.

In 2013, the review and amendment to soil lead health investigation level (HIL) in the NEPM (NEPC 2013) was derived using a human health risk assessment model with a number of input assumptions. There are two key assumptions around the absorption of lead into the body following incidental ingestion of soil. These assumptions are:

- The bioaccessibility (BAC) of the lead in the soil (i.e., once ingested, the proportion of lead dissolved from the soil).
- The absorption (Abs) of the dissolved lead (i.e., how much of the soluble lead is then taken up into the bloodstream as opposed to passing through the gut).

The soil lead HIL used in the NEPM adopts an oral RBA of 50% (i.e., the oral bioavailability of lead acetate in children which is a highly soluble form of lead) under the assumption that 100% of the lead in soil is bioaccessible (i.e., 100% BAC, all the lead in soil dissolves in the human gut), and 50% of the soluble lead is then absorbed (50% Abs).

NEPC (2013) and CRC CARE (2009) provide for further adjustment of lead screening levels based on bioaccessibility.

The arsenic HIL in the NEPM was derived adopting an arsenic bioavailability within the range of 70 – 100% (NEPC 2013). However, the NEPM states that available data from Bendigo in Victoria suggests that the bioavailability of arsenic in soil derived from mine tailings in this region commonly ranges from 10 – 20% and is generally less than 30%. This indicated a bioavailability of 70 – 100% is considered conservative. Like lead, the NEPM allows for adjustment of the arsenic screening levels based on bioaccessibility.

The NEPM does not prescribe specific IVBA for evaluating contaminant bioaccessibility. Rather the NEPM (Schedule B4) notes that there are a number of IVBA methods that may be considered as a surrogate measure of arsenic and lead relative bioavailability including the RBALP, SBRC and IVG methods. However the NEPM notes "*that the selection and use of any in vitro method should be conducted on a contaminant-specific basis where the availability, validity and limitations of available methods are considered at the time of the assessment*" (NEPC 2013).

Canada

Current regulatory guidance allows for the incorporation of bioavailability data from in vivo methods as a more accurate risk assessment on a site specific basis. Canadian regulators have yet to issue guidance on the use of in vitro bioaccessibility methods for assessing contaminant bioaccessibility. Based on Golder's experience with bioaccessibility testing in Canada, it is typical practice for Health Canada to be informed of the plan to use bioavailability and to provide a work plan documenting the method and testing details. Feedback is provided and approval to proceed is granted once matters raised in the review are addressed.

Netherlands

The National Institute for Public Health and the Environment (RIVM) recommends the use of the RIVM in vitro method for use in site specific risk assessments for lead. However, at this stage there is no formal policy or guidance on the use of in vitro bioavailability methods (Ng et al 2010).

United Kingdom

The Environment Agency's (EA) view is that bioavailability tests should be used cautiously as the relationship between measured bioaccessibility and the relative human biological availability / toxicity of contaminants remains uncertain. EA does not recommend a specific test but notes that "*provided such testing has been carried out in accordance with guidelines for good practice, we consider that the results may be useful for arsenic as part of a "lines of evidence approach" to evaluating site-specific risk including the sensitivity of any quantitative risk assessment. A "lines of evidence approach" means that no single piece of evidence, such as the outcome of an in vitro test should be solely relied on to make a decision about health risks. But alongside other investigations and considerations, such as a greater understanding of soil chemistry, in vitro tests may inform a site-specific risk evaluation*" (CL:AIRE 2016).

Denmark

The Danish Environmental Protection Agency commissioned a review of human bioaccessibility of metals/metalloids and PAHs from soil (DHI 2003). The review concluded that the use of bioavailability/bioaccessibility was suitable for deriving site specific soil quality criteria and cleanup criteria following a site specific risk assessment. However, the review considered that there was insufficient data to allow for general regulation of soil quality using revised bioavailability/bioaccessibility values. As noted by Ng et al (2010), no formal policy, position or guidance has been published since this review.

Italy

In Italy there is currently no formal guidance or position on the use of bioavailability in human health risk assessment in Italy. However, in Piedmont region generic guideline values for agricultural land use take into account bioavailability of metals only.

United States

The USEPA has provided regulatory approval for the use of the RBALP/SBRC IVBA assay to the application of lead contaminated soils. The approval was based on a study evaluating correlation between in vivo and in vitro studies for 19 lead contaminated soils (USEPA 2009). A Standard Operating Procedure (SOP) has also been developed and published by USEPA (2008, 2012) for this method.

The USEPA has not provided regulatory approval to date for other in vitro assays and for other soil contaminants. Diamond et al (2016) evaluated the application of the USEPA (2012) IVBA assay (0.4 M glycine/pH 1.5) to arsenic contaminated soils. The study suggested strong support for the application of this assay to evaluating arsenic RBA.

APPENDIX B

Summary of Bioaccessibility Test Methods

Table B1: In vitro bioaccessibility test procedures applied to arsenic in soils and compared with in vivo bioavailability data.

Reference *Method*		Substrate	Gastric phase	Intestinal phase	Analysis	Comparison
Ruby *et al.* 1996 *PBET*		Three smelter site soils, fine fractions (<31 µm), 170-3900 mg/kg As.	0.4 g soil in 40 mL pepsin / citrate / malate / lactate / acetate buffer, bubbled with argon, 37 °C, 1 hr. Separate extractions at pHs 1.3, 2.5, 4.0.	Amended with $NaHCO_3$ solution (via dialysis bag) to pH 7.0, 70 mg porcine bile, 20 mg porcine pancreatin, bubbled with argon, 37 °C, sampled 1 hr and 3 hr.	Centrifuged, ICP-MS.	Relative oral bioavailability to rabbit or cynomolgus monkey inferred from urinary excretion fraction.
Rodriguez *et al.* 1999	*IVG*	15 mining and smelter site soils, airdried, sieved to <250 µm, 400-18,000 mg/kg As.	4 g soil and 200 g dough in 600 mL 0.15 M NaCl, 1 % porcine pepsin, anti-foaming agent, pH 1.8, stirred under argon, 37 °C, 1 hr	Amended with $NaHCO_3$ to pH 5.5, 2.1 g porcine bile, 0.21 g porcine pancreatin, stirred under argon, 37 °C, 1 hr.	Centrifuged, microfiltered, acidified, ICP-HG.	Relative oral bioavailability to piglet inferred from urinary excretion fraction.
	PBET		0.4 g soil in 40 mL pepsin / citrate / malate / lactate / acetate buffer, pH 2.0, stirred under argon, 37 °C, 1 hr.	Amended with $NaHCO_3$ to pH 7.0, 70 mg porcine bile, 20 mg porcine pancreatin, stirred under argon, 37 °C, 1 hr.		
Rodriguez *et al.* 2003			1 g soil in 250 mL hydroxylamine hydrochloride buffer, shaken, 70 °C, 2 hr			
Basta *et al.* 2007 *IVG*			1 g soil with/out 50 g dough in 150 mL 0.15 M NaCl, 1 % porcine pepsin, pH 1.8, stirred, 37 °C, 1 hr	Amended with $NaHCO_3$ to pH 5.5, 0.563 g porcine bile, 0.563 g porcine pancreatin, 37 °C, 1 hr.	Centrifuged, microfiltered, ICP-AES.	
Ellickson *et al.* 2001		Mining site soil (NIST SRM 2710), sieved to <74 µm, 630 mg/kg As.	50 mg samples in 8 mL simulated saliva, followed by 100 mL simulated gastric fluid, pH 1.4, shaken, 37 °C, 2 hr. Saliva had complex formula including inorganic salts, mucin, urea. Gastric fluid 0.03 M NaCl, 0.084 M HCl, 0.32% pepsin.	Centrifugation, supernatant removed and amended with 100 mL 0.2 M $NaHCO_3$, pH 6.5, further 2 hr shaken, 37 °C.	Centrifuged, digested conc. HNO_3, microfiltered, ICP-MS.	Relative oral bioavailability to rat estimated from organs, blood, bone, urine, feces; single-dose trial.
Bruce *et al.* 2007 *PBET*		9 mining site soils, fines <215 µm or crushed to <81 µm, 42-2600 mg/kg As.	0.4 g soil in 40 mL pepsin / citrate / malate / lactate / acetate buffer, bubbled with argon, 37 °C, 1 hr. Separate extractions at pHs 1.3, 2.5, 4.0.	Amended with $NaHCO_3$ to pH 7.0, 70 mg porcine bile, 20 mg porcine pancreatin, stirred under argon, 37 °C, sampled at 1 hr and 3 hr.	Centrifuged, microfiltered, ICP-MS.	Relative oral bioavailability to rat and cattle, estimated from single-dose trials.
Juhasz *et al.* 2007a,b *SBET*		12 railway corridor, dip site, mine site and gossan soils, sieved to <250 µm, 42-1,100 mg/kg As	1 g soil in 100 mL 30 g/L glycine, pH 1.5, "suspension mixer", 37 °C, 1 hr.	None.	Microfiltered, ICP-AES or ICP-MS.	Relative oral bioavailability to swine estimated from blood: single dose trial.
Juhasz *et al.* 2009	*PBET*		1 part soil: 100 parts solution, pepsin / citrate / malate / lactate / acetate buffer, pH 2.5, "suspension mixer", 37 °C, 1 hr.	Amended with Na_2CO_3 to pH 7.0, 1.75 g/L bile and 0.5 g/L pancreatin added, "suspension mixer", 37 °C, further 4 hr.		
	IVG		1 part soil: 150 parts solution, 10 g/L pepsin, 8.77 g/L NaCl, pH 1.8, "suspension mixer", 37 °C, 1 hr.	Amended with Na_2CO_3 to pH 5.5, 3.5 g/L bile and 0.35 g/L pancreatin added, "suspension mixer", 37 °C, further 1 hr.		
	SBRC		1 part soil: 100 parts solution, 30 g/L glycine, pH 1.5, "suspension mixer", 37 °C, 1 hr.	Amended with NaOH to pH 7.0, 1.75 g/L bile and 0.5 g/L pancreatin added, "suspension mixer", 37 °C, further 4 hr.		
	DIN		1 part soil: 50 parts solution, pepsin / mucin / NaCl / KCl/ KH_2PO_4 buffer, pH 2.5, "suspension mixer", 37 °C, 2 hr.	Amended with simulated intestinal fluid to 1:100 soil:solution ratio, pH 7.5, "suspension mixer", 37 °C, further 6 hr. Complex mixture, Na_2CO_3, inorganic salts, bile, pancreatin, trypsin, urea.		
Nagar *et al.* 2009 *IVG*		One soil amended with arsenate and drinking-water treatment residual solids, aged 3 years, sieved to <250 µm, 150-450 mg/kg As	1 g soil in 150 mL 0.15 M NaCl, porcine pepsin, pH 1.8, bubbled with argon, 37 °C, 1 hr.	None	Centrifuged, microfiltered, ICP-MS	Relative bioavailability to mouse estimated from blood.

Bradham *et al.* 2011 *SBRC*		9 mining and smelting site soils, 2 ASTM standard materials, airdried, sieved to <250 µm, 170-6,900 mg/kg As	1 g soil in 100 mL 0.4 M glycine, pH 1.5, rotated, 37 □C, 1 hr.	None	Refrigerated, ICP-OES	Relative oral bioavailability to mouse estimated from urinary excretion fraction
Juhasz *et al.* 2014a	*SBRC*		1 part soil: 100 parts solution, 0.4 M glycine, pH 1.5, 37 □C, 1 hr (mixing unspecified)	Amended to pH 7.0, 1.75 g/L bile and 0.5 g/L pancreatin added, 37 □C, further 4 hr (base and mixing method unspecified).	Microfiltered, ICP-AES or ICP-MS.	
	IVG		1 part soil: 150 parts solution, 10 g/L pepsin, 8.77 g/L NaCl, pH 1.8, 37 □C, 1 hr (mixing unspecified).	Amended to pH 5.5, 3.5 g/L bile and 0.35 g/L pancreatin added, 37 □C, further 1 hr (base and mixing method unspecified).		
	DIN		1 part soil: 50 parts solution, pepsin / mucin / NaCl / KCl/ KH_2PO_4 buffer, pH 2.5, 37 □C, 2 hr (mixing unspecified)	Amended with simulated intestinal fluid to 1:100 soil:solution ratio, pH 7.5, 37 □C, further 6 hr (mixing unspecified). Complex mixture, Na_2CO_3, inorganic salts, bile, pancreatin, trypsin, urea.		
	PBET		1 part soil: 100 parts solution, pepsin / citrate / malate / lactate / acetate buffer, pH 2.5, 37 □C, 1 hr (mixing unspecified).	Amended to pH 7.0, 1.75 g/L bile and 0.5 g/L pancreatin added, 37 □C, further 4 hr (base and mixing method unspecified).		
	UBM		1 part soil: 15 parts simulated saliva, pH 6.5, mixed 15 minutes. Complex formula containing inorganic salts, urea, amylase, mucin, uric acid. Addition of simulated gastric fluid to 1:37.5, pH to 1.3, 37 □C, 1 hr. Complex formula containing inorganic salts, glucose, glucuronic acid, urea, glucosamine, bovine serum albumin, mucin, pepsin. Mixing unspecified.	Amended with simulated intestinal fluid to 1:97.5, pH to 6.3, rotated, 37 □C, further 4 hr. Complex formula containing inorganic salts, urea, bovine serum albumin, pancreatin, lipase, bile. Mixing unspecified.		

Wragg *et al.* 2011 *UBM*	11 archived smelting site soils and river sediments (refer Rodriguez *et al.* 1999) and 3 reference soils	0.6 g soil added to 9 mL simulated saliva, pH 6.5, shaken 10 s (Denys) or 30 s (Wragg). Complex formula containing inorganic salts, urea, amylase, mucin, uric acid. Addition of 13.5 mL simulated gastric fluid, pH to 1.3, rotated, 37 □C, time to 1 hr. Complex formula containing inorganic salts, glucose, glucuronic acid, urea, glucosamine, bovine serum albumin, mucin, pepsin.	Amended with 36 mL simulated intestinal fluid, pH to 6.3, rotated, 37 □C, further 4 hr. Complex formula containing inorganic salts, urea, bovine serum albumin, pancreatin, lipase, bile.	Centrifuged, preserved d. HNO_3, re-digested c. HNO_3 / H_2O_2, ICP-OES.	Relative oral bioavailability to piglet inferred from urinary excretion fraction.
Denys *et al.* 2012 *UBM*	16 mining and smelting site soils, airdried, sieved to <250 µm, 18-25,000 mg/kg As.				Relative oral bioavailability to swine calculated from urine, bone, liver and kidney endpoints.
Griffin & Lowney 2013 *RBALP-G*	20 soils including mining, smelter, river sediment, orchard and timber treatment sites, sieved to <250 µm, 16-10,000 mg/kg As.	1 g soil in 100 mL 0.4 M glycine, pH 1.5, rotated, 37 □C, 1 hr.	None	Microfiltered, acidified, refrigerated, ICP-MS	Relative oral bioavailability to swine inferred from urinary excretion fraction (Casteel *et al.* 2009)
Griffin & Lowney 2013 *RBALP-I*	17 soils including mining, smelting, pesticide plant, orchard, cattle dip and volcanic sites, sieved to <250 µm, 120-1,400 mg/kg As.	None	1 g soil in 100 mL 0.4 M glycine, 0.05 M phosphate, pH 7.0, rotated, 37 □C, 1 hr.		Relative oral bioavailability to monkey inferred from urinary excretion fraction (Roberts *et al.* 2007)

Hawkins *et al.* 2013	*IVG*	One mining soil, sieved to <250 µm, 440 mg/kg As.	1 g soil in 150 mL 0.10 M HCl, 1 % porcine pepsin, pH 1.8, stirred, 37 □C, 1 hr.	Amended with NaOH to pH 6.5, 0.56 g porcine bile, 0.56 g porcine pancreatin, stirred, 37 □C, 2 hr	Centrifuged, microfiltered, ICP-AES	Relative oral bioavailability to swine inferred from urinary excretion fraction.
	RBALP[1]		0.3 g soil in 30 mL 0.4 M glycine, rotated, 37 □C, 1 hr. Separate extractions at pHs 1.5 and 2.5.	None	Chilled, centrifuged, ICP-AES	
Juhasz *et al.* 2014b *SBRC-G*		13 herbicide and mining sites, sieved to <250 µm, 81-2,300 mg/kg As.	1 part soil in 100 parts solution, 0.4 M glycine, pH 1.5, "suspension mixer", 37 □C, 1 hr.	None	Microfiltered, ICP-AES or ICP-MS.	Relative oral bioavailability to swine estimated from blood
Bradham *et al.* 2015 *SBRC*		37 mining, smelting, agricultural, orchard, railway, cattle dip and natural soils, 3 reference materials, sieved to <250 µm, 110-6,900 mg/kg As.	1 part soil in 100 parts solution, 0.4 M glycine, pH 1.5, rotated, 37 □C, 1 hr.	None	Refrigerated, ICP-MS.	Relative oral bioavailability to mouse estimated from blood, single dose trial
Diacomanolis *et al.* 2015 *PBET*		51 mining wastes, sieved or crushed to <250 µm, 102-3130 mg/kg As.	0.4 g soil in 40 mL buffer, 1 hr. Composition, mixing method and temperature not specified. Separate extractions at pHs 1.3, 2.5, 4.0.	Amended with $NaHCO_3$ to pH 7.0, 70 mg porcine bile, 20 mg porcine pancreatin, sampled at 1 hr and 3 hr. Mixing method and temperature not specified.	Centrifuged, microfiltered (0.22 µm), frozen, diluted d. HNO_3, ICP-MS	Relative oral bioavailability (against both As^{III} and As^{V}) for 12 wastes to rat calculated from blood and from urine

[1] This paper refers to the method employed as RBALP and PBET interchangeably, although the two are rather different. From the detail, RBALP would appear to be the correct term.

Li *et al.* 2015	*SBRC*	12 farming, mining and smelting sites, sieved to <250 µm, 22-4,200 mg/kg As.	100 mL 0.4 M glycine, pH 1.5.	1.0 g soil. Shaken, 37 □C, 1 hr.	Amended with NaOH to pH 7.0, 1.75 g/L bile and 0.5 g/L pancreatin added, shaken, 37 □C, further 4 hr.	Microfiltered, ICP-MS	Relative oral bioavailability to mouse estimated from blood, single dose trial
	IVG		150 mL 0.15 M NaCl, 10 g/L pepsin, pH 1.8.		Amended with $NaHCO_3$ to pH 5.5, 3.5 g/L bile and 0.35 g/L pancreatin added, shaken, 37 □C, further 1 hr.		
	DIN		50 mL pepsin / mucin / NaCl / KCl/ KH_2PO_4 buffer, pH 2.0.		Amended with simulated intestinal fluid to 100 mL, pH 7.5, shaken, 37 □C, further 6 hr. Complex mixture, Na_2CO_3, inorganic salts, bile, pancreatin, trypsin, urea.		
	PBET		100 mL pepsin / citrate / malate / lactate / acetate buffer, pH 2.5.		Amended with $NaHCO_3$ to pH 7.0, 1.75 g/L bile and 0.5 g/L pancreatin added, shaken, 37 □C, further 4 hr.		
	UBM		15 mL simulated saliva, 10 s. Complex formula containing inorganic salts, urea, amylase, mucin, uric acid. Addition of 22.5 mL simulated gastric fluid, pH to 1.2. Complex formula containing inorganic salts, glucose, glucuronic acid, urea, glucosamine, bovine serum albumin, mucin, pepsin.		Amended with 60 mL simulated intestinal fluid, pH to 6.3, shaken, 37 □C, further 4 hr. Complex formula containing inorganic salts, urea, bovine serum albumin, pancreatin, lipase, bile.		

APPENDIX C

Evaluation Criteria for Bioaccessibility Test Methods

USEPA (2007) recommends that the twelve criteria be met before a test method is considered suitable for regulator use. The twelve criteria are as follows

1) A scientific and regulatory rationale for the test method should be available, including a clear statement of its proposed use.

2) Relationship of the test method endpoint(s) to the biologic effect of interest must be described.

3) A detailed protocol for the test method is required, including a description of the materials needed, a description of what is measured and how it is measured, acceptable test performance criteria (e.g., positive and negative controls), a description of how data will be analysed, a list of the species for which the test results are applicable, and a description of the known limitations of the test including a description of the classes of materials that the test can and cannot accurately assess.

4) The extent of within-test variability and the reproducibility of the test within and among laboratories must have been demonstrated. The degree to which sample variability affects this test reproducibility should be addressed.

5) The test method performance must have been demonstrated using reference chemicals or test agents representative of the types of substances to which the test method will be applied, and should include both known positive and known negative agents.

6) There must be sufficient data to permit a comparison of the performance of a proposed substitute test with that of the test it is designed to replace.

7) Data supporting the validity of a test method should be obtained and reported in accordance with Good Laboratory Practices (GLPs).

8) Data supporting the assessment of the validity of the test method must be available for review.

9) The methodology and results should have been subjected to independent scientific review.

10) The method should be time and cost effective.

11) The method should be one that can be harmonised with similar testing requirements of other agencies and international groups.

12) The method must provide adequate consideration for the reduction, refinement, and replacement of animal use.

The expanded evaluation criteria for evaluating the bioaccessibility test methods comprised the following:

Scientific / technical

- **Applicability of the test**

  1. Is there a detailed description of the method?
  2. What exactly does it purport to measure?
  3. Are the results in a form that can be incorporated into quantitative risk assessments of ingestion pathways?
  4. Do results commonly indicate bioavailability significantly less than 100 %?

- **Application of the test –**

5. What contaminants is it for?
6. What forms of those contaminants?
7. Can the test be applied to differing contaminant sources (e.g., anthropogenic versus geologic)?
8. In what environments?
9. At what concentrations?
10. Over what range of bioavailability values?
11. Are there positive or negative interferents?

- **Accuracy of the test –**

Is the test accurate?

12. Do results reflect human oral bioavailability and to what level of confidence?
13. How closely does the method mimic the human ingestion and digestion process – intake and uptake?
14. Has it been validated against in vivo (animal) models, and if so, are those models appropriate for comparison to human?
15. In what range of conditions has the model been validated?
16. What supporting chemical and/or epidemiological evidence is there? Have inter-lab comparative studies been undertaken?

- **Precision of the method –**

17. Are results reproducible within and between laboratories?
18. Are measures of variation low?
19. Are blanks close to zero and spike recoveries close to 100 %?
20. Have data quality objectives been achieved for a wide range of matrices, concentrations and bioavailabilities?
21. Are there well-characterised results for well-defined reference materials?
22. Do results change significantly if the method is varied – is the method stable?

Economic / practical

- **Cost of testing –**

23. Consider sample collection, transport, preparation, analysis, quality assurance and quality control, and reporting, over and above analysis for ‘total’ contaminant.
24. Consider supporting information needs including geochemical characterisation of the contaminant and matrix.
25. If resource consent could be required for sampling, consider application and monitoring costs.
26. What additional special skills are needed to design the investigation, over and above sample collection for ‘total’ contaminants?

- **Length of testing –**

  27. How long does the test take?
  28. Consider sample collection, transport, preparation and analysis, quality assurance and quality control, and reporting, over and above analysis for 'total' contaminant.
  29. Consider supporting information needs including geochemical characterisation of the contaminant and matrix.

- **Difficulty of the test –**

  30. How many steps are there in the method / procedure?
  31. What equipment is required to complete the method and what is the expected cost of the equipment
  32. What special skills or equipment are needed, carry out the laboratory work?
  33. Are these skills or equipment readily available in New Zealand?
  34. Are any special precautions required to handle or transport reagents or samples?
  35. If the method has patent protection, consider licensing fees if any.
  36. What level of analysis is required to support a business case (i.e., to cover set up and compliance cost) for a New Zealand based laboratory to adopt a method?
  37. What is the availability/capability for testing in neighbouring countries (e.g., Australia) and what test method are they using?

Social / regulatory

- **Regulatory acceptance of method –**

  38. Was the method developed by a reputable authority?
  39. Has validation been published in a peer-reviewed journal?
  40. Could the method get certification of any kind?
  41. Are all reagents approved for use in New Zealand?
  42. What is the familiarity/compatibility between regulatory bodies/approaches using in neighbouring countries (e.g., Australia)?

- **Regulatory acceptance –**

  43. Is bioavailability acceptable to New Zealand regulators?
  44. Would resource consent be required for sampling?
  45. What consent mechanisms under the Resource Management Act 1991 (RMA) exist or are required for implementation of bioavailability (and health risk assessment)?

- **Public and social acceptance –**

  46. Does undertaking the test itself pose any ethical issues or is it likely to create any nuisances?
  47. Did development or validation pose any ethical issues?
  48. Does the test pose any Te Tiriti o Waitangi / Treaty of Waitangi issues?

APPENDIX D

Assessment of Leading Arsenic Bioaccessibility Test Methods

Table 1: Multi-dimensional effectiveness assessment of leading arsenic bioaccessibility test methods.

Assessment Criterion	RBALP / SBRC / SBET	UBM
Was the method developed by a reputable authority?	The test was developed by the Solubility and Bioavailability Research Consortium, commercial researchers in the United States of America, working with regional US Environment Protection Agency staff. Much development work has also been done by staff of the University of South Australia and partner organisations.	The UBM was developed by the Bioaccessibility Research Group of Europe (BARGE), an informal collaborative group including government agencies of the United Kingdom, the Netherlands, France, Denmark and Canada, and universities.
Have validation results been published in peer-reviewed journals?	A review of available literature identified the following published validation studies: ■ In vitro assessment of arsenic bioaccessibility in contaminated (anthropogenic and geogenic) soils (Juhasz et al. 2007a). ■ Comparison of in vivo and in vitro methodologies for the assessment of arsenic bioavailability in contaminated soils (Juhasz et al. 2007b). ■ Assessment of four commonly employed in vitro arsenic bioaccessibility assays for predicting in vivo relative arsenic bioavailability in contaminated soils (Juhasz et al. 2009). ■ Relative bioavailability and bioaccessibility and speciation of arsenic in contaminated soils (Bradham et al. 2011). ■ Validation of an in vitro bioaccessibility test method for estimation of bioavailability of arsenic from soil and sediment (Griffin and Lowney 2013). ■ The effect of soil properties on metal bioavailability: field scale validation to support regulatory acceptance (Hawkins et al. 2013). ■ Variability associated with As in vivo – in vitro correlations when using different bioaccessibility methodologies (Juhasz et al. 2014a). ■ Validation of the predictive capabilities of the SBRC-G in vitro assay for estimating arsenic relative bioavailability in contaminated soils (Juhasz et al. 2014b). ■ Independent data validation of an in vitro method for the prediction of the relative bioavailability of arsenic in contaminated soils (Bradham et al. 2015). ■ In vitro bioaccessibility and in vivo relative bioavailability in 12 contaminated soils: Method comparison and method development (Li et al. 2015).	A review of available literature identified the following published validation studies: ■ An inter-laboratory trial of the unified BARGE bioaccessibility method for arsenic, cadmium and lead in soil (Wragg et al. 2011). ■ In vivo validation of the unified BARGE method to assess the bioaccessibility of arsenic, antimony, cadmium and lead in soils (Denys et al. 2012). ■ Validation of the predictive capabilities of the SBRC-G in vitro assay for estimating arsenic relative bioavailability in contaminated soils (Juhasz et al. 2014). ■ In vitro bioaccessibility and in vivo relative bioavailability in 12 contaminated soils: Method comparison and method development (Li et al. 2015).
Can the test be applied to different contaminant sources?	The test has been validated for soils subject to herbicide / pesticide application (railway corridor, cattle dip, orchard); mining and smelting waste deposition; 'farming' (no details specified); and natural sources (volcanic, gossan) (refer in particular Griffin and Lowney 2013, Bradham et al. 2015, Li et al. 2015, Diamond et al. 2016). Some of these sources used oxidised As^{V} compounds while others used As^{III} salts or sulphide minerals.	Most validation materials, and the NIST 2710 and 2710A reference materials, were soils historically contaminated by solid wastes from mining or smelting activities, although: ■ Six of the fifteen soils studied by Denys et al. (2012) were historically contaminated by air deposition from a smelter. ■ Four of the twelve soils in Li et al. (2015) were from 'farming' sites (the exact process and timing of contamination was not stated).
Can the test be applied to different contaminant forms?	Griffin and Lowney (2013) sought to validate the test for soils containing arsenic in a wide range of different binding phases, including: ■ As^{V} and As^{III} mineral forms such as arsenopyrite, arsenic trioxide, mixed arsenic oxides, lead arsenic oxide, and iron arsenate, including chemical incorporation in "slag", pyrite, lead oxides and iron sulphate. ■ Arsenic adsorbed on iron and manganese oxyhydroxides, and on clay. The test has not been validated for soils contaminated with chrome copper arsenate timber preservatives or organoarsenic herbicides. The geochemistry of the latter is likely to be sufficiently different that the test should not be applied to soils containing them.	Bradham et al. (2011) examined the mineralogy of the test materials used in that study and in Juhasz et al. (2014), and found a wide range in composition. Some of those soils were dominated by sorbed forms of As^{V} (the fully oxidised, pentavalent state of arsenic), one by the As^{V} mineral scorodite, while others featured reduced As^{III} minerals such as realgar and arsenopyrite, or a mixture of all four of these forms. Bradham et al. (1999) and Juhasz et al. (2014) found that both RBA (to mouse) and IVBA (to UBM) were markedly lower in samples rich in arsenopyrite. Tentatively, then, the UBM seems robust to some significant changes in mineralogy. The strong affinity of arsenate for ferric iron appears to be reflected in lower RBA and IVBA in validation studies. Elevated aluminium or clay content also seems to reduce RBA and IVBA.
In what environments?	No formal description of materials that the test can or cannot accurately assess has been developed. The method has been validated for soils in residential and some agricultural uses (including orchards). Soils used for validation have varied widely in clay and sand content; in aluminium, calcium, iron and lead content; in pH and phosphate content. The influence, if any, of organic matter and/or sulphur content does not appear to have been explored in validation studies.	No formal description of materials that the UBM can or cannot accurately assess has been developed. It is not clear whether the range of test materials in the four identified validation studies was sufficient to be confident that the UBM is generally applicable to all soils impacted by arsenic-containing mining and smelting wastes, let alone to arsenic-containing soils in general. It is notable that many of the validation soils contained high to very high levels of lead, and most of the soils used by Wragg et al. (2011) contained "unusual" levels of elemental sulphur. The method has been validated for soils that had been in residential use, including gardens and play areas, and in some unspecified agricultural uses. Consistent validation results were obtained in acidic (pH < 3), organic (total organic carbon > 3%), or clayey (clay up to 31 %) soils.

Assessment Criterion	RBALP / SBRC / SBET	UBM
At what concentrations?	The meta-analysis of Diamond et al. (2016) cites soils with total arsenic in the range 42-6,900 mg/kg. The bottom end of this range is somewhat higher than the arsenic residential SCS of 20 mg/kg or rural residential SCS of 17 mg/kg; however, no factors have been identified that might lead to different test performance at low arsenic concentrations. Li et al. (2015) includes three soils with lower arsenic content than Diamond et al. (2016). While the RBA for the soil with the least arsenic (22 mg/kg) was quite uncertain, it appeared to be under predicted by this test (and by other methods).	The method has been validated for concentrations similar to arsenic residential SCS by Denys et al. (2012), using soils with 18-25,000 mg/kg As, and Li et al. (2015) with 22-4,200 mg/kg. Wragg et al. (2011) used 400-18,000 mg/kg, and Juhasz et al. (2014) used 170-6,900 mg/kg As; however, no factors have been identified that might lead to different UBM performance at low arsenic concentrations. Wragg et al. (2011) found that, for their highly contaminated test materials that had complex composition, much better results (relative to performance criteria) were obtained when the UBM was modified to a 1:1,000 solid:solution ratio. They believed that this was a saturation effect and/or related to matrix interference, and expected that the ratio change would not be necessary or appropriate for moderately contaminated soils.
Over what range of bioavailability values?	The meta-analysis of Diamond et al. (2016) cites soils with an RBA range of 1.9-80.5 %. This is considered to be highly satisfactory as OSWER (2012) observed that 95 % of USEPA's database of 103 arsenic RBA values were less than 60 %.	Denys et al. (2012) explicitly sought a good coverage of the % RBA (and hence IVBA) range. They validated soils with an RBA range of 3-100 % (IVBA 3-74 %). Wragg et al. (2011) used soils with an RBA range of 4-43 %; Juhasz et al. (2014) 12-53 %; Li et al. (2015) 6-65 %. These are all considered to be highly satisfactory as OSWER (2012) observed that 95 % of USEPA's database of 103 arsenic RBA values were less than 60 %.
Are there positive or negative interferents?	Griffin and Lowney (2012) consider that soil lead exceeding 50,000 mg/kg is likely to be a negative interferent. This is not a significant drawback as such high soil lead concentrations would certainly be inappropriate for sensitive end uses. Moreover, lead also appears to decrease arsenic absorption in rat (Diacomanolis et al. 2013).	Wragg et al. (2011) found that, for their highly contaminated test materials that had complex composition, much better results (relative to performance criteria) were obtained when the UBM was modified to a 1:1,000 solid:solution ratio. They believed that this was a saturation effect and/or related to matrix interference, and expected that the ratio change would not be necessary or appropriate for moderately contaminated soils.
Can the method be applied to other contaminants?	This IVBA assay has been validated for estimating lead RBA by USEPA (2007). Diamond et al (2016) propose that the correlation model based on the reviewed data set provides confidence of adopting this as a standard extraction protocol for arsenic and lead. This would provide for potential cost savings where lead and arsenic are co-contaminants in soil.	The test can also be applied to lead and cadmium. Application to antimony is promising (Denys et al. 2012) but not yet validated for use.
How closely does the method mimic the human ingestion and digestion process?	The developers set out to create a simple, inexpensive bioaccessibility test method, not a physiologically based one. The extraction is explicitly carried out at pH, temperature, and duration approximating stomach conditions. The test focuses on readily ingestible soil particles, the < 250 µm fraction (roughly speaking, no coarser than a fine sand).	The UBM is intended to be physiologically based, incorporating mouth, stomach and intestine phases. Each phase is designed to have realistic (fasted) residence / mixing time, temperature, pH, inorganic and organic constituents, and enzymes. The UBM focuses on readily ingestible soil particles, the < 250 µm fraction (roughly speaking, no coarser than a fine sand).
Do results reflect human oral bioavailability?	Wragg et al. (2011) proposed performance criteria for a linear relationship between relative oral bioavailability (RBA) and in vitro bioaccessibility (IVBA), based on a United States of America Federal Drug Administration guideline for drug release in the body, as follows: ■ Pearson correlation coefficient $R > 0.8$ and $R^2 > 0.6$ ■ Slope $0.8 < m < 1.2$ Additionally, Denys et al. (2012) and Griffin and Lowney (2013) explicitly sought RBA-IVBA regression intercept close to zero. No quantitative control on intercept is provided; we would suggest $0 < c < 20$ %. Diamond et al. (2016) carried out a meta-analysis of paired RBA and test IVBA results for 83 sampled soils analysed in six previous studies from three different laboratories using different RBA procedures. Using a weighted linear regression taking into account uncertainty in the individual data points, and excluding one outlier for which IVBA was much greater than RBA, they developed the following linear model: $RBA\ (\%) = 0.79 \times IVBA\ (\%) + 3$: $R^2 = 0.87$ This model easily meets the correlation and intercept criteria, and is on the boundary of acceptability for slope. The implication is that raw test results generally overestimate real bioavailability, but in a consistent fashion. Diamond et al. (2016) believed that this model was robust but wanted to validate it further in future against additional data. We suggest the results of Li et al. (2015) could be used for this purpose, and note that their data indicates a similar result.	Wragg et al. (2011) proposed performance criteria for a linear relationship between relative oral bioavailability (RBA) and in vitro bioaccessibility (IVBA), based on a United States of America Federal Drug Administration guideline for drug release in the body, as follows: ■ Pearson correlation coefficient $R > 0.8$ and $R^2 > 0.6$ ■ Slope $0.8 < m < 1.2$ Additionally, Denys et al. (2012) and Griffin and Lowney (2013) explicitly sought RBA-IVBA regression intercept close to zero. No quantitative control on intercept is provided; we would suggest $0 < c < 20$ %. On this basis, only one of the four identified UBM validation studies, Li et al. (2015), was able to meet method performance criteria. Denys et al. (2012: results in graphic form only) and Juhasz et al. (2014) comfortably met correlation and intercept criteria, but in both these studies the slope of the RBA-IVBA relationship is rather less than the desired minimum of 0.8. That is, raw UBM results tend to overestimate bioavailability. *We have been unable to verify the performance data presented by Wragg et al. (2011) and are seeking clarification from the authors. This should not be taken as evidence for or against the validity of the UBM.*
What supporting evidence is there?	The test results are validated against RBA results. Where mineralogical information has been obtained (Griffin and Lowney 2012, Bradham et al. 2015), arsenic RBA and IVBA appear consistent with soil chemical and physical characteristics. Both RBA and IVBA are reported to be low in soils dominated by arsenic sulphide minerals, arsenic oxides, and slag; and high in soils where arsenic is principally associated with manganese oxides or iron sulphates.	On the limited data presented in Wragg et al. (2011: obtained by Rodriguez et al. 1999), Denys et al. (2012), and Juhasz et al. (2014: obtained by Bradham et al. 2011), UBM results are not only validated by RBA results, but also broadly consistent with the chemical and physical characteristics of the soils being studied. Both RBA and IVBA were markedly lower in soils impacted by slags and/or calcine waste and/or containing arsenopyrite.

Assessment Criterion	RBALP / SBRC / SBET	UBM
	Further, Juhasz et al. (2007a) observed that anthropogenic sources of arsenic have higher RBA and IVBA (using this test) than geogenic sources, which would seem a reasonable consequence of the method of application and the co-occurrence of elevated phosphate.	
Are results repeatable and reproducible?	Wragg et al. (2011) propose performance criteria for a linear relationship between RBA and IVBA, based on a US Federal Drug Administration guideline for drug release in the body, as follows: ■ Within-laboratory repeatability < 10 % relative standard deviation (RSD) ■ Between-laboratory reproducibility < 20 % RSD. Koch et al. (2013) report repeatability of 5-8 % RSD and reproducibility of 9-13 % for three laboratories analysing NIST 2710 by this test. Brattin et al. 2013 report repeatability of < 3 %, and reproducibility averaging 3 %, in a four-laboratory comparison [these are standard deviations, not RSDs]. Generally acceptable repeatability results were obtained by Bradham et al. (2011) and Juhasz et al. (2009, 2014b). In the meta-analysis of Diamond et al. (2016), "laboratory" variable (n = 3) accounted for 3 % of the variance in RBA. A test of heterogeneity of slopes for data from each laboratory was not significant, indicating that combined data may be described by a common slope. None of these results are entirely convincing as at least five participants are normally required to rigorously demonstrate reproducibility.	Wragg et al. (2011) propose performance criteria for a linear relationship between RBA and IVBA, based on a US Federal Drug Administration guideline for drug release in the body, as follows: ■ Within-laboratory repeatability < 10 % relative standard deviation (RSD) ■ Between-laboratory reproducibility < 20 % RSD. Wragg et al. (2011) specifically set out to demonstrate repeatability and reproducibility. Within-laboratory repeatability, with RSD of 4-7 %, readily met their 10 % performance criterion. Between-laboratory reproducibility **did not** meet its 20 % performance criterion for the standard method. Only two laboratories carried out the modified method (with the 1:1,000 solid:solution ratio), and although their results were similar, two participants is not sufficient to demonstrate reproducibility. For Denys et al. (2012) and Juhasz et al. (2014) repeatability was generally satisfactory. As single-laboratory trials they were unable to investigate reproducibility, which they consider to be the major issue still facing the UBM.
Are blanks close to zero and spike recoveries close to 100 %?	All studies used in vitro analytical blanks and spikes, which were reported to be satisfactory throughout.	Considering the RBA assessment that the UBM seeks to replicate, Denys et al. (2012) and the precursor studies Rodriguez et al. (1999) and Bradham et al. (2011) all stress that the gross clinical condition of animals in the RBA assessments was unaffected by the experiments. Denys et al. (2012) place considerable emphasis on dose-response curves for different target organs having a common near-zero intercept and linear dose-dependence, which was achieved in that paper and also by Li et al. (2015). Recoveries of the reference compound, sodium arsenate, in the animal studies were not reported in any of these papers. All studies used in vitro analytical blanks and spikes, which were reported to be satisfactory throughout.
Have data quality objectives been achieved for a wide range of samples?	Data quality does not seem greatly affected by arsenic form or matrix properties. However, RBA repeatability often appears low for samples with low arsenic content, leaving it difficult to determine whether test results are accurate in these materials. Juhasz et al. (2014b) reported one of twelve materials had notably poorer test repeatability, at 13 %. This was ascribed to pH variation although it was not clear why this particular sample should have given pH stability problems. Bradham et al. (2011) also have two of 10 samples with relatively poor repeatability.	Wragg et al. (2011) found that, for their highly contaminated test materials that had complex composition, much better results (relative to performance criteria) were obtained when the UBM was modified to a 1:1,000 solid:solution ratio. They believed that this was a saturation effect and/or related to matrix interference, and expected that the ratio change would not be necessary or appropriate for moderately contaminated soils.
Are there well-characterised results for well-defined reference materials?	Validation studies have included standard ASTM reference soils NIST 2710, 2710A and/or 2711.	The four identified validation studies included standard ASTM reference soils NIST 2710, 2710A and/or 2711 in bioaccessibility testing. Denys et al. (2012) and Juhasz et al. (2014) included the reference material in bioavailability testing as well, and obtained comparable results.
Is the method stable?	Griffin and Lowney (2012) showed that large changes in extraction pH, temperature, duration, or buffer strength all had some effect on test results for at least some soils, as did changing soil:solution ratio. Changing buffer strength, adding hydroxylamine or redox agents (at the usual pH) and changing filter pore sizes had little or no effect. Adding phosphate, silicate, or organic acids also had significant effects on test results (Griffin and Lowney 2012); however, we consider this could be a real effect, not necessarily a flaw in the test. We have not identified any studies of the effect of phosphate or other oxyanions on RBA.	A study reported in Wragg (2012) indicated that minor changes to the UBM could affect lead and cadmium bioaccessibility, but did not state any identified problems for arsenic.
How much does the test cost?	The test itself would be comparable to common commercial laboratory procedures such as synthetic precipitation leaching protocol (SPLP). However, obtaining standard soils of known bioavailability is expensive. Griffin and Lowney (2012) estimate the test should cost USD $60-$100 per sample plus a setup fee of USD $300-$500 per batch, or approximately USD $100 per sample for a moderate batch size. Golder's experience has been that this cost is reasonable even allowing for collecting some supporting information at the same time (total arsenic, lead, iron, manganese, phosphate and soil pH).	The test itself would be expensive compared to other commercial laboratory procedures, as it is relatively complex and requires many reagents, some of which are unusual and expensive. Obtaining standard soils of known bioavailability is also expensive.

Assessment Criterion	RBALP / SBRC / SBET	UBM
How long does the test take?	The test itself would take over an hour per batch to run. Air-drying, sieving, homogenising and subsampling soils, and sieving soils, and preparing the extraction solution, would add at least another day. Analysing extracts for arsenic should be a comparatively rapid step as is preparing an analytical report including quality control data. Supporting analyses for chemical and physical properties of soils could take several days.	Running the UBM itself would take a full day per batch. Air-drying, sieving, homogenising and subsampling soils, and preparing reagents, would add at least another day. Analysing extracts for arsenic should be a comparatively rapid step as is preparing an analytical report including quality control data. Supporting analyses for chemical and physical properties of soils could take several days.
Is the test complex?	The test is comparable to common commercial lab procedures such as the 'synthetic precipitation leaching protocol' (SPLP).	The UBM comprises three extraction phases using complex solutions including unusual reagents.
Does it require special equipment or precautions?	The test requires no special equipment or precautions.	The UBM requires no special equipment or precautions.
Could the method get certification of any kind?	The test could be certified.	We believe the UBM could be certified.
Regulatory acceptance	USEPA (2007b) has provided formal validation of the SBRC method for estimating lead RBA. Regulatory acceptance (i.e. by USEPA) has not been provided for other contaminants to date using this method. However, the work of Bradham et al (2015) and Diamond et al (2016) suggests a high level of confidence for adopting this IVBA assay as a standard protocol for arsenic.	The UBM has been accepted by regulators in the Netherlands, Denmark and the United Kingdom. In two English contaminated land projects, bioaccessibility testing reduced remedial costs by £7-30M and £3.75M respectively, reassured residents and restarted the stalled housing market around one site (Cave 2012).
Does undertaking the test pose any ethical or regulatory issues?	Like most bioaccessibility test methods, the test is explicitly designed to avert ethical and regulatory issues.	Like most bioaccessibility test methods, the UBM is explicitly designed to avert ethical and regulatory issues.
Did development and validation of the test pose any ethical or regulatory issues?	Animal testing on swine and mouse was required to validate the test. Monkey testing was also undertaken during development, but was not required for validation.	Animal testing on swine and mouse was required to validate the UBM.
Does the test pose any Te Tiriti o Waitangi / Treaty of Waitangi issues?	No Waitangi issues have been identified.	No Waitangi issues have been identified.

APPENDIX E

USEPA (2009) Validation Assessment of In Vitro Lead Bioaccessibility Assay

Validation Assessment of *In Vitro* Lead Bioaccessibility Assay for Predicting Relative Bioavailability of Lead in Soils and Soil-like Materials at Superfund Sites

1. Introduction

Validation and regulatory acceptance criteria articulated in EPA (2007a), as adapted from ICCVAM (1997), have been applied to an *in vitro* lead bioaccessibility (IVBA) assay described in detail in EPA (2007b). This report summarizes the basis for the Agency's determination that the IVBA method for lead has satisfied the validation (and regulatory acceptance) criteria for application of the method in an appropriate regulatory context (articulated in the cover letter to EPA, 2007b). The lead IVBA method provides a tool for characterizing site-specific RBA of lead in soils that is far less resource-intensive than the *in vivo* bioassay methods such as the immature swine bioassay (Casteel *et al*. 1997, 2006; EPA, 2007b).

2. Validation Assessment of the In Vitro Lead Bioaccessibility Assay

This section summarizes information pertinent to each of the validation criteria established in the Agency soil bioavailability guidance EPA (2007a). Because many of the criteria overlap for this assessment, the method validation and regulatory acceptance criteria were consolidated.

2.1. Scientific and regulatory rationale for the test method, including a clear statement of its proposed use, should be available.

The scientific and regulatory rationale for the lead IVBA method is presented in the following documents:

> EPA. (2007a). Guidance for evaluating the bioavailability of metals in soils for use in human health risk assessment. December 2006 OSWER 9285.7-80. May 2007 (Attachment A)

EPA (2007b). Estimation of Relative Bioavailability of Lead in Soil and Soil-like Materials Using In Vivo and In Vitro methods. OSWER 9285.7-77. May 2007. (Attachment B)

Regulatory and scientific rationale: The guidance document (EPA, 2007a) articulates the regulatory rationale for assessing bioavailability of metals soils in assessing human health risks at hazardous waste sites:

> *Accounting for potential differences in oral bioavailability of metals in different exposure media can be important to site risk assessment (U.S. EPA, 1989). This is true for all chemicals, but is of special importance for ingested metals. This is because metals can exist in a variety of chemical and physical forms, and not all forms of a given metal are absorbed to the same extent. For example, a metal in contaminated soil may be absorbed to a lesser extent than when ingested in drinking water or food. Thus, if the oral RfD or CSF for a metal is based on studies using the metal administered in water or food, risks from ingestion of the metal in soil might be overestimated. Even a relatively small adjustment in oral bioavailability can have significant impacts on estimated risks and cleanup goals* (EPA, 2007a).

The guidance also delineates the role of medium-specific bioavailability values intended for use as national default values (i.e., IEUBK Model for Lead in Children, EPA Adult Lead Methodology), from the importance of site-specific values intended to represent conditions at a specific location.

> *However, even in cases where sufficient data exist to support default medium-specific absorption factors for a chemical, site-specific data collection may also be important. Important factors that can affect the bioavailability of metals in soil can be expected to vary from site to site, or within a given site. These include the physical and chemical forms of the metal, as well as the physical and chemical characteristics of the association between the metal and soil particles. Default values for bioavailability may not accurately reflect these factors (e.g., chemistry, particle size, matrix effects) at any given*

> *site. Therefore, use of default values should not substitute for site-specific assessments of bioavailability, where such assessments are deemed feasible and valuable for improving the characterization of risk at the site (see Decision Framework, below)* (EPA, 2007a).

The technical support document (EPA, 2007b) describes in detail two methods that can be used to assess site-specific relative bioavailability (RBA) of lead in soils: 1) an *in vivo* RBA assay in a juvenile swine model; and 2) an *in vitro* bioaccessibility assay (IVBA). The term RBA refers to the ratio of the bioavailability of lead in the soil to that of water soluble lead (e.g., lead acetate). This report summarizes the results of studies that evaluate the validity of the IVBA assay to reliably predict RBA for a range of soil/lead mineral compositions found at lead mining and smelting sites.

The scientific rationale and intended use of these methods are articulated in the technical support document:

> *When reliable data are available on the absolute or relative bioavailability of lead in soil, dust, or other soil-like waste material at a site, this information can be used to improve the accuracy of exposure and risk calculations at that site. Based on available information in the literature on lead absorption in humans, the U.S. Environmental Protection Agency (U.S. EPA) estimates that relative bioavailability of lead in soil compared to water and food is about 60%. Thus, when the measured RBA in soil or dust at a site is found to be less than 60%, it may be concluded that exposures to and hazards from lead in these media at that site are probably lower than typical default assumptions. Conversely, if the measured RBA is higher than 60%, absorption of and hazards from lead in these media may be higher than usually assumed* (EPA, 2006b).

2.2. Relationship of the test method endpoint(s) to the endpoint of interest must be described

The technical support document (EPA, 2007b) describes the outcomes of studies conducted in immature swine to measure RBA for lead, and corresponding IVBA measurements, on 19 soil

samples collected from 8 different mining and smelting sites in EPA Regions 3, 7, and 8. In addition, 2 prepared materials were analyzed, including a Galena-enriched soil and a NIST paint standard. The sources of the samples are identified in Table 2-3 of EPA (2007b). The mineral composition and mineral phase of the lead in the samples (presented in Table 2-4 of EPA, 2007b), varied considerably and are thought to provide a reasonable representation of lead residues expected at residential soils and slag-impacted soil at lead and smelting sites.

2.3. A detailed protocol for the test method must be available and should include a description of the materials needed, a description of what is measured and how it is measured, acceptable test performance criteria (e.g., positive and negative control responses), a description of how data will be analyzed, a list of the materials for which the test results are applicable, and a description of the known limitations of the test including a description of the classes of materials that the test can and cannot accurately assess.

Standard Operating Protocol (SOP): A detailed description of the IVBA method and the statistical approaches used in the assessment of prediction limits of the assay (see Section 2.2) is provided in EPA (2007b). A stand-alone Standard Operating Protocol (SOP) has also been developed by the Agency (EPA, 2008).

Applicable test materials: Application of the IVBA method SOP is expected to yield predictions of RBA that fall within the prediction interval of the assay (EPA, 2007b; see Section 2.2 of this report). The prediction interval was based on results of assays of samples having a wide range of different soil types and lead phases from a variety of different sites. However, most of these samples tested were from a mining and milling sites, and it is possible that IVBA assay results of some forms of lead that do not occur at this type of site might fall within the established prediction interval. Therefore, whenever a sample containing an unusual and/or untested lead phase is evaluated by the IVBA protocol, this should be identified as a potential source of uncertainty in the resulting prediction of RBA. In the future, as additional samples with a variety of new and different lead forms are tested by both *in vivo* and *in vitro* methods, the range of applicability of the method may be further refined.

Assay limitations: Limitations of regulatory applications of the IVBA assay are identified in the Agency cover letter to the method technical support document (EPA, 2007b). These include the following limitations specific to the IVBA assay:

1. **Application to children and extrapolation to adults**. The IVBA assay was developed to predict lead RBA in children and was calibrated with estimates of RBA made from studies conducted in juvenile swine (EPA, 2007b). The juvenile swine bioassay has been utilized as an experimental methodology for predicting RBA in human children; therefore, the prediction equations for estimating RBA from results of the IVBA assay are assumed to apply to human children. While there is evidence to indicate that absolute bioavailability of soluble lead (e.g., in food or water) varies with age, the Agency is not aware of information on the age-dependence (or independence) of the RBA for lead in soil.

2. **Sample lead concentration limits:** The 19 samples tested in the development of the prediction equation and prediction interval for the IVBA assay described in EPA (2007b) ranged from 1,200-14,000 ppm lead. This validation range should be sufficient for most applications of the methodology. Although there is no basis for predicting that errors would necessarily be introduced into the estimates of RBA if sample concentrations outside this range were used in the IVBA assay, use of such samples without validating comparisons with results of the in vivo juvenile swine assay will introduce additional uncertainty into estimates of RBA. A further constraint on the lead concentration is noted in the attachment; sample concentrations used in the IVBA assay should not exceed 50,000 ppm for relatively soluble forms of lead (i.e., lead acetate, lead oxide, lead carbonate), in order to avoid saturation of the extraction fluid. However, applications of the IVBA assay to such high lead concentrations is unlikely to be relevant for improving risk management decisions; thus, this limitation is not likely to be a serious constraint for use of the methodology. Should additional data become available that would suggest modification of the above limits, the Agency will issue additional guidance. In addition, the minimum soil concentration in the sample is determined by that which is measurable in the assay using the SOP.

3. **Particle size:** All samples tested in the development of the prediction equation and prediction interval for the IVBA assay described in EPA (2007b) were sieved through a 60 mesh screen which excluded particles greater than 250 µm. Particle size can be expected to affect dissolution rates for lead that is embedded in particles and is known to affect absolute bioavailability of lead. Therefore, additional uncertainty will be associated with RBA estimates based on application of the IVBA assay to samples having particle sizes larger than 250 µm. In general, humans are believed to ingest particles that are predominantly smaller than 250 µm in diameter (Kissel *et al*., 1996; Sheppard and Evenden, 1994; Driver *et al*.,1989; Duggan and Inskip, 1985; Que Hee *et al*., 1985; Duggan, 1983), so measures of RBA on samples more coarse than this would usually not be considered relevant to risk assessment. Likewise, RBA estimates based on *in vitro* bioaccessibility assays of samples that have not been processed through a 60 mesh (or finer) sieve are generally not appropriate for quantitative use in site-specific risk assessments.

4. **Soil mineralogy:** The IVBA assay prediction equation for RBA (i.e., Equation 3, see Section 2.2 of this report) is expected to be widely appropriate to a variety of soil types and lead mineral phases. However, most of these samples tested were from a mining and milling sites, and it is possible that IVBA assay results of some forms of lead that do not occur at this type of site might fall with in the established prediction interval. Thus, whenever a sample that contains an unusual and/or untested lead phase is evaluated by the IVBA assay, this should be identified as a potential source of uncertainty.

   Available data are not yet sufficient to establish reliable quantitative estimates of RBA for each of the different mineral phases of lead that are observed to occur in the test materials. However, multivariate regression analysis between point estimate RBA values and mineral phase content of the different test materials allows a tentative rank ordering of the phases into three semi-quantitative tiers (low, medium, or high RBA), as follows:

Low Bioavailability	Medium Bioavailability	High Bioavailability
Fe(M) Sulfate Anglesite Galena Pb(M) Oxide Fe(M) Oxide	Lead Phosphate Lead Oxide	Cerussite Mn(M) Oxide
(M) = metal		

5. **Uncertainty in predicted RBA value:** As noted above, the IVBA assay for lead (U.S. EPA, 2007a) measures IVBA for a test material, and converts this to an estimate of RBA by application of a mathematical formula. The resulting prediction of RBA should be thought of as the best estimate of the central tendency estimate of RBA associated with that IVBA, but the actual RBA (if measured *in vivo*) might be either higher or lower than the prediction, due either to authentic inter-sample variability and/or to measurement error in RBA or IVBA. In general, the best estimate of RBA is the most appropriate value for use in the IEUBK model, but risk assessors and risk managers should use their professional judgment to decide if calculations using other values from within the RBA prediction interval should also be evaluated as part of an uncertainty analysis.

2.4. The extent of within-test variability and the reproducibility of the test within and among laboratories must have been demonstrated. The degree to which sample variability affects this test reproducibility should be addressed.

Within test variability: Precision of the IVBA protocol was assessed with 75 and 83 replicate analyses on each of two standard reference materials (NIST SRM 2710 and 2711, respectively) conducted within one laboratory (University of Colorado at Boulder) over several years. The mean coefficient of variation for both standards was 7% and mean IVBA values (± SD) were 75% ± 5% for SRM 2710 and 84% ± 6% for SRM 2711 (Drexler and Brattin, 2007; EPA, 2007b).

Inter-laboratory reproducibility: An inter-laboratory comparison of performance of the IVBA was conducted with four participating laboratories: ACZ Laboratories Inc.; University of Colorado at Boulder; U.S. Bureau of Reclamation Environmental Research Chemistry Laboratory; and National Exposure Research Laboratory (Drexler and Brattin, 2007; EPA, 2007b). Each participating laboratory applied the IVBA method to analyses (in triplicate) of each of the19 test samples used in the assessment of the method prediction equation (i.e., Equation 3, Section 2.2. of this report). Average within-laboratory variability (coefficient of variation, CV) ranged from 1.4 to 6.3% (Drexler and Brattin, 2007). The inter-laboratory coefficient of variation (i.e., CV for estimates from all laboratories, for each sample) ranged from 1.5% to 6.9% (mean: 3.4%) for 17 of the 19 samples (Drexler and Brattin, 2007). Two samples (*California Gulch AV Slag*, *Galena-enriched Soil*) had coefficients of variation of 18.6% and 29.7%. Mean coefficient of variation for all 19 samples was 5.6%.

Effects of sample variability: EPA (2007b) reported a prediction interval for the IVBA assay that was derived based on analysis of samples having a wide range of different soil types and lead phases from a variety of different sites, that are expected to be typical of application of the assay to mining and smelter sites (see Figure 1 and Section 2.2 of this report). The within-laboratory (University of Colorado at Boulder) coefficient of variation ranged from 0.2% to 26.7% (mean: 6.1%) for the 19 samples (based on data presented in Table 3-1 of EPA, 2007b). The high end of the range was impacted by two samples (*California Gulch AV Slag*, CV=17%; *Galena-enriched Soil*, CV=27%). Excluding the latter two samples, the coefficient of variation for the remaining 17 samples ranged from 0.2% to 11.4 % (mean: 4.2%).

2.5. The test method performance must have been demonstrated using reference materials or test materials representative of the types of substances to which the test method will be applied, and should include both known positive and known negative agents.

Performance with reference materials: Precision of the IVBA protocol was assessed with 75 and 83 replicate analyses on each of two standard reference materials (NIST SRM 2710 and 2711, respectively) conducted within one laboratory (University of Colorado at Boulder) over several years (Drexler and Brattin, 2007; EPA, 2007b; see Section 2.4 of this report).

Performance with representative materials: EPA (2007b) reports the prediction interval for the IVBA assay that was derived based on analysis of samples having a wide range of different soil types and lead phases from a variety of different sites, that are expected to be typical of application of the assay to mining and smelter sites (see Section 2.2 of this report).

2.6. Sufficient data should be provided to permit a comparison of the performance of a proposed substitute test with that of the test it is designed to replace.

The IVBA assay is intended to be used as a more cost-effective surrogate to the immature swine bioassay described in EPA (2007b). The 95% prediction interval for IVBA assay predictions of *in vivo* swine bioassay estimates of RBA is reported in EPA (2007b). The prediction interval was established from analyses of 19 samples from 12 different sites, having a wide range of different soil types and lead phases, that expected to be typical of application of the assay to mining and smelter sites (see Section 2.2).

The relationship between the test method endpoint (i.e., IVBA) and the biological effect of interest (i.e., RBA) is described in the form of a mathematical model. Several different mathematical models were tested including linear, power, and exponential. The results are summarized below (methods are detailed in Appendix D of EPA, 2007b):

Model	**a**	**b**	**c**	**R^2**	**AIC**
Linear: $RBA = a + b \cdot IVBA$	-0.028	0.878		0.924	-30.46
Power: $RBA = a + b \cdot IVBA^c$	-0.003	0.978	1.293	0.931	-29.92
2-Parameter Exponential: $RBA = a + b \cdot \exp(IVBA)$	-0.634	0.619		0.936	-33.02
3-Parameter Exponential: $RBA = a + b \cdot \exp(c \cdot IVBA)$	-0.476	0.464	1.225	0.936	-31.11
AIC, Akaike's Information Criterion; R^2, least square coefficient of determination From Appendix D (page D-14) of EPA (2007b).					

All of the models fit the data reasonably well, with the two exponential models fitting slightly better than the linear model. However, the difference in quality of fit between linear and

exponential models was not meaningful in terms of the intended application of the model to the prediction of RBA from results of the IVBA assay. Therefore, the linear model is currently considered to be the preferred model. As more data become available in the future, the relationship between IVBA and RBA can be reassessed and the best-fit model form reconsidered and revised accordingly.

Linear fitting of the data was also performed taking the error in both RBA and IVBA into account; there was nearly no difference in fit. Based on this outcome, the less complex approach (and more transparent) approach, weighted linear regression, was selected to represent the quantitative relationship between RBA and IVBA. This decision may be revisited as more data become available. The currently preferred model is (based on weighted linear regression) is as follows (Equation 3):

$$RBA = 0.878 \cdot IVBA - 0.028 \quad \text{Eq. (3)}$$

The best fit linear model for the data and corresponding 95% prediction interval are shown in Figure 1. Use of Equation 3 to calculate RBA from a given IVBA measurement will yield the *"typical*" RBA value (i.e. central estimate) expected for a test material with that IVBA, and the true RBA may be somewhat different (either higher or lower).

2.7. Data supporting the validity of a test method should be obtained and reported in accordance with Good Laboratory Practices (GLPs).

Data supporting validity of the IVBA assay are reported in detail in EPA (2007b).

2.8. Data supporting the assessment of the validity of the test method must be available for review.

Data supporting the assessment of the validity of the IVBA assay detailed in EPA (2007b) are available online (http://www.epa.gov/superfund/health/contaminants/bioavailability/guidance.htm).

2.9. The methodology and results should have been subjected to independent scientific review.

EPA (2007b), which describes in the IVBA methodology, has undergone extensive review by EPA scientists, was the subject of an EPA-sponsored workshop in April, 2003, and an independent peer review. The IVBA methodology was reported in a peer-reviewed publication (Drexler and Brattin, 2007).

2.10. The method should be time and cost effective.

Based on studies conducted in the validation of the IVBA (EPA 2007b), costs of assessment of a soil sample using the IVBA assay are expected to range from $1/10^{th}$ to $1/20^{th}$ of the costs of the immature swine bioassay. Time requirements for the IVBA assay are expected to range from $1/20^{th}$ to $1/50^{th}$ of that required to conduct the *in vivo* bioassay (i.e., days compared to weeks).

2.11. The method should be one that can be harmonized with similar testing requirements of other agencies and international groups.

Other international agencies (e.g., Canada, United Kingdom, European Union) are pursuing the development of methods for *in vitro* assessment of RBA of lead and of other metals and inorganic contaminants in soil. The IVBA assay described in the technical support document (EPA, 2007a) is directly applicable to these international programs.

2.12. The method should be suitable for international acceptance.

The IVBA assay is suitable for international acceptance.

2.13. The method must provide adequate consideration for the reduction, refinement, and replacement of animal use.

The IVBA assay is intended to replace the use of the immature swine bioassay and, therefore, widespread adoption of the method will decrease use of animals for assessing RBA of lead in soil.

3. Summary

The IVBA assay for lead has been evaluated against validation criteria established in EPA (2007a) for validation of test methods to be used in a regulatory context. All validation criteria established in EPA (2007a) have been satisfied. Scientific and regulatory rationales for the assay have been articulated. Standard Operating Protocols have been established and tested for intra-laboratory precision and inter-laboratory reproducibility. The quantitative relationship between the IVBA assay output and the test method it is intended to replace (i.e., immature swine bioassay) have been established. The description in the method SOP is expected to yield predictions of RBA that fall within acceptable prediction limits for applications in lead site risk assessment. The prediction interval is based on assays of samples having a wide range of different soil types and lead phases from a variety of different sites and, as a result, the method is expected to be widely applicable to soil typically encountered at lead waste sites. Limitations in the regulatory application of the method have been identified. Based on this assessment, EPA considers the IVBA method to be valid for predicting RBA of lead in soils in support of site-specific risk assessments. The Agency supports and encourages use of this methodology when implemented in context with the decision framework described in its soil bioavailability guidance (EPA, 2007a).

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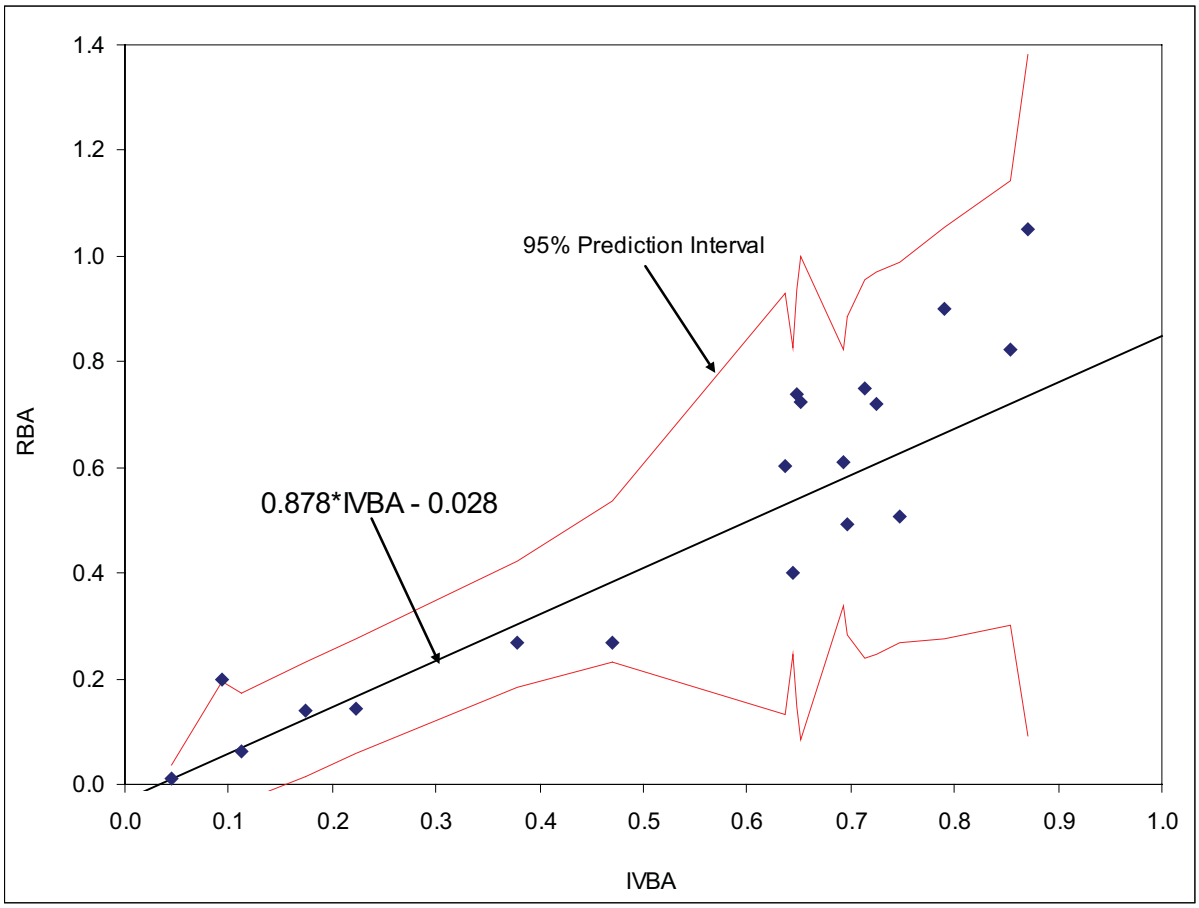

Figure 1. Prediction interval for *in vivo* RBA based on measured IVBA (from Figure D-7 of EPA, 2007b).

APPENDIX F

Report Limitations

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Report Limitations

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