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Supplemental Guidance on Human Health Risk Assessment for Oral Bioavailability of Substances in Soil and Soil-Like Media

Federal Contaminated Site Risk Assessment in Canada

Canada

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FEDERAL CONTAMINATED SITE RISK ASSESSMENT IN CANADA

SUPPLEMENTAL GUIDANCE ON HUMAN HEALTH RISK ASSESSMENT FOR ORAL BIOAVAILABILITY OF SUBSTANCES IN SOIL AND SOIL-LIKE MEDIA

June 2017

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Contaminated Sites Division
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PREFACE

The Federal Contaminated Sites Action Plan (FCSAP) is a program of the Government of Canada designed to achieve improved and continuing federal environmental stewardship as it relates to contaminated sites located on federally owned or operated properties or non-federal lands for which the federal government has accepted full responsibility. Guidance documents on human health risk assessment (HHRA) prepared by the Contaminated Sites Division of Health Canada, in support of the FCSAP, are available on our website and may also be obtained by contacting the Contaminated Sites Division at: cs-sc@hc-sc.gc.ca.

This guidance document *Federal Contaminated Site Risk Assessment in Canada: Supplemental Guidance on Human Health Risk Assessment for Oral Bioavailability of Substances in Soil and Soil-like Media* was prepared to provide guidance for custodial departments. As is common with any national guidance, this document will not satisfy all the requirements presented by contaminated sites, custodial departments or risk assessors in every case. As the practice of HHRA advances and as the FCSAP proceeds, new and updated information on various aspects of HHRA will be published. As a result, it is anticipated that revisions to this document will be necessary from time to time to reflect the new information. You should consult Health Canada at the address below to confirm that the version of the document in your possession is the most recent edition and that the most recent assumptions, parameters, etc., are being used.

This guidance document is published by the Contaminated Sites Division of Health Canada. Contributors to the report include individuals from Health Canada: Heather Jones-Otazo, Deanna Lee, Sanya Petrovic and Dr. Pat Rasmussen; Dr. Rosalind Schoof of Ramboll Environ, Inc., Ian Mitchell of Millennium EMS Solutions Ltd., and members of Bioaccessibility Research Canada.

Health Canada requests that any questions, comments, criticisms, suggested additions or revisions to this document be directed to: Contaminated Sites Division, Safe Environments Directorate, Healthy Environments and Consumer Product Safety Branch, Health Canada. E-mail: cs-sc@hc-sc.gc.ca

See also: www.canada.ca/en/health-canada/services/environmental-workplace-health/contaminated-sites.html

ABBREVIATIONS AND ACRONYMS

ABA	absolute bioavailability
ANZECC	Australian and New Zealand Environment and Conservation Council
BARC	Bioaccessibility Research Canada
BARGE	Bioaccessibility Research Group of Europe
BW	body weight
CALA	Canadian Association for Laboratory Accreditation
CCAC	Canadian Council on Animal Care in Science
CCME	Canadian Council of Ministers of the Environment
CEC	cation exchange capacity
COC	chemical of concern
COPC	chemical of potential concern
CSF	cancer slope factor
DMOE	Danish Ministry of the Environment
DQO	data quality objectives
DQRA	detailed quantitative risk assessment
EPHC	Environment Protection and Heritage Council (Australia)
FCSAP	Federal Contaminated Sites Action Plan
GE	gastric extractable
GI	gastrointestinal
GLP	good laboratory practices
HC	Health Canada
HHRA	human health risk assessment
HQ	hazard quotient
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ILCR	Incremental Lifetime Cancer Risk
IVBA	*in vitro* bioaccessibility
IVG	*in vitro* gastrointestinal
IVIV	*in vivo-in vitro*
K_{ow}	octanol-water partition coefficient
K_{oc}	organic carbon-water partition coefficient
K_d	soil-water partition coefficient
NEPC	National Environment Protection Council (Australia)
NIST	National Institute of Standards and Technology
NRC	National Research Council (U.S.)
PAH	polycyclic aromatic hydrocarbon
PCB	polychlorinated biphenyl
PQRA	preliminary quantitative risk assessment
QA/QC	quality assurance/quality control

RAF	relative absorption factor
RBA	relative bioavailability
RBALP	relative bioaccessibility leaching procedure
RIVM	Netherlands National Institute of Public Health and the Environment
SBRC	Solubility/Bioavailability Research Consortium
SEM	scanning electron microscopy
SOP	standard operating procedure
SRM	standard reference material
TDI	tolerable daily intake
TOC	total organic carbon
TRV	toxicity reference value
UBM	unified BARGE method
US DoD	United States Department of Defense
US EPA	United States Environmental Protection Agency
XAS	x-ray absorption spectroscopy
XRD	x-ray diffraction

1.0 INTRODUCTION

This guidance is intended to be used by custodians of federal contaminated sites in Canada and their consultants. The approach is specifically designed for the assessment of sites that are to remain the responsibility of federal agencies for promotion of greater consistency in risk assessment methods and the interpretation of results.

Although the guidance has a specific federal focus, it is generally consistent with international and provincial guidance, as it relies upon the same underlying principles, analytical methods and exposure equations. Risk assessors should be aware, however, that for issues requiring decisions by a private party or by provincial/territorial governments, risk assessments may have to be completed in accordance with local provincial/territorial statutes and regulations, which may differ from the standardized methods described in this guidance document. When the methods employed in such cases differ significantly from those presented here, risk assessors should identify the differences and the assumptions required by provincial/territorial agencies, and discuss the implications (for the federal custodial department) for risk characterization and interpretation.

1.1 Background

The systemic effects of an ingested substance on human health are determined by the amount of the substance absorbed into the body and subsequently available to exert toxic effects. Human health risk assessments (HHRAs) rely mostly on exposure assessments that are based on external doses, and often do not include assessments of substances absorbed into systemic circulation in the body. It is commonly assumed that the proportion of chemical absorbed following ingestion of soil at a contaminated site is equivalent to the absorption in the toxicity study used to determine human health benchmarks. This assumption is generally conservative, since laboratory toxicity studies frequently use substances in a form that is easily absorbed, which may overestimate potential exposure. The bioavailability of such substances in soil may be reduced because of 1) the presence of less soluble chemical forms in soils; 2) the chemical and physical interactions of the chemical with soil particles; or 3) the transformation of chemicals in soils over time (e.g., weathered soils). Therefore, at some contaminated sites, obtaining more precise estimates of human health risks, which include assessment of bioavailability, may be desirable before making risk management decisions.

1.2 Purpose

The purpose of this guidance document is to provide supplementary information to Health Canada's Detailed Quantitative Risk Assessment Guidance (Health Canada [HC] 2010a). This document provides guidance on how to incorporate oral bioavailability in HHRAs for sites with contaminated soils or soil-like material or media (i.e., indoor settled dust, sediment, waste materials such as mining tailings or slag). Incorporating bioavailability adjustments in risk assessments (instead of using a default absorption value of 1 in exposure dose equations) can allow for more realistic risk estimates. This can provide support for site-specific remediation targets (which may be greater than screening levels) while ensuring adequate protection of human health.

This guidance focuses specifically on the incorporation of oral bioavailability of inorganic substances in the exposure assessment component of the risk assessment. What is termed 'bioavailability' is the result of several complex processes, and many HHRAs will present unique situations not specifically addressed herein. Risk assessors are encouraged to ensure that their assessments address all relevant potential exposures to substances at a contaminated site.

This document does not address bioavailability from dermal or inhalation exposures. The general approach for dermal bioavailability is incorporated into existing Health Canada guidance (HC 2010a; 2012). Inhalation bioavailability, by definition, addresses only one exposure medium (air); however, the form (e.g., particle size distribution or metal species) of substances present in air at the site may vary from that used in the study (or studies) upon which the toxicological reference values (TRVs) are based. In addition, there are less data on inhalation bioavailability which make site-specific adjustment to inhalation bioavailability less amenable to HHRAs.

Guidance presented in this document is not designed, or intended, as a substitute for the sound professional judgment of a qualified and experienced risk assessment practitioner. However, where possible and appropriate, the guidance provided here may be used and referenced. Where alternative or unique approaches have been found necessary, these should be sufficiently documented and described to enable peer review and should be evaluated for their impact on risk estimates relative to the application of the standard methods. Oral bioavailability of substances in soil is currently an active area of research. Consequently, it is recognized that some of the underlying information and references provided may need to be updated in the future. An overriding goal is to provide a general framework and to promote use of the best available science in assessing federal contaminated sites in Canada.

Since the underlying science and methods for assessing oral bioavailability are rapidly evolving, the main body of this guidance focuses on the overall framework, approach and requirements for inclusion of oral bioavailability adjustments in HHRAs, particularly for inorganic substances. Specific technical methods considered current at the time of writing are summarized in the appendices. The risk assessor is responsible for identifying which technical methods are appropriate and/or recommended by the applicable regulatory or government agencies. This guidance was adapted from a report by ENVIRON (2011, available on request from Health Canada), which includes additional technical information.

1.3 Outline

Some basic definitions of terms used in a discussion of bioavailability are provided in **Section 2**, together with a set of equations needed for calculation of bioavailability adjustments in risk assessment. (Some of these equations are reproduced again in **Section 5.4** for use in worked examples.) **Section 2** lists the key factors affecting bioavailability (e.g., soil characteristics) and goes on to discuss important issues that arise when the feasibility of bioavailability testing and adjustment is being considered.

In the absence of site-specific testing, a literature-based relative bioavailability (RBA) value may be acceptable but justification for this approach must be provided to show how the RBA value chosen is relevant to the site under investigation. **Section 3** outlines the caveats associated with this practice.

Section 4 provides information regarding oral bioavailability studies and how to conduct them, including analysis of environmental media, and sample collection and processing. This is followed by the primary chemical forms and oxidation states for six specific metals. *In vitro* bioaccessibility and *in vivo* bioavailability test methods are described, as well as the validation of the former by comparison with the latter.

Section 5 presents the general equations used to incorporate oral bioavailability in an HHRA and provides some worked examples using these equations and others from **Section 2**.

Appendices A and **B** provide more detail about the key considerations in the use of *in vitro* and *in vivo* tests respectively. The necessary information and criteria for an *in vivo-in vitro* (IVIV) comparison (for validation of the *in vitro* method) is available in **Appendix C**. **Appendix D** lists some *in vitro* and *in vivo* methods in common use. Finally, **Appendix E** provides a summary of approaches used to evaluate oral bioavailability in the United States, United Kingdom, the Netherlands, Denmark, Australia, New Zealand and France are outlined.

2.0 USE OF ORAL BIOAVAILABILITY IN RISK ASSESSMENT

2.1 Definitions

Bioavailability is implicitly or explicitly considered whenever chemical exposures to substances are calculated, when dose–response is analyzed or when predictions of toxicity or risk are made. Exposure to substances may be characterized as intakes (i.e., administered dose) or uptakes (i.e., absorbed dose), and estimated dose rates should be clearly described to indicate whether intake or uptake is being presented. In risk assessment, both oral dose estimates and oral TRVs are typically presented as intake or administered dose.

Definitions of bioavailability vary among scientific disciplines. The National Research Council (NRC) provides a comprehensive summary of definitions (NRC 2003). In mammalian toxicology and HHRA, **bioavailability** describes the absorption and uptake of a substance into systemic circulation by an organism. More specifically, the fraction or percentage of a dose that is absorbed is called the **absolute bioavailability** (ABA) and is typically represented as the ratio of the absorbed dose to the administered dose:

(2.1) $$\text{Absolute bioavailability (ABA)} = \frac{\text{absorbed dose}}{\text{administered dose}}$$

ABA may be used to convert intake estimates to uptake estimates and to convert an administered dose to an absorbed dose (see Section 5.3).

Relative bioavailability (RBA) is a term that is used to describe the comparative difference in bioavailability between different forms of the substance (e.g., different metal compounds, or metal species with different oxidation states) or in different environmental matrices, and RBA may be stated as either a fraction or a percentage.

The bioavailability of a substance in the environmental matrix of interest relative to the dosing (i.e., reference) medium used in the critical toxicity study (i.e., the TRV study) is the most common use of the relative bioavailability concept in contaminated sites risk assessment and is shown in equation 2.2 below:

(2.2) $$\text{Relative bioavailability (RBA)} = \frac{\text{ABA}_{\text{test}}}{\text{ABA}_{\text{reference}}}$$

Where :

ABA_{test} = absolute bioavailability from test medium

$\text{ABA}_{\text{reference}}$ = absolute bioavailability from the reference medium

The oral RBA is usually less than 1.0 because the most bioavailable form of a chemical is typically used in toxicity studies, but it is also possible to have a RBA greater than 1.0 if the chemical is more readily absorbed from the environmental medium (e.g., water) than from the reference medium used in the toxicity studies (e.g., diet).

In mammalian toxicology, RBA can be calculated without any actual calculation of ABA (Schroder *et al.* 2003). For example, tissue levels achieved following exposure to different forms of a chemical, such as lead levels in bone or liver may be directly compared in order to estimate RBA.

Note that various terms are used to describe RBA. HC's Preliminary Quantitative Risk Assessment (PQRA) and Detailed Quantitative Risk Assessment (DQRA) guidance refer to relative absorption factors (RAFs) (HC 2010a; 2012). Theoretically, the RBA is the ABA (i.e., the absorbed fraction or percentage) of a substance from the environmental medium (e.g., soil or sediment) divided by the ABA (i.e., the absorbed fraction or percentage) from the dosing medium used in the toxicity study (equation 2.3).

$$\text{RBA} = \frac{\text{absorbed fraction from soil (\% or fraction)}}{\text{absorbed fraction from dosing medium used in the TRV study (\% or fraction)}} \quad (2.3)$$

When adjusting for oral bioavailability in an HHRA, it is important to understand the assumptions made in both the toxicity assessment and the exposure assessment. The default assumption in an HHRA is that the oral bioavailability of the chemical in soil is the same as in the medium in the key toxicity study used to derive the oral TRV. For example, if the TRV is derived from drinking water studies, the default assumption is that the chemical in soil has the same bioavailability of the chemical in drinking water (i.e., RBA=1); however, the chemical form or exposure medium encountered at the site may differ significantly from the chemical form or exposure medium that was used in the key toxicity study. As described in Section 5, the RBA is typically used to modify intake estimates in exposure assessments. In particular, the RBA is applied in risk assessment to account for the difference in absorption between the chemical in the environmental medium and the chemical in the dosing vehicle or the exposure medium used in the critical studies (i.e., key laboratory or epidemiology study(ies)) used to derive the TRV.

Another term that has become commonly used in association with relative bioavailability is **bioaccessibility**. This term has been used to describe the results of *in vitro* tests that measure the fraction of dissolved soluble substances from the environmental matrix (i.e., soil or sediment) that is available for absorption in simulated gastrointestinal (GI) fluid (Ruby *et al.* 1999). Since the RBA of inorganic chemicals is thought to be related to variations in physiological solubility within the GI tract, *in vitro* bioaccessibility (IVBA) is often used as a surrogate for RBA in soil; however, this is a simplistic view, as there are many variables that affect *in vivo* absorption.

2.2 Use of bioavailability within the federal risk assessment framework

This guidance on the consideration of oral bioavailability in risk assessment is intended to be consistent with and expand upon HC's DQRA guidance (HC 2010a). It is recommended that HC be consulted before a bioavailability study for a federal contaminated site is conducted, to determine whether there is a precedent for a particular chemical of concern (COC), test methodology or other issue that may affect the suitability of the proposed study and/or if there are any concerns regarding the usefulness of incorporating bioavailability in the HHRA. As previously noted, the information presented in this guidance document may require updating as the science progresses in the field.

The use of oral bioavailability adjustments in DQRA at a site should be thoroughly considered on a case-by-case basis, taking into consideration the information contained in this guidance. A brief discussion of bioavailability, including when bioavailability adjustments are appropriate, the data requirements for bioavailability/bioaccessibility studies and cautions on interpreting bioavailability/bioaccessibility data are presented in Section 4.7 of the DQRA guidance (Health Canada 2010a). Various aspects of bioavailability assessment, including pathway-specific considerations for oral, dermal and inhalation exposure assessments and the use of oral bioavailability adjustments, are discussed in the DQRA guidance.

An important part of determining the appropriateness of oral bioavailability adjustments is to ascertain the details of the toxicity study(ies) used to derive the TRV. While it is usually straightforward to determine the exposure medium (e.g., drinking water or diet) used in that study, sometimes multiple studies with multiple exposure media are relied upon. For studies conducted in laboratory animals, it is usually clear what the form of the substance was used (i.e., metal species or polycyclic aromatic hydrocarbon [PAH] mixture), but in human epidemiology studies the form of the substance(s) may not be known. For organic chemicals such as dioxins and furans, polychlorinated biphenyls (PCBs) and PAHs, which often occur as mixtures, the exact mixture associated with the toxicity study exposure may not be known. Application of an RBA in a risk assessment should be accompanied by support for the relevance of the RBA to the TRV.

The equation used to calculate the estimated dose via inadvertent ingestion of contaminated soil is given in the DQRA guidance document. The dose equation includes an RAF, which is assumed to be equal to 1.0 by default. The RAF is analogous to the RBA used in this document, and its application is shown in the following generalized dose estimation equation for inadvertent ingestion of soil:

(2.4)
$$\text{Dose (mg/kg/day)} = \frac{C_s \times IR_s \times RAF_{Oral} \times ET}{BW \times LE}$$

Where :

C_S	= concentration of chemical in soil (mg/kg)
IR_S	= soil ingestion rate (kg/day)
RAF_{oral}	= relative absorption factor from the GI tract (unitless)
ET	= exposure term (unitless) = days/week x weeks/year (x years for carcinogens)
BW	= body weight (kg)
LE	= life expectancy (years) to be employed for assessments of carcinogens only

If site-specific oral RBA data are obtained, the information can be incorporated into equation 2.4 (see worked example in section 5.4.1). The RBA is used to adjust the exposure dose (i.e., intake) to account for differences in absorption of the chemical between the exposure medium and the dosing medium of the toxicity study, so that:

(2.5)
$$\text{Intake}_{adjusted} = \text{Intake}_{unadjusted} \times RBA$$

Where :

$\text{Intake}_{adjusted}$	= exposure dose adjusted to reflect RBA (mg/kg/day)
$\text{Intake}_{unadjusted}$	= exposure dose without consideration of bioavailability (mg/kg/day)
RBA	= relative bioavailability value (unitless)

Risk estimates are calculated from intakes after adjustment for RBA (equation 2.5) (or RAF_{oral} in equation 2.4). The hazard quotient (HQ) for a threshold substance is calculated as:

(2.6)
$$HQ = \frac{\text{Intake}_{adjusted}}{TDI}$$

Where :

HQ	= hazard quotient
$\text{Intake}_{adjusted}$	= exposure dose adjusted to reflect RBA (mg/kg/day)
TDI	= tolerable daily intake (mg/kg/day)

The incremental lifetime cancer risk (ILCR), using adjusted intake, is calculated as:

(2.7) $$ILCR = Intake_{adjusted} \times CSF_{oral}$$

Where :

ILCR = incremental lifetime cancer risk

$Intake_{adjusted}$ = exposure dose adjusted to reflect RBA (mg/kg/day)

CSF_{oral} = oral cancer slope factor ((mg/kg bw/day)$^{-1}$)

2.3 Default assumptions for bioavailability

When HHRAs are being conducted and no site-specific bioavailability data are available, the default RBA is set to 1, and it is assumed that the bioavailability of substances in site media is comparable with that of the study(ies) used to derive the TRV. Note that this does not mean that the assumed ABA of a substance is 100%, as may be incorrectly stated in some risk assessments. As noted in the Introduction, there are multiple factors that may cause reduced bioavailability of substances in site soils. Although necessary when there are no site-specific bioavailability data or when the absolute absorption in the critical toxicity study is difficult to estimate, using an RBA of 1.0 may result in an overestimate of site-related health risks.

2.4 When to consider site-specific bioavailability

Bioavailability studies or a detailed evaluation of RBA are not necessarily appropriate at every site or for each chemical at a particular site. Sometimes the benefits will not be apparent until after a screening risk assessment or sensitivity analysis has been conducted.

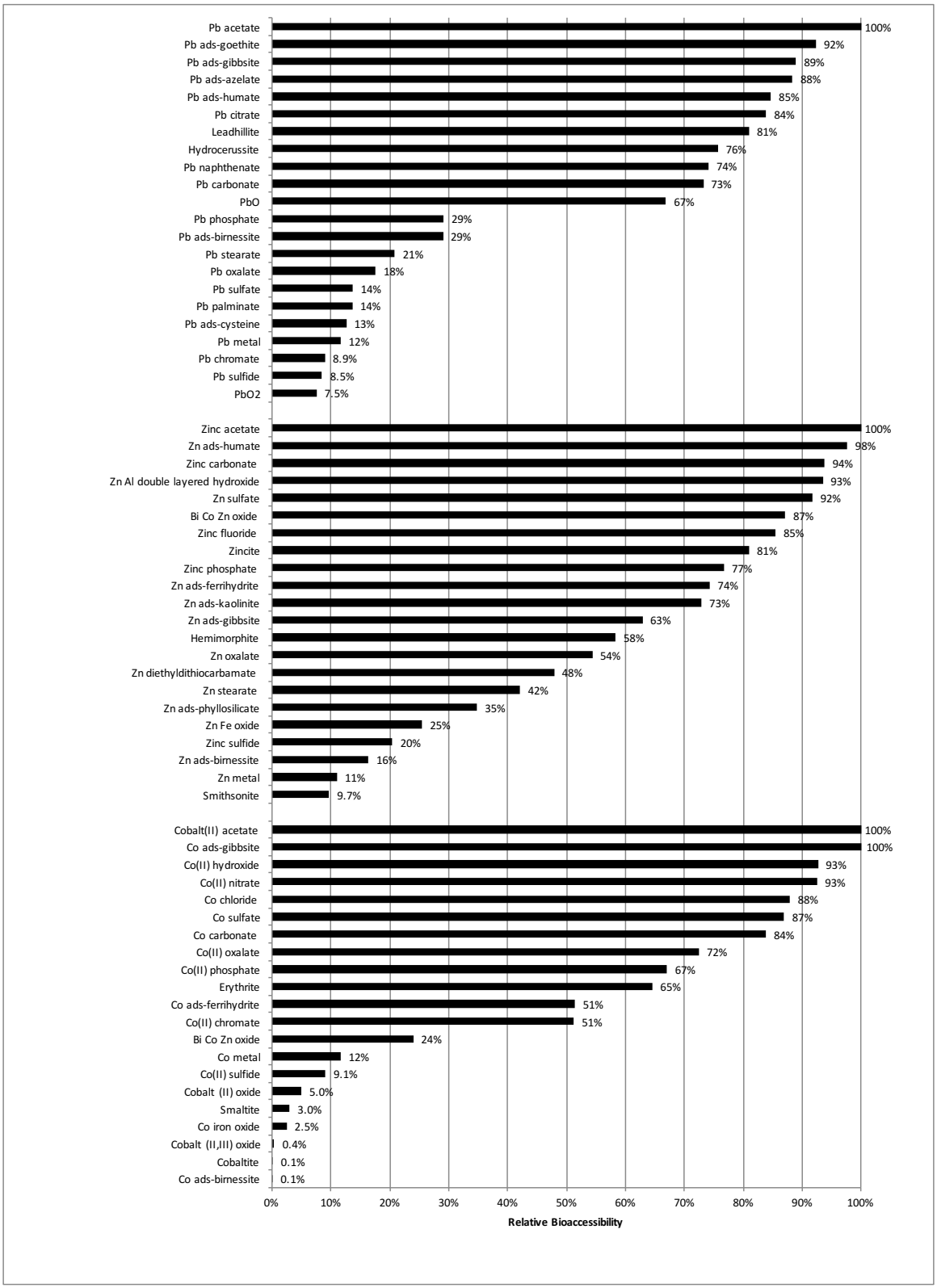
Careful consideration of the following factors will aid in assessing the usefulness of RBA evaluation for one or more substances at a site.

Chemicals of Concern and Primary Exposure Route: Relative oral bioavailability evaluations may be useful for sites where ingested substances present the greatest potential risk. Sites where risks are driven by inhalation exposure (especially where volatile compounds are present) are less likely to benefit from RBA evaluations than sites where risk is primarily driven by the soil ingestion pathway.

Existing Site Data: Site data can be used to evaluate whether the combination of contaminant ageing, contaminant properties and soil properties are likely to result in strong retention of contaminants in soils or similar media. For example, metal sulfides and elemental metals are expected to be less bioavailable than metal carbonates, whereas metal sulfates are expected to be moderately bioavailable, as indicated by a study of the relative bioaccessibility of more than 60 lead, zinc and cobalt compounds in house dust (Figure 2–1; Rasmussen *et al.* 2014). Characterization of metal phases can offer qualitative insights into relative bioaccessibility, as demonstrated by Meunier *et al.* (2010a): they found that, for arsenic, in samples with mixtures of less prevalent but more soluble phases the presence of the more soluble phases could increase the bioaccessibility *in vitro*. Brattin *et al.* (2013) demonstrated that inclusion of arsenic speciation data in models based on swine and monkeys improved predicted RBAs. A comparison of synchrotron X-ray speciation with gastric bioaccessibility extractions demonstrated a significant quantitative relationship between lead speciation and lead bioaccessibility in house dust (Rasmussen *et al.* 2011). Matrix characteristics may suggest the likelihood that chemicals will bind to soil particles. For example, binding may be greater in fine clay soils than in coarser soils as a result of the greater surface area for adsorption and other characteristics of clay soils. Greater binding to soils may alter the bioavailability of some chemicals. These types of data may also be necessary to establish that the bioaccessibility or bioavailability test selected is appropriate for the contaminant and media at the site.

The DQRA guidance includes a brief discussion of the desired characteristics of *in vitro* bioaccessibility assays and interpretation of these assays, which are also addressed in further detail in Section 4.4 and Appendix A of this document.

Figure 2–1: Bioaccessibility of lead (Pb), zinc (Zn), cobalt (Co) compounds expressed as percentages relative to their soluble reference salts used in TRV assays. Mean values for 2–5 replicates per compound (from Rasmussen *et al.* 2014).



Reproduced by permission of The Royal Society of Chemistry [Rasmussen, P.E., S. Beauchemin, L.C.W. MacLean, M. Chénier, C. Levesque and H.D. Gardner. 2014. Impact of humidity on speciation and bioaccessibility of Pb, Zn, Co and Se in house dust. J. Anal. At. Spectrom. 29: 1206–1217]

Chemical Species and Matrix Relative to Key Toxicity Value: If the critical study that supports a particular TRV was conducted using a specific form of a chemical or matrix that is different from the chemical form or soil matrix found at the site, then an RBA evaluation could demonstrate reduced exposure and potential risks. The absorbed dose of chemicals in site media may be reduced compared with absorbed doses in laboratory studies because toxicity studies are typically conducted using a soluble dosing vehicle and chemical species that are relatively bioavailable.

Time Required: The period of time needed to design, conduct and report a site-specific bioavailability study may affect the time available for evaluating some sites, such as those requiring time-critical management measures.

Cost: Costs for site-specific studies will vary by test, depending on the protocol, where the study is conducted and the level of effort required to incorporate the results into the risk assessment. Some factors that influence cost are discussed in Section 2.4.1 and 2.5. Regardless of whether an *in vitro* or *in vivo* test is being conducted, the cost of the bioavailability study is not insignificant and should be weighed against the potential savings in clean-up costs. The areal extent of contamination, chemical concentrations, magnitude of risks and/or potential for human exposure based on current and future land use should be sufficient to justify the costs associated with bioavailability testing. It may be useful to consider the costs of implementing institutional and/or other controls to limit human contact with site soils as an alternative to site-specific studies.

2.4.1 Key Factors Affecting Bioavailability

The oral bioavailability of chemicals in soil and other soil-like media is a function of four primary factors: 1) particle size and the characteristics of the soil or soil-like medium (e.g., organic carbon and clay content), 2) the form and properties of the chemicals and the nature of their interactions with soil particles, 3) the nature of the soil contact by humans (e.g., hand-to-mouth contact by a child) and 4) the key absorption processes in the GI tract for different substances.

This discussion primarily addresses soil characteristics but may also be relevant for other related exposure media (i.e., soil-like media), including indoor settled dust or sediment and waste materials such as mine tailings and slag. Indoor dust or sediment may have a higher organic content and a different range of particle sizes that adhere to skin compared with soil. The nature of the contact with soil or soil-like media is critical because it governs the particle size fraction that may be ingested.

Mine tailings and other waste materials are likely to have reduced organic content and different pH than soils, as well as highly variable particle size ranges. Consequently, when testing these other exposure media it is important to fully characterize the material being evaluated with regard to particle size distribution and other characteristics discussed in the following sections.

Clay minerals, metal oxides and organic matter have ionic functional groups to which metals and other inorganics may adsorb to form complexes. In some cases, metal complexes that are weakly adsorbed to soil components may desorb or re-adsorb with changing soil pH, while strongly adsorbed metal complexes may not desorb from soil. Those complexes that do not desorb from soil are said to be aged (i.e., weathered) and may exhibit low bioaccessibility if they also fail to desorb in simulated GI fluid. For example, arsenic in soil that is characterized by low pH (less than 6) and elevated iron oxide content has been shown to have reduced bioavailability over time (Subacz *et al.* 2007; Yang *et al.* 2002 and 2005; Cutler *et al.* 2013, 2014). In clay soils, metals also may form ionic bonds that are not pH dependent. This results in stronger bonds and lower solubility (ENVIRON 2011).

Table 2–1 qualitatively illustrates the influence of various site characteristics on metal bioavailability, but it is not a comprehensive summary. In risk assessment, site-specific testing is preferred to show how characteristics of site soils affect oral bioavailability at a site. If no site-specific testing is done, a strong scientific rationale with primary references should be provided for any assumptions of soil or soil chemistry characteristics that may affect the oral bioavailability of metals.

Table 2–1: Soil[1] and Chemical Characteristics Affecting Oral Bioavailability of Metals[2]

Oral Bioavailability			
Soil Characteristics	**Low**	**Medium**	**High**
Metal Forms:			
Sulfides	X		
Elemental (metallic)	X		
Sulfates		X	
Carbonates			X
Oxides	X (Cr, Ni & Hg)		X (As, Pb)
Particle Size:			
Fine			X
Coarse	X		
Weathered/Aged Contamination:			
Sulfides	X	X	
Elemental	X	X	
Carbonates		X	X
Oxides		X	X
Organic Compounds[3]	X		
Soil Chemistry:			
Acidic		X	
Basic (alkaline)			X (Cd, Hg, Pb, Ni)
High total organic carbon			X (Hg, Pb)
High Fe and Mn		X (As)	
Sulfide-producing soil		X (Cd, Hg, Pb, Ni)	

Notes:

[1] Soil includes soil-like media, e.g., dust, sediment and waste materials such as slag and mine tailings.

[2] ENVIRON (2011) modified from Table 3–1 in the United States Department of Defense (US DoD) *Guide for incorporating bioavailability adjustments* (US DoD 2003).

[3] Note that bioavailability of metal compounds may increase during ageing because of the formation of more bioaccessible organo-metallic species (MacLean *et al.* 2013; Rasmussen *et al.* 2014; Beauchemin *et al.* 2014).

Cr (chromium), Ni (nickel), Hg (mercury), As (arsenic), Pb (lead), Cd (cadmium), Fe (iron), Mn (manganese).

The following sections provide an overview of the role of particle size and relevant soil characteristics, the influence of inorganic metal or metalloid forms on bioavailability, and factors affecting the degradation and sequestration of organics in soil.

Particle Size and Bioavailability

Soils and dusts may vary markedly in their particle size distributions, and the RBA of particle-bound chemicals may vary with particle size for a number of reasons, such as available surface area, rates of particle dissolution in the GI tract, fraction of organic carbon and the chemical reactivity of fine particles vs. coarser particles. Many of the processes governing chemical interactions with soil particles described in the following sections will vary with particle size.

Chemical concentrations in soil are typically reported on the basis of an analysis of bulk soil, whereas bioavailability studies are often performed using a fine fraction of soil (generally <250 µm or less), which is thought to better represent the fraction likely to be contacted and ingested by people. Chemicals may be distributed non-uniformly across particle sizes for any given soil or dust (Bright *et al.* 2006), making particle size fraction an important factor to consider in characterizing exposures (Richardson *et al.* 2006). Methodological issues related to particle size fractions are discussed further in Section 4.2. The sieved samples (i.e., the fine fractions) used for the bioavailability study should be analyzed for chemical concentration in advance of the study, as the concentrations of chemicals may differ from those in the bulk soil samples analyzed as part of the environmental site assessments.

Soil Characteristics

The bioavailability of both organic and inorganic chemicals varies with different media. Soil characteristics influence chemical solubility and mobility in the environment, which in turn influence the absorption of a chemical within the GI tract. Soil organic carbon content and pH in particular have been shown to influence bioavailability (NRC 2003; Kelley *et al.* 2002; Ruby 2004; Yang *et al.* 2002; Datta and Sarkar 2005; Jardine *et al.* 2013). Amendments to soil, such as phosphate (from fertilizers) and compost, can also influence bioavailability (Cutler *et al.* 2014; Zia *et al.* 2011). These factors will vary from site to site depending on the physical make-up of the soil, which itself changes over time through weathering.

Clay minerals make up the smallest size fraction of soil particles. Because of their large surface area-to-volume ratio and highly reactive surface, clay minerals are one of the most important components to influence movement of chemicals in soil (Kelley *et al.* 2002). The negatively charged minerals in clay provide the reactive surface that is important in understanding the soil-chemical interactions influencing mobility and bioavailability. The negatively charged surface of clay is described as the cation exchange capacity (CEC) and provides important information regarding a soil's potential to bind chemicals.

Organic components in soil, including detritus and living organic matter, react with ionic and polar contaminants and tend to react with metal ions. Black carbon, an organic geological material, also reacts with non-polar chemicals. As well, organic matter contains small pore spaces that provide hydrophobic sites for chemical absorption (Kelley *et al.* 2002).

The length of time that chemicals have been in soil varies considerably among sites and can strongly influence their bioavailability. As indicated by Kelley *et al.* (2002), factors that affect the bioavailability of organic vs. inorganic compounds (including metals) are different and need to be considered separately. Over time organic compounds can diffuse into the solid soil phase, adsorb to soil particles or become sequestered within micro-pores in soil. They may also undergo biodegradation or biotransformation, forming complexes with soil particles. Often these changes can result in decreases in chemical mobility and the potential for uptake by organisms, although some reactions may also increase bioavailability (see Table 2–1). Inorganic chemicals are not degraded over time; however, their bioavailability may be affected by changes in speciation that occur with changing soil chemistry and/or changes that result from the formation of physical bonds. Soil interactions and the influence of weathering are described below for inorganic and organic chemicals.

Influence of Inorganic Chemical Forms on Toxicity and Bioavailability

The toxicity of inorganic chemicals such as metals may vary with the form of the chemical. For example, methylmercury, inorganic mercury and elemental mercury all have different characteristics and toxicities. In the absence of metal species characterization, generally the most conservative TRV is applied. Issues for selected metals are discussed in greater detail in Section 4.3 and in other HC guidance documents for contaminated sites.

The fate and transport of metals (or metalloids) in soil are greatly influenced by the chemical species or form present, which in turn affects bioavailability. The oxidation state of a metal determines its ability to form oxidation-reduction (redox) reactions with soil components (Kelley *et al.* 2002). Arsenic (III, V) and chromium (III, VI) are two metals that may change oxidation state within the terrestrial environment. While hexavalent chromium is more toxic than trivalent chromium, trivalent chromium is predominant in most soils because hexavalent chromium requires stronger oxidizing conditions than are typically present. In contrast, the oxidized form of arsenic (pentavalent arsenic) predominates under the mild oxidizing conditions typically present in soils. Other metals, including cadmium (II), lead (II), mercury (II) and nickel (II), are also able to undergo redox reactions to form complexes with soil particles, but they undergo alterations in oxidation state only rarely, or not at all (Kelley *et al.* 2002).

The physical form of the metal compounds and mineralogical features also affect RBA. Metals in soils that arise from mining and mineral processing may be part of the soil particle matrix, whereas metal compounds deposited on soil from atmospheric emissions or mixed in with soil from the use of metal-based products may be attached to particle surfaces rather than incorporated into particle matrices. There have been substantial advancements during the past decade in the tools used to characterize the interactions of metals with soil phases (NRC 2003; Scheckel *et al.* 2009). Spectroscopic techniques (i.e., X-ray diffraction [XRD], X-ray absorption spectroscopy [XAS]) offer the ability to characterize mineralogical properties of metals in soil. XAS methods are playing an increasingly important role in characterizing the interactions of inorganics with soil particles (Meunier *et al.* 2011; NRC 2003; Scheckel *et al.* 2009). Scanning electron microscopy (SEM) can be used to identify morphological features of metal–soil particle associations. When SEM is used with XRD and/or XAS (NRC 2003), both mineralogical and speciation data can be obtained from soil samples to support RBA analyses.

Soil Interactions and the Bioavailability of Organic Substances

The majority of this document focuses on inorganic compounds; however, a brief discussion of the bioavailability of organics is provided below.

Important differences exist between the interactions of non-polar and polar organic chemicals with soil. Non-polar organic compounds are mainly found in association with organic components such as soot particles and humic material, whereas polar organic chemicals are found in association with mineral components of soil (NRC 2003). Soil characteristics that affect adsorption of organic chemicals to soil particles include organic carbon and clay content.

As with inorganics, organic chemicals that form strong bonds with soil particles will be less mobile and less bioavailable than organic chemicals in solution. Non-polar organic chemicals will partition to organic carbon in soil; therefore, as the fraction of organic carbon in soil increases, bioavailability is likely to decrease. Adsorption of organic chemicals to soil particles, adherence to surfaces, formation of bonds and sequestration into pore spaces generally increase with time unless physical or chemical changes occur at the site to prevent these ageing processes (NRC 2003). Sequestration of organic chemicals in soil may occur through several processes, including partitioning, diffusion into soil pore spaces and adsorption to soil particle surfaces (NRC 2003; Alexander 2000).

Non-polar organic chemicals also may adhere to soil by forming weak physical or chemical bonds with the soil surface, particularly with organic matter or clay (NRC 2003; Alexander 2000). It is possible for organic chemicals to become encapsulated by soil organic matter or clays such that the organic chemicals become inaccessible and will not be desorbed from the soil. Organic carbon provides a substrate for adsorption of organic compounds in soil. Clay content in soil also influences adsorption, providing a charged surface area for reactions with polar organic compounds. In addition, clay particles have a small diameter (<2 µm), which allows for the creation of micropores and nanopores that sequester organic chemicals, as discussed above.

Polar organic chemicals will interact with soil constituents by many different kinds of interaction, with those that are ionizable having many characteristics in common with the interactions described above for metals (NRC 2003). Highly soluble organics may be found in greater concentrations in pore water and are likely to be more bioavailable than those that are adsorbed to soil particles.

In addition to inorganic or elemental forms of metals, some metals may be present in soils or sediments as organometallic chemicals. For example, under certain conditions, methylmercury in sediments is formed from the methylation of inorganic or elemental mercury in aquatic systems, and the application of agricultural products containing organic arsenicals may result in elevated levels of arsenic in agricultural soils.

Solubility and Environmental Partitioning Coefficients

Increased solubility of a substance generally correlates with increased potential for uptake by organisms. For this reason, quantifying the solubility of a chemical in soil can improve the understanding of the potential oral bioavailability of a chemical in a specific soil or at a particular site. Solubility or affinity for organic carbon in soil is quantified by several different partition coefficients: the octanol-water partition coefficient (K_{ow}), organic carbon-water partition coefficient (K_{oc}) and soil-water partition coefficient (K_d) (NRC 2003; Chung and Alexander 2002). K_{ow} and K_{oc} are chemical properties, whereas K_d is a site-specific parameter that will vary depending on site soil characteristics (Chiou and Kile 2000; Chiou 2002). The K_{ow} is based on a laboratory test of the partitioning of chemicals between octanol, representing soil organic matter, and water. The K_{oc} is a measurement of a chemical's affinity for organic matter in soil, and represents the ratio of the mass of the chemical that partitions or is adsorbed to soil organic carbon to the concentration that remains in water at equilibrium. K_d is a site-specific measurement that represents the ratio of a chemical concentration associated with the soil to the dissolved concentration in solution for a particular soil type, when the system is at equilibrium. Organic chemicals with high K_{ow}, K_{oc} and K_d values are expected to be more strongly adsorbed to soil particles and less soluble in GI fluid than chemicals with lower partition values. Decreased solubility correlates with a decreased potential for uptake and absorption by organisms (ENVIRON 2011).

Receptor Characteristics

In addition to soil properties, the characteristics of human receptors can have a significant effect on bioavailability. Some of the characteristics that influence bioavailability (US EPA 2007a) include the following:

- Age
- Dietary status (fasting or recently fed)
- Genetic variation
- Nutritional status (deficiency or surplus) for essential elements.

These characteristics are not normally evaluated on a site-specific basis, since the people at a site or who may use a site are frequently unknown and are expected to change over time. However, each of the characteristics has the capacity to affect the multiple complex processes that constitute absorption across the human GI tract. These processes include passive or facilitated diffusion and active transport. Further, intestinal absorption of microparticles or colloids has been demonstrated through multiple mechanisms and can be relevant to contaminants with limited solubility in GI fluids (NRC 2003).

Soil Amendments to Reduce Bioavailability

The influence of soil characteristics and weathering on the bioavailability of chemicals in soil is illustrated by studies that use various soil amendments to promote the formation of insoluble chemical phases for the long-term immobilization of soil contaminants through chemical fixation.

Chemical fixation strategies that have been most commonly evaluated to reduce the bioavailability of metals in soil, initially focused on lead and increasingly on arsenic. Appropriate chemicals or other amendments are added to the soils to alter the dominant chemical and/or mineralogic form of the metal to a less bioavailable state. The United States Environmental Protection Agency (US EPA) provides an overview of soil amendment strategies and the various considerations that must be balanced, such as impacts on plant nutrient uptake, when attempting to reduce metal bioavailability (US EPA 2007b, 2015). These processes are still experimental and have a varied record of success. They are also beyond the scope of this guidance. When bioavailability is being assessed, understanding the physio-chemical and biological processes involved in absorption is important. Rigorous peer review of the risk management plan and site testing are recommended if chemical fixation methods are included in remediation and/or risk management plans for contaminated sites.

2.5 Planning for bioavailability studies

Determining whether a bioavailability study may be useful in an HHRA (e.g., whether the bioavailability study will show there is a sufficient reduction in the RBA of a contaminant) should include consideration of relevance for the site in question, which will take into account the time required and the cost of the study. The site-specific factors described in Section 2.4 will help determine likely relevance for a particular site. It is expected that a minimum of 3–6 months might be required for planning, executing, reporting and incorporating study results into a risk assessment using well-established approaches. *In vitro* studies following established standard operating procedures (SOPs) would require less time to complete, whereas *in vivo* studies requiring detailed protocol development and stakeholder/peer review would require more time and effort. More time may be needed if new approaches are being developed or extensive consultation with government agencies is necessary.

Obtaining concurrence on the study protocol from interested parties is an important step. Submitting the protocol to government agencies, stakeholders and/or peer reviewers may facilitate the study process and endorsement of the final study results. Obtaining input before conducting the test may prevent resources from being spent on tests that might not be recommended by government agencies. It is also important to determine how the results will be incorporated into the risk assessment and how they will be used to develop clean-up goals.

Timing and costs are important considerations in deciding whether an *in vivo* or *in vitro* study will be conducted. The time and financial resources necessary for the bioavailability and/or bioaccessibility tests can also vary depending on the availability of standardized SOPs for testing the chemicals of interest. *In vitro* methods for assessing the bioaccessibility of lead, arsenic, cadmium, chromium and nickel are relatively well established (Brattin *et al.* 2013; Denys *et al.* 2012; Drexler and Brattin 2007; ENVIRON 2011; Juhasz *et al.* 2009a and 2009b; Kelley *et al.* 2002; Koch *et al.* 2013; Meunier *et al.* 2010b; Ng *et al.* 2010; US EPA 2007c) which would be lower in costs compared to *in vivo* studies. The validity of *in vitro* methods for organic chemicals, on the other hand, has generally not been well established (Rostami and Juhasz 2011). Using established protocols, *in vitro* analysis costs per sample would be expected to be consistent with those for other specialized chemical analyses. In addition to costs for chemical analyses of soil samples, costs associated with the other elements of study, such as planning, sample collection and study reporting, would need to be considered. For instance, additional costs would be associated with any mineralogical analyses performed.

In vivo studies cost considerably more than *in vitro* studies. Estimated costs should be obtained for developing the study protocol, running the study and preparing a final report. Additional costs (primarily associated with achieving adequate stakeholder consultation and peer review) would be incurred if the study design is modified from, or is different from, those previously used. It may also be advisable to conduct a pilot study to demonstrate that the proposed model is appropriate.

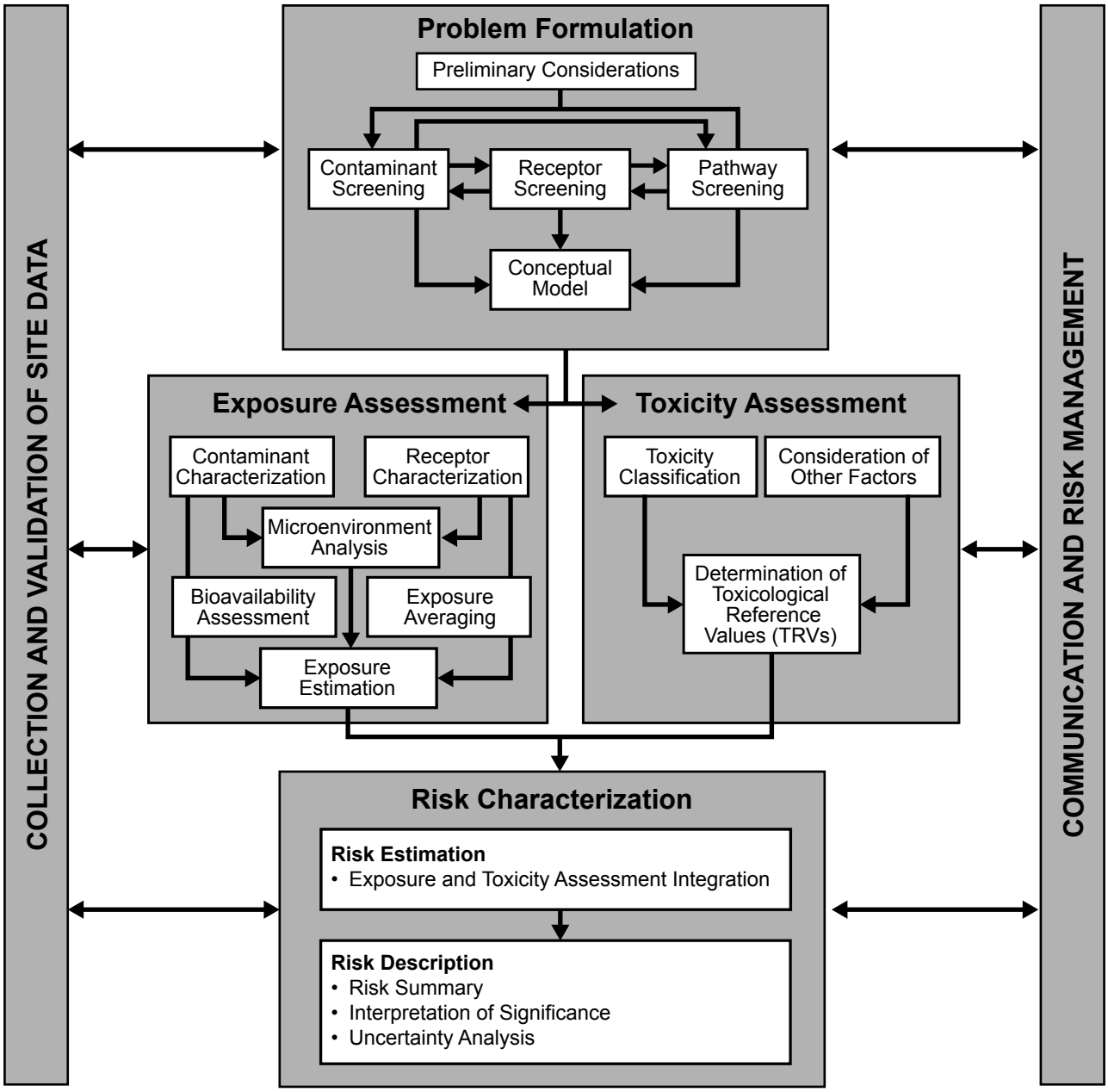
Selection of an appropriate laboratory with a quality assurance project plan in place (which follows or meets international or national quality assurance/quality control [QA/QC] standards) is important in conducting a bioavailability study that will produce reliable, defensible results.

2.6 Decision-making framework for developing and evaluating bioavailability adjustments

HC DQRA guidance (HC 2010a) provides the framework for decisions and steps in an HHRA. A brief discussion is provided below which relates to the steps to decisions made related to bioavailaibilty and/or bioaccessibility testing. Before a risk assessment is conducted, substances in soil are screened against the Canadian Council of Ministers of the Environment (CCME) human health soil quality guidelines (CCME 1999) or other appropriate human health screening levels as part of the environmental site investigation(s). If soil concentrations are below appropriate screening levels, there is no need to proceed with an HHRA. If screening levels are exceeded, the site owner may decide to proceed to remediation to numerical guideline values or to use a risk-based approach to assess, remediate and/or manage the site. Bioavailabilty adjustments may be used in an HHRA to support risk-based approach. The HHRA framework used in a risk-based approach is illustrated in Figure 2–2.

HHRAs are completed using an iterative approach. The incorporation of site-specific RBA adjustments may be considered in the exposure assessment of a DQRA (Figure 2–2). The decision to incorporate bioavailability in a risk assessment is typically made after determining whether target risk levels would be exceeded in an HHRA with no consideration of bioavailability adjustments. If target levels are not exceeded, there is no benefit to incorporating bioavailability in the HHRA.

Figure 2–2: Human health risk assessment framework*



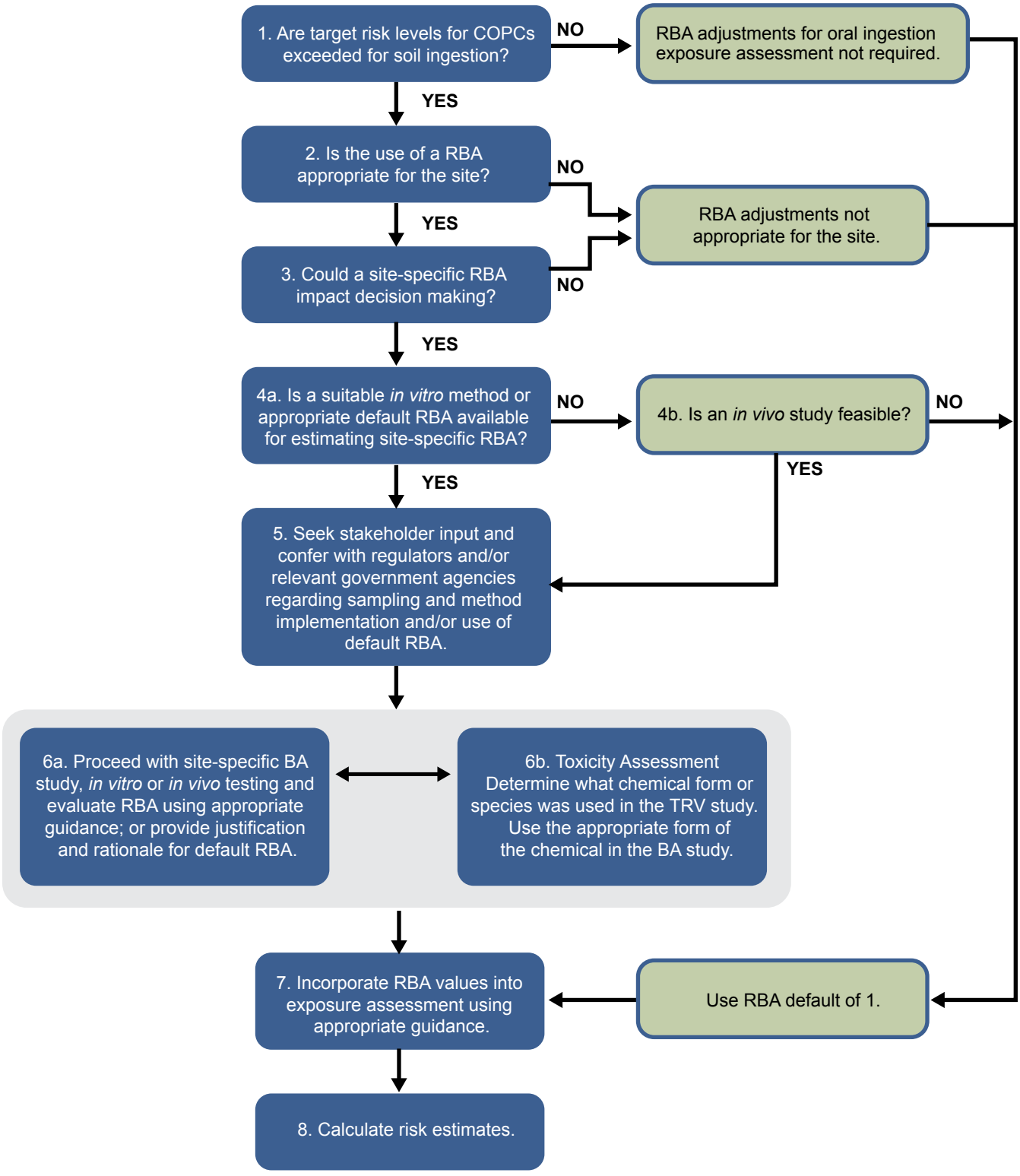
*From HC (2010a).

When considering whether to derive site-specific RBA information for a site, the premise is that *in vitro* tests will be preferentially selected over *in vivo* studies if suitable methods are available for chemicals of potential concern (COPCs), since bioaccessibility tests are more cost-effective than bioavailability tests.

Figure 2–3 lists important considerations for assessing the feasibility of *in vivo* studies if no suitable *in vitro* method is available. The risk assessment process is an iterative one, and more data may need to be collected if or when data gaps are discovered. The conceptual site model in the problem formulation step should be revisited as the risk assessment proceeds, to ensure that the conceptual site model remains valid (i.e., verify that COPCs are still relevant and that operable exposure pathways still exist). The final component of the risk assessment is risk characterization, in which exposure and toxicity assessments are integrated to provide an evaluation of the estimated risks resulting from exposure to chemicals from the contaminated site.

Figure 2–3 also highlights key considerations when determining the feasibility of incorporating bioavailability in the exposure assessment phase of a risk assessment. The key considerations for deriving site-specifc bioavailability (BA) adjustments from *in vivo* (bioavailability) or *in vitro* (bioaccessibility) studies are discussed in the following pages (pg. 16–20). More information on bioaccessibility/bioavailability testing can be found in Appendices A to C.

Figure 2–3: Considerations for the incorporation of bioavailability/bioaccessibility in a DQRA*



*Adapted from Decision Framework developed by Bioaccessibility Research Canada (BARC) in 2014, ENVIRON (2011),US EPA (2007a) and HC (2010a).
BA = Bioavailability.

Screening Human Health Risk Assessment

1. Develop a conceptual site model and evaluate the potential exposure routes for all receptors and COPCs identified for the site.

- Are the concentrations of COPCs in soil identified in the site investigation representative of likely exposure at the site?
  - » Evaluate whether soil samples were collected from representative areas of the site where people may contact soils and whether COPC concentrations in the soil samples are representative of these areas.
- Identify humans who may frequent the site and potential exposure routes through which they may come into contact with COPCs.
- Does an HHRA indicate potential risk from soil ingestion associated with a COPC?
- Are target risk levels for COPCs exceeded for soil ingestion? If the oral exposure route is not a major contributor to total risk, *oral bioavailability studies may not be useful.*

Considerations for Bioavailability Adjustments

2. Is the use of an RBA evaluation appropriate for the site?

- Are there acceptable methods available to estimate site-specific RBA for the COPCs?
  - » Has an applicable regulatory or government agency developed a default RBA value for the COPC? If no methods are identified and no default RBA value has been developed for the COPC, assume a default RBA of 1.
- Are the levels of exposure of the COPC from soil ingestion in the range in which bioavailability adjustments may be useful?
  - » If the use of RBA would still result in unacceptably high exposure and risk estimates and inclusion of RBA adjustments will not result in any changes in risk management at the site, there is no benefit to conducting an RBA study.

3. Could RBA adjustment affect decision-making?

- Do available *in vitro* and/or *in vivo* studies in the literature suggest that the COPCs from site soils will be substantially less bioavailable than what is assumed in the critical toxicity study?
  - » Do factors such as concentration of COPCs, RBA value and contaminant source indicate that bioavailability adjustments may be of value?
- Do site-specific characteristics, chemical speciation and mineralogy of site soils suggest that bioavailability adjustments may be of value?
  - » A comparison of site soil characteristics and chemical properties with those of studies conducted at other sites may provide an indication of the utility of conducting a site-specific bioavailability study.
- What are the costs versus benefits of obtaining an RBA value?
  - » Determine the costs of obtaining an RBA value, the range of values likely obtained and the potential benefits of this range of values in a site-specific risk assessment. If the benefits outweigh the costs, then use of an RBA adjustment may be appropriate for the site.
- Is there time available to allow for designing, undertaking and reporting on a site-specific bioavailability study before decisions need to be made?

4a. Is a suitable *in vitro* method available for estimating site-specific RBA?

- Is the *in vitro* protocol acceptable? Is the study protocol consistent with methods reported in the literature for the chemical of interest?
- Was the protocol validated?
  - » Use of validated methods is preferred, and information about validation will increase confidence in study findings, especially for *in vitro* studies. The "burden of proof" requirements for methods that have not been validated can be expected to be high. HC should be consulted if a protocol that has not been validated is being considered for use.
- Refer to Section 4 for a review of *in vitro* bioavailability approaches, Appendix A for key considerations for *in vitro* testing and Appendix D for information on commonly used approaches at the time of writing.
- Is there a default RBA value (less than 1) for the COPC accepted by an applicable regulatory or government agency?
  - » Is it appropriate for the site? Site-specific testing is always preferred because the bioavailability of substances in soils is expected to be variable, depending on soil matrix and chemical speciation. Site-specific bioavailability or bioaccessibility tests are suggested to refine risk estimates at the site. Use of a default RBA value less than 1, or data from the literature should include a rationale based on soil matrix and chemical speciation to show the relevance of the bioavailability adjustment factor chosen for the site. Typically, site-specific studies are required, but in some cases arguments may be presented to support use of values from the literature or from other similar sites. The use of literature-based bioavailability assessments is discussed further in Section 3.

4b. If no *in vitro* test method or default RBA value is available for the COPC, is an *in vivo* study feasible?

- Is the *in vivo* protocol acceptable?
  - » Is the study protocol consistent with methods reported in the literature for the chemical of interest?
- Is the approach being used to determine RBA clearly explained?
  - » RBA adjustments may be based on either comparison of ABA or on direct comparison of measures such as tissue concentrations (e.g., blood, liver or urine). Practically, as long as the dosing regimens used in the reference studies are similar, the RBA value can be calculated by direct comparison of measured levels in tissue (e.g., ensuring that a positive control is used, with chemical species and dosing medium similar to that used in the TRV study). For example, for chemicals that are absorbed in the body and then largely excreted in the urine, the urinary excretion fractions may be used to estimate RBA.
- Is the animal model relevant for humans and the exposure scenario?
  - » The study protocol should include a justification of the animal model selected with a discussion of anatomical and physiological characteristics of the selected model compared with those of humans, with consideration of the specific age groups or most sensitive age groups that may be present at the site and a rationale that shows why the proposed animal model is relevant to site exposures.
- For *in vivo* studies, are the excreta/tissues appropriate for the chemical of interest, and is the sample collection period appropriate?
  - » On the basis of available toxicokinetic studies, assess the absorption, distribution and excretion for the chemical of interest to determine whether the appropriate excreta/tissues have been selected to measure absorption.

- Are the numbers of replicates/dose groups included in the study design adequate for calculation of the RBA value?
  - » An adequate number of replicates and dose groups to represent various doses and adequate information for calculation of the RBA value are needed (see Oomen *et al*. 2002; ENVIRON 2011 for a review of study protocols).

  Refer to Section 4.6 and Appendix D for a review of in vivo bioavailability approaches and for information on commonly used approaches. See Appendix B for key considerations for an in vivo test.

- If a suitable *in vivo* procedure is feasible, proceed to Step 5; if no suitable *in vivo* study is feasible, assume that the RBA value is 1 and proceed with Step 7.

  See Appendix A for key considerations for an *in vitro* test and Appendix C for a list of minimum criteria that will need to be met for *in vivo* validation of an *in vitro* method.

In summary, decisions to proceed with bioavailability assessments are made using an iterative approach to an HHRA after it has been determined that RBA adjustments may help to refine exposure estimates and thereby provide more realistic indicators of potential risk. Bioavailability adjustments are typically considered in detailed HHRAs where target risk levels may be exceeded without bioavailability adjustments. Professional judgment is required as well as consideration of site-specific factors.

5. Seek stakeholder input and confer with applicable government agencies regarding sampling and method implementation.

- Is there stakeholder acceptance of the bioavailability study methods, data analysis and data application?
  - » Community, regulatory and government agency agreement on the approach (i.e., design, data analysis and data application) is important for successful risk assessments and remediation.
- Are the investigators familiar with the design of bioavailability studies?
  - » Assess the experience level of the investigator; determine whether a peer review of the study protocol by an experienced investigator is necessary. Determine whether there is adequate justification for any deviation from published study protocols.
- Is the laboratory familiar with the proposed study protocol?
  - » Can references be provided to verify the laboratory experience in conducting bioavailability/bioaccessibility studies?
- For *in vivo* studies, is the laboratory accredited by recognized authorities*?*
  - » For laboratory animal studies, the principal accreditation organization in Canada is the Canadian Council on Animal Care in Science (CCAC), an independent body created to oversee the ethical use of animals in science in Canada.
- Are the QA/QC procedures acceptable and, if applicable, are good laboratory practices (GLP) rules followed*?*
  - » For *in vivo* studies commercial laboratories may follow GLP, while university research laboratories may need to develop a project-specific quality assurance project plan. If possible, request that applicable government agencies review proposed laboratory procedures in advance.

6a. Proceed with RBA study and evaluate RBA using appropriate guidance.

- Obtain soil samples from various areas of environmental concern that are frequented by people, and ensure that a sufficient number of samples are obtained from each of these areas. Confirm that the samples collected are sufficient for both bioaccessibility/bioavailability testing and chemical analyses.
- Conduct bioaccessibility/bioavailability testing and chemical analyses on soil samples that have been dried and sieved to <150 µm particle size fractions (or otherwise justified particle size).
  - » Ensure that the appropriate form of the substance is used in the testing (see 6b below). Calculate the bioaccessibility/bioavailability results according to the standard operating procedure of the test.
- Evaluate the RBA study.
  - » Review the laboratory report to ensure that the study was done according to accepted test methods and SOPs. Confirm that all QA/QC requirements were met. Review the bioaccessibility and RBA results and calculations. Review the RBA study to ensure that the methods and data interpretation are consistent with available guidance and literature.
- How do the results compare with those of similar studies reported in the literature?
  - » Note any differences in study design and results compared with studies reported in the literature. Do variable site characteristics (physical or chemical characteristics of soil, chemical species) explain variability between study results?
- Have uncertainties in the study design and application of the RBA been adequately identified?
  - » The uncertainties associated with the study design and data analysis should be included in the risk assessment and their impact on the risk estimates addressed.

6b. Toxicity assessment

- Determine the appropriate form of the substance (based on the TRV study) that should be used in the *in vitro* or *in vivo* studies.
- How does the species or form of the COPC differ from that in the critical toxicity study?
  - » If the chemical species used in the toxicity study is known to be the same or more soluble than the form(s) found on site, information on bioavailability may be useful to assess the difference in amount of chemical absorbed from site soil/sediment.

7. Incorporate RBA values in the exposure assessment using appropriate guidance.

- Calculate the RBA value from bioaccessibility or bioavailability testing and incorporate it into the exposure estimates.

8. Calculate risk estimates and complete risk characterization.

- Guidance on incorporation of RBA into exposure estimates is provided in Section 5 of this document.
  - » The calculated RBA value is used to adjust the estimated exposure from soil ingestion in place of the default RBA (or RAF_{oral}) of 1.

3.0 LITERATURE-BASED BIOAVAILABILITY ASSESSMENTS

Generally, use of a literature-based RBA is considered acceptable only when there is a sufficient body of high-quality, site-specific data to identify a reasonable upper-bound value, as may be the case for lead or arsenic in soil. Such a default value should be expected to be equal to or higher than the site-specific value in at least 95% of tested soil samples. Derivation of a site-specific RBA value is generally preferred over a default or literature-based value because RBA has been shown to vary substantially across sites. As discussed in Section 2.4.1, site-specific factors may cause variability of RBA even when contaminant sources are similar at different sites. Soil types and co-contaminants may also vary, affecting the interactions of chemicals with soil constituents. Application of a literature-based value is most likely to be applicable when the basis for variation in RBA among sites is well understood.

3.1 Minimum requirements for use of RBA from literature

At a minimum, the following requirements are suggested if an RBA value from a literature review is being considered for use in a contaminated site risk assessment:

The chemical form and exposure medium for the critical toxicity study(ies) used to establish the TRV must be established. For both *in vitro* and *in vivo* studies, results must be included for a reference material or reference compound (e.g., NIST standard reference material) comparable to the exposure medium and chemical form used in the TRV study. The RBA value will be based on comparison of the reference results with the exposure medium or chemical form being tested.

Multiple studies must be available specific to the COPC and site exposure medium (e.g., soil).

- The exposure medium (e.g., soil) should be characterized in the literature studies as well as at the site, and the literature studies must match the site characteristics. For example, if soil is the exposure medium, the literature studies should include the same soil type with similar properties (e.g., organic carbon content, clay content). Samples tested should have been sieved to <250 µm or finer fraction (preferably the soil and literature fraction should match) and the chemical concentration determined in the sieved samples. The origin of the contamination in the tested samples should be known and should be relevant to the site being evaluated.
- Studies used to characterize bioavailability should be of high quality, follow GLP or equivalent standards and be published either in peer-reviewed literature or by an applicable government agency (e.g., HC, US EPA).

The selected bioavailability estimate for the site exposure medium should represent the high end of values from the literature. Where data are sufficient to relate bioavailability to measured soil properties, they should reflect the high end of values for soils similar to the site soils (i.e*., use a conservative approach).*

3.2 Use of regression or correlation equations

Regression or correlation equations have been developed for two purposes:

- To correlate bioaccessibility (*in vitro)* results with RBA values derived from *in vivo* studies (*in vivo-in vitro* [IVIV] comparisons), or
- To predict or correlate RBA or bioaccessibility with measureable site characteristics, such as soil properties.

Regression equations based on IVIV comparisons have been used to predict lead and arsenic RBA values from bioaccessibility data (US EPA 2007c; Brattin *et al.* 2013). The regression equation for lead is discussed in Section 4.4.1 and 5. Further information on IVIV validation can be found in Appendix C.

If an equation has been endorsed by an applicable government agency (e.g., US EPA), it can be considered for a site with the following provisions:

- The underlying studies used to develop the equation encompass the conditions found at the site. For example, they must reflect the same metal species/form, and site characteristics such as soil properties must be within the range included in the correlation.
- Any other limitations or requirements, either implicit or explicit, in the use of the equation are met. For example, if an equation is developed for mining-associated metals contamination, it should be used only for mining contamination unless a technical justification and sufficient rationale for peer review can be made for extrapolating it to other situations. An equation derived from mining-related contaminated soils may not be relevant to assessing the same substance from paint contamination, given differences in speciation, etc.
- The characteristics of soils from the site that are used to derive the equation must be adequately described.
- Where soil properties are variable across the site, values producing a conservative (high) estimate of bioavailability or bioaccessibility should be used. If only a small number of values are available from the site, then a conservative value should be used, such as a maximum value. If the site is thoroughly characterized, then a more representative value may be justifiable with rationale for the choice of the statistic used.

Examples of bioaccessibility predictions based on soil properties include predicting arsenic bioaccessibility from soil pH and/or iron content (Subacz *et al.* 2007; Yang *et al.* 2002 and 2005; Cutler *et al.* 2014; Whitacre *et al.* 2013) or chromium and total organic carbon (TOC) (Jardine *et al.* 2013). Most of these studies involved investigation of the influence on bioaccessibility of weathering or the addition of soil amendments under limited experimental conditions. As such, they are unlikely to yield broadly generalizable regression equations.

3.3 Chemical speciation

A literature-based bioavailability value may be appropriate if the chemical species present at a site has a relatively low bioavailability. All the general requirements for determining whether bioavailability adjustments should be considered in a HHRA apply when determining whether a literature-based bioavailability value can be used. In addition, the literature must clearly show that bioavailability varies by chemical species, even when other factors are considered, and the site data must show that the lower bioavailability species is the dominant form of chemical species across the site. The influence of more soluble forms must be accounted for in a conservative manner if they are present in more than trace amounts. As shown by Meunier *et al.* (2010a), minor amounts of a soluble arsenic phase can affect the bioaccessibility of the sample.

4.0 CONDUCTING BIOAVAILABILITY STUDIES

Bioavailability assessments of chemicals at a site should include a review of current practice/literature. As described in Section 2, when incorporated into complex risk assessments RBA data are used to correct for differences in the bioavailability of a chemical in the site exposure medium being assessed relative to its bioavailability in the toxicity studies supporting the TRV. These differences may arise because of differences in the mineral forms of metals (i.e., carbonates, oxides, sulfides) or congener mixture (for organics) and the exposure media used in the toxicity studies supporting the TRV in comparison to what is found at test sites. When oral TRVs are based on intake rather than absorbed dose, RBA adjustments may be applied directly to make the intake estimates comparable with the TRV. Assessing TRV study(ies) in planning for use of bioavailability adjustments in risk assessment is discussed in Section 2.2 and 2.4.

A variety of methods have been used to estimate the RBA of chemicals in soil, including both *in vivo* tests with laboratory animals and *in vitro* extraction tests that are intended to represent chemical dissolution from soils in the GI tract. A variety of *in vivo* approaches are needed because of differences in the toxicokinetic behaviour of the chemicals being evaluated. Most of these approaches are modifications of methods used routinely to measure the bioavailability of chemicals dissolved in water, mixed with diet or, in the case of non-polar organics, mixed with corn oil.

The *in vivo* approaches are generally accepted so long as they are proven to provide reliable measures of RBA, and are properly designed and conducted. Tests should be relevant to human physiology. Critical peer review of *in vivo* approaches is recommended before the tests are conducted to obtain technical input, so that the results will be applicable and relevant to the risk assessment.

4.1 Characterization of environmental media

Characterization of sieved site soils and soil-like media used in bioavailability tests are helpful to interpret site-specific studies. Physical and chemical soil parameters that may be useful include pH, moisture content, TOC, CEC, particle size distribution and available anions (for studies of cationic metals and nonpolar organics). However, insight into RBA variation may be gained by a review of soil parameters that are routinely analyzed in the unsieved soils as part of the site investigation.

Speciation to determine oxidation states (e.g., trivalent rather than hexavalent chromium or nickel oxidation states) or chemical forms (e.g., elemental vs. inorganic vs. methylated forms) is necessary for metals with multiple TRVs, unless there is strong evidence indicating the chemical form that is present. Detailed mineralogical analysis can provide a line of evidence to support RBA estimates, but it is difficult to develop defensible estimates solely from mineralogy. Similarly, analysis for elements that are important in soil alteration reactions, such as iron, manganese, calcium and phosphorous, may provide another line of evidence to explain RBA results or variation.

As discussed in Section 2, in addition to soils, other exposure media that may be of interest at a contaminated site include house dust, waste materials such as mine tailings and slag, and sediment. These media may vary from soils in characteristics such as particle size distribution, TOC and pH. Despite these variations, the same methods used to evaluate soils may be used to assess the RBA of other related exposure media with several caveats.

- House dust may contain large fractions of household debris and organic material that may complicate sieving, and therefore hair and large debris particles should be removed (as much as possible) from the dust sample prior to sieving (Rasmussen *et al.* 2013).
- House dust is typically less dense than soils because of its higher TOC (28% in dust vs. 5% in soil; Rasmussen *et al.* 2008). This high TOC, in addition to its increased bulk, may make it physically difficult to administer a sufficient dose to animals in an *in vivo* study. Some recent studies have evaluated *in vitro* methods for assessing bioaccessibility from house dust, including Dodd *et al.* (2013), who have commented on the marked heterogeneity of metal sources in house dust and the associated variety of mechanisms governing dissolution in extraction tests, as well as the need to use higher fluid-to-solid ratios when metal concentrations are high.
- Metal compounds in house dust arise from indoor sources in addition to outdoor sources (Walker *et al.* 2011; Beauchemin *et al.* 2011) and are transformed during ageing under different indoor weathering conditions (Rasmussen *et al.* 2014; Beauchemin *et al.* 2014); thus metal bioaccessibility in house dust can differ significantly from (and is often higher than) metal bioaccessibility in adjacent soil (Rasmussen 2004; Rasmussen *et al.* 2008).
- Studies on bioavailability in sediments have primarily focused on measuring the bioavailability of metals to ecological receptors. Techniques developed for ecological receptors (e.g., sequential extraction techniques and measuring of acid volatile sulfides) may not be relevant for HHRA. Instead, for the latter, standard methods for soils may be applied to predict oral RBA for sediments.

When characterizing site soils or other environmental media, the same analytical method should be used to measure total concentrations of chemicals in bioaccessibility test extracts and corresponding soil samples in the site investigation, so that chemical concentrations in the sieved soils used in the bioaccessibility tests and bulk soils can be compared.

4.2 Sample collection and processing

Some sample preparation requirements apply generally to all methods of assessing RBA:

- Weathered site soils should be used for the testing since weathered soils provide the best representation of the behaviour of aged compounds found at a contaminated site.
- Soil samples should be sieved to remove particles that are too large to readily adhere to skin and be subsequently ingested, unless the main concern is absorbed dose from pica (e.g., deliberate soil ingestion). Typically, a 60-mesh sieve (250 µm) has been used to remove larger particles (Kelley *et al.* 2002). Fractions less than 250 µm have been shown to adhere to skin and are thought to be more representative of the soil fraction that is ingested through hand-to-mouth contact than bulk soil. In the U.S., the 250 µm size fraction has become the standard for conducting *in vivo* studies of relative arsenic and lead bioavailability from soil (see US EPA 2007c; Freeman *et al.* 1995; Maddaloni *et al.* 1998). Most soil bioavailability studies conducted to date have used this size fraction (Kissel *et al.* 1996; Meunier *et al.* 2011). There is evidence that smaller particles are the most critical (Siciliano *et al.* 2009): a review of the variation of chemical concentrations across particle sizes by Bright *et al.* (2006) suggests that increasing attention should be paid to even smaller size fractions. Richardson *et al.* (2006) propose some additional particle size considerations for *in vitro* studies, but it should be noted that these suggestions may not yield sufficient sample sizes for *in vivo* studies. Work by Meunier *et al.* (2011) provides support for continued use of fractions less than 250 µm, rather than smaller fractions. In 2016, the US EPA recommended that the particle size used to represent the dominant particle size fraction that is likely to be adhered to hands and incidentally ingested be revised to <150 µm (US EPA 2016). Subsequently, the *in vitro* bioaccessibility assay for lead and arsenic published by the US EPA was revised to specify the use of <150 µm particle size fraction as part of the standard operating procedure for this test (US EPA 2017a).The chemical concentrations in both the sieved soil fractions used for bioavailability studies and bulk soils from the site assessment should be analyzed.

- Soil samples should not be ground prior to use in the study (i.e., they should be handled using protocols similar to those used during laboratory analysis), and an appropriate amount of soil should be collected to allow for the bioaccessibility/bioavailability testing and chemical analyses required.

Soil collection and characterization should be designed according to the expected nature of exposures to the soil. In a residential setting, people will generally have contact primarily with surface soils. Specific activities such as gardening may lead to exposure to deeper soils as well; some regulatory jurisdictions require consideration of deeper soils to protect against these activities or future site changes. Selection of a sample depth should be identified in the risk assessment report, with supporting rationale.

In general, if there is surficial contamination at a site, at a minimum, the surficial soils should be collected to represent the material to which most human exposure is anticipated to occur. The number of samples collected for testing will depend on a variety of factors. Samples should be representative of the different soil or waste material types believed to be present at the site. For mineralogical and *in vitro* studies at small sites, 5 to 10 soil samples may be adequate for characterization of mineralogy and bioaccessibility in a given exposure area. The number of samples required may vary depending on site conditions and results (e.g., if there is large variability in analytical results). However, for *in vivo* studies, evaluation of fewer soil samples is more realistic because of the greater cost of testing and analysis. If a site is large and heterogeneous, it may be desirable to conduct an *in vivo* study using a few soil samples from the areas where exposure is most likely and to couple those with additional *in vitro* studies of other areas. If soils are being collected for *in vitro* bioaccessibility or *in vivo* bioavailability testing, HC may be contacted for site-specific advice.

4.3 Specific considerations for selected metals

For inorganic substances, the forms present in soil may be characterized by methods that identify both mineral forms present and the morphology of the metal–soil particle associations. A survey of these methods is provided by the NRC (2003). Electron microprobe analyses have been frequently used to characterize metal species and the manner in which they are associated with soil particles, and SOPs have been developed (Kelley *et al.* 2002; US DoD 2003). XAS methods are playing an increasingly valuable role in characterizing the interactions of inorganics with soil particles (Meunier *et al.* 2011; NRC 2003; Scheckel *et al.* 2009; Walker *et al.* 2011; MacLean *et al.* 2011; Beauchemin *et al.* 2014; Rasmussen *et al.* 2011 and 2014).

If speciation data are desired, a generalized Microprobe SOP (ENVIRON 2011) can be considered in addition to the methods listed above to evaluate the forms of various metals such as arsenic, cadmium, lead, nickel and non-elemental inorganic forms of mercury. Other techniques are available to obtain speciation data for metals, such as sequential extraction procedures.

The primary chemical forms and oxidation states of specific metals in soil are briefly described below. Additional discussion of mineralogy for a variety of metals can be found in Kelley *et al.* (2002) and in individual research reports. The US EPA (2007c) provides a summary for lead.

4.3.1 Arsenic

Trivalent (III) and pentavalent (V) inorganic arsenic compounds predominate in soils, occurring as discrete mineral phases of widely varying solubility and as ionic forms that may be sorbed to soil constituents. All inorganic arsenic compounds induce toxic effects by the same mechanism regardless of their valence state, so all forms of inorganic arsenic may be considered together when assessing bioavailability. Speciation studies that determine the oxidation state of arsenic present at a site are not a critical requirement for a bioavailability study, but mineralogical analysis can provide a useful line of evidence to support data interpretation.

4.3.2 Chromium

Chromium occurs in soil in the trivalent (III) and hexavalent (VI) oxidation states with very different TRVs. Trivalent chromium hydroxide, which has low solubility, is the most prevalent form of natural chromium in soils, hexavalent chromium mostly being a result of anthropogenic sources (Kelley *et al.* 2002). Characterization of chromium oxidation states is recommended if it is necessary to establish that hexavalent chromium is not present. Default risk-based clean-up levels based on ingestion of soils containing Cr (III) are higher than those for Cr (VI) and can only be applied if Cr (VI) is not present. If a mixture is present, Cr (VI) is expected to be more bioaccessible than Cr (III) (Jardine *et al.* 2013). Derivation of an RBA value will be complicated in such situations.

4.3.3 Mercury

Mercury is usually present in soils as inorganic mercury, either as elemental mercury (Hg^0) or as one of two non-elemental ionic forms: mercurous (Hg^{+1}) or mercuric (Hg^{+2}). Elemental mercury has different toxic endpoints from the other inorganic compounds of mercury, and therefore a speciation study may be useful as an alternative to using the most conservative TRV. Organic mercury compounds usually are not present in significant quantities in soil or dust in the absence of a specific manufacturing process that generated such compounds. When evaluating sediments, however, methylmercury is likely to be present and should be considered.

Sequential extraction procedures have been developed to quantitatively evaluate forms of mercury in soil. Welfringer and Zagury (2009) present a comprehensive approach to assessing both mercury speciation and bioaccessibility. Sequential extraction methods are advantageous because they are relatively easy to perform compared with other highly specialized analytical techniques. The method is useful for distinguishing elemental mercury from various other inorganic forms (i.e., mercuric sulfide, carbonates, hydroxides, oxides and chlorides) as well as quantifying the amount of organic mercury in the soil. This procedure is recommended prior to designing and conducting *in vitro* or *in vivo* bioavailability studies for mercury.

4.3.4 Lead

Inorganic lead occurs in numerous mineral forms; however, all of the inorganic forms that occur in soil have the same toxic endpoint (US EPA 2007c). Therefore, all forms may be considered together when assessing bioavailability. Nevertheless, because the lead phases vary widely in their solubility and bioaccessibility, detailed mineralogical analysis can provide a useful line of evidence to support RBA data interpretation.

4.3.5 Cadmium

Cadmium occurs in soil in discrete mineral phases that range in solubility from sparingly soluble (e.g., sulfides) to highly soluble (e.g., carbonates) and in ionic forms sorbed to soil constituents. However, all inorganic forms of cadmium found in soils induce chronic toxic effects by the same mechanism after ingestion. Consequently, speciation studies are not needed to distinguish the specific cadmium compounds present at a site, and all forms may be considered together when assessing bioavailability. Although cadmium phases vary widely in their solubility, soil cadmium bioaccessibility is often high (Oomen *et al*. 2002; Schoof and Freeman 1995), and detailed mineralogical analysis may not provide a useful line of evidence to support RBA data interpretation.

4.3.6 Nickel

Nickel occurs in soil sorbed to soil constituents and as discrete mineral phases that range in solubility from poorly soluble (e.g., sulfides and sulfates) to moderately soluble (e.g., carbonates). HC (2010b) has established separate oral TRVs for different forms of nickel. In the absence of speciation information, the most conservative of these TRVs is applied in an HHRA. Because the nickel forms vary in toxicity, as well as solubility and bioaccessibility, detailed mineralogical analysis is especially useful in assessing soil nickel RBA data.

4.4 Applying in vitro approaches

The most commonly used *in vitro* tests (as described in Appendix D) are laboratory extraction tests that simulate the dissolution of chemicals in the GI tract. They can be single compartmental (i.e., stomach) or multi-compartmental (i.e., GI) models, and they are used to estimate bioaccessibility (the fraction of dissolved soluble substances from the environmental media [i.e., soil] that is available for absorption in the stomach or GI tract [see Section 2.1]). For *in vitro* studies, the test method should include a reference material (e.g., NIST standard reference material) that is the same as the chemical form used in the TRV study and/or a comparable soluble form of the chemical. Use of a reference material that is the same as that used in the TRV study is favoured.

The bioaccessible fraction of the soil measured from the *in vitro* tests is also called the *in vitro* bioaccessibility (IVBA) assay result and is calculated using equation 4.1.

It should be noted that the relationship between *in vitro* and *in vivo* data may not be 1:1, and thus whenever sufficient data are available IVBA values may require adjusting before being used as a surrogate for *in vivo* RBA, as in the case of lead discussed below.

In the absence of a robust database of *in vitro* and *in vivo* data for the same samples, it has typically been assumed that bioaccessibility determined as dissolution of test material vs. dissolution of the reference chemical may be used directly as an RBA value to adjust intake estimates. The assumption is that the test will provide an estimate of the relative amount of the chemical available for absorption in the GI tract from the test material relative to a soluble form of the chemical.

For *in vitro* tests, the ratio of the concentration of the chemical in the extraction fluid to the concentration in soil may have a large influence on the results of the bioaccessibility tests, for at least some contaminants. For compounds that are sparingly soluble, a low fluid-to-mass ratio may result in the saturation limit being reached, which is not an accurate measure of bioaccessibility (i.e., it would underestimate bioaccessibility). Low extract concentrations with low soil concentrations will result in high bioaccessibility values. Unless the assay has been validated in the literature for a particular contaminant, it is important that several ratios of simulated gastric fluid and soil mass are tested to make sure that measured bioaccessibility is relatively independent of the fluid-to-mass ratio, or that an appropriate, conservative ratio is selected (Richardson *et al.* 2006).

Use of bioavailability adjustments in risk assessment should be supported by a detailed, scientifically based rationale addressing the general bioavailability considerations outlined in Section 3, chemical-specific considerations and any additional relevant information. A particularly important consideration is whether the *in vitro* bioaccessibility method used can be considered validated *in vivo* (Step 4a in Section 2.6; see Appendix C for minimum criteria for valid *in vitro-in vivo* comparisons); if it cannot, then reliable data may not be obtained for the site. The onus is on risk assessors to provide sufficient rationale to support bioavailability adjustments for chemicals in a risk assessment. Note that appropriate QA/QC analyses should be completed before applying IVBA results for use in an HHRA.

4.4.1 Lead and Arsenic

For lead, the correlation between *in vivo* and *in vitro* data is often observed to be strong, and certain *in vitro* methods are considered to be "validated" against *in vivo* data. The use of the US EPA (2007c) regression equation (Equation. 4.2 below) is a reasonable and scientifically defensible approach for lead. The equation was developed from the regression of *in vivo* RBA data (from studies in swine) on *in vitro* bioaccessibility data for 19 different mine-impacted soils using the University of Colorado Relative Bioaccessibility Leaching Procedure (RBALP), detailed in Drexler and Brattin (2007). The RBALP is also known as the Solubility/Bioavailability Research Consortium (SBRC) Procedure for Stomach Phase Extraction (SBRC-G) and is described in US EPA (2012a) as the IVBA assay for lead (see Appendix D). The US EPA (2017a) protocol specifies 100 mL of extraction fluid to 1 g of soil. For both lead and arsenic, equation 4.1 is used to calculate the IVBA result. Equation 4.2 is used to calculate the RBA for lead, while equation 4.3 is used to calculate the RBA for arsenic:

$$\text{(4.1)} \qquad \text{IVBA (\%)} = \frac{\text{Concentration in extraction fluid (mg/L)} \times \text{Volume of fluid (L)} \times 100}{\text{Concentration in soil (mg/kg)} \times \text{Mass of soil (kg)}}$$

$$\text{(4.2)} \qquad \text{RBA}_{\text{lead}} = 0.878(\text{IVBA, unitless fraction}) - 0.028 \qquad (r^2 = 0.92)$$

$$\text{(4.3)} \qquad \text{RBA}_{\text{arsenic}} = 0.79(\text{IVBA}) + 0.03 \qquad (r^2 = 0.87)$$

Note that in equations 4.2 and 4.3, RBA and IVBA are expressed as fractions while in equation 4.1, IVBA is expressed as a percentage. There are some specific limitations associated with the US EPA IVBA regression equation for lead:

- The method is intended to predict RBA in children (i.e., the method was designed to mimic a child's stomach), although the results are often extrapolated to adults.
- The soil lead concentrations used to validate the Drexler and Brattin (2007) IVBA and the IVIV relationship ranged from 1,200 to 14,000 mg/kg, and use of soils outside this range may introduce uncertainty into the analysis.
- Soils used in the validation study were sieved to obtain the size fraction less than 250 µm in diameter. The particle size fraction specified in the US EPA's *in vitro* bioaccessibility standard operating procedure for lead and arsenic has since been revised to specify a particle size of less than 150 µm as this size fraction is considered most relevant to soil-to-skin contact and subsequent ingestion via hand-to-mouth contact (US EPA 2017a). Rationale for the use of other particle sizes other than those specified in validated *in vitro* bioaccessibility test methods should be provided and discussed with Health Canada prior to conducting any *in vitro* bioaccessiblity testing. Soils larger than 250 µm in diameter are not considered relevant to risk assessment and may not yield reliable results using this method.
- This equation was found to apply to a wide range of different soil types and forms of lead from a variety of different sites; however, the IVBA was validated using mostly soils from mining and milling sites. If unusual or untested lead phase soils are to be used with this method, some uncertainty may be introduced to the analysis.
- The data were generated using the RBALP (and described in Appendix D). The relationship would not necessarily apply to other *in vitro test methods.*
- The linear regression equation that describes the relationship between the *in vivo* and *in vitro* data is intended to predict a central tendency estimate of RBA in fasted swine, but the actual RBA may be higher or lower than the predicted value.

When used with HC DQRA guidance (HC 2010a) and the decision framework listed in Section 2.6 or US EPA (2007a), the IVBA is expected to yield reliable and more realistic estimates of bioavailability in humans for use in an HHRA, with reduced impacts on time and resources compared with the use of *in vivo* bioavailability measurement methods.

4.4.2 Other Substances

Bioaccessibility/bioavailability adjustments for metals other than lead are associated with more uncertainty and should be discussed with HC.

In the case of arsenic, the current HC TRV (Health Canada 2010a) is based on a relatively soluble form of the chemical. Thus, once an appropriate *in vitro* method is identified with validation from *in vivo* studies, the *in vitro* result can be adjusted using correlation data as required (see Section 3.2). The onus is on the risk assessor to determine whether supplemental guidance on this or other studies is available in this rapidly evolving field of study. In recent years, validation studies for arsenic have been conducted and published by various researchers (Brattin *et al.* 2013; Juhasz *et al.* 2014; Bradham *et al.* 2015). It is up to the risk assessor to determine whether validation studies published in the literature are appropriate and relevant to the site under investigation.

RBA results for chemicals derived from *in vitro* studies adjusted using a regression or correlation equation can then be applied directly to the exposure assessment or risk characterization. RBA (or RAF_{oral}) results can be applied to intake estimates for any chemicals with a TRV regardless of whether the TRV is based on administered dose or absorbed dose.

If a TRV is based on absorbed dose, RBA-adjusted intakes ($Intake_{adjusted}$) will need to be converted to uptake prior to calculating the risk estimate.

In vitro tests for organic chemicals have not been developed as fully as those for metals; however, *in vitro* bioaccessibility tests have been reported for PAHs, PCBs, dioxins/furans and one chlorinated pesticide, lindane (e.g., Ruby 2004; Finley *et al.* 2009; James *et al.* 2011). The *in vitro* test methods for organic chemicals are designed to mimic the human GI system. Whereas it has been assumed that desorption of metals from soil in low pH solution provides a conservative estimate of bioaccessibility in *in vitro* test methods, reaction of organic chemicals with lipids and/or proteins and/or passive diffusion across intestinal epithelial cells are the primary methods used for estimating the bioaccessibility of organic chemicals.

In vitro tests for organic chemicals typically include lipids and proteins meant to represent bile salt micelles, which are hydrophobic lipid balls that are encased in bile salts or include cell lines to mimic the intestinal epithelium. In the GI system, it is thought that organic chemicals partition to lipids, and then the bile salts from the small intestine facilitate absorption across the GI walls or organic chemicals transverse the intestinal epithelium by passive diffusion. A variety of chemicals have been used in extraction tests to mimic the bile salt micelles. Wittsiepe *et al.* (2001) used powdered milk in a study of dioxin/furan bioaccessibility, Oomen *et al.* (2001) and Ruby *et al.* (2002) used oleic acid in a study of PCBs and dioxin/furan bioaccessibility, and Holman (2000) has patented a mixture of oleic acid, monoolein, diolein and lecithin. Use of "bile salts" and lipids greatly increases the bioaccessibility of organic chemicals in soil. Gron (2005) used chicken and potato baby food in a study of PAH bioaccessibility, while James *et al.* (2011) used a C18 lipid membrane to determine whether a lipid sink improved the predictive ability of *in vitro* tests in comparison with *in vivo* tests for PAHs. Cavret *et al.* (2003) investigated *in vitro* transepithelial transport of PAHs using Caco-2 cells. Ruby *et al.* (2016) found that TOC in soils were inversely related to bioaccessibility in existing studies regarding oral bioavailability and bioaccessibility of carcinogenic PAHs but bioaccessiiblity results were highly variable, depending on the *in vitro* bioaccessibilitly test method used and the substrates tested.

In vitro tests have historically not always correlated well with *in vivo* tests for organic chemicals (Rostami and Juhasz 2011), and therefore the technical rationale for applying any *in vitro* test for organic chemicals should be developed before proceeding.

4.5 Establishing the validity of in vitro tests

A test is considered valid for a particular set of conditions if there is sufficient evidence to demonstrate that it will reliably (or at least conservatively) determine bioavailability or bioaccessibility. The *in vitro* test must represent the entire range of conditions for which it will be used and must have been demonstrated to be effective (e.g., by comparison with established *in vivo* methods). Please refer to Section 4.6 and Appendix D for further information on current *in vivo* approaches used.

In the U.S., the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) developed a formal approach to evaluate new or modified toxicological methods (ICCVAM 1997). The Organization for Economic Cooperation and Development has also played a significant role in directing and monitoring the development of new test methods. While the Drexler and Brattin (2007) *in vitro* test for evaluating the bioaccessibility of metals has undergone a formal validation process for lead in soil (Ruby *et al.* 1999; Ruby 2004; US EPA 2009), it is important that validation should not be the only criterion used to judge the acceptability of relative bioavailability data for application in risk assessment (Schoof 2004). Critical scientific review should be a component of accepting the use of data from newly developed methods.

Key considerations for *in vitro* tests and *in vivo* tests are provided in Appendices A and B, respectively, and minimum criteria for a valid *in vivo–in vitro* comparison is provided in Appendix C.

Juhasz *et al.* (2013) discuss considerations and list the criteria that should be considered in the *in vivo–in vitro* validation (i.e., the goodness of fit between *in vivo* and *in vitro* observations) and performance validation, which refers to the assessment of the agreement between the model predictions and an independent set of data that were not used to construct the model.

4.6 Applying in vivo approaches

ENVIRON (2011) provides a review of *in vivo* approaches used to measure RBA. The method used to derive the RBA value may vary with the design of the RBA study. Detailed discussions about the estimation of RBA for lead and arsenic, based on swine and/or mouse studies, are provided in US EPA (2007c; 2010; 2012a; 2012b and 2012c). A summary of current *in vivo* approaches can be found in Appendix D.

4.7 Quality assurance/quality control (QA/QC)

QA consists of those activities needed to ensure that a defined standard of data quality with a stated level of confidence is met. A project's data quality objectives (DQOs) should be defined at the outset of the project to establish acceptable levels of data precision, bias, representativeness, completeness, comparability and detection limits. QA procedures, including the collection of field QC samples and their required frequencies, should be established in order to monitor whether the DQOs are being met.

QC samples are collected and analyzed to evaluate the precision and/or bias of the sampling and analysis process. In general, QC samples would include the following:

- Duplicate/replicate samples to evaluate precision;
- Blank samples to ensure that the results are not caused by cross-contamination or handling problems; and
- Reference samples (e.g., samples with known bioaccessibility/bioavailability) where possible.

US EPA (2012a; 2017a) lists expected mean IVBA results and acceptable ranges for National Institute of Standards and Technology (NIST) standard reference material (SRM) 2710, 2710a, 2711 and 2711a, which are used as control soils for metals.

The British Geological Survey has prepared a UK reference sample that can be used to provide QA for the unified BARGE (Bioaccessibility Research Group of Europe) method (UBM) bioaccessibility and total element determinations (https://www.bgs.ac.uk/barge/reference.html).

Percentage bioaccessibility values exceeding 100% are commonly reported and arise from analytical uncertainty in both the numerator ("bioaccessible metal" in ppm determined in a subsample using the *in vitro* extraction) and denominator ("total metal" in ppm determined on another subsample). Deciding on the best approach for handling bioaccessibility results exceeding 100% requires an understanding of the cause, which is usually related to the heterogeneous distribution of metals and minerals in the soil or dust matrix (Rasmussen *et al.* 2014). Such heterogeneity can result in a bimodal or polymodal bioaccessibility distribution within a single sample. Follow-up replicate analyses of samples yielding >100% can provide valuable information, but in practice the ability to investigate samples displaying >100% bioaccessibility can be limited by lack of adequate sample material and/or resources. In datasets in which a large percentage of values approach the limit of detection, it is recommended to apply the more rigorous quality criterion (limit of quantitation) to minimize the occurrence of such outliers (Rasmussen *et al.* 2014).

The collection and analysis of appropriate QC samples, as part of a QA/QC program, can help ensure that the quality of the data collected is known, and that it meets a project's data quality objectives.

QC results should be reviewed and interpreted on an ongoing basis and the QA procedures modified as necessary. At project completion, an evaluation of the project data quality should be presented in a report.

5.0 INCORPORATING BIOAVAILABILITY ADJUSTMENTS INTO RISK ASSESSMENTS

5.1 Introduction

The application of bioavailability adjustments of a COPC in soils or other environmental media at a contaminated site is used to refine the COPC exposure estimates in the exposure assessment, by comparing the bioavailability of the COPC in the soil (or other environmental media) with its bioavailability of the COPC in the reference (i.e., dosing) medium used in the toxicity assessment. The complete risk assessment should be conducted in accordance with HC's DQRA guidance (Health Canada, 2010a).

5.2 Uncertainty and variability

At most contaminated sites, contaminant sources, natural variation in soil properties, contaminant concentrations and speciation will cause variability in the RBA of substances in soil. In one extreme case the range of reported bioaccessibility values for arsenic in soils from one site varied over 10 fold (Meunier *et al.* 2010a). Four-fold differences in bioavailability for lead in soils have been reported from one study (Graziano *et al.* 2001). Uncertainty is also associated with bioavailability data. Sources of uncertainty are related to the reliability of the *in vitro* and *in vivo* methods used and the representativeness of the samples tested with regard to anticipated human exposures. For example, Koch *et al.* (2013) reported a wide range of bioaccessibility results from laboratories testing the same SRM, using variations of *in vitro* methods. For these reasons, using the mean relative bioavailability or bioaccessibility of contaminants in soil is not typically considered adequately protective of human health. An upper bound estimate of RBA is recommended, based on the range of test results, sample number and test reliability.

Some researchers have been reporting the results of bioaccessibility studies in terms of adjusted soil concentrations (i.e., applying the RBA adjustment to the soil concentration and reporting a reduced soil concentration), rather than reporting the RBA as a percentage or fraction. This format has some usefulness when regulatory or government authorities accept a modified soil concentration as a point of comparison with risk-based screening levels; the format has also proven useful in presenting the results of inter-laboratory comparisons. However, as a general practice its use is discouraged because it does not allow for an understanding of possible variation of bioaccessibility of chemicals in soil. Assessments that incorporate bioaccessibility should clearly state all results and assumptions to enable application of the information.

5.3 General equations

The general equations used in an HHRA that are presented in Section 2 and Section 4.4 are included here, followed by worked examples to show how RBA is incorporated into HHRA.

RBA results can be applied to intake estimates for any chemical with a TRV. Exposure dose adjustment using an RBA value to account for differences between exposure medium and toxicity study dosing medium is calculated as follows:

(2.5) $$\text{Intake}_{\text{adjusted}} = \text{Intake}_{\text{unadjusted}} \times \text{RBA}$$

Where :
$\text{Intake}_{\text{adjusted}}$ = exposure dose adjusted to reflect relative bioavailability (i.e. RBA adjusted Intake) (mg/kg/day)
$\text{Intake}_{\text{unadjusted}}$ = exposure dose without consideration of bioavailability (mg/kg/day)
RBA = relative bioavailability value (unitless)

The RBA-adjusted intake can then be used in hazard and risk equations using the administered-dose tolerable daily intake or slope factor, so that for threshold substances:

(2.6) $$\text{HQ} = \frac{\text{Intake}_{\text{adjusted}}}{\text{TDI}}$$

Where :
HQ = hazard quotient
$\text{Intake}_{\text{adjusted}}$ = exposure dose adjusted to reflect RBA (mg/kg/day)
TDI = tolerable daily intake (mg/kg/day)

and for non-threshold substances:

(2.7) $$\text{ILCR} = \text{Intake}_{\text{adjusted}} \times \text{CSF}_{\text{oral}}$$

Where :
ILCR = incremental lifetime cancer risk
$\text{Intake}_{\text{adjusted}}$ = exposure dose adjusted to reflect RBA (mg/kg/day)
CSF_{oral} = oral cancer slope factor $((\text{mg/kg-bw/day})^{-1})$

If a TRV is based on absorbed dose, administered intakes will need to be converted to uptake (see Section 2.1) prior to calculating the risk estimate by multiplying the intake by the ABA, so that:

(2.1) $$\text{Uptake} = \text{Intake} \times \text{ABA}$$

Where :
Uptake = absorbed dose (e.g., mg/kg-bw/day)
Intake = administered dose (e.g., mg/kg-bw/day)
ABA = absolute bioavailability value (unitless)

For threshold substances, conversion of the TDI based on an administered dose to a toxicity value based on an absorbed dose is calculated from the following equations:

(5.1) $$TDI_{absorbed} = TDI_{administered} \times ABA$$

Where :
$TDI_{absorbed}$ = tolerable daily intake as absorbed dose (mg/kg-bw/day)
$TDI_{administered}$ = tolerable daily intake as administered dose (mg/kg-bw/day)
ABA = absolute bioavailability value (unitless)

and the hazard quotient based on an absorbed dose is calculated as:

(5.2) $$\text{Hazard Quotient (HQ)} = \frac{\text{Uptake}}{TDI_{absorbed}}$$

Similarly, for carcinogens, if the CSF is based on an absorbed dose, the CSF based on an administered dose is calculated using the following equation:

(5.3) $$CSF_{absorbed} = CSF_{administered} \times ABA$$

Where :
$CSF_{absorbed}$ = slope factor from absorbed dose $((mg/kg\text{-}bw/day)^{-1})$
$CSF_{administered}$ = slope factor from administered dose $((mg/kg\text{-}bw/day)^{-1})$
ABA = absolute bioavailability value (unitless)

5.4 Worked Examples

The following examples are intended to demonstrate how bioavailability adjustments might be calculated and reported. The numbers used are purely for illustrative purposes and do not reflect actual bioaccessibility or bioavailability values.

5.4.1 Application of RBA adjustment derived from in vitro testing for lead bioaccessibility

An HHRA is being conducted for a residential site with lead contamination in soils, at concentrations up to 1200 mg/kg. Without consideration of bioavailability and using a default of 1 for the RAF_{oral} (or RBA), the estimated maximum exposure for a toddler would be:

(2.4)

$$\text{Dose} = \frac{C_s \times IR_s \times RAF_{oral} \times ET}{BW}$$

$$= \frac{1200 \times 0.00008 \times 1 \times 1}{16.5}$$

$$= 0.0058 \text{ mg/kg/day}$$

In vitro testing using the RBALP method yielded a bioaccessibility of 70%. Using the US EPA RBA equation for lead (equation 4.2 in Section 4.4.1), this would correspond to a relative bioavailability value of:

(4.2)

$$\text{RBA} = 0.878 \text{ (IVBA)} - 0.028$$

$$= 0.878 \text{ (0.70)} - 0.028$$

$$= 0.59 \text{ or } 59\%$$

Incorporating the calculated RBA value from equation 4.2 into the exposure equation (in place of the default RAF_{oral} of 1) and using receptor characteristics from HC (2012) for a toddler would result in an adjusted dose of:

(2.4)

$$\text{Dose} = \frac{C_s \times IR_s \times RAF_{oral} \times ET}{BW}$$

$$= \frac{1200 \times 0.00008 \times 0.59 \times 1}{16.5}$$

$$= 0.0034 \text{ mg/kg/day}$$

Where :

Dose = $Intake_{adjusted}$ (i.e., RBA adjusted dose) (mg/kg/day)
C_s = soil concentration (1200 mg/kg)
IR_s = soil ingestion rate of a toddler (0.00008 kg/day)
RAF_{oral} = RBA = 0.59 (unitless) from RBA equation above
ET = exposure term (unitless) for residential scenario
BW = body weight of a toddler (16.5 kg)

Without adjusting for bioavailability (i.e., RBA =1), the calculated estimated exposure is 0.0058 mg/kg/d compared to an estimated exposure of 0.0034 mg/kg/d, when the calculated RBA value of 0.59 from equation 4.2, is incorporated in equation 2.4.

5.4.2 In vivo study example using RBA

As part of the HHRA for a residential area, the bioavailability of cadmium is being assessed using an *in vivo* study. Soil with a Cd exposure point concentration of 20 mg/kg is used in the study.

Without considering bioavailability, and using a default of 1 for the RAF_{oral} (or RBA) in equation 2.4, the estimated exposure to Cd from soil ingestion of a threshold substance for a toddler would be:

(2.4)

$$\text{Dose} = \frac{C_s \times IR_s \times RAF_{oral} \times ET}{BW}$$

$$= \frac{20 \times 0.00008 \times 1 \times 1}{16.5}$$

$$= 9.7 \times 10^{-5} \text{ mg/kg/day}$$

Where :

C_s = concentration of contaminant in soil (20 mg/kg)
IR_s = receptor soil ingestion rate (0.00008 kg/day)
RAF_{oral} = relative absorption factor from the GI tract = 1 (unitless)
ET = exposure term (unitless) = 1 for residential scenario
BW = body weight of a toddler (16.5kg)

A review of the toxicological information on cadmium indicates that the oral absorption of Cd in humans is low, ranging from 1% to 10% (Agency of Toxic Substances and Disease Registry [ATSDR] 2012) or 3% to 7% estimated by WHO (2011). A review of the key studies used to derive the oral TDI listed in HC (2010b) indicated that the TDI is based on epidemiology studies with exposure to Cd from dietary exposure. The results of the *in vivo* study using juvenile swine suggest as a conservative estimate, absorption of 4.5% of Cd from the test soils compared to 5.0% assumed absorption from food used to derive the TRV (WHO 2011).

Using equation 2.3, the relative bioavailability of Cd from the *in vivo* study is calculated as:

(2.3)

$$\text{RBA} = \frac{\text{Absorbed fraction from soil}}{\text{Absorbed fraction from food}}$$

$$= \frac{0.045}{0.05}$$

$$= 0.90$$

When the *in vivo* RBA of 0.9 (from equation 2.3) is substituted in equation 2.4, the estimated exposure from soil ingestion of Cd in the example above for a toddler, using receptor characteristics from HC (2012), would be:

$$\text{Dose} = \frac{C_s \times IR_s \times RAF_{oral} \times ET}{BW}$$

$$= \frac{20 \times 0.00008 \times 0.9 \times 1}{16.5}$$

$$= 8.7 \times 10^{-5} \text{ mg/kg/day}$$

Where :

C_s = concentration of contaminant in soil (20 mg/kg)
IR_s = receptor soil ingestion rate (0.00008 kg/d)
RAF_{oral} = relative absorption factor (RBA) from the GI tract = 0.9 (unitless)
ET = exposure term (unitless) = 1 for residential scenario
BW = body weight of a toddler (16.5 kg)

In the case of Cd illustrated here, absorption in both the test soils and the reference medium (i.e., food) are similar, resulting in a high RBA value. This illustrates the importance of incorporating the RBA adjustments on a substance- and site-specific basis. At some sites, a high RBA value may not result in a change in exposure or risk characterization.

5.5 Estimating risk

After the exposure estimate is calculated with adjustment for RBA (using equation 2.4), risk is calculated as an HQ or ILCR using equation 2.6 or 2.7, respectively. Refer to HC 2010a, 2012 for further detail.

5.6 Conclusions

Application of oral bioavailability in an HHRA can reduce uncertainties and assist in obtaining better estimates of potential risk associated with exposure to chemicals in soils at contaminated sites. This can have an impact on potential costs associated with site management and conclusions regarding potential health risks.

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APPENDIX A

KEY CONSIDERATIONS FOR *IN VITRO* TESTS

The following is a summary of the criteria and information to be considered in evaluating the validity of *in vitro* test methods. This list of key considerations for *In Vitro Tests* was modified from Bioaccessibility Research Canada (BARC) (2014) and includes criteria and additional information from ENVIRON (2011), Juhasz *et al.* (2013) and Wragg *et al.* (2011).

Criterion	Supplementary Information
Use an appropriate *in vitro* test method that is considered to have been validated against an *in vivo* method to study the bioaccessibility of COCs. At present, US EPA IVBA, relative bioavailability leaching procedure RBALP (also known as SBRC-G), SBET, *in vitro* gastrointestinal (IVG) assay or UBM can be used to study the bioaccessibility of As and/or Pb; other methods and elements require justification.	Good repeatability and reproducibility have been demonstrated using these methods for a range of As, Pb, Cd and Cr contaminated soils. Bioaccessibility results are generally consistent with *in vivo* results for these soils for As and Pb and, for some methods, Cd (e.g., Table 2 in Koch and Reimer 2012; BARC 2014). The laboratory analyzing the extracts and soils for contaminant concentrations should be certified for that work by the Canadian Association for Laboratory Accreditation (CALA) or a similar organization such as the Programme d'accréditation des laboratoires d'analyse (PALA) in Quebec. See Appendix D for *in vitro* test methods currently in common use.
Soil is sieved to <150 um particle size fraction if using the US EPA IVBA SOP (US EPA, 2017a) or other appropriate particle size fraction (e.g., <250 um) specified in the validated *in vitro* method used. Justification for other particle sizes needs to be provided.	It is important to recognize the difference between particle size that is defined by a particular sieve size and the mean particle size that adheres to human hands. The latter is the size fraction considered to be relevant for risk assessment, because it is thought to best represent the soil fraction ingested through hand-to-mouth contact. Particle size defined by a particular sieve size includes all soil fractions that can pass through that sieve. The <150 µm and <250 µm soil fractions are considered appropriate for use in testing because the mean particle size fraction is expected to fall within the range of soil particle sizes that adhere to hands. Ruby *et al.* (1996) reported a geometric mean particle size range of 19–42 µm for soils that were sieved to <250 µm. This is within the range of 34–105 µm found to adhere to hands (Siciliano *et al.* 2009). If soils were screened using a smaller sieve size, such as 45 µm, this would most likely result in a mean particle size outside that range and therefore, would not be appropriate for use in risk assessment US EPA (2017a) specifies sieving soils to <150 µm. The effect of particle size (obtained by sieving) on bioaccessibility has been tested in a few studies, but reports are conflicting and the number of analytes and samples are limited in most cases (Morman *et al.* 2009; Morrison and Gulson, 2007; Smith *et al.* 2009; Shock *et al.* 2007; Madrid *et al.* 2008). When the samples analyzed for bioaccessibility are part of a larger set for which another standard fraction has been analyzed, such as <2 mm, it may be necessary to establish relationships between the other fraction and the <250 µm fraction with respect to total contaminant concentrations. The experimental details for this type of study should be discussed between the laboratory conducting the bioaccessibility testing and the users of the data.

Criterion	Supplementary Information
Ensure that the method is free of saturation effects and addresses established guidelines.	Additional guidance is provided in HC 2010a. Agitation must be adequate to ensure that there is good contact of test material with the solution (see Comparison of Mixing Parameters by L. Meunier, available on request from BARC, for a description of mixing with respect to As bioaccessibility). Therefore, if a method is altered in any way it must be verified that the method is still free of saturation effects. For As bioaccessibility, the literature reports the robustness of the physiologically based extraction test and IVG methods to saturation effects (Meunier *et al.* 2010b; Makris *et al.* 2008). For the RBALP method, a discussion of when saturation effects might occur for Pb can be found in US EPA (2012a) and Drexler and Brattin (2007). End-over-end agitation is recommended by US EPA (2017a) to ensure that there is effective surface area contact of the test material and extraction fluid for the dissolution of the contaminant in the fluid.
The water soluble reference compound (e.g., blank spike, positive control) is related to the TRV used in the risk assessment.	An appropriate water soluble reference compound (positive control, blank spike) should be incorporated into the bioaccessibility testing. The reference compound should be the same as or similar to the compound or form of the contaminant in the TRV study that is used in the risk assessment, in order to minimize differences between the *in vitro* test and the TRV study.
Include adequate QA/QC samples. Other quality control testing included in each bioaccessibility testing batch are blanks (e.g., reagent blanks, bottle blanks) and positive control samples (blank spike or method spike and matrix spikes).	At least 10% of the samples for both the extraction fluid and the test soils should be analyzed in duplicate or triplicate with the number of replicates decided by discussions between the laboratory conducting the bioaccessibility testing and users of the data. Batch sizes should be no more than 10 samples. A blank consists of all reagents except for samples carried through the entire bioaccessibility test. Certified reference materials, which may be available from NIST, with known expected recoveries and expected RBAs (e.g., from US EPA 2017a) should be used as positive control samples. In the absence of certified reference materials (or SRM), laboratory-established control limits should be used. If control limits are not available for the chemical being tested, an alternative QC measure could include the analysis of other elements for which control limits are available. The laboratory analyzing the extracts and soils for contaminant concentrations should be certified for that work by the Canadian Association for Laboratory Accreditation (CALA) or a similar organization like the Programme d'accréditation des laboratoires d'analyse (PALA) in Quebec. Consult specific *in vitro* SOPs for QA/QC requirements.

APPENDIX B

KEY CONSIDERATIONS FOR *IN VIVO* TESTS

The following is a summary of the criteria and information to be considered in evaluating the validity of *in vivo* test methods for application in HHRA. The list of key considerations for *In Vivo Tests* was modified from BARC (2014) and includes criteria and additional information from ENVIRON (2011), Juhasz *et al.* (2013), Wragg *et al.* (2011) and US EPA (2012c).

Criterion	Supplementary information
The bioavailability study protocol should consider previously published methods for the COC.	In the planning stages of the bioavailability study, project managers should review the literature reporting methods for the contaminants under study, and they should compare the details (some of which are discussed below) of the planned study with those in the literature. If appropriate, the planned study protocol should be consistent with previous studies, but project managers may consider the method reported in the literature to be inappropriate or not applicable to their study. If this is the case, it is advisable to review these points, along with the comparisons generally, in a literature review section of the report for the bioavailability study. A compilation of some of the *in vivo* bioavailability studies from the peer-reviewed literature can be found in Koch and Reimer (2012), which can be used as a starting point.
Unless otherwise justified, soil is sieved to the <150 µm or <250 µm particle size fraction, which provides the best characterization of the risk of exposure from contaminated soil ingestion.	It is important to recognize the difference between particle size that is defined by a particular sieve size and the mean particle size that adheres to human hands. The latter is the size fraction considered to be relevant for risk assessment, because it is thought to best represent the soil fraction ingested through hand-to-mouth contact. Particle size defined by a particular sieve size includes all soil fractions that can pass through that sieve. The < 150 µm and <250 µm soil fractions are considered appropriate for use in testing because the mean particle size fraction is expected to fall within the range of soil particle sizes that adhere to hands. Ruby *et al.* (1996) reported a geometric mean particle size range of 19–42 µm for soils that were sieved to <250 µm. This is within the range of 34–105 µm found to adhere to hands (Siciliano *et al.* 2009). If soils were screened using a smaller sieve size, such as 45 µm, this would most likely result in a mean particle size outside that range and therefore, would not be appropriate for use in risk assessment.
Justification is provided for the choice of animal model.	For inorganic chemicals, a variety of animal (mice, rabbits, dogs, swine and primates) models have been used. The pros and cons of different animal models are discussed in the discussion paper Considerations for bioavailability testing by BARC (2011) and CRC CARE technical report no.14 by Ng *et al.* (2010). A comparison of animal models for arsenic studies is provided in US EPA (2012c). Juvenile swine is the most well developed model. Some researchers consider it the model of choice for the assessment of soil chemical bioavailability in human children because of the similarities with human GI physiology and the weight of a young child. Monkey GI physiology is closer to that of humans, but monkeys are not readily available research animals. For these reasons, HC recommends the use of juvenile swine unless justification is provided for using a different animal model. An example of such justification may be that the bioavailability model aims to replicate the conditions used in the study on which the TRV is based, and a different animal model was used (e.g., rats), or the RBA estimates developed using a mouse model provide a statistically similar RBA estimate as that derived from a monkey or swine model.

Criterion	Supplementary information
It is demonstrated, possibly through a preliminary pilot test, that the dosing regimen is free of saturation effects.	The dosing regimen (dose, number of dose groups, dosing frequency, etc.) should produce results that fall in the linear zone of the dose–response curve, where a dose–response curve is established by plotting a response (e.g., liver concentration of the chemical) against the dose given to the animal, with a minimum of three doses. For contaminants for which the dose dependency of bioavailability may be an issue, the bioavailability study design must be justified; in most cases a minimum of three doses will be required. Bioavailability testing is more useful when the range of soil concentrations is restricted to environmentally relevant concentrations that may provide results that are useful for risk assessment. In all cases, dosing regimens and soil selection will be limited by the amount of soil that can be introduced to an animal model and the contaminant detection limits achievable in the tissues being analyzed.
Justification is provided for the target organs/tissues selected to measure absorption for a given COC.	The selection of an appropriate animal model will be influenced by the endpoint used to measure absorption. Frequently used biological endpoints for assessing soil chemical bioavailability are blood, urine, feces and organs such as the kidney and liver.
Positive controls (i.e., reference compounds) in the *in vivo* study mimic the positive controls used in the critical toxicity study as closely as possible.	For acceptable correlations and meaningful incorporation of test results in the risk assessment process, the dosing medium and reference material used in the bioavailability study should closely match the reference compound used in the critical study used to derive the TRV. Justification should be given for the choice of reference compound in the bioavailability study, especially for deviations from conditions in the TRV study. Dosing considerations are also important. For example, absorption of some chemicals may be influenced by factors such as fasting or non-fasting conditions and the form of the chemical used as the reference material (BARC 2011, Ng *et al.* 2010, ENVIRON 2011). Absorption may be decreased in non-fasted animals compared with fasted animals. Absorption of some chemicals is also affected by the solubility of the chemical. For chemicals that may have multiple TRVs and different solubilities, based on different forms (e.g., nickel), there should be a check that the appropriate TRV study is used. For example, an elemental mercury toxicity value cannot be used to assess inorganic mercury compounds (ENVIRON 2011).
RBA along with the RBA uncertainty is reported.	A worked example of how RBA uncertainty was calculated should be provided. See the protocol recommended by BARC (2011). If funds and the study design permit, a positive intravenous dosing control should be included to enable reporting of ABA as well.
The animal experiment is approved by an animal care committee in accordance with the CCAC and conducted by a laboratory experienced in animal testing.	The CCAC website is www.ccac.ca/en_/assessment

APPENDIX C

MINIMUM CRITERIA FOR A VALID *IN VIVO-IN VITRO* (IVIV) COMPARISON

The following is a summary of the minimum criteria and information for a valid IVIV comparison prior to application of *in vitro* bioaccessibility data in HHRA. This list of criteria is not HC policy, but it does present criteria summarized from BARC (2014), ENVIRON (2011), Juhasz *et al.* (2013) and Wragg *et al.* (2011) that were relevant at the time of publication.

Criterion	Supplementary information
A minimum number (8–12) of well-characterized soils has been used.	The sources and characteristics of the soils should be well documented.
A range of contaminant concentrations and bioavailabilities are considered.	Include different contaminant sources (mining, agriculture, landfill, etc.) and soil types per method per contaminant to obtain a good range of concentrations and bioavailabilities. Discussion of this point is further elaborated in Juhasz *et al.* (2013).
R^2>0.64 (r >0. 8) or a statistically significant correlation is obtained.	A compilation of some of the IVIV comparison studies from the peer-reviewed literature can be found in Koch and Reimer (2012).
A slope value of 0.8 to 1.2 is obtained.	Wragg *et al.* (2011). Other slope values should be justified.
Bioaccessibility repeatability (within-laboratory variation) and reproducibility (between laboratory variability) can be proven.	Wragg *et al.* (2011). Bioaccessibility repeatability determined by a median value of 10% relative standard deviation (RSD). Reproducibility (between laboratories) is determined by a median value of 20% RSD.
The *in vitro* method should be shown to predict RBA for soils independent of the initial study used to validate the *in vitro* method against *in vivo* methods.	The performance of a predictive model may be overestimated if it is tested only with the samples used to construct the model. The prediction of the model using data independent of those used to construct the model should be evaluated (Juhasz *et al.* 2013). How do results compare with similar studies reported in the literature? Do variable site conditions (physical or chemical characteristics of soil chemical species) explain variability between study results?

APPENDIX D

COMMONLY AVAILABLE BIOAVAILABILITY AND BIOACCESSIBILITY TESTS

The tests summarized in this appendix are commonly available for use at the time of writing and are considered by some government agencies (such as US EPA) or research organizations (such as BARGE or BARC) to be validated, so long as they are used for the purposes and within the constraints described below. However, other test methods may exist for which risk assessment practitioners can provide sufficiently rigorous evidence of validation. Practitioners should ensure, however, that any methods used are current and appropriate before applying them at any specific site, whether they are based on those described below, adopted from other sources or newly developed. Koch *et al.* (2013) provides a comparison of 17 current methods and the variability among these methods for inorganic substances.

D.1 In Vitro Methods

D.1.1 In Vitro Bioaccessibility Assay for Lead and Arsenic in Soil

Overview

The US EPA (2017a) SOP for an *In Vitro* Bioaccessibility Assay for Lead and Arsenic in Soil provides guidance on the analytical procedures needed to conduct validated *in vitro* bioaccessiblity testing for lead and arsenic in soils. This method has been revised from earlier SOPs issued by the US EPA (2008) for lead (US EPA 2012a). The method is used to measure the fraction of lead and/or arsenic that is solubilized in an extraction solvent resembling gastric fluid; the fraction of lead or arsenic that is soluble in the *in vitro* system, called the *in vitro* bioaccessibility (IVBA), is used as an indicator of *in vivo* RBA. The IVBA result using this method has been shown to be a good predictor of *in vivo* RBA of lead and arsenic applicable to a wide range of soil types and lead and arsenic phases. The US EPA's IVBA SOP is a refinement of earlier gastric phase *in vitro* tests, such as SBET and RBALP. The intent of the SOP is to provide guidance to users so that the results they generate meet the data quality objectives for the intended application of IVBA and can be used to estimate the bioavailability of lead in soil.

The test involves leaching samples (screened to <150 µm) with a glycine-buffered extraction fluid adjusted with HCl to pH 1.5 and heated to 37ºC to simulate stomach conditions. The extract is analyzed for concentrations of lead and/or arsenic. A duplicate soil sample is analyzed for total lead and/or total arsenic, bioaccessibility of the metals are calculated from the ratio of concentrations of each metal in the extract versus the solid.

Validation Status

The method has been validated by the US EPA and has been found to correlate well with the RBA for lead and arsenic-bearing soils tested in US EPA *in vivo-in vitro* studies. The IVBA (i.e., bioaccessibility result) is used in the lead and arsenic IVBA correlation equation to calculate RBA for lead and arsenic.

Limitations

Validation has only been confirmed for lead and arsenic, and use for other chemicals would require appropriate support. The method only simulates gastric extraction. The RBA equation developed from the IVBA method for lead and arsenic are applicable to a wide range of soil types; however, the IVBA result does not correlate with the results from juvenile swine *in vivo* assays for phosphate-amended soils, and therefore the method is not considered validated, nor is it recommended for assessing the RBA of lead in phosphate-amended soils. US EPA also states that phosphate amendments should also be avoided in arsenic contaminated soils and that the role of phosphate on arsenic IVBA and RBA is not known (US EPA 2017a). If the IVBA method is used for soils that contain unusual or untested lead phases, this should be identified as a potential source of uncertainty, because it is unknown whether the tested soils might follow the observed correlation established by the RBA correlation equation.

D.1.2 Solubility/Bioavailability Research Consortium (SBRC) Procedure for Stomach Phase Bioaccessibility Extraction Test

Overview

The SBRC developed an SOP for measuring bioaccessible lead or arsenic in soils and solid waste materials, along with a recommended quality assurance program. The gastric phase of the SBRC (SBRC-G) is essentially identical to the RBALP.

The test involves leaching samples (screened to <250 µm) with the HCl-glycine extraction fluid buffered at pH 1.5 and heated to 37°C to simulate stomach conditions (as described for IVBA above). The extract is analyzed for concentrations of lead and/or arsenic. A duplicate soil sample is analyzed for total lead and/or arsenic, bioaccessibility being calculated from the ratio of concentrations in the extract versus the solid.

The SOP is described in detail in ENVIRON (2011) and US EPA (2012a).

Validation Status

The test has been found to correlate well with RBA for lead and arsenic-bearing soils tested in US EPA *in vivo-in vitro* studies and is the basis for the lead and arsenic IVBA correlation equations (US EPA 2007c; Diamond *et al.* 2016; US EPA 2017a).

Limitations

The method does not specifically identify limitations, but validation has only been confirmed for lead and arsenic, and use for other chemicals would require appropriate support. The method only simulates gastric extraction.

D.1.3 SBRC Procedure for Stomach and Small Intestinal Phase Extraction

Overview

The SBRC developed an SOP for measuring the bioaccessibility of inorganics in soils and solid waste materials, along with a recommended quality assurance program. The approach was based on the physiologically based extraction test (PBET) method published by Ruby *et al.* (1996) but with an updated test cell and mixing method. It was designed to replicate the GI tract of a child, with consideration of pH and chemical factors in the stomach and small intestine, soil-to-solution ratio, stomach mixing and stomach emptying rate.

Two separate extraction phases are used. The first extraction phase (SBRC-G) involves a gastric solution at pH 2; after 1 hour, the solution is brought to pH 7.0, and bile salts and pancreatin are added. Samples are collected after the stomach phase extraction and after 3 hours of intestinal phase extraction.

The SOP is described in detail in ENVIRON (2011).

Validation Status

No specific validation data are described in ENVIRON (2011). The test is based on Ruby *et al.* (1996), which has been tested against *in vivo* methods for lead and arsenic but has not been formally accepted by US EPA at this time. For lead, samples of mine waste materials, residential soils in the vicinity of historical smelters, mine tailings and a stream channel sample affected by historical mining and milling activities were evaluated using this approach and a rat model; the test was found to correlate very well with the rat bioavailability results. For arsenic, residential soil and a house dust sample near a historical copper smelter were evaluated using this test and either New Zealand White rabbits (soil) or Cynomolgus monkeys (house dust). The test was found to over-predict bioavailability, suggesting that it is conservative. The SOP references chromium and mercury, but no information on validation for these metals was provided.

Limitations

The method does not specifically identify limitations, but validation has been confirmed only for lead and arsenic and primarily for soils affected by smelters and mining waste (lead only); its use for other chemicals would require appropriate support.

D.1.4 Ohio State University (OSU) In Vitro Gastrointestinal Method for Determination of the Bioaccessibility of Select Metals and Metalloids in Soil and Geomedia (IVG)

Overview

The method simulates the human GI tract and is a two-step sequential extraction test that includes a gastric solution extraction followed by an intestinal solution extraction. The test is used to determine the percentage bioaccessibility of lead, arsenic and cadmium in soils. The bioaccessibility of these metals in soils has been shown to be correlated with *in vivo* bioavailability tests using immature swine.

For the gastric phase, the test involves heating a gastric solution to 37ºC to simulate stomach conditions, to which dried and screened soil (<250µm) is added. The gastric solution is pH adjusted and maintained at a pH of 1.8. The extract is filtered and refrigerated for preservation prior to analyses, and the contaminants extracted from the gastric phase are expressed as gastric extractable (GE).

For the intestinal phase, the remaining solution from the gastric phase is adjusted to a pH of 6.1, and porcine bile extract and porcine pancreatin are added. The filtered extracts are refrigerated for preservation prior to analysis. Contaminants extracted in the intestinal phase are expressed as intestinal extractable.

Validation Status

The SOP states that the measured percentage of bioaccessible Pb, As and Cd have been shown to be correlated with *in vivo* data from dosing trials using immature swine. Schroder *et al*. (2003) reported that the GE can be used to estimate the RBA of lead, arsenic and cadmium.

Limitations

The method description and SOP do not specifically limit the application of this test.

D.1.5 The BARGE Unified Bioaccessibility Method

Overview

The BARGE modified an *in vitro* method originally developed by the Netherlands National Institute of Public Health and the Environment (RIVM) (Oomen *et al*. 2003) for use in HHRA of contaminated sites and prepared an SOP for the method.

The process simulates the dissolution and absorption of the chemical in ingested material as it moves through the human GI tract after 5 minutes in the mouth, 1 hour in the stomach and 4 hours in the small intestine with synthesized saliva, gastric fluid, duodenal fluid and bile.

The results of the test can be expressed in either milligrams of bioaccessible chemical per kilogram of solid matrix or percentage bioaccessible.

Details of the method, including the SOP and supporting studies, are available at www.bgs.ac.uk/barge/ubm.html

Validation Status

Validation studies were conducted by comparing results with an *in vivo* analysis using a juvenile swine model. It was successfully validated for arsenic, cadmium and lead using 16 soils contaminated from smelting or mining activities (Denys *et al.* 2012). An attempt to validate the method for antimony was unsuccessful as a result of consistently low bioavailability and bioaccessibility.

The method has been successfully applied for cadmium, lead and zinc from smelter emissions in urban and agricultural soils from France (e.g., Roussel *et al*. 2010), and for arsenic, chromium and lead contamination in urban UK soils (e.g., Broadway *et al*. 2010).

Limitations

The method description and SOP do not specifically limit the application of this test. The validation studies included both smelting and mining contamination. At this time BARGE considers it validated only for arsenic, cadmium and lead; its use for other inorganics should include appropriate support.

D.2 In vivo Methods

D.2.1 Protocol for the Determination of the Bioavailability of Arsenic in Soil Following Oral Administration in Cynomolgus Monkeys

Overview

The protocol, described in detail by ENVIRON (2011), involves administering capsules of soil containing arsenic or soluble arsenic to cynomolgus monkeys. A series of blood (optional) and urine samples are then used to determine relative bioavailability. An optional intravenous dose group can be used to determine ABA. Each treatment group includes three monkeys.

ABA is estimated by comparing arsenic excreted in urine in the capsule vs. the intravenous group. ABA may also be calculated by evaluating plasma arsenic concentrations over time for oral and intravenous exposures. RBA is determined either by dividing the ABA of soil arsenic by the ABA of soluble arsenic, or by comparing urinary excretion or plasma concentrations over time directly for soil and soluble arsenic if the intravenous group is omitted.

Validation Status

Cynomolgus monkeys are considered to have close anatomical and physiological similarities to those of humans, and this procedure has successfully been used to estimate arsenic bioavailability in humans (Charbonneau *et al.* 1978; ATSDR 2007; ENVIRON 2011).

Limitations

This method involves live monkeys (but does not require that the monkeys be killed at the end of the study), resulting in high costs and ethics requirements. The test may be suitable for other chemicals with a similar toxicokinetic profile to that of arsenic, i.e., high ABA and rapid excretion via kidneys, but detailed justification of the method's applicability should be provided. As well, monkeys may not be readily available test animals in comparison with juvenile swine or mice.

D.2.2 Protocol for Bioavailability Study of Arsenic and Lead in Soil Following Oral Administration Using Juvenile Swine

Overview

The protocol, described in ENVIRON (2011), uses male juvenile swine to estimate the relative oral bioavailability of lead and arsenic in soil. Animals are dosed over 15 days with site soils or with sodium arsenate (for arsenic) and lead acetate (for lead). In the described method, soil is encapsulated in dough balls, although alternative dosing methods, such as administration of soil slurries via gavage, may be considered (Juhasz *et al.* 2007*).*

The test uses a total of 50 juvenile swine if both lead and arsenic are being evaluated. Each of the groups (control, 3 lead acetate dose groups, 3 sodium arsenate dose groups and 3 site soils) contain 5 animals per treatment group. Blood, urine, tissue and bone samples are collected and used to determine relative lead and arsenic bioavailability.

Validation Status

Juvenile swine have similar nutritional requirements, bone development and mineral metabolism, and size to those of young children, and there are previous studies evaluating lead and arsenic bioavailability from oral exposure in these animals.

Limitations

The test requires the use of live animals, which are sacrificed upon completion of the test, resulting in relatively high costs and ethics requirements. The test is considered applicable for children and not necessarily adults. Use of the test for chemicals other than lead and arsenic in soil requires appropriate support.

D.2.3 Protocol for Bioavailability Study of Arsenic in Soil Following Oral Administration Using Mice

Overview

The protocol, described in Bradham *et al*. (2011), uses 4–6-week old female C57BL/6 mice maintained on AIN-93G purified rodent diet and fed soil-amended diets with a 1% (wt/wt) soil:diet ratio. Twelve mice (three per metabolic cage) are dosed for 10 days. The ABA of arsenic from ingestion of a soil- or sodium arsenate-amended diet is calculated as the ratio of cumulative excretion of arsenic in urine and cumulative arsenic intake from the amended diet. RBA is calculated as the ratio of the ABA for arsenic in a specific soil-amended diet to the ABA for arsenic in a diet containing sodium arsenate.

Validation Status and Advantages

Mice are well characterized physiologically, and available data on GI absorption of ingested arsenicals support use of the mouse as a test species. As described by Bradham *et al.* (2011), similarities between mice and humans in metabolism and disposition of arsenicals are sufficient to permit use of mouse data to create physiologically based pharmacokinetic models to estimate arsenic bioavailability for humans. Low purchase and husbandry costs, ease of handling, improved predictive value of data because of the feasibility of an increased sample size in assays, and the potential for widespread use of a mouse-based assay in many laboratories are cited as additional reasons to consider this animal model.

Limitations

This method involves live animals, resulting in relatively higher costs and ethics requirements. Use of the test for chemicals other than arsenic would require appropriate support.

D.2.4 Protocol for Bioavailability Study of Cadmium in Soil Following Oral Administration Using Sprague-Dawley Rats

Overview

The RBA of cadmium in soils (compared with soluble cadmium) is evaluated in this protocol, described by ENVIRON (2011). Soil is administered in gelatin capsules; soluble cadmium is administered as an oral gavage dose of cadmium chloride. Three groups of rats are used: a control, a cadmium chloride group and a soil-exposed group; the recommended number of animals is 32 per group (96 total).

Blood samples are collected from the rats over 6 days, and the area under the cadmium concentration versus time curve for soil-exposed vs. cadmium chloride rats is used to determine relative oral bioavailability.

Validation Status

Sprague-Dawley rats are recognized by US EPA for chemical safety evaluation, and there are previous studies of soil cadmium bioavailability in this species.

Limitations

This method involves live animals, resulting in high costs and ethics requirements. Use of the test for chemicals other than cadmium would require appropriate support.

D.2.5 Protocol for Bioavailability Study of Mercury in Soil Following Dosed Feed Administration Using Weanling Sprague-Dawley Rats

Overview

The protocol, described in detail by ENVIRON (2011), involves dosing weanling Sprague-Dawley rats with soil or soluble mercury ($HgCl_2$) in order to determine the RBA. The test soil is mixed with rat feed.

Five male and five female rats are used in each treatment group, including an untreated control group, an intravenous soluble mercury group, an oral soluble mercury group and a group fed site soil. Blood mercury concentrations are used to determine relative bioavailability.

Validation Status

Sprague-Dawley rats are recognized by US EPA for use in chemical safety evaluation testing, and previous studies have been conducted on mercury absorption in this species.

Limitations

This method involves live animals, sacrificed at the end of the study, resulting in high costs and ethics requirements. Use of the test for chemicals other than mercury would require appropriate support.

APPENDIX E

INTERNATIONAL APPROACHES TO EVALUATING BIOAVAILABILITY

A number of international government agencies allow the use of bioavailability adjustments in risk assessments. Guidance to support development of such adjustments is in various stages of development. There are variations among countries in definitions of terms, test methods that are deemed acceptable, reporting requirements, regulatory frameworks and guidance on specific chemicals. A brief summary of recommendations by government agencies in the United States, United Kingdom, the Netherlands, Denmark, Australia, New Zealand and France is provided in this appendix as a point of reference (noting that the information is current at the time of preparation of this report). Table E–1 lists international information sources for the use of bioavailability testing and incorporation of bioavailability adjustments in quantitative HHRA.

Table E–1: Bioavailability Information Sources for International Environmental and Health Agencies

Information Source	Web Links
US EPA, Soil Bioavailability at Superfund Sites	https://www.epa.gov/superfund/soil-bioavailability-superfund-sites
US EPA, Soil Bioavailability at Superfund Sites: Guidance	https://www.epa.gov/superfund/soil-bioavailability-superfund-sites-guidance
US EPA (2012b), Recommendations for Default Value for Relative Bioavailability of Arsenic in Soil	https://semspub.epa.gov/work/11/175338.pdf
US EPA (2016), Recommendations for Sieving Soil and Dust Samples at Lead Sites for Assessment of Incidental Ingestion. OLEM 9200.1-129	https://semspub.epa.gov/work/HQ/100000133.pdf
US EPA (2017a), Standard Operating Procedures for an *In Vitro* Bioassessibility Assay for Lead and Arsenic in Soil and Validation Assessment of the *In Vitro* Arsenic Bioaccessibility Assay for Predicting Relative Bioavailability of Arsenic in Soils and Soil-like Materials at Superfund Sites. OLEM 9200.2-164.	https://semspub.epa.gov/work/HQ/196750.pdf
The Netherlands RIVM, Bioaccessibility of Contaminants from Ingested Soil in Humans (Sips *et al.* 2001)	www.rivm.nl/bibliotheek/rapporten/711701012.pdf
The Netherlands RIVM, The Bioaccessibility of and Relative Bioavailability of Lead from Soils for Fasted and Fed Conditions (Hagens *et al.* 2008)	www.rivm.nl/bibliotheek/rapporten/711701080.pdf
Australia Environment Protection and Heritage Council (EPHC), Guideline on Site Specific Health Risk Assessments	www.nepc.gov.au/system/files/resources/93ae0e77-e697-e494-656f-afaaf9fb4277/files/schedule-b4-guideline-site-specific-health-risk-assessments-sep10.pdf
Ng *et al.* 2010. Contaminant Bioavailability and Bioaccessibility. Part 1: A Scientific and Technical Review. CRC CARE Technical Report no. 14, CRC for Contamination Assessment and Remediation of the Environment, Adelaide, Australia.	www.crccare.com/files/dmfile/CRCCARETechReport14-Part1-Contaminantbioavailabilityandbioaccessibility2.pdf
Bioaccessibility Research Canada (BARC)	www.bioavailabilityresearch.ca
Bioaccessibility Research Group of Europe (BARGE)	www.bgs.ac.uk/barge/home.html

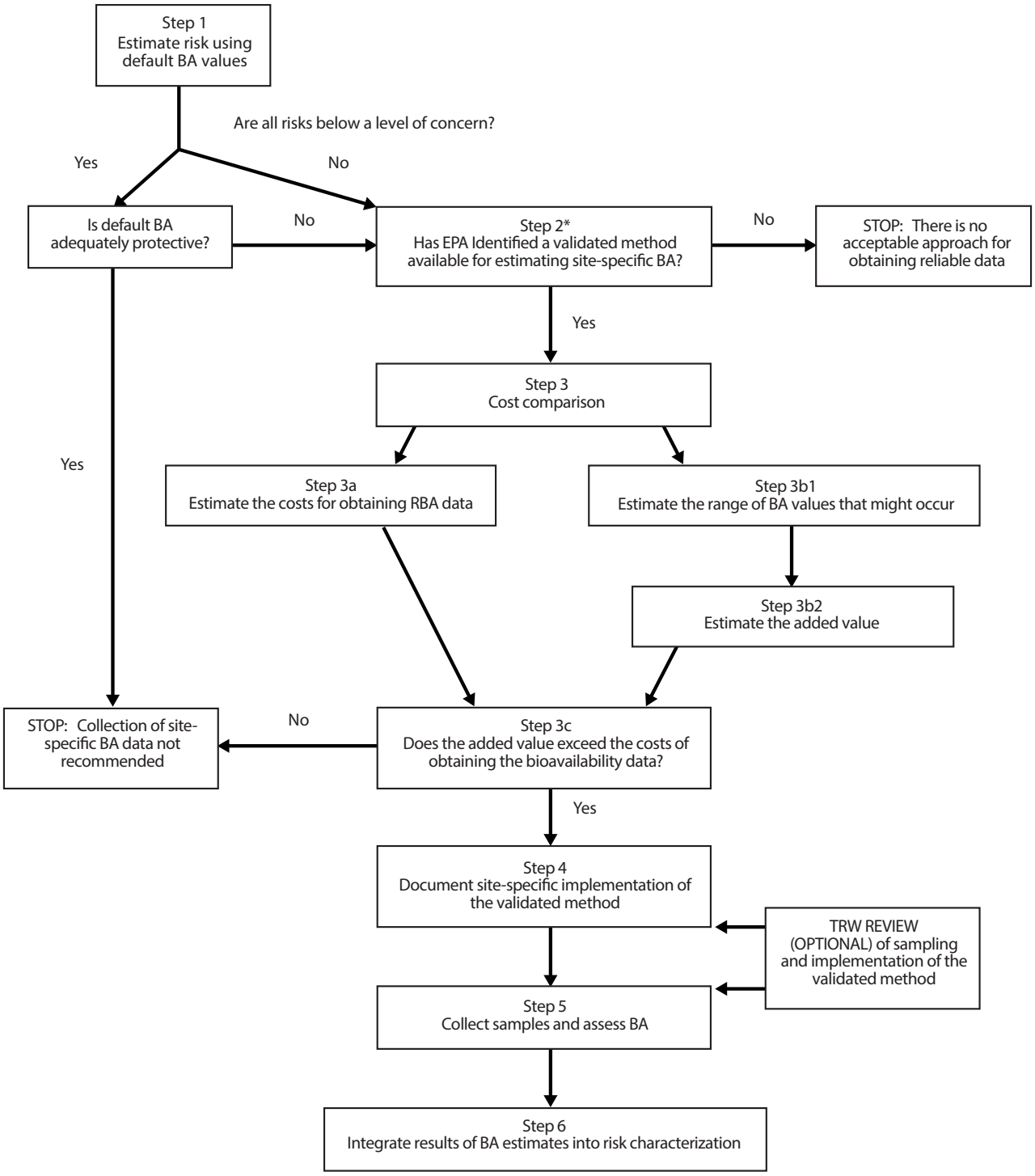
E.1 United States

Since the development of health risk assessment guidance in 1989 to evaluate contaminated sites for the Superfund program, the United States has formally acknowledged the potential disparity between chemical absorption in TRV studies vs. site exposure media (US EPA 1989). US EPA provides a default RBA value of 0.6 for lead (US EPA 1999) and recommended a default RBA value of 0.6 for arsenic (US EPA 2012b).

In 2007, US EPA issued guidance on how to assess the site-specific oral bioavailability of metals in soils for use in HHRA. The guidance document reviews existing guidance about how to incorporate bioavailability adjustments into risk assessments and provides recommended processes for deciding when to collect site-specific information on the oral bioavailability of metals in soils and for documenting the data collection and analysis. General criteria that the US EPA normally will use to evaluate whether a specific bioavailability method has been validated for regulatory risk assessment purposes are also described. The guidance is intended to provide technical and policy guidance to US EPA staff on making risk management decisions for contaminated sites. It also provides information to the public and to the regulated community on how US EPA intends to exercise its discretion in implementing its regulations at contaminated sites.

The US EPA guidance includes a recommended decision framework on how to evaluate and incorporate site-specific oral bioavailability information into risk-based decision-making. The recommended decision framework is intended to improve risk estimates at specific sites where the framework is applied, as well as to encourage the expansion of a knowledge base that can be applied to future assessments of the bioavailability of metals in soil at all sites. The US EPA decision framework (summarized in the flowchart shown in the Figure E.1) uses evaluation criteria and an ordered process for considering these criteria in the assessment of site-specific bioavailability of metals. Their recommended decision framework is intended to help ensure that 1) decisions about when to collect site-specific data are well thought out and documented; and 2) when data are collected, these data will be of appropriate quality to support site-specific risk assessment and risk management decision-making. Each step of the decision framework is described in greater detail in the US EPA guidance.

Figure E–1: Recommended decision framework for assessing oral bioavailability (BA) of metals at contaminated sites (US EPA 2007a)



US EPA has issued detailed guidance for estimating the RBA of lead in soil and soil-like materials (US EPA 2007c) using either an *in vivo* model or an *in vitro* model (see Appendix D). The guidance describes the evaluation of oral bioavailability of lead in soil from a variety of lead-contaminated sites using both *in vivo* (juvenile swine) and *in vitro* models. US EPA (2007b) concludes that the juvenile swine model is useful for evaluating lead absorption from soil and soil-like materials in children, and the *in vitro* method described in their report correlates well with the *in vivo* model results. A regression equation is provided to convert the *in vitro* bioaccessibility results to an RBA.

US EPA published an *in vitro* protocol, *Standard Operating Procedure for an In vitro Bioaccessibility Assay for Lead in Soil* in 2008, which has since been updated (US EPA 2012a; 2017a). The US EPA IVBA protocol is based on previous work conducted by Ruby *et al.* (1993; 1996) and Drexler and Brattin (2007). Validation of the IVBA method for arsenic is documented in Diamond *et al.* (2016) and US EPA (2017b).

US EPA has an active program developing methods for evaluating the RBA of arsenic in soil. Juvenile swine has been US EPA's preferred *in vivo* model for estimating arsenic bioavailability, and a US EPA-approved protocol and SOP have been published (US EPA 2010). More recently, a mouse model was developed and the results were published by US EPA scientists (Bradham *et al.* 2011). Current *in vitro* methods have been found to correlate with *in vivo* measurements for swine, mice and monkey (e.g., Bradham *et al.* 2011; Brattin *et al.* 2013), and a validation program for arsenic is currently being conducted. US EPA has also established a default bioavailability adjustment of 0.6 for arsenic, based on an upper percentile, but recommends using site-specific data where feasible (US EPA 2017a).

US EPA does not currently have approved *in vivo* or *in vitro* protocols for other metals or organic compounds. Some regional US EPA and state regulatory offices provide additional guidance on use of bioavailability adjustments in risk assessment.

US EPA (2007a) acknowledges that differences in absorption between a chemical present in site media and the dosing vehicle used in toxicity studies can be quite large, particularly for metals that may exist in a variety of chemical and physical forms. When these differences in absorption are considered through the use of bioavailability adjustments, the resulting impact on risk estimates and clean-up goals can be significant. Nevertheless, in the absence of data supporting an alternative assumption, US EPA recommends that the bioavailability of a chemical in contaminated media should be assumed to be equal to that in the toxicity study associated with that chemical (US EPA 2007a).

If risks are above a level of concern and there is a potential for significant added value in collecting site-specific bioavailability data, then US EPA recommends determining whether or not a validated method is available for estimating site-specific bioavailability.

US EPA guidance (2007a) states that bioavailability assessment relies on validated *in vivo* or *in vitro* models that estimate absorption in the human GI tract. While absorption of metals such as lead is affected by the type of minerals present (e.g., lead as cerussite, galena, oxides), US EPA does not accept use of mineralogy data alone as a basis to quantify the bioavailability in soil and soil-like materials, because other factors also affect the RBA (US EPA 2007c).

E.2 United Kingdom

In 2005, the United Kingdom (UK) Environment Agency assembled a workshop attended by UK regulators and researchers, as well as international experts in the field of bioavailability, for the purpose of evaluating the appropriateness of using bioaccessibility test results in HHRA. At that time, the Environment Agency recognized the usefulness of bioavailability adjustments in HHRA but could not recommend the use of bioaccessibility data without "suitable justification" (UK Environment Agency 2005). Suitable justification is described as demonstration of the following: a detailed description of the sample collection, preparation, analysis and quality assurance methods; an understanding of the uncertainties in the test method; application of the bioaccessibility data only to the soil and dust ingestion pathway; no extrapolation of data from one chemical to another; and potential for planned land use changes that could affect oral bioaccessibility by causing changes in chemical sequestration. The Environment Agency is particularly interested in the use of bioaccessibility test results to evaluate elevated levels of naturally occurring arsenic in soils as part of a "multiple lines of evidence" approach to HHRA (UK Environment Agency 2011). Although application of bioavailability adjustments is allowable under limited conditions, the Environment Agency does not provide a recommendation for specific *in vivo* or *in vitro* test methods (UK Environment Agency 2007; 2011).

E.3 The Netherlands

The Netherlands' National Institute for Public Health and the Environment (RIVM) has developed an *in vitro* bioaccessibility test method, Bioaccessibility of Contaminants From Ingested Soil in Humans, Method Development and Research on the Bioaccessibility of Lead and Benzo(a)pyrene (Sips *et al.* 2001). The *in vitro* method is based on the digestive system of a child and represents a worst case model by assuming a fasted state (gastric pH of 1). An update to this method allows for evaluating the bioaccessibility of lead under an "average physiological state" by conversion between only fasted or only fed physiological states (Hagens *et al.* 2008). While the RIVM recommends use of the *in vitro* method for evaluating the bioaccessibility of lead in soil, it appears that the Dutch Environment Assessment Agency has not yet formally incorporated the use of the RIVM method in evaluating lead-contaminated soils (Brand *et al.* 2009).

E.4 Denmark

Limited English-language information is available on-line from the Danish Ministry of the Environment (DMOE) website, and so readily accessible information was obtained from a variety of sources outside the DMOE. DMOE commissioned a series of studies assessing various *in vitro* bioaccessibility testing methods, including evaluation of the RIVM method (Sips *et al.* 2001) and the *in vitro* protocol described by Kelley *et al.* (2002). A great deal of variability was observed between methods, and ultimately the RIVM method under fasted conditions was recommended for evaluating the oral bioaccessibility of metals (DHI Water and Environment 2005). The DMOE supports the use of bioaccessibility results for lead in HHRA, but no formal policy is known to be available at the time of this report (Oomen *et al.* 2006).

E.5 Australia and New Zealand

In 2001, the Australian and New Zealand Environment and Conservation Council (ANZECC) ceased to exist, and the environmental programs managed under ANZECC were taken over by the Environment Protection and Heritage Council (EPHC). The EPHC incorporates Australia's National Environment Protection Council (NEPC)[1]. The EPHC/NEPC Guideline on Health Risk Assessment Methodology (NEPC 1999) provides for the use of bioavailability factors when calculating health-based soil criteria, but no direction is provided regarding selection of bioavailability test methods or methods for use of bioavailability test data. Since issuance of the health risk guideline, Ng *et al.* (2009; 2010) completed Contaminant Bioavailability and Bioaccessibility, a review of bioaccessibility testing protocols and methods for application of bioavailability adjustments in HHRA. In this review, conducted on behalf of the NEPC, Ng *et al.* (2009; 2010) provided recommendations on how to incorporate bioavailability information into HHRA and how to convert *in vitro* test results to *in vivo* values for arsenic and lead; as well, they directed HHRA practitioners to US EPA (2007a) for further guidance on selection of *in vitro* and *in vivo* test methods. No guidance is provided for evaluating the bioavailability of organic compounds or metals other than lead and arsenic. However, in a presentation to BARC on October 5, 2010, Dr. Albert Juhasz of the Centre for Environmental Risk Assessment and Remediation, University of South Australia, identified multiple *in vitro* assays under development for evaluation of dichlorodiphenyltrichloroethane, PAHs, cadmium and arsenic (Royal Military College 2010).

E.6 France

Researchers at the French National Institute for Industrial Environment and Risks, with BARGE, have developed and validated a bioaccessibility protocol, the Unified BARGE Method (UBM), to assess the bioavailability of arsenic, antimony, cadmium and lead in soil (Denys *et al.* 2012*). (*www.bgs.ac.uk/barge/ubm.html).

[1] The NEPC is a special council comprising environment ministers from the Australian government and from each state and territory.