CRC for Contamination Assessment and Remediation of the Environment

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Part 2: Guidance for industry

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Part 2: Guidance for industry

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Background

When determining the impact of an ingested chemical on human health risk assessment, the chemical's toxicity is influenced by the degree to which it is absorbed from the gastrointestinal tract into the body (i.e. its bioavailability). As oral references doses (RfDs) and cancer slope factors (CSFs) are generally expressed in terms of ingested dose, rather than absorbed dose, the variability in absorption between different exposure media, chemical forms etc. may significantly influence risk calculations. In Australia, NEPM health investigation levels (HILs) are highly conservative and derived using a bioavailability default value of 100%. However, the assumption that 100% of the soil-borne contaminant is bioavailable may overestimate exposure thereby influencing risk calculations. As a result, assessment of contaminant bioavailability may help refine exposure modelling for Tier 2 human health risk assessment.

This guidance document is limited to evaluating the bioavailability of contaminants via the incidental soil ingestion pathway and is based on a comprehensive review (Ng et al. 2010) undertaken as part of recommendation 24 of the National Environmental Protection Measure (NEPM) five-year statutory review. The majority of bioavailability research has focused on inorganic contaminants such as arsenic and lead, which forms the basis of this guidance document.

Definitions

The term 'bioavailability' has many different meanings across various disciplines of toxicology and pharmacology (see NRC 2003). Taking into consideration contaminant exposure via multiple pathways, bioavailability for the purpose of this guidance document is defined as '*the amount of a contaminant that is absorbed into the body following dermal contact, ingestion or inhalation*'.

More specific definitions for bioavailability may include:

Absolute bioavailability (ABA): the fraction of a compound which, following ingestion, inhalation or dermal contact, is actually absorbed and reaches systemic circulation.

$$\text{ABA} = \frac{\text{Absorbed dose}}{\text{Ingested dose}}$$

Equation [1]

Relative bioavailability (RBA): the comparative bioavailability of different forms of a chemical or for different exposure media containing the chemical. RBA is the ratio of the absorbed fraction from an exposure medium (e.g. soil) to the absorbed fraction from a reference dose (e.g. lead acetate). RBA is used in place of default bioavailability assumptions when reliable site-specific data is obtained.

$$RBA = \frac{ABA_{test}}{ABA_{reference}}$$

Equation [2]

A related term, pertinent to bioavailability assessment, is 'bioaccessibility'.

***Bioaccessibility*:** the fraction of a compound that is soluble following gastrointestinal extraction and is therefore available for absorption.

This term is specifically referred to when in vitro[1] chemical assessment models are used for estimating the relative bioavailability of a contaminant.

Relative bioavailability assessment

In the absence of human studies or the availability of suitable epidemiological data, the RBA of soil-borne contaminants may be assessed using in vivo[2] methods. Bioavailability assessment using an in vivo model is considered to be the most reliable method for refining exposure models for Tier 2 human health risk assessment. While a variety of animal models have been used for the assessment of RBA (see Ng et al. 2010), standard operating procedures for these in vivo models are currently unavailable. However, the US EPA have developed a guidance document for evaluating the bioavailability of metals in soil for use in human health risk assessment (US EPA 2007b), while Rees et al. (2009) detail an in vivo swine assay for the determination of relative arsenic bioavailability in contaminated soil and plant matrices. The use of juvenile swine for the assessment of RBA is prescribed by the US EPA, however, other in vivo models (e.g. rodents, primates) may be utilised if deemed suitable for the contaminant of interest. Bioavailability endpoints may include the determination of the contaminant in blood, organs, fatty tissue, urine and faeces, urinary metabolites, DNA adducts and enzyme induction (e.g. cytochrome P_{450} mono-oxygenases). CRC CARE Technical Report no. 14: Part 1 (Ng et al. 2010) provides details of bioavailability endpoints including advantages and disadvantages of each method, in addition to contaminant bioavailability data from national and international studies.

Bioaccessibility assessment

Several in vitro methods have been developed for the prediction of contaminant relative bioavailability (Ng et al. 2010). These in vitro methodologies do not attempt to replicate conditions found in vivo, but mimic key processes such as contaminant dissolution. In vitro assays have the potential to overcome the time and expense limitations of in vivo studies thereby providing a surrogate measurement of bioavailability that is quick and inexpensive compared to animal models. However, in order for an in vitro bioaccessibility test system to be useful in predicting the in vivo relative bioavailability of a test material, it is necessary to establish empirically that a strong correlation exists between the in vivo and in vitro results across a variety of sample types.

[1] Outside the living body in an artificial environment.
[2] Within a living organism.

Inorganic contaminants

A limited number of studies have established the relationship between in vivo RBA and in vitro bioaccessibility. For inorganic contaminants, these studies have been limited to arsenic (Basta et al. 1999; Juhasz et al. 2007, 2009a; Rodriguez et al. 1999) and lead (Drexler & Brattin 2007; Juhasz et al. 2009b; Schroder et al. 2004; US EPA 2007a) with only the US EPA study (US EPA 2007a) gaining regulatory acceptance. Data is emerging for other inorganic contaminants (e.g. cadmium, nickel), however the correlation between RBA and bioaccessibility is lacking or limited information is available which precludes confidence in the determination of in vivo - in vitro relationships.

Arsenic

Arsenic RBA may be predicted using the gastric phase of the SBRC (Juhasz et al. 2007, 2009a) or IVG (Rodriguez et al 1999; Basta et al. 2007) in vitro methods.

Arsenic RBA (%) = 0.992 x SBRC $_{gastric}$ (%) + 1.66 (Juhasz et al. 2009a) *Equation [3]*

Arsenic RBA (%) = 0.85 x IVG $_{gastric}$ (%) + 0.97 (Basta et al. 2007) *Equation [4]*

In a comparison study, Juhasz et al. (2009a) demonstrated that the in vitro assay encompassing the SBRC gastric phase provided the best prediction of in vivo arsenic RBA compared to other in vitro assays including IVG, PBET and DIN assays.

Lead

The US EPA study (US EPA 2007a) identified that the IVBA[3] in vitro methodology was able to accurately predict lead RBA for a wide range of lead species found typically at mining and milling sites.

Lead RBA (%) = 0.878 x IVBA (%) – 0.028 *Equation [5]*

The use of this equation will yield 'typical' RBA values which may vary (either higher or lower) than values obtained from in vivo analysis. However, the reliability of the methodology for predicting lead RBA of other lead species found in other soils is questionable as highlighted by the study of Juhasz et al. (2009b) and Marschner et al. (2006). This uncertainty should be considered if the methodology is applied to soils dissimilar to those in US EPA (2007a). In addition, the IVBA method was found to be unsuitable for the assessment of lead bioaccessibility in phosphate amended soils as excess phosphate in the sample medium caused interferences (US EPA 2008).

Organic contaminants

A number of in vitro methodologies have been developed for the determination of organic contaminant bioaccessibility, however the correlation between bioaccessibility and RBA is lacking or limited information is available which precludes confidence in the determination of in vivo-in vitro relationships. Consequently, in vitro assays should not be used as a surrogate assay for predicting relative bioavailability for organic contaminants.

[3] The IVBA methodology is also referred to the Solubility Bioaccessibility Research Consortium (SBRC) gastric phase or the Simplified Bioaccessibility Extraction Test (SBET).

Using bioavailability and bioaccessibility data for risk characterisation

Figure 1 illustrates when and how bioavailability and bioaccessibility can be incorporated into the risk assessment framework for the incidental soil ingestion exposure pathway. The recommended decision framework is intended for the collection of data to inform site-specific risk-based decisions. The decision framework prevents the use of invalidated models for site-specific risk assessment.

The < 250 µm soil particle size fraction is used for RBA and bioaccessibility assessment as this particle size fraction is representative of that which adheres to the hands of young children (Ruby et al. 1996). Following drying at 40°C, the < 250 µm soil particle size fraction may be obtained by sieving soil through a 60 mesh stainless steel screen. Samples should be thoroughly mixed prior to RBA and bioaccessibility assessment to ensure sample homogeneity.

RBA may be determined using the following methodologies or other in vivo methodologies that are deemed suitable for the contaminant of interest:

- Guidance for evaluating the oral bioavailability of metals in soils for use in human health risk assessment (US EPA 2007b)
- Principles and application of an in vivo swine assay for the determination of arsenic bioavailability in contaminated matrices (Rees et al. 2009).

Arsenic and lead RBA may be determined by assessing their bioaccessibility in contaminated soil using the gastric phase of the SBRC assay (Juhasz et al. 2007, 2009a, 2009b; US EPA 2007a) and incorporating bioaccessibility data into Equations [3] and [5]. For quality assurance and quality control, it is recommended that a reagent blank, bottle blank, blank spike, duplicate sample and a control soil (NIST 2711) be included in the bioaccessibility procedure at a frequency of 1 in 20 samples (minimum 1 per batch).

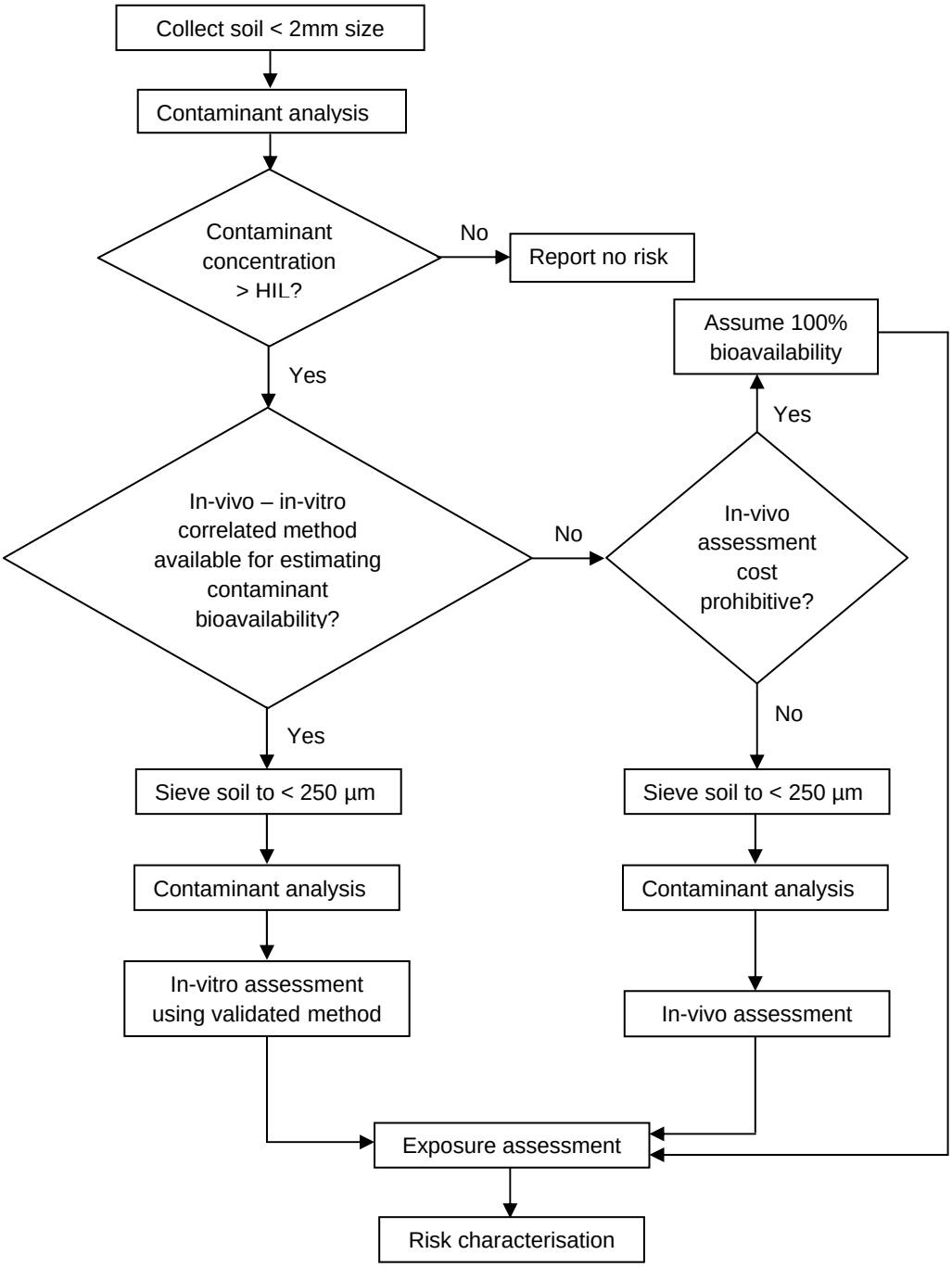

Figure 1. Schematic diagram for the determination of contaminant bioavailability and bioaccessibility for human health risk assessment.

References

Basta, NT, Foster, JN, Dayton, EA, Rodriguez, RR & Casteel, SW 2007, 'The effect of dosing vehicle on arsenic bioaccessibility in smelter-contaminated soils', *Journal of Environmental Science and Health Part A* 42: pp. 1275-1281.

Drexler, JW & Brattin, WJ 2007, 'An in vitro procedure for estimation of lead relative bioavailability: with validation', *Human and Ecological Risk Assessment* vol. 13, iss. 2, pp. 383-401.

Juhasz, AL, Smith, E, Weber, J, Rees, M, Rofe, A, Kuchel, T, Sansom, L & Naidu, R 2007, 'Comparison of in vivo and in vitro methodologies for the assessment of arsenic bioavailability in contaminated soils', *Chemosphere* vol. 69, iss. 6, pp. 961-966.

Juhasz, AL, Smith, E, Weber, J, Naidu, R, Rees, M, Rofe, A, Kuchel, T & Sansom, L 2009a, 'Assessment of four commonly employed in vitro arsenic bioaccessibility assays for predicting in vivo arsenic bioavailability in contaminated soils', *Environmental Science and Technology* vol. 43, no. 24, pp. 9487-9494.

Juhasz, AL, Smith, E, Weber, J, Naidu, R, Rees, M, Rofe, A, Kuchel, T & Sansom, L 2009b, 'Evaluation of SBRC-gastric and SBRC-intestinal methods for the prediction of in vivo relative lead bioavailability in contaminated soils', *Environmental Science and Technology* vol. 43, no. 12, pp. 4503-4509.

Marschner, B, Welge, P, Hack, A, Wittsiepe, J & Wilhelm, M 2006 'Comparison of soil Pb in vitro bioaccessibility and in vivo bioavailability with Pb pools from a sequential soil extraction', *Environmental Science and Technology* vol. 40, no. 8, pp. 2812-2818.

Ng, J, Juhasz, A. L, Smith, E and Naidu, R 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.

NRC (National Research Council) 2003, *Bioavailability of contaminants in soils and sediments: Processes, tools and applications,* The National Academies Press, Washington, DC.

Rees, M, Sansom, L, Rofe, A, Juhasz, AL, Smith, E, Weber, J, Naidu, R & Kuchel, T 2009, 'Principles and application of an in vivo swine assay for the determination of arsenic bioavailability in contaminated matrices', *Environmental Geochemistry and Health* vol. 31, suppl. 1, pp. 167-177.

Rodriguez, R, Basta, NT, Casteel, SW & Pace, LW 1999, 'An in vitro gastrointestinal method to estimate bioavailable arsenic in contaminated soil and solid media', *Environmental Science and Technology* vol. 33, no. 4, pp. 642-649.

Ruby, MV, Davis, A, Schoof, R, Eberle, S & Sellstone, CM 1996, 'Estimation of lead and arsenic bioavailability using a physiologically based extraction test', *Environmental Science and Technology* vol. 30, iss. 2, pp. 422-430.

Schroder, JL, Basta, NT, Casteel, SW, Evans, TJ, Payton, ME & Si, J 2004, 'Validation of the in vitro gastrointestinal (IVG) method to estimate relative bioavailable lead in contaminated soils', *Journal of Environmental Quality* vol. 33, pp. 513-521.

US EPA 2007a, *Estimation of relative bioavailability of lead in soil and soil-like materials using in vivo and in vitro methods,* US EPA OSWER 9285.7-77, Office of Solid Waste and Emergency Response, US Environmental Protection Agency, Washington, DC 20460.

US EPA 2007b, *Guidance for evaluating the oral bioavailability of metals in soils for use in human health risk assessment,* US EPA OSWER 9285.7-80, Office of Solid Waste and Emergency Response, US Environmental Protection Agency, Washington, DC 20460.

US EPA 2008, *Standard operating procedure for an in vitro bioaccessibility assay for lead in soil,* EPA 9200.1-86, Office of Solid Waste and Emergency Response, US Environmental Protection Agency, Washington, DC 20460.

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