Checklist of minimum criteria for *in vivo* bioavailability studies

Criteria	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)[1], and which can be used as a starting point.
Unless otherwise justified, soil is sieved to the less than 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 discrepancy between particle size, which is defined by a particular sieve size, and the mean particle size that adheres to human hands, which is the best representation of the soil fraction that is ingested through hand to mouth contact and is relevant for risk assessment. In some soil samples, the <250 µm fraction can have *mean* particle sizes (19-42 µm) (Ruby et al 1996)[2] within the range found to adhere to hands (e.g., 34-105 µm) (Siciliano et al 2009)[3]. In these cases, using a small particle size fraction, like <45 µm, would probably result in a much smaller mean particle size in the sample that does not represent that of concern.

[1]Koch, I., Reimer, K.J. "Bioaccessibility Extractions for Contaminant Risk Assessment." In Comprehensive Sampling and Sample Preparation Volume 3; Pawliszyn, J.; Le, X. C.; Li, X-F.; Lee, H. K.; Eds; Elsevier, Academic Press: Oxford, UK, pp 487–507, 2012.

[2] Ruby, M. V.; Davis, A.; Schoof, R.; Eberle, S.; Sellstone, C. M. Environ.Sci.Technol. 1996, 30, 422-430.

[3] Siciliano, S. D.; James, K.; Zhang, G. Y.; Schafer, A. N.; Peak, J. D. Environ.Sci.Technol. 2009, 43, 6385-6390.

Criteria	Supplementary information
Justification is provided for the choice of animal model.	For inorganic contaminants, mouse, rabbits, dogs, swine and primates have all been used as animal models. The pros and cons of different animal models are discussed in the discussion paper C*onsiderations for bioavailability testing* by Casteel and Koch 2010, and *CRCCARE technical report no.14* by Ng et al 2010. Juvenile swine is the most well developed model; it is considered the model of choice for the assessment of soil contaminant bioavailability in human children because of the similarities with the human gastro-intestinal physiology and weight of a young child. BARC 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 toxicity/ toxicological reference value (TRV) is based, and a different animal model was used (e.g., rats).
It is demonstrated, possibly through a preliminary pilot test, that the dosing regime 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 contaminant) against the dose given to the animal, with a minimum of 3 doses. For contaminants where dose dependency may be an issue, the bioavailability study design must be justified; in most cases a minimum of three doses will be required. If bioavailability results are to be compared with bioaccessibility results, consideration should be given to the range of test concentrations and how it relates to the range of relevant bioaccessibility concentrations. For example, bioaccessibility testing may be considered to be irrelevant for very high concentrations of contaminants, especially if characteristic percent bioaccessibilities are very high. In such cases, concentration ranges in soils used for bioavailability testing should be restricted to lower values where bioaccessibility testing gives results that are useful for risk assessment. In all cases, dosing regimes 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 contaminant bioavailability are blood, urine, feces, and organs such as the kidney and liver.

Criteria	Supplementary information
Positive controls in the in-vivo study mimic the positive controls used in the critical toxicity study as closely as possible.	Absorption of some chemicals may be influenced by factors such as fasting or non-fasting conditions, the form of the chemical used, and toxicity endpoint measured (Casteel and Koch 2010, Ng et al 2010, Environ 2010). For acceptable correlations and meaningful incorporation of test results in the risk assessment process, the dosing regime, reference material and biological endpoint used in the bioavailability study should closely match the controls used in the toxicity study. For contaminants of interest for risk assessment in Canada, Health Canada (2010)[4] publishes a summary of toxicity study conditions, and components of these studies should be considered when matching positive controls to the toxicity study. Justification should be given for the choice of positive control used in the bioavailability study, especially for deviations from conditions in the toxicity study.
RBA along with the RBA uncertainty is reported.	A worked example of how RBA uncertainty was calculated should be provided. See protocol developed by Stan Casteel for how to correctly calculate and report RBA uncertainty in C*onsiderations for bioavailability testin*g. If funds 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 (ACC) in accordance with the Canadian Council on Animal care (CCAC), and conducted by a laboratory experienced in animal testing.	The website for CCAC is http://www.ccac.ca/en_/assessment.

[4] Health Canada, Contaminated Sites Division, 2010. Federal Contaminated Site Risk Assessment in Canada, Part II: Health Canada Toxicological Reference Values (TRVs) and Chemical-Specific Factors, Version 2.0, pp 1-69, see Appendix A.