Checklist of minimum criteria for *in vitro* bioaccessibility tests

Criteria	Supplementary information
Use RBALP (also known as SBRC or SBET), PBET, IVG, or UBM to study bioaccessibility of As and 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 consistent with *in vivo* results for these soils for As and Pb, and for some methods, for Cd (e.g., Table 2 in Koch and Reimer 2012)[1]. The analysis of the extract should be carried out by an accredited laboratory if possible. *SOPs for the four methods are available*.
Unless otherwise justified, soil is sieved to the less than 250 µm particle size fraction.	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. The effect of particle size (obtained by sieving) on bioaccessibility has been tested in a few studies, but reports are conflicting and number of analytes and samples are limited in most cases.**Error! Bookmark not defined.**[4] In circumstances where 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.

[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.

[4] Morman, S. A.; Plumlee, G. S.; Smith, D. B. *Appl.Geochem.* 2009, 24, 1454-1463; Morrison, A. L.; Gulson, B. L. Sci.Total Environ. 2007, 382, 30-42; Smith, E.; Weber, J.; Juhasz, A. L. Environ.Geochem.Health 2009, 31, 85-92;

Criteria	Supplementary information
Ensure method is free of saturation effects and addresses established guidelines.	Draft guidelines that may be considered were published in 2009 by Health Canada, Contaminated Sites Division, Safe Environments Programme, Federal Contaminated Site Risk Assessment in Canada Part V: Guidance On Complex Human Health Detailed Quantitative Risk Assessment For Chemicals (DQRACHEM). Agitation must be adequate to ensure good contact of test material with the solution (see *Comparison of Mixing Parameters* by L. Meunier, available on request, 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. For As bioaccessibility, the literature reports the robustness of the PBET and IVG methods to saturation effects.[5,6] For the RBALP method, a discussion of when saturation effects might occur for Pb can be found in Drexler and Brattin 2007.[7]
Totals and subsamples are run in triplicate (depending on the number of samples).	At least 10% of the samples for both the totals analysis and bioaccessibility analysis should be analyzed in duplicate or triplicate with the number of replicates decided by discussions between the laboratory conducting the bioaccessibility testing and the users of the data. If the number of samples is less than 5, all samples should be analyzed in triplicate. The method used to analyze for total contaminant concentrations for the bioaccessibility test should also be used to analyze total contaminant concentrations for the rest of the site.
Spike or reference compound is related to the TRV used in the risk assessment.	Communications must take place prior to any bioaccessibility testing between the testing laboratory and the data user to confirm the TRV that is being used in the risk assessment, so that an appropriate spike can be incorporated into the bioaccessibility testing. For contaminants of interest for risk assessment in Canada, Health Canada (2010)[8] publishes a summary of toxicity study conditions, and components of these studies should be considered when matching an appropriate spike to the toxicity study.

Shock, S. S.; Bessinger, B. A.; Lowney, Y. W.; Clark, J. L. Environ.Sci.Technol. 2007, 41, 4813-4820; Madrid, F.; Biasioli, M.; jmone-Marsan, F. Arch.Environ.Contam.Toxicol. 2008, 55, 21-32.

[5] Meunier, L.; Wragg, J.; Koch, I.; Reimer, K. J. J.Environ.Sci.Health Part A-Tox./Haz.Sub.Environ.Eng. 2010, 45, 517-526.

[6] Makris, K. C.; Quazi, S.; Nagar, R.; Sarkar, D.; Datta, R.; Sylvia, V. L. Environ.Sci.Technol. 2008, 42, 6278-6284.

[7] Drexler, J. W.; Brattin, W. J. Hum.Ecolog.Risk Assess. 2007, 13, 383-401.

[8] 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.

Criteria	Supplementary information
Other quality control testing included in each bioaccessibility testing batch are a blank and a control sample, preferably one that has certified bioaccessibility values.	Batch sizes should be no more than 10 samples. A blank consists of all reagents except for samples carried through the entire bioaccessibility test. Control samples with certified values may available from NIST. In the absence of certified reference materials, laboratory-established control limits should be used. If such limits are not available for the contaminant being tested, an alternate quality control measure could include analyzing for other elements for which control limits are available.