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Variability of bioaccessibility results using seventeen different methods on a standard reference material, NIST 2710

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Bioaccessibility is a measurement of a substance's solubility in the human gastro-intestinal system, and is often used in the risk assessment of soils. The present study was designed to determine the variability among laboratories using different methods to measure the bioaccessibility of 24 inorganic contaminants in one standardized soil sample, the standard reference material NIST 2710. Fourteen laboratories used a total of 17 bioaccessibility extraction methods. The variability between methods was assessed by calculating the reproducibility relative standard deviations (RSDs), where reproducibility is the sum of within-laboratory and between-laboratory variability. Whereas within-laboratory repeatability was usually better than (<) 15% for most elements, reproducibility RSDs were much higher, indicating more variability, although for many elements they were comparable to typical uncertainties (e.g., 30% in commercial laboratories). For five trace elements of interest, reproducibility RSDs were: arsenic (As), 22–44%; cadmium (Cd), 11–41%; Cu, 15–30%; lead (Pb), 45–83%; and Zn, 18–56%. Only one method variable, pH, was found to correlate significantly with bioaccessibility for aluminum (Al), Cd, copper (Cu), manganese (Mn), Pb and zinc (Zn) but other method variables could not be examined systematically because of the study design. When bioaccessibility results were directly compared with bioavailability results for As (swine and mouse) and Pb (swine), four methods returned results within uncertainty ranges for both elements: two that were defined as simpler (gastric phase only, limited chemicals) and two were more complex (gastric + intestinal phases, with a mixture of chemicals).

Keywords: Bioaccessibility, variability, multiple methods, NIST 2710.

Introduction

Regulations and environmental criteria used in contaminated site assessment and associated remediation are often based on minimizing human health risks. In most cases, the total concentration of a target substance in a particular

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substrate (e.g., soil, sediment or water) has been used to derive the regulations and environmental criteria, for example, in Canada.[1] For soil, this leads to a default assumption that 100% of a contaminant is bioavailable, where bioavailability is the extent to which a substance is absorbed by an organism into its systemic circulation and can potentially cause an adverse effect.[2] Although chemicals that are dissolved in water may be readily available for uptake by various organisms, this may not be the case for soils and sediment where the contaminant may be tightly bound to the soil matrix. *In vivo* (usually animal) studies are used to measure bioavailability but less costly laboratory (*in vitro*) tests have been developed to model exposure in the gastrointestinal environment; the amount of the contaminant that dissolves in such tests is referred to as the bioaccessible portion. Bioaccessibility is often used as a surrogate for bioavailability, which can be used in site-specific risk assessments.

The approach to measuring bioaccessibility in at least two jurisdictions includes a focus on a single specific method. The United States Environmental Protection Agency (US EPA) is one such jurisdiction and has published guidance on the use of an "*In Vitro* Bioaccessibility Assay for Lead in Soil,"[3,4] which has been assessed as a "reliable predictor of *in vivo* relative bioavailability of lead (Pb) in a wide range of soil types and lead phases from a variety of different sites."[4] In Europe, the Bioaccessibility Research Group of Europe (BARGE) has developed a single method, the "Unified BARGE Method" (UBM). The UBM is available as a "validated" methodology based on *in vivo* comparisons to swine for arsenic (As), cadmium (Cd), and Pb (http://www.bgs.ac.uk/barge/ubm.html).[5]

In Canada, an international research consortium, partly supported by the Contaminated Sites Division of Health Canada, named Bioaccessibility Research Canada (BARC) exists to help provide a scientific basis for evaluating and predicting inorganic and organic contaminant bioaccessibility and/or bioavailability in soils found at contaminated sites in Canada. The approach of this group is not to focus on a single bioaccessibility method but to develop guidance for the selection of a method and the appropriate application of bioaccessibility results to risk assessment.

BARC identified that a number of methods have been developed independently over the years and are being used by Canadian laboratories for risk assessment purposes, and thus there was a need for a review and comparison of soil bioaccessibility measurement methods. Several reviews are available (e.g.,[6,7]) and comparative studies between different research groups and methods have focused on one or more of four elements in soils (As, Cd, nickel (Ni) and Pb).[8,9,10,11] To compare methods on a larger scale – within Canada and internationally – and for a larger number of elements, a round-robin study was undertaken to compare soil bioaccessibility data obtained by Canadian and international laboratories using a standard reference material (SRM). The main objective was to quantify the variability surrounding such measurements when different methods are used. A comparison of the results with *in vivo* relative bioavailability (RBA) data for the SRM was also undertaken.

Materials and methods

Study design

The study design, summarized in Figure 1, was determined by consensus of participants (listed as co-authors). The methods and conditions were those used at the time in each participating laboratory; changes have since been made to some methods such as the UBM.[5] Elements of interest were those that the participating laboratories routinely analyze, with As and Pb being the most common among individual laboratories and considered (by consensus) to be of greatest relevance,[12] Additionally, an expanded suite of elements routinely analyzed at the central laboratory was included to obtain bioaccessibility data for elements not commonly reported (e.g., copper (Cu), zinc (Zn), strontium (Sr), barium (Ba), etc.). The chosen soil was the SRM produced by the National Institute of Standards and Technology (NIST), Montana High, number 2710 (hereafter referred to as NIST 2710) (particle size <74 μm). This soil was chosen because of its high concentrations of many elements (including As, Pb, Cu, and Zn) and because a large stock of the soil was readily available for the study.

The number of replicates was five for NIST 2710 and three each of a mixed spiking solution for blanks and extraction blanks. Sample preservation was a dilution (1:10) of the sample extract with 0.1 M HNO_3 (environmental grade) prior to shipping, along with storage at 0–8°C following extraction and prior to analysis. To minimize uncertainty in results, analysis of all bioaccessibility extracts was at a single commercial accredited laboratory (hereafter referred to as the central laboratory) within 48 h of receiving the sample extracts. In addition, 12 of 14 participating laboratories conducted independent chemical analysis of the extracts in-house or at other commercial accredited laboratories.

Materials

SRM 2710, Montana High was sourced from NIST. All subsamples were obtained from a single bottle, which was opened from the sealed state (as sent by NIST), measured out for all laboratories at once, sealed into 5 mL plastic containers and shipped to each participating laboratory. No other sample manipulation was performed.

Spiking solutions were made up by diluting three certified inductively coupled plasma (ICP) standard solutions of mixed elements (1000 mg/L each element, 33 mL of each solution) (C00-063-306, C00-063-308 and C00-063-309

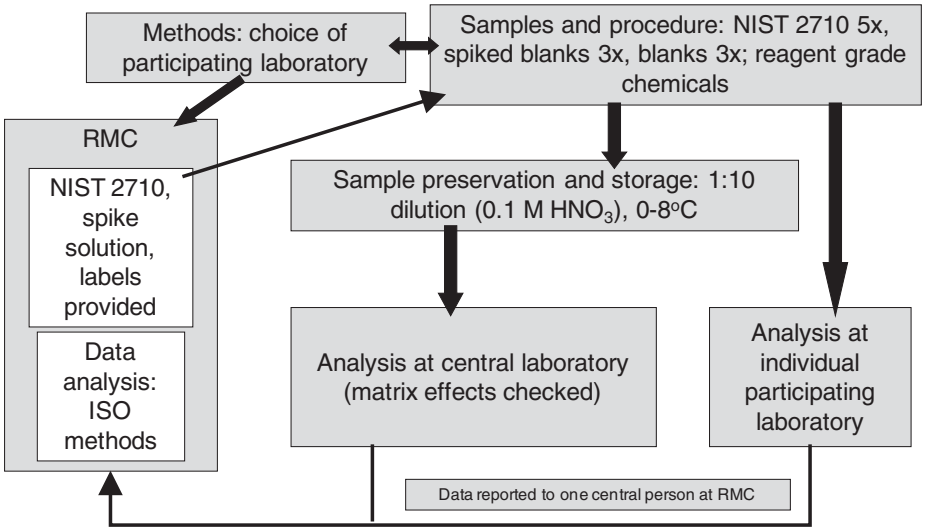


Fig. 1. Experimental design of the round-robin study. Analysis at individual participating laboratories occurred in 12 of the 14 participating laboratories. RMC = Royal Military College, where all data analysis took place and to whom methods were provided. The central laboratory was ASU, the Analytical Services Unit (see text for details). NIST = National Institute of Standards and Technology; ISO = International Standards Organization.

from SCP Science) to obtain a mixture of aluminum (Al), As, boron (B), Ba, Cd, cobalt (Co), chromium (Cr), iron (Fe), manganese (Mn), Ni, Pb, selenium (Se), Sr, vanadium (V), and Zn. The spiking concentrations in the final bioaccessibility extracts were 250 μg/L (based on the concentration of As in the final diluted extract of NIST 2710, using a mid-range percent bioaccessibility estimate). Cu was not included in the spiking solutions because the available ICP solutions did not include it; nevertheless calculations of this element in the NIST 2710 were carried out.

Bioaccessibility methods

The laboratories in the BARC round-robin study and their method(s) of choice are summarized in Table 1. Similarities in methods allowed similar abbreviations to be used in some cases (e.g., RBALP[1], PBET), but only one method (RBALP) was carried out by more than one laboratory; all other methods, even with similar details (PBET), differed by one or more chemicals and/or chemical concentrations. Methods were loosely generalized as simple (Simp) or complex (Comp), based on the differentiation between methods that had simpler steps (e.g., one phase, fewer chemicals, such as the Health Canada, HC, method) and more complex steps (e.g., two or more phases, many chemicals, such as the UBM) based on human physiological conditions. The HC method has been developed for house dust research [13,14] and is not a standard protocol for regulatory purposes. Anonymity has been preserved for two laboratories, and a total of 17 methods in 14 laboratories were tested, with saliva, gastric, intestinal and colon phases all represented. All methods model the fasted state. The methods, including analysis of bioaccessibility extracts in individual participating laboratories, are detailed in the Supporting Information.

Bioaccessibility extract analysis at the central laboratory

The Analytical Services Unit (ASU) of Queen's University (Kingston, ON) is the Canadian Association for Laboratory Accreditation (CALA)-accredited central laboratory that analysed the bioaccessibility extract samples. Each sample was clearly labelled and stored in a secured area (before and after analysis) in a refrigerator at 4°C. Extracts were analyzed directly for silver (Ag), Al, As, B, Ba, beryllium (Be), Cd, Co, Cr, Cu, Fe, Mn, molybdenum (Mo), Ni, Pb, antimony (Sb), Se, tin (Sn), Sr, titanium (Ti), thallium (Tl), uranium (U), V, and Zn using a Varian VISTA AX CCD Simultaneous ICP-AES. Sample analysis batches of 11–22 samples included a minimum of one instrumental blank, two instrumental duplicates (one for each phase), and one calibration check.

Where blank sample volume was sufficient, a matrix spike was prepared with the blank sample (seven methods), using eight elements of interest (As, Cd, Co, Cr, Cu, Ni, Pb and Zn). For duplicate analyses of extracts, the majority of RSDs were less than 10% (a maximum relative standard deviation (RSD) of 39% was obtained for one element, Sr).

[1]The Relative Bioavailability Leaching Procedure (RBALP) is also referred to as the Solubility and Bioavailability Research Consortium (SBRC) method, the Simple Bioaccessibility Extraction Test (SBET), and the *In Vitro* Bioaccessibility Assay (IVBA) for Pb in soil.

Table 1. Summary of bioaccessibility methods.

Lab ID	*Method abbreviation*	*General*	*Volume: mass*	*pH*	*Brief details*
OSU	IVG	Comp	150:1	1.8, 6.1	Simple gastric (10 g/L pepsin, 0.1M salt), intestinal (3.75 g/L bile extract and pancreatin)
EPM	IVG	Comp	150:1	1.8, 5.5	Simple gastric (10 g/L pepsin, 0.15M salt), intestinal (3.3 g/L bile extract, 0.3 g/L pancreatin)
RRU	PBET	Comp	100:1	2, 7	Gastric (1.25 g/L pepsin, 0.5 g/L citrate and malate, 0.42 mL/L lactic acid, 0.5 mL/L acetic acid), intestinal (1.75 g/L bile extract, 0.5 g/L pancreatin)
RMC	PBET	Comp	100:1	1.8, 7	Gastric (1.25 g/L pepsin, 0.5 g/L citrate and malate, 0.5 mL/L acetic acid, 0.15 M NaCl), intestinal (1.75 g/L bile extract, 0.5 g/L pancreatin)
UG	PBET	Comp	100:1	2.5, 7	Gastric (0.12 g/L pepsin, 0.5 g/L citrate and malate, 0.42 mL/L lactic acid, 0.5 mL/L acetic acid), intestinal (0.36 g/L bile extract, 0.03 g/L pancreatin, buffers and 9 g/L BSA)
SFU	PBET	Comp	100:1	2.5, 7	Gastric (0.13 mM pepsin, 0.5 g/L citrate and malate, 0.42 mL/L lactic acid, 0.5 mL/L acetic acid), intestinal (0.36 g/L bile extract, 0.03 g/L pancreatin, buffers and 0.9 g/L BSA)
BGS	PBET	Comp	100:1	2.5, 7	Gastric (1.25 g/L pepsin, 0.5 g/L citrate and malate, 0.42 mL/L lactic acid, 0.5 mL/L acetic acid), intestinal (1.75 g/L bile extract, 0.5 g/L pancreatin)
US	SHIME	Comp	40:1, 60:1, 100:1	1.5, 6.2, ca 7	Gastric (0.033 g/L pepsin, 0.04% glycerol), intestinal (complex), colon (micro-organisms)
RIVM	RIVM	Comp	1000:1	1.5, 6	Saliva + gastric (complex mixture), intestinal (complex duodenal +bile fluids)
BGS	UBM	Comp	40:1, 60:1	1.2–1.4, 6.3	Same as RIVM
RRU	RBALP	Simp	100:1	1.5	0.4 M Glycine
Lab A	RBALP	Simp	100:1	1.5	0.4 M Glycine
Lab B	RBALP	Simp	100:1	1.5	0.4 M Glycine
Exponent	Gly	Simp	100:1	2.5	0.4 M Glycine
Exponent	Phos	Simp	100:1	2.5	0.4 M Phosphate
SGS	Mod TCLP	Simp	20:1	1	0.17M HCl 0pt1,177.9pt
HC	HC	Simp	2000:1	1.5	0.07 M HCl

Simp = simple; Comp = complex; denotion of mixtures as "complex" in details column indicates that supporting information should be consulted. Where two or more pHs are listed, two or more phases were represented. BSA = bovine serum albumin; other abbreviations can be found in text.

Reagent blank results were less than the instrumental reporting limits (Al, B, Fe, Se = 0.05 mg/L; As = 0.015 mg/L; Ba, Co, Cr, Cu, Sr = 0.01 mg/L; Cd = 0.002 mg/L; Mn, Ni, Pb, V = 0.005 mg/L; Zn = 0.02 mg/L) except for a single result (Fe at 0.12 mg/L). Percent recoveries for calibration checks ranged from 89 to 107% (average ± standard deviation 99 ± 3%). Matrix spike recoveries were in a similar range (86–112%, average ± standard deviation 101 ± 5%).

Statistical analysis

All data were analyzed at the Royal Military College of Canada (RMC). Bioaccessibility extract concentrations provided by the central laboratory were used along with masses and volumes provided by each participating laboratory to calculate bioaccessible element concentrations (mg/kg) and percent bioaccessibility (bioaccessible element concentration/total certified element concentration × 100%). Data from individual laboratories were provided as bioaccessible element concentrations and total element concentrations, along with percent bioaccessibility in most cases. Most statistical calculations were carried out in Microsoft® Excel, using equations in ISO 5625-2,[15] similar to the approach in Wragg et al.[11]

The ISO document should be consulted for details but the various parameters are simplified as follows: the general mean can be thought of as the mean of laboratory means. The repeatability standard deviation can be thought of as the mean of laboratory standard deviations. The reproducibility standard deviation is the most complicated statistic, as the sum of repeatability variance and between-laboratory variance, but it is similar to the standard deviation of laboratory means. The above descriptions are not exact; for example the calculations allow for changes to the number of data points, such as when points are removed as outliers. In all cases means are arithmetic, and all percent RSDs were calculated with respect to the general mean.

Analysis of variance (ANOVA) procedures were applied to the data using Systat® 10. Results from one laboratory (École Polytechnique de Montréal, IVG method) were excluded because the results obtained were outside the range of results normally obtained and published by the laboratory (personal communication with G.J. Zagury, 2008).[16,17] Within individual laboratories, the Grubb's test was applied to data where percent RSD values were more than 20%, and outliers were excluded according to guidance in ISO 5625-2. For NIST 2710 calculations, when fewer than three values in a set of five were detectable, no calculations were carried out for that set. When three or more values were detectable, the non-detectable values were replaced with half the detection limit.[18]

Results and discussion

In the following discussion of results, the terminology "gastric phase" will be used to denote the bioaccessibility extraction phase representing the stomach, and "gastric + intestinal phase" will be used for the phase that includes both sequential stomach and intestine steps, where results are obtained from the extract produced at the end of the sequence. For two methods (BGS UBM and RIVM method) the gastric phase was preceded by a saliva phase, and the gastric + intestinal phase includes sequential saliva, gastric and intestine phases, but saliva was never analyzed as its own phase. The US SHIME method also has a "colon" phase, which includes sequential gastric, intestine and colon phases.

Quality control results: Blanks and spikes

Blank results were for the most part lower than the instrumental detection limits provided by the central laboratory, with overall only 12% of all average blank values detectable (results and a more detailed discussion are given in Supporting Information). Cu and Zn were the most commonly detected elements, but they are not commonly assessed for bioaccessibility and likely laboratories did not control for these elements. Pb contamination was also found in 7 (of 27) methods (see Supporting Information), but no As or Co concentrations were above the detection limits in any extracts. When extracts were analyzed in individual laboratories, detectable results were obtained in blank solutions at a frequency of 15%, and as for the central laboratory analysis, Pb, Zn, and Cu were most commonly detected.

Spike recoveries generally straddled 100% (results are tabulated in the Supporting Information), with a slightly high bias overall (61% of all spike recoveries were greater than 100%). In the gastric + intestinal and the colon phases 65% of recoveries were less than 100%, and this was thought to be attributable to the higher pH conditions in these phases, since at higher pH some cationic species may be removed from solution. For example, Pb and Cd solubilities are strongly pH dependent: they have higher solubility in acidic conditions but their complexation by biomolecules or chemical precipitation in a higher pH environment (such as in intestinal fluid) has been proposed.[19] All general mean results for gastric and gastric + intestinal phases were in the range of 70–130%. The results reported for spike samples by the individual laboratories were similar to those obtained when the central laboratory was used.

Variability in the bioaccessibility results for NIST 2710

One of the main objectives of the round-robin experiment was to assess the variability in bioaccessibility results when different methods are used. The variability was determined using statistical analysis methods described in ISO 5725–2,[15] modeled on the approach that is applied to trials of a single method used in different laboratories. Although a single method was not used in the present study, the objective was to determine the variability between methods and thus they were considered together. The method involves calculating variances, standard deviations and RSDs for repeatability (within-laboratory) and reproducibility (between-laboratory) scenarios, and percent RSDs obtained in this way are used as measurements of variability.

Lower values of percent RSDs indicate better repeatability or reproducibility.

Bioaccessibility results can be reported as bioaccessible concentrations in mg/kg soil, or percent bioaccessibility (the concept of relative bioaccessibility will be described more fully later). For percent bioaccessibility, a total soil concentration for each element is incorporated into the calculation. For the calculation of percent bioaccessibility from the central laboratory analysis of extracts, the certified or guidance concentrations were used (for convenience, values are provided in Table 2, and more information is available from NIST[20]). This resulted in the same variances, standard deviations and RSDs for bioaccessible concentrations and percent bioaccessibility; therefore only results for percent bioaccessibility are presented (Table 2). Individual laboratories measured total concentrations of elements using their own methods and these values were used to calculate percent bioaccessibility from individual laboratory results.

Table 2. General means (percent bioaccessibility, %BA), repeatability and reproducibility percent relative standard deviations (% RSDs) obtained from a single central laboratory.

Parameter	*Al*	*As*	*Ba*	*Cd*	*Co*	*Cr*	*Cu*	*Fe*	*Mn*	*Ni*	*Pb*	*Sr*	*V*	*Zn*
Concentrations (mg/kg) in NIST 2710	64,400	626	707	21.8	10	39	2950	33,800	10,100	14.3	5532	330	76.6	6952
Commercial Laboratory – Gastric														
# Methods[a]	15	15	14	15	1	2	15	15	15	5	15	15	12	15
General mean (% BA)	1.6	42	15	73	36	38	63	5.4	24	23	59	12	12	28
% RSD (repeatability)	7.1	4.7	13	8.8	1.5	3.7	6.8	5.2	6.3	37	6.1	8.5	14	6.7
% RSD (reproducibility)	50	41	95	18	na	126	25	55	32	70	45	31	34	23
Minimum (% BA)	0.56	8.8	nd	27	nd	nd	31	0.85	12	nd	13	3.6	nd	15
Maximum (% BA)	3.4	70	60	107	37	74	90	11	41	51	101	20	25	40
Commercial Laboratory – Gastric + intestinal														
# Methods[a]	8	10	8	9	0	0	10	9	10	1	10	10	2	10
General mean (% BA)	1.2	31	3.2	45	nd	nd	50	4.4	16	62	17	12	6.1	13
% RSD (repeatability)	4.6	7.1	18	15	nd	nd	6.7	6.6	6.6	93	7.4	22	24	8.7
% RSD (reproducibility)	31	44	78	38	nd	nd	24	46	38	na	83	113	27	56
Minimum (% BA)	nd	2.8	nd	nd	nd	nd	20	nd	4.9	nd	0.44	2.8	nd	0.32
Maximum (% BA)	1.9	59	10	90	nd	nd	73	6.7	30	150	51	60	7.6	25

[a]With detectable results. na = not applicable for calculation of variance because only one laboratory had detectable results; nd = not detectable (see supporting information for detection limits for different methods).

Consequently, the variances, standard deviations and RSDs differed for bioaccessible concentration and percent bioaccessibility; thus both sets of results are presented (Table 3). Within-laboratory repeatability (related to intralaboratory precision) was based on the repeatability obtained by each laboratory from five replicate extractions of NIST 2710. For results obtained in the central laboratory, the RSDs were less than 15% for 21 out of 26 (81%) results (all phases combined, Table 2). Repeatability RSDs were generally better (smaller values) for individual laboratory results. For example, RSDs were less than 5% for approximately 66% of results and less than 10% for almost 90% of the results for individual laboratories (Table 3). In all cases, the RSDs were better in the gastric phase than in the gastric + intestinal phase. For individual laboratories, RSDs were also slightly lower for bioaccessible concentrations compared with percent bioaccessibility (Table 3).

The data suggest that, not surprisingly, incorporation of the total concentration (as measured by each laboratory) into the calculation (i.e., percent bioaccessibility) increased the within-laboratory variability. The overall reproducibility (the sum of within-laboratory repeatability and between-laboratory variation, related to interlaboratory precision) was compromised by low numbers of detectable values for some elements, specifically for Cr and Ni in the gastric phase. Excluding these elements, the least reproducible elements (central laboratory results, largest percent RSD reproducibility in Table 2) were Ba and Fe in the gastric phase (Ba 95%, Fe 55%), and Sr, Pb and Ba in the gastric + intestinal phase (Sr 112%, Pb 83%, Ba 78%).

For the main trace elements of interest, the percent reproducibility values in the gastric phase were as follows: As 41%; Cd 18%; Cu 25%; Pb 45%; and Zn 23%. In the gastric + intestinal phase the percent reproducibility values were as follows: As 44%; Cd 38%; Cu 24%; Pb 83%; and Zn 56%. Notably, Cd, Zn, and especially Pb reproducibilities were worse in the gastric + intestinal phase. This may be attributable to variable losses at the higher pHs in the intestinal phase (lower percent bioaccessibility values) through precipitation or adsorption reactions. This trend was seen in individual laboratory results as well (Table 3).

The percent reproducibility values for the main trace elements of interest are summarized in Table 4 to facilitate comparisons between phases, result type (bioaccessible concentrations and percent bioaccessibility) and result source (individual laboratory or central laboratory results). We hypothesized that the increased sources of variability between laboratories would cause the measured variability to be higher, resulting in increased RSDs for individual laboratory results compared with those obtained in the central laboratory. The opposite trend appears to be true: in nearly all cases, the RSDs obtained were lower in the individual

Table 3. General means (mg/kg and percent bioaccessibility, %BA), repeatability and reproducibility percent relative standard deviations (% RSDs) obtained from individual laboratories.

Parameter	*Al*	*As*	*Ba*	*Cd*	*Co*	*Cr*	*Cu*	*Fe*	*Mn*	*Ni*	*Pb*	*Sr*	*V*	*Zn*
						Individual laboratories, mg/kg – Gastric								
# Methods[a]	2	12	3	10	5	3	6	3	3	2	13	3	2	7
General mean (mg/kg)	1180	280	99	15	2.7	2.1	1950	1890	2340	2.9	3030	42	7.9	1800
% RSD (repeatability)	2.4	3.1	3.5	2.2	2.8	17	2.9	3.5	2.0	2.6	2.9	2.1	4.2	2.0
% RSD (reproducibility)	84	36	81	11	22	78	28	40	28	24	46	30	21	18
Minimum (mg/kg)	470	146	30	12	1.9	0.70	1070	1380	1630	2.3	831	31	6.7	1340
Maximum (mg/kg)	1910	452	195	18	3.6	4.5	2424	2900	3060	3.4	4820	57	9.7	2460
						Individual laboratories, mg/kg – Gastric + intestinal								
# Methods[a]	1	8	2	5	1	1	4	2	3	0	9	2	1	4
General mean (mg/kg)	1410	206	25	10	1.5	1.2	1580	1830	2060	nd	988	32	4	1160
% RSD (repeatability)	2.2	5.1	4.3	18	1.8	8.4	5.1	2.7	6.4	nd	8.4	2.0	7.5	5.5
% RSD (reproducibility)	na	22	54	41	na	na	18	14	28	nd	69	5	na	34
Minimum (mg/kg)	1375	152	14	5	2	1	1259	1591	1562	nd	114	30	4	761
Maximum (mg/kg)	1441	319	37	20	2	1	2027	2046	2939	nd	2614	33	5	1754
						Individual laboratories,% BA – Gastric								
# Methods[a]	1	12	3	10	5	3	6	2	2	2	13	2	2	7
General mean (% BA)	2.5	48	20	76	35	8.6	74	6.3	31	19	61	28	13	30
% RSD (repeatability)	1.9	3.0	3.1	2.2	2.9	13	3.1	3.1	1.1	2.6	3.2	1.5	3.5	2.0
% RSD (reproducibility)	na	38	52	13	34	56	30	31	7.9	24	48	49	12	19
Minimum (% BA)	2.4	23	8.0	58	21	3.0	42	4.9	29	16	20	18	11	22
Maximum (% BA)	2.5	79	28	91	54	15	100	8.2	33	23	106	38	14	41
						Individual laboratories,% BA – Gastric + intestinal								
# Methods[a]	0	8	2	5	1	1	4	1	2	0	9	1	1	4
General mean (% BA)	nd	34	7.6	51	17	4.9	58	5.9	28	nd	19	32	8.7	19
% RSD (repeatability)	nd	5.1	4.2	17	1.8	8.4	5.6	3.5	6.3	nd	11	1.4	7.5	5.7
% RSD (reproducibility)	nd	24	64	38	na	na	17	na	9.4	nd	67	na	na	27
Minimum (% BA)	nd	24	3.8	27	16	4.5	45	5.6	26	nd	2.2	32	8.2	13
Maximum (% BA)	nd	52	12	97	17	5.4	71	6.1	32	nd	49	33	10	27

[a]With detectable results. nd = not detectable (see supporting information for detection limits for different methods); na = not applicable for calculation of variance because only one laboratory had detectable results.

laboratory results, although in many cases, the differences were less than 20%.

One reason for this result may be that the individual laboratories reported only elements that were associated with a high level of control within their laboratory conditions. The inclusion of elements in the larger central laboratory data set that are not normally monitored, and thus may not have been in control in a particular laboratory, may have led to a higher degree of uncertainty. However, the inclusion of total element concentrations into the percent bioaccessibility results for individual laboratories did not result in large differences in between-laboratory reproducibilities compared with those associated with bioaccessible concentrations (mg/kg) and thus it is difficult to discern a trend.

Table 4. Comparison of reproducibility percent relative standard deviation (%RSD) for trace elements of interest obtained by individual laboratories (denoted bioaccessible concentration and percent bioaccessibility) and the commercial laboratory (%RSDs were the same for bioaccessible concentration and percent bioaccessibility).

Percent RSDs (reproducibility),%	*As*	*Cd*	*Cu*	*Pb*	*Zn*
Gastric					
Bioaccessible concentration – individual	36	11	28	46	18
Percent bioaccessibility – individual	38	13	30	48	19
Commercial laboratory	41	18	25	45	23
Gastric + intestinal					
Bioaccessible concentration – individual	22	41	18	69	34
Percent bioaccessibility – individual	24	38	17	67	27
Commercial laboratory	44	38	24	83	56

Bioaccessibility results: General means

The general mean for a given element is the overall mean obtained from all laboratories. They are summarized (as percent bioaccessibility) in Tables 2 and 3, and for ease of comparison, in Figure 2. Percent bioaccessibility results were generally higher when they were provided by the individual laboratories compared with those obtained using the central laboratory and certified values (on average 26% higher). Interestingly, only small differences were seen in the bioaccessible concentration general means (mg/kg, figure not shown), with the individual laboratory results being, on average, only 1.3% lower than the central laboratory results.

This indicates that including the total element concentrations determined by each laboratory, which tended to be lower than the certified values (used to calculate central laboratory percent bioaccessibility results), into the percent bioaccessibility results may introduce a positive bias. Hence for samples with unknown element concentrations, such a bias can be considered conservative, or protective of exposure.

The lowest percent bioaccessibilities were measured for Al (<3.4%) and Fe (<11%), both major elements in the NIST 2710 soil and common major elements in most soils. Their bioaccessibility has not been studied to a great degree, but the present results are comparable to results for road and mine soils,[21] as well as dusts.[21,22] The general means of the two phases for the main trace elements of interest were as follows (central laboratory – individual laboratory results): As 42–48% (gastric) and 31–34% (gastric + intestinal); Cd 73–76% and 45–51%; Cu 63–74% and 50–58%; Pb 59–61% and 17–19%; and Zn 28–30% and 13–19%. The Pb bioaccessibility in this sample is similar to the 60% RBA (relative bioavailability) for soils in general discussed by US EPA for children and adults.[3,23]

For almost all elements the gastric + intestinal general means were lower than the gastric general means. Pb and Ba were most affected by this, with bioaccessible concentrations and percent bioaccessibilities lowered by around 70%. On average, the other elements were lower by only 30%. As mentioned previously, the intestinal conditions likely caused reactions, such as precipitation and adsorption, which are possibly responsible for the loss of elements in solution. The lower bioaccessibilities in intestinal conditions seen here for Pb and Ba were not observed to the same degree in the spike results (presented in the Supporting Information: Pb 107% in gastric and 73% in gastric + intestinal phase; Ba 110% and 87%), suggesting that the soil components of the SRM 2710 material enhanced the afore-mentioned reactions.

Method trends

The main objective of the round-robin experiment was to assess the variability in bioaccessibility measurements between different laboratories, using, in most cases, different methods. The use of different methods also allows the data to be inspected for trends according to methods, but a systematic approach could not be used since slight differences existed between nearly all methods. Only pH could be examined as constant variable between all methods (a detailed description of such method trends that could be seen is given in the Supporting Information).

A plot of mean percent bioaccessibility results for each method vs. pH is shown in Figure 3 for five elements of interest (As, Cd, Cu, Pb and Zn) in NIST 2710. The plot indicates a negative trend with increasing pH, which is expected for most of the elements examined, and has been seen before.[8]

Statistical analysis of the relationship between pH and percent bioaccessibility (detailed results are given in the Supporting Information) revealed that the strongest correlations (highest absolute R values) were statistically significant ($\alpha = 0.05$) and occurred for Pb ($R = -0.789$, $N = 27$), Zn ($R = -0.803$, $N = 25$) and Cd ($R = -0.771$, $N = 24$) overall. They remained strong and significant when only gastric phase results were examined. For the majority of elements the correlations were stronger when only the gastric phase results were examined. Negative correlations, meaning that percent bioaccessibility decreases with increasing pH, were confirmed for all elements.

The negative slope is not as pronounced for As, Cu and Zn. In particular, the As values do not change much when the pH is changed from acidic values in the gastric phase to neutral values in the intestinal phase for some methods. However, lower initial (gastric) pHs generally give higher bioaccessibilities; initial gastric pH has been targeted as a factor influencing bioaccessibility by others.[9] Similarities in values between the gastric and gastric + intestinal phase in the present study should not be interpreted as negating the need for the intestinal phase, since pronounced differences have been seen for other samples, and are sample-dependent.[24]

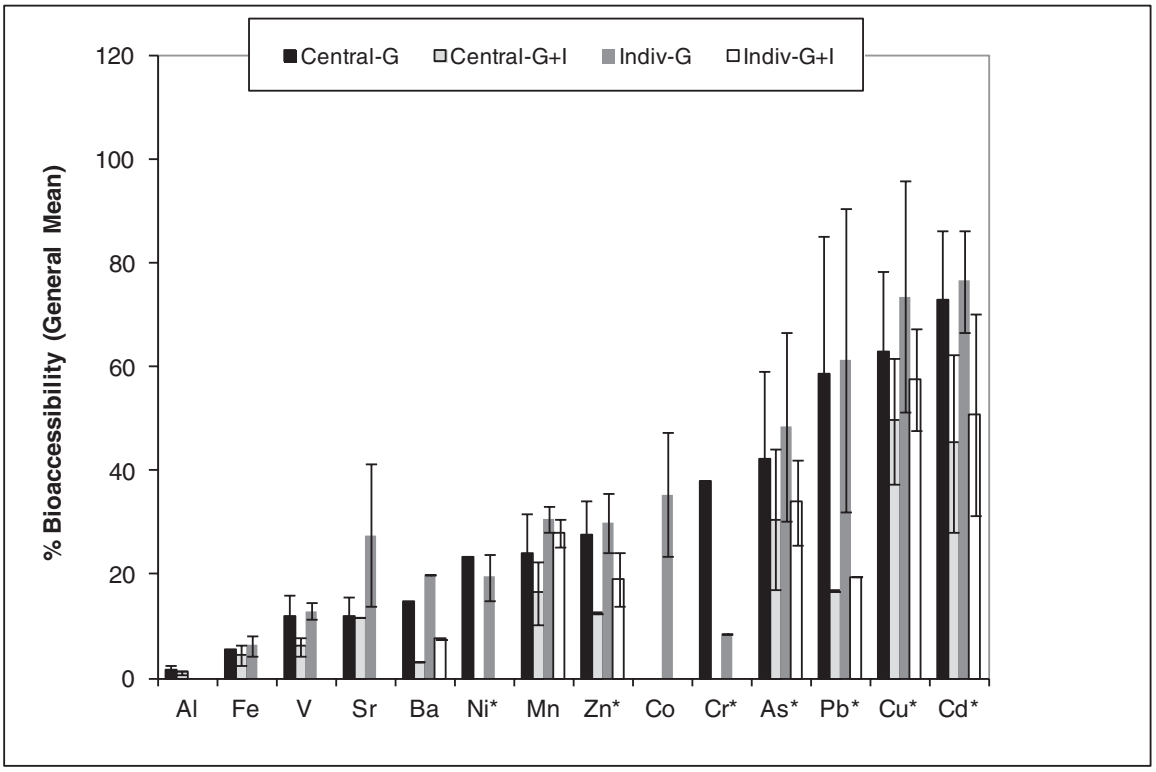


Fig. 2. General means of percent bioaccessibility (all methods combined) of elements organized by increasing percent bioaccessibility values. Central-G = central laboratory (Analytical Services Unit) results, gastric phase; Central-G+I = central laboratory results, gastric + intestinal phase; Indiv-G = individual laboratory results, gastric phase; Indiv-G+I = individual laboratory results, gastric + intestinal phase. Missing bars indicate results based on one data point or where no results were detected or available (n is reported in Tables 2 and 3). Error bars are reproducibility RSD; where no error bars are shown, RSD > 50% of general mean. *Indicates elements identified by participants as important for risk assessment.

Comparison of As and Pb results to In Vivo *relative bioavailability (RBA)*

Bioaccessibility results are commonly assessed by comparison to RBA, and this is especially useful when bioaccessibility results vary widely,[10] as they do in the present study. A favourable comparison could mean agreement (within experimental uncertainty) between bioaccessibility and bioavailability results. When only one soil is used, however, as it is in the present study, such agreement should not be misinterpreted as indicating a predictive method. More commonly, a favourable comparison is a predictable relationship between a series of bioaccessibility and bioavailability results; such comparisons have been used by others to indicate "validation" of laboratory bioaccessibility methods, such as for the RBALP[25] and UBM.[5]

Such comparisons are carried out between percent bioaccessibility and RBA (the proportion, usually expressed as a percentage, of the contaminant that is bioavailable, relative to the proportion of a standard or control substance that is bioavailable). To account for experimental conditions of the bioaccessibility test not exactly matching the gastrointestinal conditions used in the dose-response study, or indeed, any gastrointestinal conditions, bioaccessibility results can be corrected to the recovery of a control to give a relative bioaccessibility value;[26] often this value is used for "validation."[5] A bioaccessibility control representing the conditions used in the bioavailability experiments was not included in the experimental design of the present round-robin study so instead the spike results for As and Pb were used for this purpose.[2]

That is, the bioaccessibility results were corrected with the mean of the spike results for each laboratory or method ($\text{mean}_{\%\text{bioaccess}}/\text{mean}_{\%\text{spike recovery}}$) to obtain a relative bioaccessibility. One of the limitations of using a single spike concentration for this purpose might be the possibility that different concentrations of spikes can give different

[2]Spikes were prepared from $Pb(NO_3)_2$ and As_2O_3, rather than $Pb(CH_3COO)_2$ and NaH_2AsO_4 used in the bioavailability studies. Although the Pb^{2+} species are not expected to differ much in solubility behaviour, the use of the spike from the present study for As is not ideal, since the neutral As(III) species ($As(OH)_3$) used in the spike may be less reactive with solution components (e.g., organic biomolecules) than anionic As(V) species ($H_2AsO_4^- \leftrightarrow HAsO_4^{2-}$).

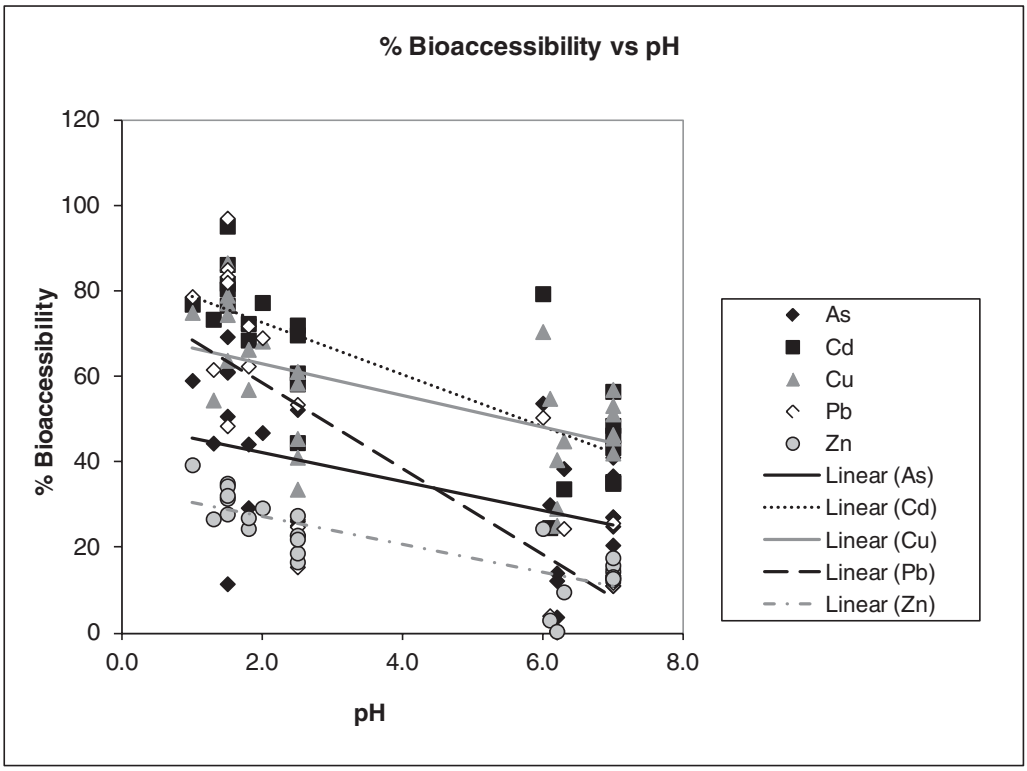


Fig. 3. Percent bioaccessibility vs. pH (gastric and gastric + intestinal phases) for some trace elements of interest.

spike recoveries under certain conditions, such as that seen for Pb [27] and Cr.[28]

As and Pb RBAs in NIST 2710 were determined at the Institut national de l'environnement industriel et des risques (INERIS) according to methods published elsewhere.[5] Swine were used and the positive controls were sodium arsenate (NaH_2AsO_4) and lead acetate ($Pb(CH_3COO)_2$). An As RBA value in NIST 2710 was also obtained from the average of three measurements reported in Bradham et al.,[29] using mice. Mean RBAs and experimental details are summarized in Table 5.

Bioaccessibility and relative bioaccessibility results obtained in the central laboratory and individual laboratories are compared with the bioavailability ranges in Figures 4 and 5. The swine bioavailability ranges were very small: for As the 95% confidence interval is 6% of the RBA (Fig. 4), and for Pb the 95% confidence interval is 4% (Fig. 5); the mouse range for As was higher with a combined RSD on the mean RBA (3 trials of 4 replicates) of 15% (Fig. 5). For As the methods for which at least one bioaccessibility result was within the RBA range (mouse and/or swine results) were: RRU-PBET, RMC-PBET, BGS-UBM, Exponent-phosphate, SGS modified TCLP, and RIVM (Fig. 4), as well as Lab A-RBALP. The results from the other two laboratories performing the RBALP were outside the range, as was the general mean for the three RBALP results shown in Figure 4 (53–63% for the central laboratory, with between-laboratory reproducibility SD of 9.5–13%, and 73% for individual laboratories with reproducibility SD of 5–8%). These results should not be assumed to indicate that the RBALP method is not predictive of bioavailability since only one sample is used in the present study.

For Pb the methods giving results within the swine range were: RMC-PBET (gastric only), SGS modified TCLP, and HC-HCl (Fig. 5), as well as Lab B-RBALP. As stated previously, the Pb swine RBA uncertainty was considered to be small since at <4%, it was substantially lower than typical uncertainties associated with Pb RBA results, such as the average of about 27% in Casteel et al.[30] Thus an additional uncertainty range was included for Pb that represents the (maximum) repeatability RSD estimated by this round-robin experiment for bioaccessibility measurements (11%), represented as 11% of the RBA in Figure 5. Seven of the 16 methods were in this larger range.

All three of the RBALP trials were within this range, which is consistent with the good agreement of the RBALP method with *in vivo* results for Pb in a series of residential soils, tailings, and slags from mining related waste sites across the United States.[25] The general means for the RBALP method are shown in Figure 5 and it can be seen that only the relative bioaccessibility results using the central laboratory (75% with reproducibility SD 15%) are within the range; absolute bioaccessibility and individual laboratory results are not in the range. The other four methods within the larger uncertainty range were RMC-PBET,

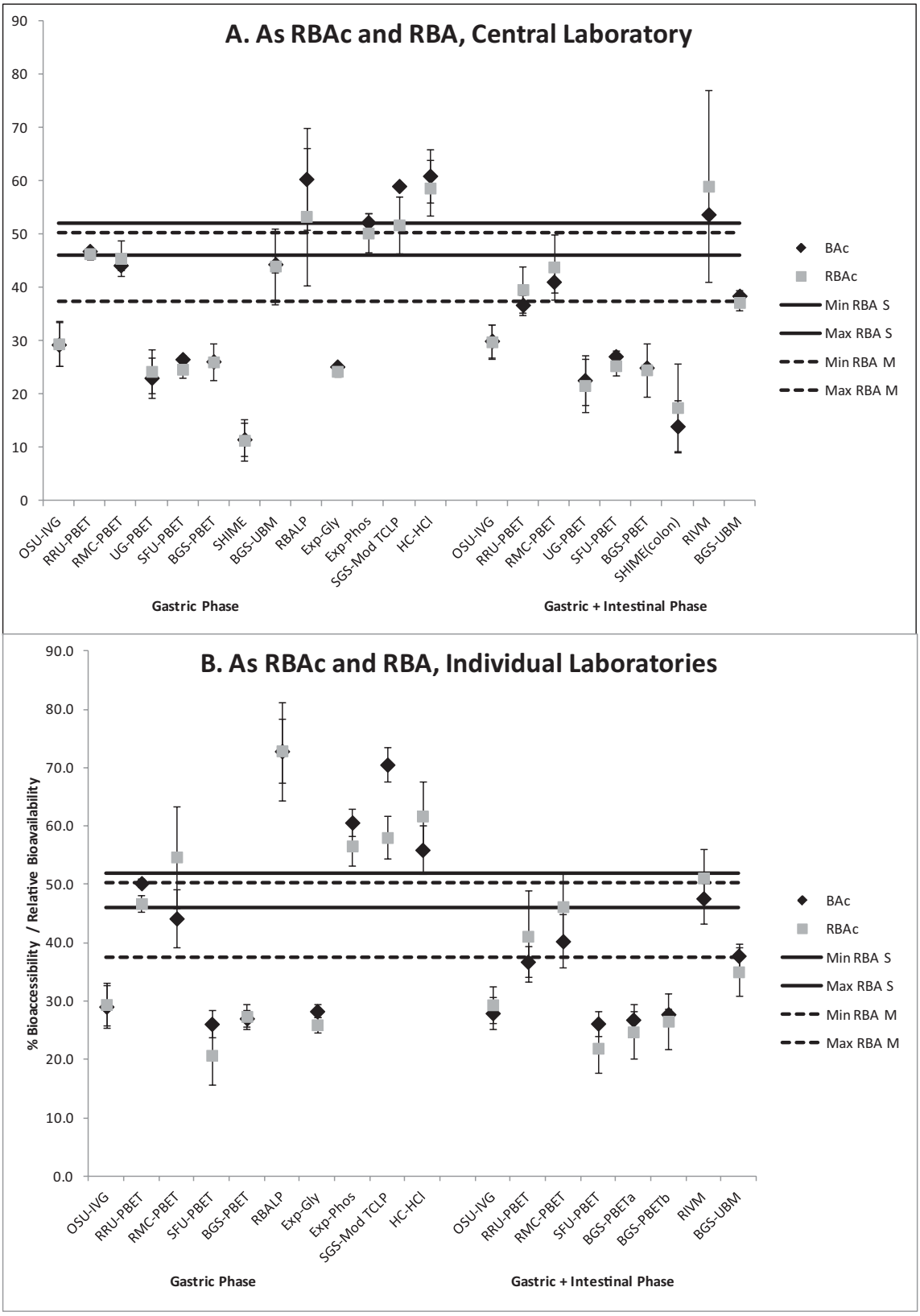


Fig. 4. Comparison of arsenic (As) bioaccessibility results from (A) central laboratory data and (B) individual laboratory data to ranges of relative bioavailability (RBA) data from swine (indicated by min RBA S to max RBA S) and mice (indicated by min RBA M to max RBA M), BAc = % bioaccessibility; RBAc = % relative bioaccessibility (bioaccessibility corrected to spike recovery). Error bars are repeatability standard deviation for each data point, except for RBALP, where they are reproducibility standard deviation.

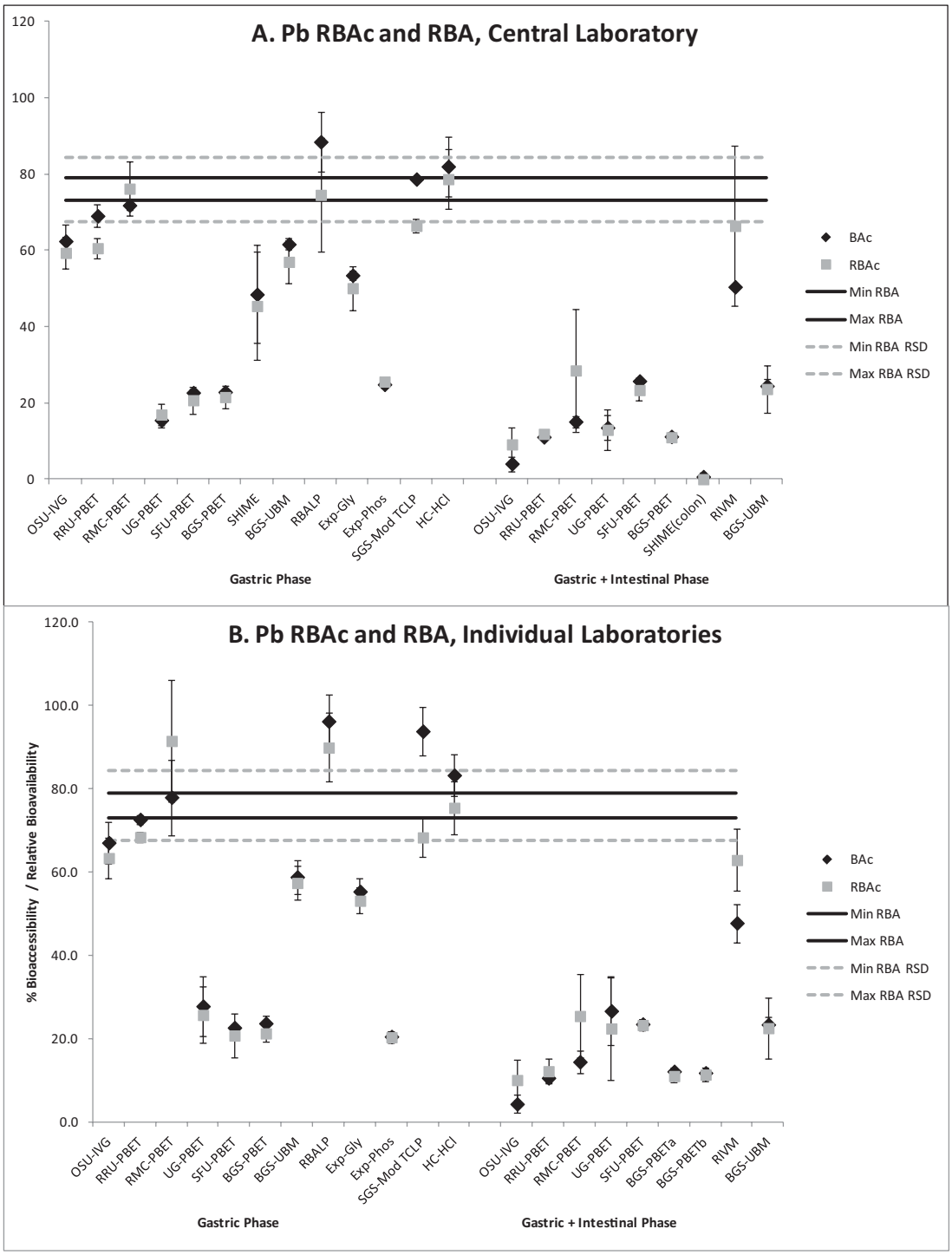


Fig. 5. Comparison of lead (Pb) bioaccessibility results from (A) central laboratory data and (B) individual laboratory data to ranges of relative bioavailability (RBA) data from swine (indicated by min RBA S to max RBA S) repeatability RSD (indicated by min RBA RSD to max RBA RSD). BAc = % bioaccessibility; RBAc = % relative bioaccessibility (bioaccessibility corrected to spike recovery). Error bars are repeatability standard deviation for each data point except for RBALP, where they are reproducibility standard deviation.

HC-HCl, SGS-Modified TCLP, and RRU PBET. Three methods not in this group have, however, shown good correlations with RBA values for Pb in other studies: the BGS UBM,[5] the RIVM method,[26] and the OSU-IVG.[31] Four methods were within RBA uncertainty ranges for both As and Pb: RRU-PBET, RBALP (Lab A but not general mean), RMC-PBET, and SGS-modified TCLP. Generally the bioaccessibility results for the methods outside the RBA

Table 5. Relative bioavailability (RBA) results for arsenic (As) and lead (Pb) in NIST 2710 from *in vivo* studies, and experimental details.

Element	*As*		*Pb*
Data Source	INERIS	Bradham et al. 2011	INERIS
Animal	Swine	Mouse	Swine
Control	NaH_2AsO_4	NaH_2AsO_4	$Pb(CH_3COO)_2$
Matrix	Urine	Urine	Kidney
RBA (%)	49	44	76
RBA range (%)	46–52	37–50	73—79
Range source	95% CI	1SD (variances added from 3 measurements)	95% CI

CI = confidence interval; SD = standard deviation.

uncertainty ranges were lower than the RBAs, except for RBALP.

The comparison described in the present study is, of course, not sufficient to assess the different methods and their validity with respect to bioavailability data; more soils should be used for such a comparison. A larger number of soils allows correlations between bioaccessibility and bioavailability data to be assessed, and this approach has been used in studies that examine a single method (e.g., [5,11,25,27,32,33]). Comparisons of multiple methods to each other and bioavailability results, on the other hand, are not as common; a 2007 study [10] involved comparison to a single soil with associated Pb bioavailability data, and another more recent one has compared 4 methods to a suite of 12 soils for As [34] and 7 soils for Cd.[35] All four methods gave bioaccessibility results that were linearly correlated with bioavailability results with coefficients (R) greater than 0.6. Clearly the use of more than one soil with bioavailability results is a valuable study design variable.

Conclusion

The BARC round-robin experiment was successful in measuring the variability of bioaccessibility measurements in different laboratories and using different methods for a single soil material. Within-laboratory repeatability was good, measuring less than 10% for most elements, and it was generally slightly higher for most elements after the gastric + intestinal phase than after the gastric phase. Percent reproducibility (sum of within-laboratory repeatability and between-laboratory variability) for many elements was comparable to typical uncertainties (e.g., 30% in commercial laboratories). The use of a single central laboratory was hypothesized to reduce the uncertainty in the data, but reproducibilities were not significantly better, probably because individual laboratories reported results for which they had a high level of control within their laboratory conditions.

When results were examined with respect to method variables, pH helped to explain the trends: increasing pH resulted in decreasing bioaccessibility for elements in NIST 2710. Bioaccessibility results were compared with bioavailability results (obtained from animal *in vivo* testing) for As (swine and mouse) and Pb (swine only) in NIST 2710. For As, three simpler and four more complex methods were within the uncertainty range of swine and/or mouse *in vivo* results. For Pb, five simple and two complex methods were close enough to the *in vivo* results that the difference was within the bioaccessibility measurement error.

Although the present study was not designed to generate conclusions about preferred methods, the results have generated much-needed discussions within BARC concerning general guidance on the factors that constitute an acceptable *in vitro* method in Canada (with international input). A second round-robin experiment was designed with this feedback to address some of these key issues. In particular, the experimental design included testing of fewer (four) methods, with more replication of methods between laboratories, and with three soils that have been tested for *in vivo* bioavailability. The results will be presented in a future publication. The requirements for deeming a bioaccessibility method adequately valid for predicting bioavailability, and hence for use in risk assessment, is part of the assessment process that requires further discussion and consensus.

Abbreviations

ASU, Analytical Services Unit (Queens University, Kingston, ON), referred throughout as the central laboratory; BGS, British Geological Survey; EPM, École Polytechnique de Montréal; Exp, Exponent; Gly, Exponent method that uses glycine; Indiv, Individual laboratory, indicates data provided by laboratories; INERIS, Institut National de l'Environnement Industriel et des Risques, France; IVG, *In vitro* gastrointestinal; Lab A, Lab B, two laboratories that elected to remain anonymous; OSU, Ohio State University; PBET, Physiologically based extraction test; PBETa is gastric + intestinal result for extract collected halfway through the intestinal extraction time (2 h with a total of 3 h extraction); PBETb is at the end of the intestinal extraction time (4 h with a total of 5 h extraction; Phos, Exponent method that uses phosphate; RBALP, Relative bioaccessibility leaching procedure; RIVM,

Rijksinstituut voor volksgezondheid en milieu: National Institute for Public Health and the Environment, Netherlands; RMC, Royal Military College; RRU, Royal Roads University; SFU, Simon Fraser University; SGS, SGS Lakefield; SHIME, Simulator of the human intestinal microbial ecosystem; TCLP, Toxicity characteristic leaching procedure; UBM, Unified BARGE Method; UG, University of Guelph; US, University of Saskatchewan.

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