1 Method Variables Affecting the Bioaccessibility of Arsenic in Soil

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3 Louise Meunier[1], Joanna Wragg[2], Iris Koch[1], and Kenneth J. Reimer[1]*,

4 1. Environmental Sciences Group, Royal Military College, P.O. Box 17 000, Station

5 Forces, Kingston, Ontario K7K7B4, Canada

6 2. British Geological Survey, Keyworth, Nottingham NG12 5GG, United Kingdom

7 * Corresponding author phone: (613)541-6000, ext 6161; fax: (613)541-6596; e-mail:

8 reimer-k@rmc.ca.

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10 ABSTRACT

11

12 Arsenic bioaccessibility tests are now being commonly used in risk assessment. However,

13 concerns remain about the reliability of such tests because the bioaccessibility of arsenic

14 from soil may be susceptible to soil composition (including iron concentration), as well as

15 method considerations such as varying liquid-to-solid ratios and the chosen buffer system.

16 In this study, arsenic-contaminated tailings and soils were tested to compare two

17 bioaccessibility methods: one that uses glycine as a buffer, and a second that is more

18 physiologically based. With the glycine-buffered method, arsenic and iron bioaccessibility

19 increased in the presence of a higher buffer concentration at higher liquid-to-solid ratios,

20 whereas the results of physiologically-based tests were unaffected by variations in these

21 parameters. In the glycine-buffered system, interactions between iron and glycine may

22 influence the concentration of arsenic in solution, which may not be consistent with human

1 gastrointestinal conditions. The choice of a physiologically-based method may be more

2 appropriate to achieve representative arsenic bioaccessibility values toward estimating

3 risks to human health.

4

5 Keywords: bioaccessibility; arsenic; glycine; contaminated soil; tailings

6

7 INTRODUCTION

8

9 Traditional human health risk estimates are based on the total concentration of

10 contaminants in soils,[1, 2] but several regulatory bodies worldwide now recognize that this

11 may overestimate the actual risk.[3-5] Instead, using the bioavailable fraction, or the fraction

12 of a substance that can be absorbed and reach systemic circulation,[6] may provide a more

13 representative estimation. This is especially important when the bioavailable fraction is

14 potentially much smaller than the total concentration of a contaminant. Such is the case for

15 arsenic in soils, which is considered only one-fifth as bioavailable as soluble (aqueous)

16 inorganic arsenic.[7]

17

18 Arsenic bioavailability data may be obtained by measuring arsenic concentrations in the

19 blood and tissues of animals following their ingestion of arsenic-contaminated substrates.

20 Several in vivo ingestion studies have indicated that arsenic soil bioavailability depends on

21 soil type and contaminant source, and varies from 2 to 48%.[8-12] Because bioavailability

22 varies greatly, site and soil-specific measurements are required.

1

2   Although the use of in vivo models may provide representative bioavailability data for

3   estimating risks, the costs and ethics of using these models has led to the development of

4   laboratory-based in vitro extraction tests that simulate human in vivo conditions. Results

5   from these tests provide an estimation of bioaccessibility, which is defined as the fraction

6   of a substance that is soluble in the gastrointestinal environment and is available for

7   absorption.[13] Several methods have been developed to assess the bioaccessibility of soil

8   contaminants but the results vary between methods, and concerns remain regarding the

9   reliability of such measurements.

10

11   Ruby et al.[11, 14] published one of the first studies examining a physiologically-based

12   extraction test (PBET). This test was based on rabbit gastrointestinal conditions and is

13   similar to paediatric conditions. It takes into consideration gastric and intestinal chemistry,

14   extraction pH, soil mass and fluid volume, stomach mixing and emptying rate, and small

15   intestinal transit time. This test is considered to be a valid predictor of oral lead

16   bioaccessibility.[15, 16] Rodriguez et al.[17] indicated that the arsenic bioaccessibility data

17   obtained by such an in vitro gastric method is strongly correlated with in vivo

18   bioavailability results, although the test slightly underestimated bioavailability. Several

19   additional studies have used variations of this physiologically-based method to measure the

20   bioaccessibility of metals, or to simplify the method.[17-19]

21

1   Parallel method development has been carried out by various agencies including the

2   Solubility/Bioavailability Research Consortium (SBRC), which has established a simple in

3   vitro test using glycine as a buffer.[20] The SBRC calls for 0.4 M glycine, which is a much

4   higher concentration than the < 1 mM concentrations of glycine typically found in

5   biological fluids.[21] Although not physiologically representative, the gastric phase of the

6   SBRC method – also entitled the Relative Bioaccessibility Leaching Procedure – has been

7   validated against in vivo lead bioavailability data.[22] The USEPA recommends this method

8   to determine the relative bioavailability of lead for the purposes of risk assessment in

9   Region 8 (Colorado Area).[23] The in vivo database for arsenic is not as extensive as that

10   for lead,[20] and a glycine-buffered test has yet to be validated for estimating arsenic

11   bioavailability.[24]

12

13   In addition to the inherent variability introduced by any chosen method and their related

14   parameters (e.g. extraction pH, liquid-to-solid ratios), the bioaccessibility of arsenic may

15   be affected by arsenic speciation[25] and soil physicochemical properties. Specifically,

16   higher soil iron oxide content and lower soil pH both tend to yield lower

17   bioaccessibility.[26-28] In some cases, the bioaccessibility is negatively correlated with

18   arsenic concentration in soil,[25, 29] but other studies have found the bioaccessibility of

19   arsenic to be independent of soil arsenic concentration.[19] The negative dependence of

20   bioaccessibility on soil arsenic concentration may be an indication of solubility saturation,

21   and Richardson et al.[30] recommend carrying out tests at various liquid-to-solid ratios to

22   rule out this possibility. In some cases (where glycine was used as a buffer), the arsenic

1   bioaccessibility increased with an increased ratio,[31] whereas other studies (that did not use

2   glycine) have shown arsenic bioaccessibility to be independent of liquid-to-solid ratio.[25]

3   Thus the choice of buffer in estimating arsenic bioaccessibility may influence the results.

4

5   In this study, arsenic-contaminated tailings, soils and reference materials representing a

6   range of iron to arsenic molar ratios were subjected to bioaccessibility tests using both a

7   physiologically-based test (Method P) and a glycine-buffered test (Method G) under

8   simulated gastric and gastric followed by intestinal conditions. The results were examined

9   to determine the effects of extraction pH, liquid-to-solid ratio, buffer choice and

10   concentration, and arsenic and iron concentrations on the bioaccessibility of arsenic. The

11   objective was to evaluate the variability introduced by the methods and sample

12   characteristics in measuring the bioaccessibility of arsenic.

13

14   MATERIALS AND METHODS

15

16   Sample Description and Preparation

17

18   The test materials consisted of soils and tailings samples, reference materials, and solid

19   matrices. The soils and tailings samples were taken from arsenic-contaminated abandoned

20   gold mine sites in Nova Scotia, Canada,[32] and are described by Meunier et al.[25] The

21   standard reference materials selected were NIST2710[33] and NIST2711[34], and a reference

22   soil from the British Geological Survey (BGS 102) was also tested.[35] Two more samples

1   consisted primarily of arsenic minerals: a mine tailings sample rich in scorodite

2   ($FeAsO_4 \cdot 2H_2O$) collected at Lower Seal Harbour (LSH), Nova Scotia, Canada,[32] and a

3   sample of arsenopyrite (FeAsS) from Niñas de Panasqueira, Portugal (Ward's Natural

4   Science). Ironite® (Mineral Supplement 1-0-0), a commercial fertilizer that contains high

5   concentrations of arsenic and iron from mine waste was also tested. All tailings samples

6   were dried and sieved to a <150 μm particle size. The arsenopyrite and LSH tailings

7   samples were ground and homogenized, using a mortar and pestle, to a <150 μm particle

8   size. Ironite® was sieved to <150 μm. Reference materials and soil were used as received

9   (the particle size for NIST2710 and NIST2711 is <74 μm, and the BGS102 particle size is

10   <50μm).

11

12   Sample Elemental Composition

13

14   The certified arsenic and iron concentrations of the standard reference materials NIST2710

15   and 2711 were used.[33, 34] All other samples were analyzed for major and trace elements by

16   aqua regia digestion. Analytical results for the Nova Scotia samples are described in detail

17   by Parsons et al.[32] Digestions of the remaining samples were performed according to

18   Method 200.7[36] and analyzed by the Analytical Services Unit, Queen's university,

19   Kingston, ON using inductively coupled plasma-optical emission spectrometry (Varian

20   AX-Vista Pro). The results for the total arsenic concentration in BGS102 (94 mg•kg^{-1}) is

21   comparable to unpublished data for this sample (102 mg•kg^{-1}).[37]

22

1   Bioaccessibility Extractions

2

3   For each sample, the bioaccessibility of arsenic and iron was measured using two different

4   methods and two liquid-to-solid ratios (i.e. 100:1 and 2000:1). The glycine-buffered

5   Method G was based on a SBRC method modified from Kelley et al.[20] The

6   physiologically-based Method P was modified from Ruby et al.[11] and Rodriguez et al.,[17]

7   and is similar to tests carried out by Cave et al.[38] and Koch et al.[39] These in vitro

8   experiments consisted of a gastric phase (phase 1, denoted P1), and a gastric followed by

9   an intestinal phase (phase 2, denoted P2).

10

11   Experiments were carried out in batches of 8 to 12 samples, along with two duplicate pairs,

12   one blank and one reference material (NIST2710 or NIST2711) for each phase. All

13   chemicals and reagents used in the bioaccessibility tests were analytical grade or better,

14   and all solutions were prepared with distilled deionised water (DDW, Barnstead E-pure

15   reverse osmosis/ion exchange apparatus Water Purification System, minimum resistance

16   18 MΩ•cm).

17

18   Gastric solution compositions are described in detail by Koch et al.[39]. Briefly, the gastric

19   solution of Method G contained 0.4 M glycine (Sigma) in DDW acidified with

20   hydrochloric acid (HCl Fisher) to a pH of 1.5 ± 0.05 (Acumet Excel XL15). For Method G,

21   an additional series of tests was performed at the 2000:1 liquid-to-solid ratio using a more

22   dilute (0.02 M) glycine solution. In Method P, the gastric solution includes porcine pepsin

7

1 (Sigma-Aldrich), sodium citrate (Caledon), malic acid (Sigma-Aldrich), glacial acetic acid

2 (Fisher), and sodium chloride (Fluka) in DDW (concentrations can be found in Koch et

3 al.[39] The solution was similarly acidified to a pH of 1.80 ± 0.05.

4

5 Test procedures are identical for both methods. In the 100:1 liquid-to-solid ratio tests, two

6 subsamples were tested for each sample (representing P1 and P2). Measured amounts of

7 gastric solution (20 ml) were poured into 50-ml polyethylene specimen containers and

8 heated to 37 °C. A previously measured quantity of solid sample (0.2 g) was added to this

9 prepared solution and the test containers were secured in a temperature-controlled flatbed

10 rotation incubator (New Brunswick Scientific Innova 4230) at 37 °C and 150 rpm under

11 aerobic conditions. The pH was measured after 30 minutes (and acidified as required), and

12 again at the end of the one-hour gastric phase. Samples were stirred over a hot plate (37

13 °C) while pH measurement took place, and the time taken to measure pH (less than six

14 minutes per sample) was included in the overall incubation time. At the end of the gastric

15 stage of the test, samples used in the gastric phase only (P1) were removed from the

16 sample set.

17

18 The remaining samples were retained for the gastric followed by intestinal phase of the test

19 (denoted P2). At the beginning of P2, the solutions were modified to simulate intestinal

20 conditions by raising the pH to 7.0 ± 0.2 using a saturated NaOH (Sigma-Aldrich) solution

21 for Method G,[20] and a 10 M solution of Na_2CO_3 (Fluka) for Method P. Porcine bile

22 (Sigma) and porcine pancreatin (Sigma-Aldrich) was also added to all intestinal solutions.

1   Test containers were returned to the incubator. The pH was measured after two hours (and

2   adjusted as required), and again at the end of the four-hour intestinal stage of the test. The

3   time taken to measure pH (less than 12 minutes during the intestinal stage of the test) was

4   included in the overall incubation time.

5

6   In the 2000:1 liquid-to-solid ratios tests, a single subsample was prepared for each solid

7   sample (to be used for both P1 and P2). Measured amounts of gastric solution (300 ml)

8   were poured into 1-L polyethylene specimen containers and heated to 37 ºC. A previously

9   measured quantity of solid sample (0.15 g) was added to this prepared solution. Test

10   containers were heated and shaken, and the pH was measured and adjusted as described

11   above. At the end of the gastric portion of the test, a solution aliquot (representing the P1

12   extract) was removed by syringe and filter (0.45 μm, PVDF membrane, Milipore), and

13   replaced with fresh gastric solution. The intestinal stage of the test was conducted as

14   described above.

15

16   At the end of each experiment, extracts were transferred to centrifuge tubes, centrifuged at

17   3800 rpm (2970•g) for 20 minutes, and the supernatant was filtered (0.45 μm, PVDF

18   membrane, Milipore).

19

20   All collected extracts from Method G and Method P were stored frozen (to -18 °C) and

21   reserved for analysis. Thawed aliquots of each extract were diluted with 2% nitric acid

22   (HNO_3 Fisher) solution and analyzed for total arsenic and iron concentrations by

1 inductively coupled plasma – mass spectrometry (Thermo Electron Corporation X-Series[II])

2 in collision cell mode as described by Smith et al.[40] Bioaccessibility results are expressed

3 as a concentration or as a percentage. The quantity of a substance (mg•kg^{-1} dry weight)

4 extracted during a bioaccessibility test is defined as the bioaccessible concentration.

5 Dividing this quantity by the total concentration in a given sample and multiplying by 100

6 gives the percent bioaccessibility.

7

8 Quality Assurance and Quality Control

9

10 Quality control tests and results are summarized in Table 1. Tests include blanks,

11 duplicates and a standard reference material (SRM), NIST2710, used in bioaccessibility

12 extractions, as well as matrix spikes and a calibration check solution for ICP-MS analysis.

13 The ICP-MS instrument detection limit was based on three standard deviations of eight

14 replicate measurements of a low concentration solution (1 and 10 µg•L^{-1} for arsenic and

15 iron respectively); 88 of the 101 blanks returned numbers below the arsenic and iron

16 instrument detection limit (1 µg•L^{-1} for arsenic; 10 µg•L^{-1} for iron). All blanks were below

17 the limit of quantification (3 µg•L^{-1} for arsenic; 20 µg•L^{-1} for iron). All SRM arsenic and

18 iron results were within laboratory control limits (Table 1). Given the low variability in

19 SRM iron bioaccessibility between all methods and all liquid-to-solid ratios, these results

20 were combined and are shown as a single entry in Table 1. The average of the relative

21 percent difference (RPD) between extraction duplicates was less than 17%. However, RPD

22 values as high as 76% were recorded for duplicates associated with one sample (MG6),

1 and may be attributed to heterogeneity of this particular sample for reasons unknown at

2 this time.

3

4 RESULTS AND DISCUSSION

5

6 The 19 samples used in this study cover a wide range of arsenic concentrations (from 94 to

7 420 000 mg•kg^{-1}) and iron to arsenic molar ratios (from 1.2 to 2600). Of these, the 13

8 Nova Scotia tailings samples (identified by the prefixes CAR-, GD-, MG-, and NB-) were

9 included in a previous study investigating the effects of soil composition and mineralogy

10 on arsenic bioaccessibility from a suite of 29 tailings and soil samples.[25] These 13

11 samples represent the wide range of arsenic concentrations and physical characteristics

12 encountered throughout the Nova Scotia gold mining districts. They were selected for

13 further experiments in the present study to compare the results previously obtained using

14 Method P (at the 100:1 and 2000:1 liquid-to-solid ratios) with the arsenic bioaccessibility

15 values obtained by Method G. The results of both methods are presented in Table 2

16 (arsenic bioaccessibility), and Table 3 (iron bioaccessibility). The percent arsenic

17 bioaccessibility ranges from near zero to 79%. The range of iron bioaccessibility results is

18 much smaller, varying from near zero to a maximum of 19%.

19

20 As previously indicated, physicochemical soil properties and arsenic speciation may result

21 in large variations within methods. Furthermore, variations between methods may be

22 attributed to a number of parameters including extraction pH, liquid-to-solid ratio, method,

1   and buffer concentration. First, the results are examined with respect to the extraction pH

2   to determine if the choice of buffer affects pH stability, how the bioaccessibility pH

3   conditions affect the bioaccessibility. The discussion then addresses the effects of liquid-

4   to-solid ratio, buffer concentration, and iron on the bioaccessibility.

5

6   Effect of Buffer on the Stability of Method pH

7

8   The ease with which pH is measured and maintained throughout a bioaccessibility test can

9   be an important factor in designing a test that is simple and economical, while providing a

10   representative measure of the soluble fraction of a contaminant.[41] Buffers that are

11   effective at gastric (pH < 2) and intestinal (pH ~ 7) pH are desirable. Glycine (used in

12   Method G, pK_as 2.3 and 9.6), as well as the combination of citrate, malate and acetate

13   (used in Method P, pK_as 3-3.4, 5-6.4) meet this requirement, since the solutions are

14   diprotic with pK_as near the appropriate pHs.

15

16   During the gastric stage of the test, pH measurements (before adjustment) varied by ±0.07

17   for Method G (using the 0.4 M glycine concentration), which was a smaller variation than

18   that observed for Method P (±0.15). Under intestinal conditions, greater variations in pH

19   measurements were recorded, and Method G (0.4 M glycine) once again demonstrated

20   more stability (±0.17) than Method P (±0.87). However, when the dilute 0.02M glycine

21   concentration was used, wider variations were recorded under gastric and intestinal

22   conditions (±0.16 and ±1.1 respectively). Therefore, at its higher 0.4 M concentration, the

1   glycine buffer provides a more effective buffering capacity than both Method G at the

2   dilute 0.02 M glycine and Method P. This suggests that the higher buffer concentration

3   provides useful pH control, but the rest of the study examines other associated effects. The

4   next section outlines how the pH in each buffer mixture affects the bioaccessibility results.

5

6   Effect of Gastric and Intestinal Extraction Conditions on the Bioaccessibility of Arsenic

7

8   Bioaccessibility tests are intended to represent a worst-case scenario for the solubility of a

9   contaminant in a simulated gastro-intestinal tract.[41] These tests thereby provide more

10   conservative (protective) adjustments in human health risk assessments. Since the

11   bioaccessibility of arsenic may be influenced by the extraction pH,[11] bioaccessibility tests

12   were carried out under both gastric (acidic pH) and intestinal (neutral pH) conditions. In

13   more than 70 percent of the results (all methods tested), the bioaccessibility of arsenic is

14   greater in P2 compared to P1 (Table 2). Inspection of the results in Table 2 reveals that

15   several tailings samples (NB11A, NB6B, GD2, LSH Tailings, CAR 1, and GD1) returned

16   a higher arsenic bioaccessibility result in P2 for all 5 treatments. The Ironite® and

17   NIST2710 sample exhibited the reverse trend of having a higher P1 arsenic

18   bioaccessibility in all cases. For the lower 100:1 liquid-to-solid ratios, variations between

19   P1 and P2 were relatively small (paired t-tests, $\log_e$ transformed data, $p > 0.52$), but these

20   variations were statistically significant when the higher 2000:1 liquid-to-solid ratio was

21   used (paired t-tests, $\log_e$ transformed data, $p < 0.011$) for both Methods G and P (Table 2).

22   Variations between P1 and P2 were not as apparent in the iron bioaccessibility results

1 (Table 3). In four of the five treatments, statistical analysis reveals that the extraction pH

2 did not significantly affect the bioaccessibility of iron (paired t-tests, $\log_e$ transformed data,

3 $p > 0.064$). The only exception is for Method G at the 100:1 liquid-to-solid ratio, where the

4 P1 results were greater than the P2 iron bioaccessibility for all but one sample (paired t-

5 tests, $\log_e$ transformed data, $p = 0.009$).

6

7 For this type of experiment, we have determined that the acceptable within-laboratory

8 repeatability is ±30%, which is consistent with the uncertainty estimated for total arsenic

9 concentration results from accredited laboratories[42] for such environmental samples.

10 Therefore, two results that are within 30% of each other are no more distinguishable than

11 two replicate analyses. The 30% uncertainty was used as a benchmark for comparison of

12 P1 and P2 values in Tables 2 and 3, where the higher result of either P1 or P2 is indicated

13 in bold only where the relative percent difference (RPD) between the two results is greater

14 than the 30%. The RPD varied by more than 30% in 53 of the 95 arsenic bioaccessibility

15 measurements, and in 62 of the 90 iron bioaccessibility measurements.

16

17 The predominance of higher P2 results for arsenic, illustrates that carrying out

18 bioaccessibility measurements in one phase only, as is typically done when the gastric

19 phase (P1) is assumed to represent worst-case conditions, would not necessarily provide

20 the most conservative estimates, especially in the case of arsenic-contaminated mine

21 tailings. In the next sections, the higher value of P1 or P2 (typically chosen as a worst-case

1   scenario in risk assessments) for each sample was used in the comparisons between

2   methods.

3

4   Effect of Liquid-to-Solid Ratios

5

6   Previous arsenic bioaccessibility results reveal that, for the 13 Nova Scotia mine tailings

7   samples included in the present study, Method P was insensitive to liquid-to-solid ratios

8   ranging from 100:1 to 5000:1.[25] To test the effects of this variable, bioaccessibility

9   extractions were carried out on an additional six samples (Table 2). There were no

10   significant differences between the two liquid-to-solid ratios tested by Method P (paired t-

11   tests, $\log_e$ transformed data, $p = 0.74$ for the entire set of 19 samples, and $p = 0.37$ when

12   comparing only the six additional samples). This finding is consistent with other results

13   from physiologically-based bioaccessibility tests.[43, 44] The results are illustrated Figure 1,

14   where similarities between the two liquid-to-solid ratios are apparent for Method P, with

15   exceptions noted only for samples BGS102 and NIST2710 (higher arsenic bioaccessibility

16   at the higher liquid-to-solid ratio) and GD5 (higher arsenic bioaccessibility at the lower

17   liquid-to-solid ratio). In such a case, the bioaccessibility results obtained at the 100:1

18   liquid-to-solid ratio with Method P would be used for risk assessment purposes.[30]

19   Conversely, large variations were observed in the case of Method G (Figure 1). Compared

20   with the 100:1 liquid-to-solid ratio, the arsenic bioaccessibility results were significantly

21   higher when the higher 2000:1H (0.4 M glycine) ratio was used (paired t-tests, $\log_e$

22   transformed data, $p < 0.0010$). In such a case, the values obtained using the higher liquid-

15

1 to-solid ratio should be retained.[30] However at this 2000:1 H liquid-to-solid ratio, a

2 greater amount of buffer is available, and we hypothesize that the buffer concentration may

3 affect arsenic bioaccessibility.

4

5 Effect of Buffer Concentration

6

7 Further experiments were conducted to determine whether the bioaccessibility of arsenic

8 was influenced by the liquid-to-solid ratio, or by the amount of glycine present. An

9 additional series of tests was therefore performed using 0.02 M glycine at the 2000:1

10 liquid-to-solid ratio. At this dilute glycine concentration, the ratio of glycine to solid

11 sample is the same as in the 100:1 (0.4 M glycine) liquid-to-solid ratio test. In other words,

12 for this experiment, the amount of glycine used in the 100:1 ratio was diluted to a 2000:1

13 ratio. This test is denoted 2000:1L to differentiate it from the higher 0.4 M glycine

14 concentration test identified as 2000:1H.

15

16 The results for Method G at 2000:1H (0.4 M glycine) generally give higher

17 bioaccessibility results than the other two method G treatments, for which the results are

18 similar, as shown in Figure 1. This trend is observed for a large majority of samples (15 of

19 the 19 samples), and suggests that a higher amount of glycine increases arsenic

20 bioaccessibility, even though statistical analysis did not reveal significant differences (1-

21 way ANOVA with Bonferroni adjustment for the three Method G treatments, $p = 0.17$; and

22 1-way ANOVA of all five treatments with Bonferroni adjustment, $p = 0.45$). A larger data

1   set may reveal statistically significant differences, which are only apparent in pair-wise

2   comparisons of the results for individual samples. In paired t-tests, results for Method G at

3   0.4 M glycine and the 2000:1H ratio are significantly different from the results obtained in

4   the other four treatments (paired t-tests, $\log_e$ transformed data, $p < 0.0010$). The higher

5   bioaccessibility may result from glycine stabilizing cationic species in solution, including

6   iron (as described by Castillo and Ramirez),[45] and arsenic associated with this iron may

7   be released (i.e. when iron remains in solution, so does arsenic). Therefore, in the Method

8   G experiments, it is likely the amount of glycine present and the influence of a glycine-iron

9   complex that affects the arsenic bioaccessibility, rather than variations in the liquid-to-solid

10   ratios. In the following section, the relationship between iron and arsenic is further

11   examined by comparing bioaccessibility results and iron to arsenic concentrations.

12

13   Effect of Iron Concentration on the Bioaccessibility of Arsenic

14

15   The arsenic bioaccessibility results presented in Figure 1 are shown in decreasing order of

16   iron to arsenic molar ratio for each sample, and the results appear generally lower as this

17   ratio decreases, although this trend is not statistically significant (linear regression, $r <$

18   $0.23, p > 0.19$). However, for all five treatments, the percent arsenic bioaccessibility is

19   negatively correlated with the total arsenic concentration (linear regression, $r > 0.50, p <$

20   0.033) and with the total iron concentration (linear regression, $r > 0.49, p < 0.035$). This is

21   consistent with previous findings for similar tailings samples.[25, 29] As demonstrated in the

22   previous sections, this negative relationship is not associated with solution saturation.

1   Previous findings have established that the highest arsenic concentrations in the tailings

2   samples are associated with sparingly soluble arsenic minerals,[25] and the decreased

3   bioaccessibility is likely associated with an increased proportion of these minerals.

4

5   When the percent arsenic and iron bioaccessibilities are compared, the only method that

6   differs slightly from the others is Method G at the higher 0.4 M glycine and 2000:1H ratio,

7   for which the results show a weak positive correlation (linear regression, $r = 0.22$) (Figure

8   2). While this slope (0.13) is not significantly different from zero ($p = 0.36$), it is higher

9   than that for the other four treatments (slopes < 0.073, $r < 0.32$, $p > 0.51$). Thus, when a

10 higher liquid-to-solid ratio is used with a 0.4 M glycine concentration, higher bioaccessible

11 arsenic concentrations appear to be associated to the greatest extent with higher

12 bioaccessible iron concentrations. This result supports the hypothesis that extraction in the

13 presence of greater amounts of glycine is conducive to solubilising more iron (and

14 associated arsenic) than amounts that would be extracted under more physiologically

15 representative conditions.

16

17 CONCLUSION

18

19 For the series of soils and tailings studied, the bioaccessibility of arsenic estimated by

20 Method P is not significantly affected by varying solid-to-liquid ratios and changes in pH,

21 whereas differences in bioaccessibility results were observed for the glycine-buffered

22 Method G. The bioaccessible arsenic concentration was also higher when a greater amount

18

1   of glycine was available in solution, which may be influenced by the interaction between

2   glycine and iron. The consequence is that using a glycine buffer in arsenic bioaccessibility

3   extractions may unduly influence the results by introducing methodological artefacts that

4   do not mimic the human gastro-intestinal conditions. At this time, neither Method P nor

5   Method G has been accepted by any regulatory agency as a validated method for arsenic

6   (against in vivo bioavailability data),[46] although some comparisons between Method G

7   and modified Method P arsenic bioaccessibility results with bioavailability results are

8   available[47, 48 and other references therein, 49]. In vivo hamster arsenic data[50] are also available for

9   one of the samples in the present study (Ironite®). For this sample, results from all

10 methods tested (28-32% for Method P; 25-39% for Method G) compared equally well with

11 arsenic bioavailability values ($31 \pm 6\%$) and did not vary significantly between methods.

12 For this reason, it is not possible to unambiguously distinguish between methods based on

13 in vivo data; clearly more bioavailability data are required to compare and validate

14 bioaccessibility methods.

15

16 The present study demonstrates that the practicality of a method and the potential for

17 artefacts resulting from that same method must be balanced. Method G has been accepted

18 as a useful method to estimate lead bioaccessibility, and hence bioavailability, but its

19 application to other elements and soil samples may require careful consideration and study.

20 The choice of a bioaccessibility method that is both physiologically based and robust with

21 respect to changes in method variables, such as Method P, may provide more

1   representative arsenic bioaccessibility results for the purpose of estimating risks to human

2   health.

3

4   ACKNOWLEDGEMENTS

5

11

12 REFERENCES

13

14 [1] Canadian Council of Ministers of the Environment (CCME). Canadian soil quality

15 guidelines for the protection of environmental and human health: arsenic (inorganic) fact

16 sheet. CCME. **1997,** 1-7.

17 [2] United States Environmental Protection Agency (USEPA). Risk Assessment Guidance

18 for Superfund Volume I: Human Health Evaluation Manual (Part A), Interim Final. **1989,**

19 EPA/540/1-89/00.

20 [3] Health Canada (HC). Bioaccessibility Workshop, Summary Report. Delta Chelsea

21 Hotel, Toronto, Canada. **2005,** 22p.

1   [4] United States Environmental Protection Agency (USEPA). Estimation of relative

2   bioavailability of arsenic in soil and soil-like materials by in vivo and in vitro methods,

3   Review Draft. **2005,**

4   [5] United Kingdom Environment Agency (UKEA). Science Update on the use of

5   Bioaccessibility Testing in Risk Assessment of Land Contamination. February **2005**,

6   http://www.environment-agency.gov.uk/static/documents/2-science_update_1284046.pdf

7   (accessed August 2007).

8   [6] Schoof, R.A. Bioavailability of Soil-Borne Chemicals: Method Development and

9   Validation. *Human Ecol. Risk Assess.* **2004,** *10,* 637-646.

10   [7] National Environmental Policy Institute (NEPI). Bioavailability policy project phase II:

11   metals task force report. NEPI. Washington, DC, USA. **2000.**

12   [8] Groen, K.V.H.A.M.G.; Liest, J.J.G.; de Boer, J.L.M.; van Ooik, T.; Timmerman, A.;

13   Vlug, R.F. Bioavailability of inorganic arsenic from bog ore-containing soil in the dog.

14   *Environ. Health Perspect.* **1994,** *102,* 182-184.

15   [9] Freeman, G.B.; Johnson, J.D.; Killinger, J.M.; Liao, S.C.; Davis, A.O.; Ruby, M.V.;

16   Chaney, R.L.; Lovre, S.C.; Bergstrom, P.D. Bioavailability of Arsenic in Soil Impacted by

17   Smelter Activities Following Oral Administration in Rabbits. *Fund. Appl. Toxicol.* **1993,**

18   *21,* 83-88.

19   [10] Freeman, G.B.; Schoof, R.A.; Ruby, M.V.; Davis, A.O.; Dill, J.A.; Liao, S.C.; Lapin,

20   C.A.; Bergstrom, P.D. Bioavailability of Arsenic in Soil and House Dust Impacted by

21   Smelter Activities Following Oral Administration in Cynomolgus Monkeys. *Fund. Appl.*

22   *Toxicol.* **1995,** *28,* 215-222.

1   [11] Ruby, M.V.; Davis, A.; Schoof, R.; Eberle, S.; Sellstone, C.M. Estimation of Lead

2   and Arsenic Bioavailability Using a Physiologically Based Extraction Test. *Environ. Sci.*

3   *Technol.* **1996,** *30,* 422-430.

4   [12] Rodriguez, V.M.; Dufour, L.; Carrizales, L.; Díaz-Barriga, F.; Jiménez-Capdeville, M.

5   Effects of oral exposure to mining waste on *in vivo* dopamine release from rat striatum.

6   *Environ. Health Perspect.* **1998,** *106,* 487-491.

7   [13] Ruby, M.V.; Schoof, R.; Brattin, W.; Goldade, M.; Post, G.; Harnois, M.; Mosby,

8   D.E.; Casteel, S.W.; Berti, W.; Carpenter, M.; Edwards, D.; Cragin, D.; Chappell, W.

9   Advances in Evaluating the Oral Bioavailability of Inorganics in Soil for Use in Human

10   Health Risk Assessment. *Environ. Sci. Technol.* **1999,** *33,* 3697-3705.

11   [14] Ruby, M.V.; Davis, A.; Link, T.E.; Schoof, R.; Chaney, R.L.; Freeman, G.B.;

12   Bergstrom, P. Development of an in vitro screening test to evaluate the in vivo

13   bioaccessibility of ingested mine-waste lead. *Environ. Sci. Technol.* **1993,** *27,* 2870-2877.

14   [15] Basta, N.T. and Gradwohl, R. Estimation of cadmium, lead and zinc bioavailability in

15   smelter-contaminated soils by a sequential extraction procedure. *J. Soil Cont.* **2000,** *9,* 149-

16   164.

17   [16] Ruby, M.V. Bioavailability of Soil-Borne Chemicals: Abiotic Assessment Tools.

18   *Human Ecol. Risk Assess.* **2004,** *10,* 647-656.

19   [17] Rodriguez, R.R.; Basta, N.T.; Casteel, S.W.; Pace, L.W. An In Vitro Gastrointestinal

20   Method to Estimate Bioavailable Arsenic in Contaminated Soils and Solid Media. *Environ.*

21   *Sci. Technol.* **1999,** *33,* 642-649.

1   [18] Palumbo-Roe, B.; Cave, M.R.; Klinck, B.; Wragg, J.; Taylor, H.; O'Donnell, K.;

2   Shaw, R. Bioaccessibility of arsenic in soils developed over Jurassic ironstones in eastern

3   England. *Environ. Geochem. Health* **2005,** *27,* 121-130.

4   [19] Wragg, J.; Cave, M.; Nathanail, P. A Study of the relationship between arsenic

5   bioaccessibility and its solid-phase distribution in soils from Wellingborough, UK. *J.*

6   *Environ. Sci. Health, Pt A.* **2007,** *42,* 1303-1315.

7   [20] Kelley, M.E.; Brauning, S.E.; Schoof, R.A.; Ruby, M.V. Assessing oral

8   bioavailability of metals in soil. Batelle Press, Columbus, OH, USA. **2002,** 124p.

9   [21] Komorowska, M.; Szafran, H.; Popiela, T. and Szafran, Z. Free amino acids of human

10   gastric juice. *Acta Physiol. Pol.* **1981,** *32,* 559-567.

11   [22] Drexler, J.W. and Brattin, W.J. An *In Vitro* Procedure for Estimation of Lead Relative

12   Bioavailability: With Validation. *Human Ecol. Risk Assess.* **2007,** *13,* 383-401.

13   [23] United States Environmental Protection Agency (USEPA). Estimation of relative

14   bioavailability of lead in soil and soil-like materials using in vivo and in vitro methods.

15   May **2007,** OSWER 9285.7-77.

16   [24] United States Environmental Protection Agency (USEPA). Human Health Risk

17   Assessment: Bioavailability – Region 8. In vitro studies on lead and arsenic. **2008,**

18   http://www.epa.gov/Region8/r8risk/hh_rba.html#ars (accessed October 2008).

19   [25] Meunier, L.; Walker, S.R.; Koch, I.; Wragg, J.; Parsons, M.B.; Jamieson, H.E.;

20   Reimer, K.J. Effects of soil composition and mineralogy on the bioaccessibility of arsenic

21   from tailings and soil in gold mine districts of Nova Scotia. Manuscript in preparation.

1   [26] Yang, J.; Barnett, M.O.; Jardine, P.M.; Basta, N.T.; Casteel, S.W. Adsorption,

2   Sequestration, and Bioaccessibility of As(V) in Soils. *Environ. Sci. Technol.* **2002,** *36,*

3   4562-4569.

4   [27] Yang, J.; Barnett, M.O.; Zhuang, J.; Fendorf, S.E.; Jardine, P.M. Adsorption,

5   Oxidation, and Bioaccessibility of As(III) in Soils. *Environ. Sci. Technol.* **2005,** *39,* 7102-

6   7110.

7   [28] Subacz, J.L.; Barnett, M.O.; Jardine, P.M.; Stewart, M.A. Decreasing arsenic

8   bioaccessibility/bioavailability in soils with iron amendments. *J. Environ. Sci. Health, Pt*

9   *A.* **2007,** *42,* 1317.

10   [29] Laird, B.D.; Van, d.W.; Corriveau, M.C.; Jamieson, H.E.; Parsons, M.B.; Verstraete,

11   W.; Siciliano, S.D. Gastrointestinal Microbes Increase Arsenic Bioaccessibility of Ingested

12   Mine Tailings Using the Simulator of the Human Intestinal Microbial Ecosystem. *Environ.*

13   *Sci. Technol.* **2007,** *41,* 5542-5547.

14   [30] Richardson, G.M.; Bright, D.A.; Dodd, M. Do Current Standards of Practice in

15   Canada Measure What is Relevant to Human Exposure at Contaminated Sites? II: Oral

16   Bioaccessibility of Contaminants in Soil. *Human Ecol. Risk Assess.* **2006,** *12,* 606-616.

17   [31] Yang, J.; Barnett, M.O.; Jardine, P.M.; Brooks, S.C. Factors Controlling the

18   Bioaccessibility of Arsenic(V) and Lead(II) in Soil. *Int. J. Soil Sediment. Contam.* **2003,** *12,*

19   165-179.

20   [32] Parsons, M.B.; Walker, S.R.; Jamieson, H.E.; Hall, G.E.M.; Vaive, J.E.; LeBlanc,

21   K.W.G. Chemical and mineralogical characterization of arsenic and associated elements in

1   tailings and soils from five historical gold mining districts in Nova Scotia - Open File. In

2   press.

3   [33] National Institute of Standards and Technology (NIST). Certificate of analysis -

4   Standard reference material 2710: Montana Soil, Highly Elevated Trace Element

5   Concentrations. Gaithersburg, MD, USA. **2003,** 6p.

6   [34] National Institute of Standards and Technology (NIST). Certificate of Analysis -

7   Standard Reference Material 2711: Montana Soil, Moderately Elevated Trace Element

8   Concentrations. Gaithersburg, MD, USA.**2003,** 6p.

9   [35] National Environment Research Council (NERC). BARGE (the Bioaccessibility

10   Research Group of Europe), BGS102 Reference Soil. **2008**.

11   http://www.bgs.ac.uk/barge/reference.html (accessed May 2009).

12   [36] United States Environmental Protection Agency (USEPA). Method 200.7 –

13   Determination of metals and trace elements in water and wastes by inductively coupled

14   plasma-atomic emission spectrometry. Environmental Monitoring Systems Laboratory,

15   Office of Research Development, Cincinnati, OH, USA. **1994.** Revision4.4.

16   [37] Wragg, J., Environmental Scientist, British Geological Survey, Nottingham, United

17   Kingdom. Personal Communication. August **2007.**

18   [38] Cave, M.R.; Wragg, J.; Palumbo, B.; Klinck, B.A. Measurement of the

19   bioaccessibility of arsenic in UK soils. **2002,** P5-062/TR02, 103p.

20   [39] Koch, I.; Sylvester, S.; Lai, V.W.-.; Owen, A.; Reimer, K.J.; Cullen, W.R.

21   Bioaccessibility and excretion of arsenic in Niu Huang Jie Du Pian pills. *Toxicol. Appl.*

22   *Pharmacol.* **2007,** *222*, 357-364.

1   [40] Smith, P.G.; Koch, I.; Reimer, K.J. Uptake, transport and transformation of arsenate

2   in radishes (Raphanus sativus). *Sci. Total Environ.* **2008,** *390,* 188-197.

3   [41] Grøn, C.;Andersen, L. Human bioaccessibility of heavy metals and PAH from soil.

4   Technology Programme for Soil and Groundwater Contamination. DHI - Water and

5   Environment. **2003,** Environmental Project No. 840 2003.

6   [42] Rutter, A. Director, Analytical Services Unit, Queen's University Personal

7   Communication. August **2009.**

8   [43] Hamel, S.C.; Buckley, B.; Lioy, P.J. Bioaccessibility of Metals in Soils for Different

9   Liquid to Solid Ratios in Synthetic Gastric Fluid. *Environ. Sci. Technol.* **1998,** *32,* 358-

10 362.

11 [44] Ollson, C.A. The Importance of Bioavailability. PhD Thesis, Faculty of Chemistry

12 and Chemical Engineering. Royal Military College of Canada, Kingston, ON, Canada.

13 **2003.**

14 [45] Castillo, M. and Ramirez, E. Synthesis and spectral properties of new complexes

15 between glycine and titanium(III), vanadium(III), chromium(III), iron(III), cobalt(II),

16 nickel(II) and copper(II). *Transition Met. Chem.* **1984,** *9,* 268-270.

17 [46] Yeow, A. United States Environmental Protection Agency (USEPA) In vivo in vitro

18 arsenic bioavailability comparison; Personal Communication. June **2009.**

19 [47] Bruce, S.; Noller, B.; Matanitobua, V.; Ng, J. In Vitro Physiologically Based

20 Extraction Test (PBET) and Bioaccessibility of Arsenic and Lead from Various Mine

21 Waste Materials. *J. Toxicol. Environ. Health, Pt A.* **2007,** *70,* 1700-1711.

1 [48] Juhasz, A.L.; Smith, E.; Weber, J.; Rees, M.; Rofe, A.; Kuchel, T.; Sansom, L.;

2 Naidu, R. In vitro assessment of arsenic bioaccessibility in contaminated (anthropogenic

3 and geogenic) soils. *Chemosphere* **2007,** *69,* 69-78.

4 [49] Juhasz, A.L.; Smith, E.; Weber, J.; Naidu, R.; Rees, M.; Rofe, A.; Kuchel, T.;

5 Sansom, L. Effect of soil ageing on in vivo arsenic bioavailability in two dissimilar soils.

6 *Chemosphere* **2008,** *71,* 2180-2186.

7 [50] Aposhian, M.M.; Koch, I.; Avram, M.D.; Chowdhury, U.K.; Smith, P.G.; Reimer,

8 K.J.; Aposhian, H.V. Arsenic in Ironite fertilizer: The absorption by hamsters and the

9 chemical form. *Toxicol. Environ. Chem.* **2009,** *91,* 1115-1124.

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Table 1. Results of quality control tests[a].

Medium	Measurement	Method	Experiment	N	Range	Average
Blank[b]	Arsenic concentration	Method P (P1, P2)	All L:S	19	$< 3.0\ \mu g \cdot L^{-1}$	–
Blank	Iron concentration	Method P (P1, P2)	All L:S	22	$< 20\ \mu g \cdot L^{-1}$	–
Blank	Arsenic concentration	Method G (P1, P2)	All L:S	30	$< 2.0\ \mu g \cdot L^{-1}$	–
Blank	Iron concentration	Method G (P1, P2)	All L:S	30	$< 20\ \mu g \cdot L^{-1}$	–
Spike recovery[c]	Arsenic recovery (%)	Method P (P1, P2)	L:S = 100:1	6	93–116%	98%
Calibration check[d]	Arsenic recovery (%)	Instrument	–	36	85–101%	93%
Calibration check	Iron recovery (%)	Instrument	–	36	95–119%	103%
SRM NIST2710	Bioaccessible arsenic (%)	Method P (P1)	Control limits[e] (L:S = 100:1)	23	24–57%	40%
SRM NIST2710	Bioaccessible arsenic (%)	Method P (P2)	Control limits (L:S = 100:1)	37	23–49%	36%
SRM NIST2710	Bioaccessible arsenic (%)	Method G (P1)	Control limits (L:S = 100:1)	37	29–70%	40%
SRM NIST2710	Bioaccessible arsenic (%)	Method G (P2)	Control limits (L:S = 100:1)	29	4.0–38%	21%
SRM NIST2710	Bioaccessible iron (%)	All methods	Control limits (All L:S)	19	0.29–15%	4.5%
SRM NIST2710	Bioaccessible arsenic (%)	Method P (P1)	L:S = 100:1	5	23–45%	39%
SRM NIST2710	Bioaccessible arsenic (%)	Method P (P2)	L:S = 100:1	5	31–38%	38%
SRM NIST2710	Bioaccessible arsenic (%)	Method G (P1)	L:S = 100:1	5	42–56%	49%
SRM NIST2710	Bioaccessible arsenic (%)	Method G (P2)	L:S = 100:1	5	16–37%	25%
SRM NIST2710	Bioaccessible iron (%)	All methods	All L:S	10	0.36–12%	4.8%
Duplicate pairs	RPD, bioaccessible arsenic (%)	Method P (P1, P2)	All L:S	38	0.22–20%	7.2%
Duplicate pairs	RPD, bioaccessible iron (%)	Method P (P1, P2)	All L:S	30	0.70–59%	17%
Duplicate pairs	RPD, bioaccessible arsenic (%)	Method G (P1, P2)	All L:S	52	0.23–47%	11%
Duplicate pairs	RPD, bioaccessible iron (%)	Method G (P1, P2)	All L:S	52	0.42–76%	12%

a. Abbreviations: N = number of replicates; L:S = liquid-to-solid ratio; P1 = gastric phase; P2 = gastric + intestinal phase; Method P = physiologically-based; Method G = glycine-buffered; SRM = standard reference material; RPD = relative percent difference, calculated as $100 \cdot (\text{result of primary sample} - \text{result of duplicate sample}) \cdot \text{average}^{-1}$. All other abbreviations are described in the text.

b. Instrument detection limit is $1\ \mu g \cdot L^{-1}$ for arsenic and $10\ \mu g \cdot L^{-1}$ for iron. Results presented in this table represent limits of quantification.

c. Spike recovery of $100\ ppb \cdot L^{-1}$ potassium arsenate (KH_2AsO_4, Fluka reagent grade) in blank PBET solution added immediately before analysis.

d. Calibration check solutions used in the ICP-MS analysis include both 50ppb and 750ppb multi-element solutions (PlasmaCAL), prepared from a different source solution than the ICP-MS calibration solutions.

e. All control limits for SRM are average ± three standard deviations for all laboratory results recorded (between 2001 and 2009 for arsenic; between 2006 and 2008 for iron), excluding the results of the present study.

1

Table 2. Percent arsenic bioaccessibility[ab].

Sample	Molar Ratio Fe:As	Total Arsenic mg•kg^{-1}	Method P[b] L:S=100:1 P1	P2	L:S=2000:1 P1	P2	Method G[c] (0.4 M) L:S=100:1 P1	P2	(0.4 M) L:S=2000:1H P1	P2	(0.02M) L:S=2000:1L P1	P2
BGS102	2600	94	2.3	2.8	12	9.5	2.8	2.1	8.4	9.7	3.5	**5.2**
NIST2711	360	110	47	37	45	38	40	34	45	51	45	44
MG6	250	320	**2.5**	1.4	3.9	3.5	**2.6**	0.33	10	**14**	3.3	**5.0**
NB7	79	460	31	34	25	29	33	26	43	58	35	39
NIST2710	72	630	**33**	23	47	36	**42**	17	**79**	35	48	39
NB6A	54	740	16	18	12	14	21	21	37	30	23	26
Ironite®	37	4300	**28**	4.7	32	30	**25**	0.39	39	30	**39**	3.7
NB11A	18	5800	14	16	6	**13**	12	**18**	10	**24**	7.4	**15**
NB6B	11	7200	3.9	**5.6**	2.8	**4.5**	3.3	**4.5**	4.1	**9.7**	2.2	**4.8**
NB12	9.4	8200	13	**29**	12	**26**	16	15	24	**54**	17	**33**
GD5	5.3	7200	47	49	32	37	**44**	26	58	61	48	43
MG3	3.3	24 000	11	12	**10**	7.2	**16**	8.4	31	35	21	22
MG4	3.3	21 000	2.0	2.7	0.99	**1.8**	4.1	3.3	9.0	**13**	2.1	**3.8**
GD2	2.5	19 000	2.4	**4.1**	0.82	**2.4**	7.8	**16**	10	13	2.9	**7.1**
CAR2	1.6	310 000	**0.62**	0.27	0.10	**0.19**	0.48	0.52	0.44	0.54	0.44	0.41
LSH Tailings	1.4	200 000	1.0	**1.6**	0.62	**0.84**	0.97	**2.6**	1.1	**3.7**	0.83	**2.8**
CAR1	1.3	77 000	2.1	**5.0**	0.50	**1.6**	2.4	**3.3**	2.4	**9.8**	2.1	**8.8**
GD1	1.3	210 000	0.13	**0.32**	0.05	**0.42**	0.13	**2.2**	0.27	**2.8**	0.22	**0.93**
Arsenopyrite	1.2	420 000	0.17	0.22	0.24	0.31	**0.23**	0.16	0.24	**0.36**	0.34	0.36

1 a. Results are presented in order of decreasing molar iron to arsenic ratio. Numbers in bold indicate the
2　 higher of either P1 or P2 result where the relative percent difference, calculated as 100 • (P1 – P2) •
3　 average^{-1} is greater than the acceptable laboratory repeatability (30%).
4 b. Abbreviations: L:S = liquid-to-solid ratio; P1 = gastric phase; P2 = gastric + intestinal phase;
5　 Method P = physiologically-based; Method G = glycine-buffered.
6 c. For Method G, the first two extractions used a 0.4 M glycine concentration, and a third extraction was
7　 performed using 0.02 M glycine.

Table 3. Percent iron bioaccessibility[ab].

| Sample	Molar Ratio Fe:As	Total Iron $mg \cdot kg^{-1}$	Method[c] L:S=100:1 P1	P2	L:S=2000:1 P1	P2	Method G[d] (0.4 M) L:S=100:1 P1	P2	(0.4 M) L:S=2000:1H P1	P2	(0.02M) L:S=2000:1L P1	P2
BGS102	2600	180 000	0.50	0.39	1.2	1.2	**0.92**	0.03	**1.2**	0.41	**1.4**	0.07
NIST2711	360	29 000	1.9	2.4	2.3	2.1	**2.5**	0.16	**3.0**	1.3	**2.8**	0.82
MG6	250	60 000	**0.83**	0.01	0.82	**1.4**	**1.9**	0.13	**2.8**	1.7	**0.86**	0.28
NB7	79	27 000	**1.5**	0.57	1.3	1.8	**4.1**	0.38	3.3	3.7	**3.5**	1.8
NIST2710	72	34 000	0.36	**4.0**	4.2	3.7	**6.1**	0.84	**12**	6.0	**6.3**	4.4
NB6A	54	30 000	**0.74**	0.45	0.57	**0.87**	**2.7**	0.63	**3.1**	1.6	**2.4**	0.28
Ironite®	37	120 000	5.2	**15**	14	12	**14**	0.06	**18**	12	**19**	0.50
NB11A	18	92 000	0.99	1.2	0.44	**0.96**	**2.2**	0.22	1.3	**1.9**	**1.3**	0.83
NB6B	11	60 000	0.87	0.69	0.60	**0.87**	**3.1**	0.25	1.9	1.5	**1.7**	0.79
NB12	9.4	64 000	n/a	n/a	2.8	**4.8**	**9.1**	2.2	8.2	9.7	8.3	6.3
GD5	5.3	29 000	n/a	n/a	5.5	6.4	**13**	1.1	16	13	**16**	5.2
MG3	3.3	59 000	n/a	n/a	2.2	**7.6**	**8.6**	0.43	**14**	10	**10**	1.5
MG4	3.3	53 000	1.1	1.1	1.1	1.1	**4.8**	0.81	7.2	5.9	**3.4**	0.92
GD2	2.5	36 000	0.89	**2.2**	0.92	1.2	**6.6**	1.1	**7.3**	4.0	2.6	2.3
CR2	1.6	360 000	0.23	0.23	0.09	0.10	**0.38**	0.10	**0.32**	0.19	**0.35**	0.05
LSH Tailings	1.4	210 000	0.67	0.89	0.42	0.35	**0.94**	0.35	0.91	**1.6**	0.85	0.66
CR1	1.3	75 000	n/a	n/a	0.41	**0.83**	**2.6**	1.5	2.5	**6.0**	**2.5**	0.81
GD1	1.3	200 000	n/a	n/a	0.16	**0.37**	0.28	**0.47**	0.43	**0.69**	0.42	0.44
Arsenopyrite	1.2	380 000	0.18	0.22	0.25	0.29	**0.32**	0.09	0.26	**0.37**	**0.48**	0.27

3 a. Results are presented in order of decreasing molar iron to arsenic ratio. Numbers in bold indicate the
4 higher of either P1 or P2 result where the relative percent difference, calculated as $100 \cdot (P1 - P2) \cdot$
5 $average^{-1}$ is greater than the acceptable laboratory repeatability (30%).
6 b. Abbreviations: L:S = liquid-to-solid ratio; P1 = gastric phase; P2 = gastric + intestinal phase;
7 Method P = physiologically-based; Method G = glycine-buffered.
8 c. Items marked n/a were not analyzed for iron concentrations.
9 d. For Method G, the first two extractions used a 0.4 M glycine concentration, and a third extraction was
10 performed using 0.02 M glycine.

11
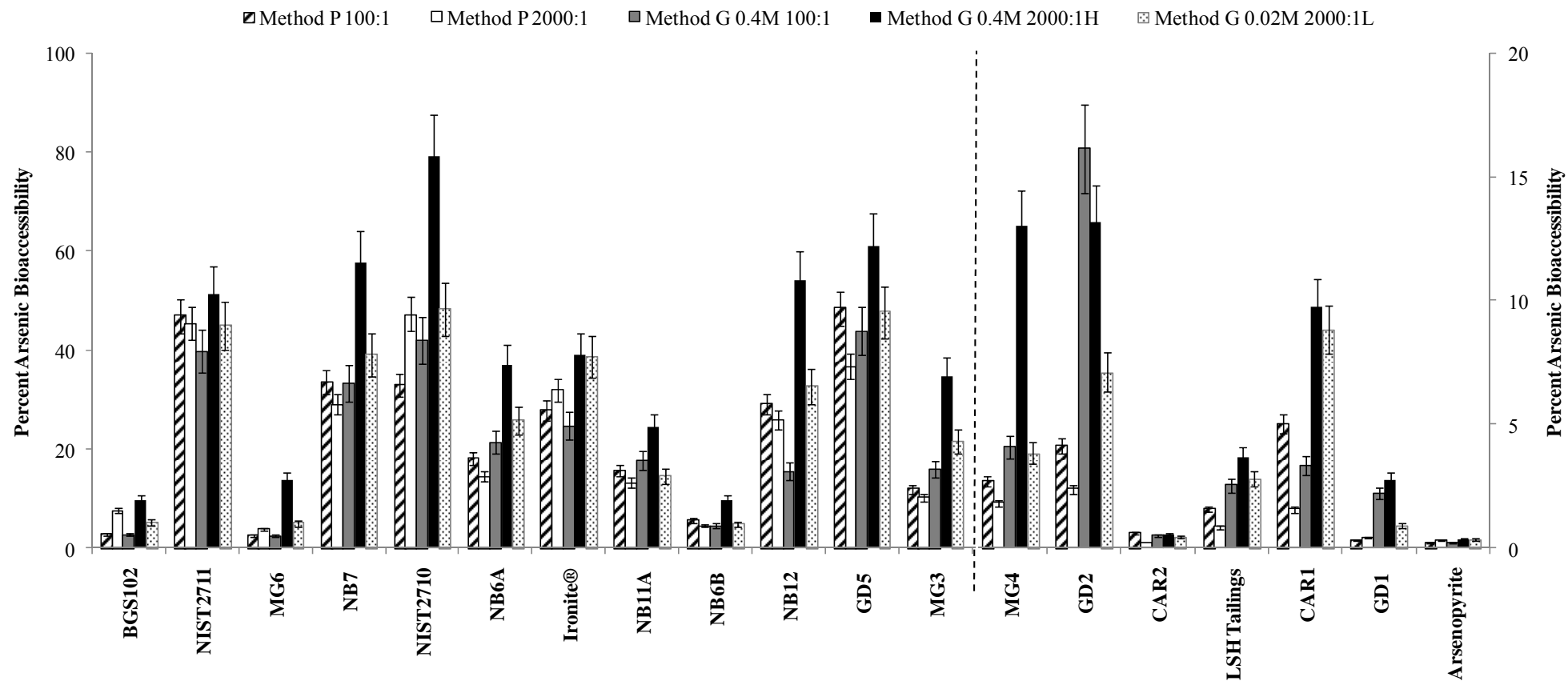
Figure 1: Percent arsenic bioaccessibility. The higher result of either the gastric phase or the gastric + intestinal phase is shown for each method in decreasing order of iron to arsenic molar ratio. Results on the right side of the dotted line are associated with the vertical axis shown on the right.

1
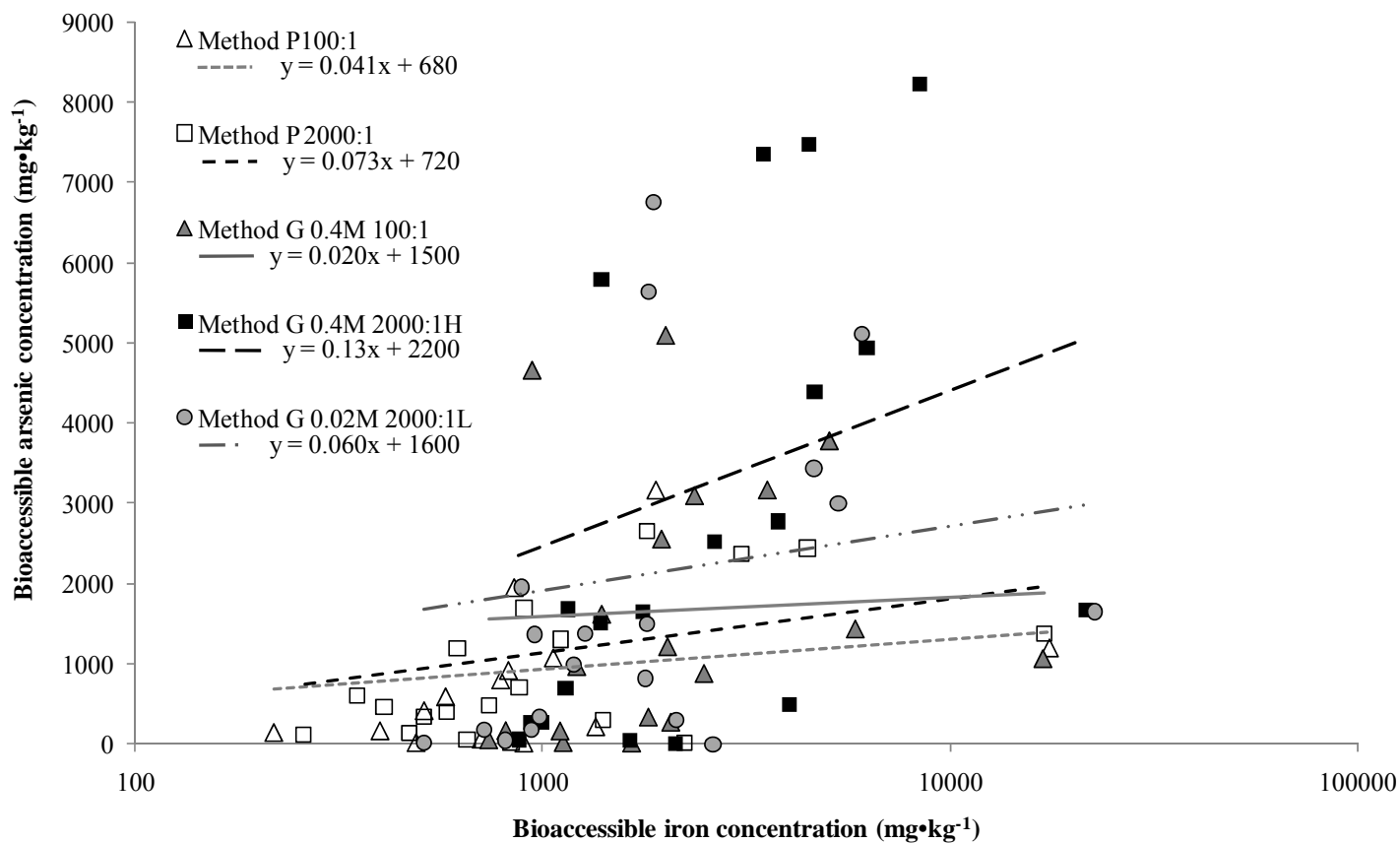
Figure 2: Bioaccessible arsenic and iron concentrations (mg•kg^{-1}) for all samples under both Method P and

Method G at the two liquid-to-solid ratios (100:1 and 2000:1). For Method G at the 2000:1, a 0.4 M glycine

concentrations was used in the 100:1 and 2000:1H extraction, and a 0.02 M glycine concentration was used

in the 2000:1L extraction.