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Journal of Environmental Science and Health, Part A
Toxic/Hazardous Substances and Environmental Engineering

ISSN: 1093-4529 (Print) 1532-4117 (Online) Journal homepage: http://www.tandfonline.com/loi/lesa20

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To cite this article: Brian D. Laird , Kyle J. James , Tom R. Van de Wiele , Matt Dodd , Stan W. Casteel , Mark Wickstrom & Steven D. Siciliano (2013) An investigation of the effect of gastrointestinal microbial activity on oral arsenic bioavailability, Journal of Environmental Science and Health, Part A, 48:6, 612-619, DOI: 10.1080/10934529.2013.731357

To link to this article: https://doi.org/10.1080/10934529.2013.731357

Published online: 26 Feb 2013.

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Journal of Environmental Science and Health, Part A (2013) **48**, 612–619
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ISSN: 1093-4529 (Print); 1532-4117 (Online)
DOI: 10.1080/10934529.2013.731357

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An investigation of the effect of gastrointestinal microbial activity on oral arsenic bioavailability

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In vitro gastrointestinal (GI) microbial activity in the colon compartment facilitates the arsenic release from soils into simulated GI fluids. Consequentially, it is possible that *in vitro* models that neglect to include microbial activity underestimate arsenic bioaccessibility when calculating oral exposure. However, the toxicological relevance of increased arsenic release due to microbial activity is contingent upon the subsequent absorption of arsenic solubilized in the GI lumen. The objectives of this research are to: (1) assess whether microbes in the *in vitro* small intestine affect arsenic solubilization from soils, (2) determine whether differences in the GI microbial community result in differences in the oral bioavailability of soil-borne arsenic. *In vitro* GI microbial activity in the distal small intestine increased arsenic release from soils; however, these effects were unlikely to be relevant since they were transient and demonstrated small effect sizes. *In vivo* arsenic absorption for juvenile swine was unaffected by antibiotic treatment. Therefore, it appears that microbial effects on arsenic release do not result in increased arsenic bioavailability. However, it remains to be seen whether the results for the limited set of soils described herein can be extrapolated to arsenic contaminated sites in general.

Keywords: Absolute bioavailability, arsenic, soil contamination, ingestion exposure, colon microorganisms.

Introduction

Elevated soil arsenic (As) concentrations can present health risk to affected communities via the incidental ingestion of contaminated soil. The calculation of such oral exposures are driven by three variables: soil concentration (C_s), soil ingestion rate (IR_s), and bioavailability (RBA).[1–3] Arsenic RBA is defined as the fraction of ingested As from contaminated soil that reaches systemic circulation relative to the fraction of ingested As from sodium arsenate that reaches systemic circulation. Preliminary Quantitative Risk Assessments typically require the conservative and protective assumption that arsenic RBA is 100%.[3] However, in more detailed risk assessments (e.g., Site Specific Risk Assessments or SSRA), risk assessors may account for limitations on gastrointestinal (GI) absorption when calculating oral exposure.[4]

Arsenic RBA is traditionally measured using in vivo dosing trials to derive the arsenic Urinary Excretion Fraction (UEF), which represents the fraction of ingested As excreted into the urine of exposed animals.[2,5] The RBA of arsenic is then calculated by dividing the UEF of animals exposed to As from soil by the UEF of animals exposed to sodium arsenate. The ratio of the UEF_{soil}:$UEF_{arsenate}$ is equivalent to As RBA since the extent of tissue binding, biliary excretion, and excretion in hair is assumed equivalent between the soil and sodium arsenate treatment groups.

Arsenic bioaccessibility is defined as the fraction of ingested As solubilized into GI fluids that is maximally available for intestinal absorption.[6] *In vitro* models (e.g., Physiologically Based Extraction Test or PBET, Ohio State University in vitro Gastrointestinal Method or OSU-IVG, Unified BARGE Method or UBM, Simulator of the Human Intestinal Microbial Ecosystem or SHIME) are often used for measuring As bioaccessibility.[7–11] Each of these methods listed above simulate a subset of the physical, chemical, and enzymatic processes of the human GI tract. However, only the SHIME incorporates the function of aerobic and anaerobic microorganisms present in the distal GI tract.[12] This could be of particular importance for estimating As bioaccessibility since GI microorganisms may influence As speciation,[13,14] thereby altering As toxicokinetics.[15] In addition, *in vitro* GI microbial activity

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often increases As bioaccessibility, as demonstrated for mine tailings, soils, and other test materials.[16–18] Consequently, *in vitro* GI models that do not include microbial activity may underestimate As bioaccessibility for human health risk assessment.

There is little evidence as to whether As that is mobilized by microbial activity is, thereafter, absorbed from the intestinal lumen into systemic circulation. Recent publications describing the role of GI microbial activity on As bioaccessibility have utilized microbial communities and physiological conditions representative of those found in the human colon.[16–18] However, it is the small intestine that is thought to be the dominant site of GI As absorption due to its large surface area and high efficiency of phosphate absorption,[19] which is a surrogate for As absorption.[20,21]

However, even without colonic As uptake, GI microbial activity may contribute to As bioavailability since the distal small intestine (e.g., ileum) also possesses a large and diverse community of aerobic and anaerobic bacteria under reducing conditions.[22–24] Furthermore, the sizeable surface area and phosphate transporter (NaP_iIIb) expression [25] would likely make microbially mediated increases in As bioaccessibility to be of utmost toxicological relevance in the ileum.

To evaluate whether it is necessary for measures of As bioaccessibility to account for GI microbial activity, we conducted *in vitro* and *in vivo* experiments to evaluate the toxicological relevance of GI microbes to As bioaccessibility and bioavailability. For the *in vitro* experiment, we compared As bioaccessibility with and without an active distal small intestinal microbial community to assess the role of microbes in a GI stage known to contribute to bioavailable As. For the *in vivo* experiment, we compared the As bioavailability of juvenile swine (with and without oral antibiotic treatment) orally exposed to soil-borne As.

Experimental

Soils

For the in vitro ileum SHIME experiment, 20 contaminated soils were collected from urban residential neighborhoods in Hoboken, Belgium. Three of the Hoboken soils (H5, H18, and H50) containing elevated As concentrations were selected for the research described in this manuscript. The soils were dry-sieved (<100 μm) to better reflect the size fraction most likely to adhere and then be ingested.[26,27] The limited quantity of Hoboken soils available precluded their use in the in vivo juvenile swine dosing trial. Instead, for the *in vivo* dosing trial, overburden from the open pit at an abandoned gold and silver mine in Mount Nansen, Yukon was collected and sieved to <250 μm. The overburden (TM1) was collected as part of a comprehensive human and environmental risk assessment conducted for the site. Arsenic concentrations in each of the soils are listed in Table 1. The use of different test materials for the in vitro and in vivo components of this research prevented any attempt to validate the SHIME methodology according to As relative bioavailability.

Table 1. Arsenic concentrations (mg kg^{-1}) in the three Hoboken Soils (H5, H18, and H50), one standard reference soil (BGS 102), and one mine overburden (TM1).

	H5	*H18*	*H50*	*BGS 102*	*TM1*
Bulk Soil	33	27	48	101	3940
100 μm Sieved	35	35	115		

The dynamic SHIME

The microbial community from the human GI tract was cultured using the SHIME according to previously published procedures.[12] In brief, the SHIME microbial community was established by mixing human fecal matter, which was freshly voided by an adult male volunteer, in a phosphate/thioglycolate buffer. The fecal mixture was subsequently centrifuged and the supernatant (40 mL) was added to a carbohydrate-based nutrition solution (600 mL) in the third compartment of a series of double-jacketed reactor flasks.

The carbohydrate nutrition contained per liter of distilled water: 5.01 g $KHCO_3$, 2.92 g NaCl, 0.5 g arabinogalacatan, 1.0 g pectin, 0.5 g xylan, 2.1 g potato starch, 0.2 g glucose, 1.5 g yeast extract, 0.5 g peptone, 2.0 g mucin, and 0.25 g L-cysteine hydrochloride. The SHIME microbial community was fed by pumping 100 mL of pH 2 nutrition into the first compartment (i.e., stomach stage) of the double-jacketed reactor flask. After 2 h in the simulated stomach stage, the solution was pumped into the second compartment (i.e., duodenum stage). Therein, the carbohydrate solution was mixed with a pancreatic enzyme solution (50 mL) containing per liter: 12.5 g $NaHCO_3$, 6.0 g Oxgall (dehydrated bile salts), and 0.9 g pancreatin. Following an additional 2 h in the simulated duodenum stage, the mixture was pumped into the third compartment (i.e., colon stage) containing the human fecal microbial inoculum which adapted to the *in vitro* colon conditions imposed. This feeding schedule was repeated every 8 h.

The pH of the simulated colon compartment was maintained between 5.9 and 6.3 using a pH controller (Consort R305). The temperature of the SHIME was set to 37°C

Table 2. Mean (n = 3; *std err*) percent bioaccessibility of four test materials (H5, H18, H50, and TM1) extracted using the SHIME.

GI Stage	*H5*	*H18*	*H50*	*TM1*
Stomach SHIME	24.6 (0.6)	16.5 (0.8)	66.4 (4.8)	6.1 (0.6)
Duodenum SHIME	15.7 (1.6)	13.8 (3.2)	36.8 (5.5)	5.1 (0.3)
Ileum SHIME	23.5 (0.5)	21.0 (0.4)	67.6 (1.5)	NA[a]
Colon SHIME	26.4 (1.0)	28.5 (1.1)	77.3 (3.8)	15.1 (0.3)

[a]Not available.

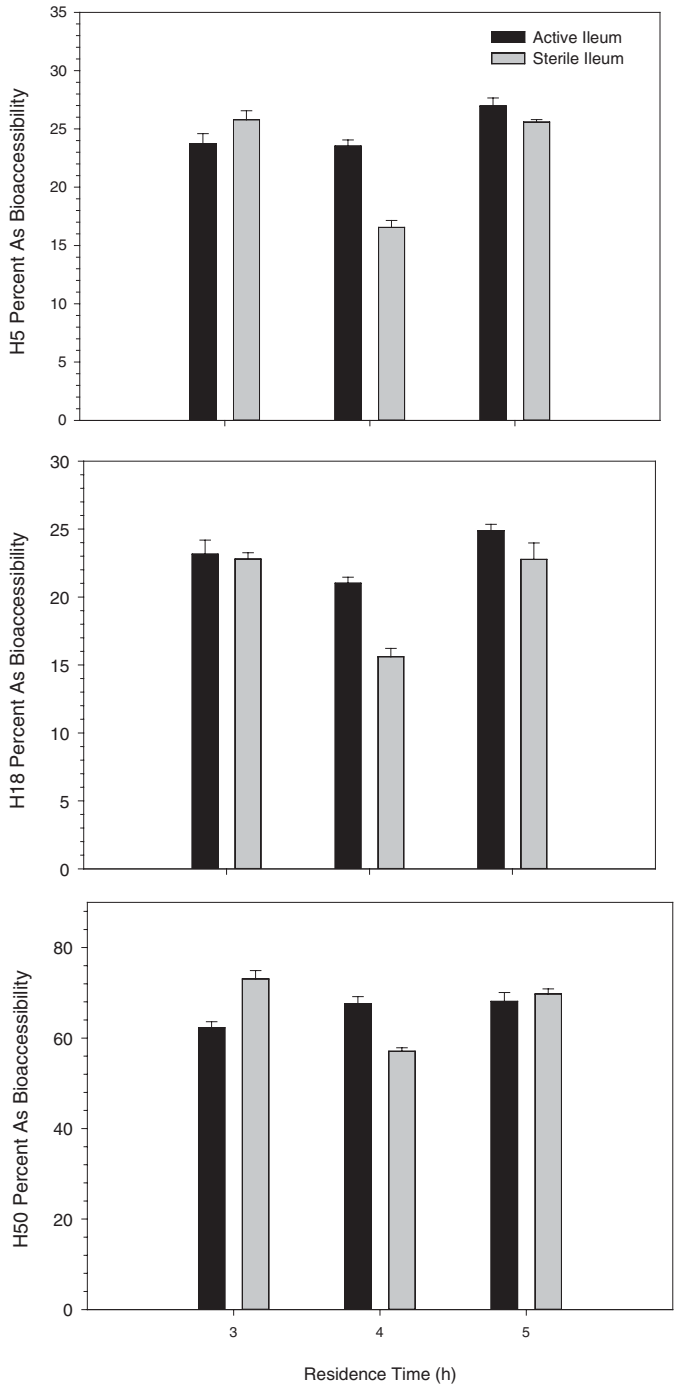


Fig. 1. Mean percent As bioaccessibility (± std err) from 3 Hoboken soils (H5, H18, H50; n = 3) following sample extraction in the Ileum SHIME compared to autoclaved controls (Sterile Ileum).

using a circulating heated water bath and the anaerobic status (−196 ± 9 mV) of the system was maintained via daily headspace purges with N_2. The colon microbial community contained 8.7 log Colony Forming Units (CFU) mL^{-1} total aerobes and 8.8 log CFU mL^{-1} total anaerobes and included Lactobacilli, Fecal Coliforms, Clostridia, Staphylococci, Enterococci, Bifidobacteria, Klebsiella, and Veillonella.

Table 3. The residence time and redox (*std err*) of the SHIME digests of BGS 102.

GI stage	*Redox (mV)*	*Residence time (hr)*
Ileum SHIME	-30.7 (3.2)	4
Colon SHIME	-305.0 (6.2)	18

The static SHIME

Arsenic bioaccessibility of the three Hoboken soils was measured in four stages of the SHIME: the stomach SHIME, duodenum SHIME, ileum SHIME, and colon SHIME. Of these four stages, only the ileum and colon stages of the SHIME received aliquots of intestinal microbial suspension. Previously published SHIME procedures were modified to include a novel microbially-active stage representative of the distal human small intestine (i.e., ileum).[10,18] Each soil (0.3 g; n = 3) was added to 150 mL glass bottles for the four stages of the SHIME. For the stomach SHIME, 30 mL of pH 1.4 HCl was added to each bottle and the pH of the soil slurry was then adjusted to 1.5 ± 0.1 via the dropwise addition of 0.5 M HCl/NaOH. The bottles were then capped, flushed with N_2 (30 min, 1 bar), and then incubated at 37°C with horizontal shaking (130 rpm). After 2 h in the stomach SHIME, 15 mL of pancreatic enzyme solution was added to each bottle and the pH was adjusted to 6.5 ± 0.2. The bottles were then flushed with N_2 and shaken for an additional 1, 2, or 3 h for the duodenum SHIME.

Thereafter, 15 mL of ileum suspension (8.6 g L^{-1} K_2HPO_4, 6.8 g L^{-1} KH_2PO_4, 3 g L^{-1} Oxgall, 1 g L^{-1} mucin, 0.5 g L^{-1} L-cysteine HCl, and 10 mL L^{-1} Dynamic SHIME colon suspension) was added to each bottle. The pH of the solution was adjusted to 6.8 ± 0.2 and the bottles were flushed and incubated for an additional 5, 4, or 3 h for the ileum SHIME. Three residence time treatment combinations were performed (e.g., 3 h duodenum + 3 h ileum SHIME, 2 h duodenum + 4 h ileum SHIME, or 1 hr duodenum + 5 h ileum SHIME) to determine whether increasing microbial activity duration increases As bioaccessibility. After the completion of the ileum SHIME, 30 mL of Dynamic SHIME colon suspension was added to each bottle and the pH was adjusted to 6.3 ± 0.2. The bottles were capped, flushed with N_2, and incubated for a final 18 h.

After each of the 4 stages of the SHIME (stomach, duodenum, ileum, and colon), bioaccessible arsenic was separated with syringe microfiltration (0.45 μm, Whatman, GMF) and a 10 mL aliquot of the bioaccessible fraction was acidified via 0.5 mL of 12 M HCl (EMD, OmniTrace) and frozen until chemical analysis.[28] To test the role of microbial activity in the determination of arsenic bioaccessibility, the ileum SHIME and colon SHIME treatments were then repeated using Dynamic SHIME suspension that was previously autoclaved and cooled.

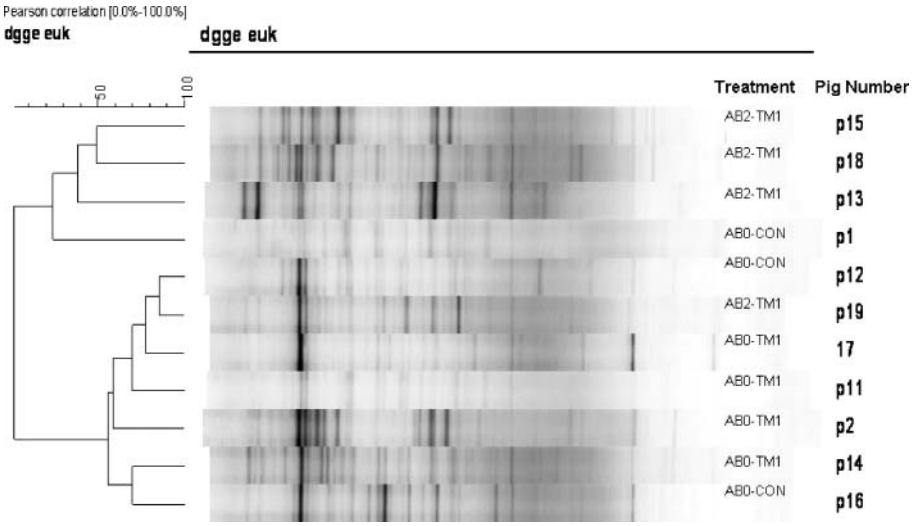


Fig. 2. Pearson correlation cluster analysis of DGGE results for the microbial community of the juvenile swine proximal colon. Lanes marked P (e.g., P1, P2, P11, etc) denote lanes containing microbial DNA extracted from the proximal colon of individual swine. The As/antibiotic treatments of each lane is also noted: AB0-CON (neither antibiotic nor As), AB2-TM1 (both As and antibiotic), and AB0-TM1 (As but not antibiotic).

Animals, housing and feed

The *in vivo* As dosing procedures were based upon methods developed by Dr. Stan W. Casteel at the University of Missouri.[2,4,9] Eleven healthy male swine (8-week-old; ~24 kg) were obtained from the Prairie Swine Center (Saskatoon, SK). The animals were housed in individual metabolic cages (1.5 × 1.5 × 0.9 m) equipped with screened collection pans to facilitate the separation and quantitative collection of urine. The pens were maintained in the thermo-neutral zone of the pigs with the lights on a 14:10 h (light:dark) cycle. The swine were weaned onto an antibiotic-free stage 3 starter ration pig chow and municipal drinking water was provided to each animal *ad libitum*. During the 3-week experiment, each animal was fed two equal portions of feed daily in the morning and evening. Their daily feed intake was restricted to 4% body weight to encourage them to completely consume their oral dose, which was incorporated into a molasses-flavored treat.

In vivo arsenic exposures

The health of the animals was monitored for 6 days prior to the onset of As exposure to ensure each animal successfully adjusted to their metabolic cages. After the 6-day acclimation period, the animals were randomly assigned to 1 of 3 treatment groups: As plus antibiotics (AB2-TM1; n = 4), As without antibiotics (AB0-TM1; n = 4), and a negative control group (AB0-CON; n = 3). The negative control group animals were administered neither antibiotic nor As. The molasses treats used to administer the antibiotics/As were made by thoroughly mixing equal portions of pig chow, rolled oats, and molasses into a thick malleable dough-ball. These molasses-flavored treats were used as a dosing vehicle rather than the more commonly used dough-ball since molasses was better able to mask the bitter flavor of the antibiotics.[9]

The antibiotic treatment procedure used to modify the GI microbial communities of the juvenile swine was designed in consultation with Dr. Chris Clark (Department of Veterinary Medicine, University of Saskatchewan). Neomycin (Soluble Neomix Powder) was selected because it is commonly given orally to pigs to treat enteric infections and is broadly effective against gram-negative bacteria and some gram-positive bacteria. Furthermore, neomycin was previously used to successfully show the importance of GI microorganisms for Hg toxicokinetics.[29] Metronidazole (Apotex Inc, DIN 00545066) was also included in the antibiotic treatment regimen due to its high efficacy against anaerobic intestinal microflora.[30]

Because this same group of microbes (i.e., faculative anaerobes, namely Dissimilatory Iron Reducing Prokaryotes or DIRPs) was thought to be responsible for the mobilization of metals from contaminated sediments,[31] metronidazole was deemed likely to cause microbial community shifts relevant to As toxicokinetics. Metronidazole is not approved for treatment of swine since it is not food-safe. Therefore, special approval was obtained to treat the swine with metronidazole on the condition that all animals would be destroyed at the end of the study and not enter the food supply. The dosages of the two antibiotics were initially set at 30% above the therapeutic dose for neomycin (30 mg kg^{-1} d^{-1}) and metronidazole (53 mg kg^{-1} d^{-1}).[32] After 7 days of treatment, the antibiotic dosages were increased to twice the therapeutic doses for neomycin (45 mg kg^{-1} d^{-1}) and metronidazole (80 mg kg^{-1} d^{-1}) to

maximize the effects upon the GI microbial communities of the juvenile swine.

For animals in the AB2-TM1 treatment group, neomycin and metronidazole were hand-mixed into the molasses treat. Similarly, TM1 was added to the molasses treat for animals in the AB2-TM1 and AB0-TM1 treatment groups. Animals in the AB2-TM1 and AB0-TM1 treatment groups were orally exposed to 160 μg As kg BW^{-1} day^{-1} from TM1 mixed into the molasses-balls. The molasses treat mixture containing antibiotic and/or As doses were divided into two equal portions, the first of which was administered (by hand) prior to morning feeding whereas the second was administered prior to evening feeding. This dosing procedure was continued for 14 days to allow urinary As excretion to reach steady-state.

During the 14-day exposure period, the total urine output was collected from each pig over 3 separate 48-h intervals (D7-8, D10-11, and D13-14) into large polypropylene containers. The volume of excreted urine for each animal was measured for each of the 3 urine collection periods. Subsequently, two 50-mL aliquots of urine were transferred into disposable polypropylene centrifuge tubes and were acidified (2% HCl) and frozen until microwave digestion and As quantification. After the 14-day exposure period, the animals were euthanized using a captive-bolt gun followed by exsanguination. The animals were dissected immediately and a 10-cm segment of the proximal colon was excised. Three 40-mL subsamples of the intestinal contents of the proximal colon segment were transferred into disposable polypropylene centrifuge tubes. The tubes were immediately flash-frozen in liquid nitrogen and stored at -80°C until denaturing gradient gel electrophoresis (DGGE).

Arsenic quantification

The acidified SHIME fluids were microwave digested prior to As quantification. The microwave digestion procedure required 2 mL of the acidified SHIME samples to be added to 10 mL of 12 M OmniTrace HCl in Teflon-coated tubes (Xpress, CEM). H_2O_2 (30%, 1 mL) was added to each tube to initiate the oxidation of organic matter and then, after allowing 15 min for the reaction to subside, another 1 mL aliquot of H_2O_2 was added to each tube. The tubes were capped and then digested at 100% power (1600 Watts, Time = 50 min) in a MARS-5 microwave (CEM). The microwave digested SHIME solutions were diluted and transferred to disposable polypropylene centrifuge tubes for analysis via Hydride Generation Atomic Fluorescence Spectroscopy (HGAFS). Inorganic As in the microwave digested samples was reduced to arsenite (As^{III}) via the addition of a KI solution (50% w/v) containing 10% ascorbic acid.[33] Thereafter, the aqueous As concentration in each of the microwave digested SHIME samples was measured using a Millenium Excalibur (PS Analytical). Blank corrected percent As bioaccessibility was calculated according to the equation:

$$\text{Bioaccessibility}(\%) = \frac{\text{Arsenic Released in Solution } (\mu g)}{\text{Arsenic Present in Soil Sample } (\mu g)} \times 100\% \quad (1)$$

The acidified swine urine collected during the in vivo As dosing trial was microwave digested prior to quantification via Inductively Coupled Plasma Mass Spectrometry (ICPMS) to ensure complete recovery of methylated As metabolites present in the swine urine. Pig urine (2 mL) was added to 3 mL of HNO_3 (16 M, EMD OmniTrace) and 0.5 mL of H_2O_2 in Teflon-coated digestion tubes (Xpress, CEM). The tubes were capped and transferred into MARS-5 microwave (CEM) and digested at 100% power (1600 Watts, Time = 45 min). The digested samples were transferred to 15-mL polypropylene centrifuge tubes and diluted to 2% HNO_3 for ICPMS analysis. Arsenic absolute bioavailability (ABA) excretion was calculated according to the equation:

$$ABA(\%) = \frac{Volume_{urins}\,(ml/48\,hr) \times [As]\,(\mu g/ml)}{Total\ Ingested\ As\,(\mu g/48\,hr)} \times 100\% \quad (2)$$

Statistics

Normality (Kolmogorov-Smirnov, $P > 0.05$) and homogeneity of variance (Levene Median, $P > 0.05$) assumptions for As bioaccessibility and urinary As excretion were confirmed using SigmaStat v3.1. Two-way Analysis of Variance (ANOVA) was used to evaluate the effects of microbial activity and ileum residence time on the bioaccessibility of As from each of the 3 Hoboken soils (SigmaStat v3.1). Pairwise multiple comparisons were performed using the Holm-Sidak method (overall significance level = 0.05). Similarly, a two-way ANOVA was used to evaluate the role of

Table 4. The effect of antibiotics on the As absolute bioavailability (*std err*) of swine exposed orally to overburden (TM1) of an abandoned mine over three 48-h collection periods (Coll Period #1: Day 7–8; Coll Period #2: Day 10–11; Coll Period #3: Day 13–14).

		Absolute As Bioavailability (%)		
Treatment Group	*Description*	*Coll Period #1 D7-D8*	*Coll Period #2 D10-D11*	*Coll Period #3 D13–14*
AB0-TM1	−Antibiotics+As	12.1 (0.9)	14.2 (0.3)	13.2 (1.0)
AB2-TM1	+Antibiotics+As	10.2 (2.1)	15.6 (1.7)	13.9 (0.8)

antibiotic co-administration and urine collection period on the As bioavailability of swine orally exposed to TM1.

Quality assurance/quality control

The standard reference material BGS 102 was digested in the SHIME as a positive control for each treatment batch of Hoboken soils to check the consistency of As bioaccessibility results with those of previous studies. Each SHIME treatment batch also included a blank and sodium arsenate spike. The methodological detection limit (MDL = $\sigma_{n=35} * t_{n-1;\,\alpha=0.01}$) of As in the SHIME fluid was 181 ng L^{-1}. Average spike recovery of sodium arsenate was 81 ± 4.8%. Instrumental precision was monitored by re-measuring one for every 15 samples; mean deviation of duplicates was 3.0%. The spike recovery of monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) in swine urine measured via ICPMS was 118 ± 3 and 121 ± 0.5%, respectively. The MDL of As in swine urine (MDL = $\sigma_{n=10} * t_{n-1;\,\alpha=0.01}$) collected at the PSCI was 292 ng L^{-1}.

Results and discussion

Arsenic bioaccessibility of the three Hoboken soils ranged between 17–66% in the stomach SHIME, 14–51% in the duodenum SHIME, 21–68% in the ileum SHIME, and 26–77% in the colon SHIME (Table 2). Also, i*n vitro* bioaccessibility tests indicated that colon microbial activity tripled the bioaccessibility of As in TM1 relative to sterile controls (Active colon SHIME: 15.1%; Sterile colon SHIME: 5.3%). Notably, the As bioaccessibility of TM1 in the Active colon SHIME was similar to previous measurements using PBET (13.5%).

When the ileum SHIME residence time was 4 hours, microbial activity increased the As bioaccessibility in H5 (Holm-Sidak, $P < 0.001$), H18 (Holm-Sidak, $P = 0.001$) and H50 (Holm-Sidak, $P = 0.004$) (Fig. 1). This microbial activity was associated with the formation of black precipitate, as previously observed in the colon SHIME,[16] despite the ileum SHIME's considerably shorter residence time and less negative redox status compared to the colon SHIME (Table 3). The size of the microbial effect upon As bioaccessibility, ranging between 5 and 11%, after the 4-h incubation was smaller than typically observed (up to 50%) in the colon SHIME.[16–18]

These microbially-mediated increases in ileum SHIME As bioaccessibility were not consistently observed when residence times were reduced to 3 h or extended to 5 h. Therefore, any microbial effects upon As bioaccessibility under small intestinal conditions are not only small but also transient. As such, small intestinal GI microbial activity is unlikely to play an important role in As bioaccessibility.

The GI microbial community of 3 of the 4 juvenile swine (p15, p18, and p13) treated with oral antibiotics (AB2-TM1) clustered separately from the microbial communities of swine not treated with antibiotics (AB0-TM1) (Fig. 2). There was, however, considerable inter-individual variation in the DGGE banding-patterns of the GI microbial communities in the swine proximal colon. For example, there was less than 50% similarity in the banding-patterns of the oral-antibiotic treated swine. Furthermore, the banding-patterns of the microbial communities of untreated swine diverged as much as 40% (Fig. 2). In previous work investigating the role of microbes in Mercury (Hg) toxicokinetics, the authors asserted that the GI microbial community was eliminated within 3 days of oral antibiotic treatment. [29] In contrast, the oral antibiotic treatment described in this study altered the composition of swine GI microbial community but certainly did not eliminate it.

Despite this, it is reasonable to assume that the structural changes observed in the GI microbial community were relevant to As toxicokinetics since metronidazole targets the anaerobic bacteria likely responsible for alterations in As bioaccessibility.[30] The As bioavailability of TM1 for swine in AB2-TM1 (i.e., As + antibiotics) was equivalent (Holm-Sidak, $P = 0.930$) to animals in AB0-TM1 (i.e., As - antibiotics) (Table 4). This observation supports the conclusion that the antibiotic-induced changes in the GI microbial community did not affect As bioavailability.

The positive control (e.g., sodium arsenate) dosing group was dropped from the experimental design once it became apparent that antibiotic treatment did not alter As urinary excretion. Including the positive control treatment groups, required for the calculation of As RBA, would have doubled the number of animals euthanized, while not augmenting our ability to test whether microbes contribute to As bioavailability. Because in vitro results showed microbes increased As bioaccessibility of TM1 2.9-fold, the in vivo results suggest that microbes do not affect As bioavailability even when they alter bioaccessibility. These results may prove to be specific to TM1, since only one soil was included in the experimental design.

Conclusions

This research provides the strongest evidence to date indicating that GI microbial activity is of limited relevance to the calculation of soil As bioaccessibility in human health risk assessment. This conclusion is in agreement with inter-laboratory comparisons that demonstrated that the As bioaccessibility of standard reference soils and the Yukon overburden as measured using PBET (without microbes) is equivalent to that of the SHIME (with microbes).[17] This indicates that *in vitro* GI models that neglect to include a microbially active intestinal stage do not necessarily underestimate the health risks posed by soil As contamination.

These results, however, may prove specific to the small number of soils used in the in vitro and in vivo experiments described in this manuscript. In addition, it is not immediately apparent whether in vitro models that do not include microbial activity adequately reflect the health risks

realized by the biotransformation of inorganic As to more toxic forms (trivalent methylated species or AsH_3 gas). Therefore, further work will be necessary before it is possible to discount the importance of GI microorganisms for the calculation of As bioaccessibility and bioavailability.

Acknowledgments

The research described herein was supported by the Natural Sciences and Engineering Research Council of Canada. We thank Kevin Banman, Roman Nosach and the staff at the Prairie Swine Center for their assistance conducting this project. We also thank Ellen Verbeke, Dr. Willy Verstraete, and the rest of LabMET for their efforts and very kind hospitality.

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