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An In Vitro Gastrointestinal Method To Estimate Bioavailable Arsenic in Contaminated Soils and Solid Media

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A method was developed to simulate the human gastrointestinal environment and to estimate bioavailability of arsenic in contaminated soil and solid media. In this in vitro gastrointestinal (IVG) method, arsenic is sequentially extracted from contaminated soil with simulated gastric and intestinal solutions. A modified IVG-AB method, where iron hydroxide gel is used to simulate the absorption of arsenic, was also evaluated. Fifteen contaminated soils collected from mining/smelter sites ranging from 401 to 17 460 mg As kg^{-1} were analyzed. In vitro results were compared with in vivo relative bioavailable arsenic (RBA) determined from dosing trials using immature swine which ranged from 2.7 to 42.8% RBA. Arsenic extracted by the IVG and IVG-AB methods was not statistically different than RBA arsenic measured by the in vivo method. Arsenic extracted by the IVG stomach and intestinal phases was linearly correlated ($r = 0.83$ and 0.82, respectively) with in vivo arsenic ($P < 0.01$). Similarly, the IVG-AB method was linearly correlated ($r = 0.79$) with in vivo bioavailable arsenic ($P < 0.05$). All IVG methods extracted similar amounts of arsenic and provided estimates of bioavailable As in contaminated media. The IVG method may aid in the design and cost-effectiveness of remedial strategies of arsenic-contaminated sites.

Introduction

Arsenic is ubiquitous in soils with natural background concentrations ranging from 0.1 to 40 mg kg^{-1} (*1*). Arsenic contamination of soil may result from mining, milling, and smelting of copper, lead, and zinc sulfide ores (*2*, *3*); raw and spent oil shale (*4*); and coal fly ash (*5*, *6*). Chronic exposure to arsenic may result in skin and internal organ cancers, impaired nerve function, kidney and liver damage, and skin lesions (*7*). Arsenic has been found at high levels (10 000−20 000 mg kg^{-1}) that present risk to human health from the incidental ingestion of soil (*8*, *9*). Incidental soil ingestion by children is an important pathway in assessing public health risks associated with exposure to arsenic-contaminated soils. Incidental ingestion of soil results from normal hand-to-mouth activities and represents the principal direct pathway for exposure to nondietary sources of arsenic in contaminated areas. Soil ingestion by children as a health issue fully illustrates the importance of this pathway in terms of subsequent chemical exposure (*10*−*14*).

Most risk from arsenic is associated with the forms of arsenic that are biologically available for absorption into systemic circulation or "bioavailable" to humans. Presently, methods are not available to quantify the percentage of bioavailable arsenic in soils or to estimate risk from incidental ingestion of arsenic-contaminated materials. Some baseline risk assessments developed for contaminated sites have used the conservative assumption that all (i.e., 100%) of the arsenic present in soils and wastes is bioavailable. However, arsenic may exist in many geochemical forms (e.g., oxides, sulfides) and physical forms (e.g., flue dust, slag, tailings, calcine, waste ore) at hazardous waste sites contaminated by mining and smelter wastes. These waste forms vary in their solubilities and geochemical stabilities to the extent that many are not likely to be very bioavailable and therefore may pose only minimal risks to humans.

The bioavailability of metals, especially lead and arsenic, in some mining wastes have been assessed by conducting expensive and lengthy dosing trials using animal models. The animal model of choice for investigating the enteric bioavailability of arsenic in children requires selection based on similar age and anatomical and physiological characteristics. Pigs are remarkably similar to humans with respect to their digestive tract, nutritional requirements, bone development, and mineral metabolism (*15*). Also, pigs, like humans, tend to ingest food intermittently allowing the stomach to evacuate periodically. This physiology is consistent with the way children most likely ingest arsenic-contaminated materials, between meals when the gastric pH is lowest. Immature pigs have therefore been used successfully as a model for the gastrointestinal function of children (*16*, *17*).

To overcome some of the difficulties and expenses associated with animal dosing trials used to assess bioavailability of lead in soils, a research effort has been directed toward development of in vitro chemical methods that simulate the gastrointestinal environment. One such method is the physiologically based extraction test (PBET) (*18*). The PBET method is a good predictor of lead bioavailability. However, PBET research with arsenic-contaminated materials has been limited to only a small number of materials, and the ability of PBET to predict arsenic bioavailability is not known.

The gastrointestinal digestive processes are quite complicated and difficult to simulate in vitro. Several studies in the area of human nutrition have reported in vitro methods to assess bioavailable iron in foodstuffs (*19*−*22*). Many of these procedural steps are based upon the medical and biochemical scientific literature to gain an understanding of the digestive process, especially in terms of digestive solution volumes produced in response to food intake volume, pH conditions during digestive phases, and quantities of digestive juices and enzymes produced such as pepsin, bile acids, pancreatin, etc. (*23*, *24*).

There are two predominant mechanisms involved during digestion of metals-contaminated soil: the solubility of the metal from the soil matrix and the uptake (absorption) of the metal across the intestinal membrane. Previous in vitro type studies have looked at the solubility of metals under gastrointestinal conditions as an indicator of potential

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TABLE 1. Chemical Element Content and Select Properties of Soils and Solid Media

properties	Soils and Solid Media 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
pH[a]	2.6	2.6	3.1	3.1	5.7	7.4	7.7	7.1	7.4	7.4	3.9	4.6	7.5	7.3	7.6
TOC (%)[b]	0.36	0.22	0.58	0.41	0.61	0.89	3.13	1.58	3.38	3.22	0.81	1.52	2.28	0.23	4.58
% <2 μm[c]	5.7	10.1	13.4	9.8	7.4	6.5	18.4	8.4	7.3	7.5					
% <50 μm[c]	51.6	49.8	57.4	45.5	49.4	30.2	59.1	36.5	45.1	45.6					

properties	Soluble Anions[d] mg kg^{-1} 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
chloride	2550	2950	1240	1080	1710	944	1170	2200	976	1130	4220	9870	912	2450	14200
sulfate	158300	83120	23400	224300	219800	1310	3290	10510	2900	2530	221100	347180	2480	1360	9450
nitrate	103	160	100	49.4	297	147	942	507	552	628	324	2470	53.8	77.5	876

properties	Major Elements[e] % 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Si	17.4	17.0	18.0	18.0	22.7	16.8	21.3	23.0	20.1	20.5	22.6	28.8	16.7	12.8	26.5
Al	1.19	1.17	1.60	1.80	3.02	2.43	3.62	3.20	2.64	2.74	7.56	4.64	1.73	2.48	3.56
Ca	1.20	0.68	0.41	2.90	2.86	12.1	9.64	8.57	7.50	6.07	2.43	3.98	12.1	14.0	8.43
Fe	29.7	31.7	28.5	25.0	16.6	20.9	11.7	16.6	17.2	18.3	7.07	2.01	20.4	22.5	6.17

properties	Trace Elements[f] mg kg^{-1} 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Pb	11070	12100	10980	8430	5530	8740	6840	3510	12600	11530	9200	214	11800	12060	3680
Zn	1600	1610	1650	1660	4740	3850	1560	2510	4045	3400	11200	5650	4400	6290	10770
Cu	385	318	384	524	997	1810	1610	2210	4230	4010	975	8240	2210	2550	954
Ni	39.1	35.9	31.4	32.2	37.4	24.5	24.4	31.9	35.3	34.0	14.8	17.3	36.7	24.5	24.3

[a] 1:1, soil:0.01 $MCaCl_2$ (*29*). [b] Total organic carbon (*30*). [c] Pipette method (*31*). [d] 1 g soil:10 mL of H_2O, shake 1 h, filter 0.45 μm. [e] X-ray diffraction (*32*). [f] SW 846, method 3050 (*33*).

bioavailability (*18*, *25*), but in vitro gastrointestinal methods that simulate the mechanism of absorption have not been reported. Arsenate and the chemically similar phosphate have been shown to have a high affinity for amorphous iron hydroxide gel (*26*−*28*). Incorporation of iron hydroxide gel in an in vitro procedure to simulate intestinal absorption is also evaluated in this study.

The primary objective of our study is to develop a method to measure the bioavailable fraction of arsenic in soil and waste which is correlated with the bioavailable arsenic, as measured in vivo (per pig dosing trials). A second objective is to compare results from our in vitro methods with those from the PBET method.

Experimental Methods

Contaminated Soils and Solid Media. Two matrices were collected for this study from a typical mining/smelter site in the western U.S. where wastes were deposited between 20 and 50 years ago. These aged and weathered wastes included a calcine material (a waste product which results from the roasting and smelting of arsenopyrite ore for the extraction of arsenic) and an iron slag material (a waste product that results from the smelting of ores for lead which is also high in iron). Five calcine (soils and solid media 1−5) and five iron slag (soils and solid media 6−10) samples are fairly consistent in their respective chemical and physical properties for each matrix (Table 1); however, they differ in their total arsenic concentrations (Table 2), ranging from 401 to 17 456 mg kg $^{-1}$. Mineralogical composition of one calcine (soil 4) and one slag (soil 9) was determined by microprobe analysis for the various and arsenic-bearing solid phases. Soil 4 contained 38% of total arsenic as an arsenic jarosite analogue and 60% arsenic associated with Fe and Mn oxides. Soil 9 contained 17% of total arsenic as an arsenic jarosite analogue, 53% associated with Fe and Mn oxides, and 30% of total arsenic associated with lead oxide.

Approximately 20 kg of each soil was collected, air-dried under ambient conditions, and sieved to collect the particle size fraction <250 μm which adheres to fingers and is thus available for incidental ingestion. Soils were thoroughly homogenized/mixed prior to use and stored in secured, air-tight containers. Five more contaminated materials (soils and solid media, soils 11−15) were included in the study to test the in vitro method over a broader range of matrices. These materials, consisting of soils and slags, had been archived from previous studies involving chemical analyses and pig dosing trials.

Immature Pig Dosing Trial. Standard operating procedures developed by Dr. Stan Casteel of the University of Missouri−Columbia Veterinary Medical Diagnostic Laboratory, approved by the U.S. Environmental Protection Agency (EPA) Region 8 (*34*), were utilized in the immature pig trials. Intact male pigs weighing 10−12 kg were randomly assigned to treatment groups consisting of a calcine dosing group, slag dosing group, negative control group (no soil), and positive control group (oral Na_2AsO_4). Five pigs were used per treatment group, with the exception of three pigs per negative control group. All pigs were individually housed in arsenic-free cages and fed water and a grower ration formulated by the University of Missouri feed mill. After a three-day acclimation period, the pigs were exposed to soil/treatment doses. Pigs were dosed with 6.25 mg of soil per kg body weight per day with one-half of the dose administered 2 h before feeding in the morning and the remaining half given 2 h before the afternoon feeding. Soil doses were placed in the center of a 5 g portion of moistened low-arsenic/low-lead diet material (Ziegler Bros., Inc., Gardners, PA) and hand administered to each animal. The soil mass per dose

TABLE 2. Chemical Content and Bioavailability of Arsenic in Soils and Solid Media

arsenic	soils and solid media														
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
total (mg kg^{-1})[a]	11300	17500	13500	11500	6250	405	450	1180	5020	4650	331	233	799	1460	401
TCLP (mg L^{-1})[b]	2.97	3.01	3.03	2.94	3.00	2.96	2.78	2.91	3.17	2.93	2.65	2.75	2.61	2.73	2.95
TCLP (mg kg^{-1})[b]	59.4	60.3	60.5	58.8	60.0	59.2	55.6	58.1	63.4	58.6	53.0	55.1	52.2	54.5	59.0
bioavailable (%)[c]	2.7	3.3	8.3	22.1	30.1	bdl[d]	bdl[d]	28.7	30.1	16.4	6.2	42.8	29.1	18.7	36.5

[a] SW 846, method 3050 (*33*). [b] SW 846 method 1311 (*33*). [c] Relative bioavailable arsenic from in vivo swine study. [d] Below detection limit.

varied from 23 to 30 mg initially and was adjusted every 3 days to account for growth of the pigs. Soil masses delivered during the final 3 days of the dosing trial ranged from 41 to 45 mg. Every 3 days thereafter, for five collection periods, 24-h excretions of urine were collected from each pig. The urine samples were filtered (Whatman 2), placed into plastic bottles, and preserved to pH 2 with concentrated HCl. Urinary samples were packed securely in coolers on ice and shipped by overnight carrier under chain-of-custody procedures to Oklahoma State University (OSU) for arsenic analysis. Following an additional filtering through 0.45 μm filters, arsenic analysis was performed by a Thermo-Jarell Ash Inductively Coupled Plasma (ICP) (Maxim) utilizing Hydride Generation (HG). To prepare the urine samples for hydride generation, 10.0 mL aliquot of urine was placed into a test tube and mixed with 3.3 mL of concentrated HCl and 4.0 mL of a solution containing 10% potassium iodide and 1% ascorbic acid. After a reaction period of at least 1 h, arsenic was determined by ICP-HG. Adequate blanks, duplicates, and matrix spikes were analyzed to meet quality assurance and quality control requirements.

In Vitro Procedures. Bioavailable arsenic was estimated in our study by two separate in vitro methods and compared to the in vivo study results (Figure 1). An additional comparison of our in vitro results was made with another previously published in vitro procedure (*18, 35*). Canning jars (1 L) were used as reactor vessels because of their wide-mouth and heavy glass composition. All in vitro procedures were conducted in a water bath at body temperature (37 °C), anaerobic conditions were maintained by constantly diffusing argon gas through the solution, and the pH of the in vitro solutions was monitored constantly and adjusted as necessary throughout the procedure. Constant mixing was maintained throughout the procedures (to simulate gastric mixing) by use of individual paddle stirrers at a speed of approximately 100 rpm. A schematic diagram depicting the in vitro reactor design is illustrated in Figure 2.

The in vitro methods were conducted in two sequential phases: (1) gastric phase, low pH by adjustment with trace metal grade HCl, followed by (2) intestinal phase, pH raised by adjustment with a saturated solution of $NaHCO_3$. Throughout the gastric and intestinal phases, a small amount of antifoam agent was added (e.g., decanol) to control excessive foaming due to constant argon gas diffusing through the solutions. A 40 mL sample was collected using a new Luer-lock syringe at the end of each phase (1 h). Samples were centrifuged at 10 000 rpm for 15 min; and the supernatant was filtered through a 0.45 μm filter, acidified to pH 2 with concentrated HCl, and analyzed for arsenic by ICP-HG (following preparation for hydride generation as described above for urine). All in vitro tests were performed in triplicate for each contaminated material. The three in vitro methods studied are presented below. Adequate blanks, duplicates, and matrix spikes were analyzed to meet quality assurance and quality control requirements. The experimental parameters for each of the in vitro methods along with their respective literature references are presented in Table 3.

(1) In Vitro Gastrointestinal Method (IVG). Gastric phase solution was 0.15 M NaCl and 1% porcine pepsin (Sigma Chemical Co., St. Louis, MO. Soil (4 g) was added to 600 mL of gastric solution. An equivalent amount of the dosing vehicle (200 g dough) was added to the gastric solution to mimic the in vivo dosing of 100 mg of soil to 5 g of dough. Gastric solution pH was adjusted to 1.8 following the addition of soil. The gastric phase solution was modified to the intestinal phase solution by adjusting the pH to 5.5 with a saturated solution of $NaHCO_3$ followed by addition of porcine bile extract (2.10 g; Sigma Chemical Co., St. Louis, MO, cat. no. B8631) and porcine pancreatin (0.21 g; cat. no. P1500).

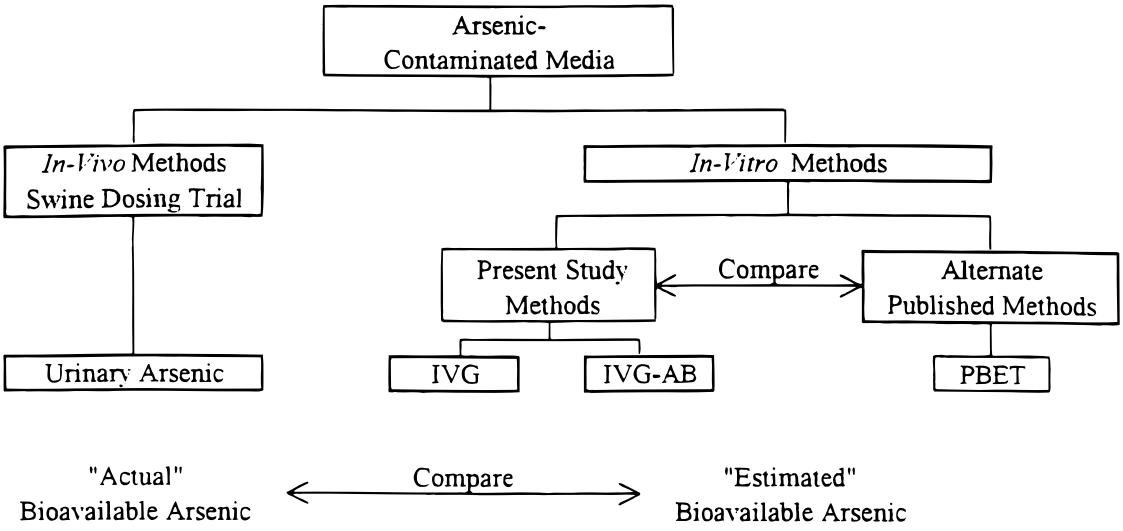

FIGURE 1. Conceptual approach to the in vitro method evaluation.

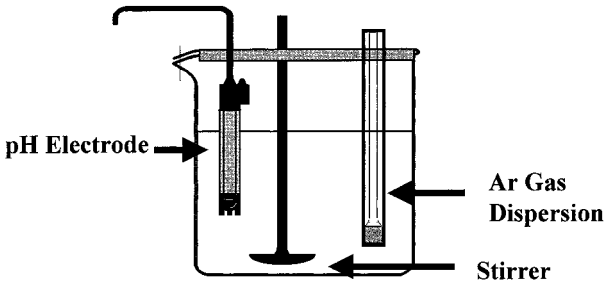

FIGURE 2. In vitro reactor design.

TABLE 3. In Vitro Experimental Parameters and Literature References

	IVG		IVG-AB		
parameter	method	reference	method	reference	PBET[a]
		Gastric Solution			
pH	1.8	24	1.8	24	2
NaCl	0.15 M	22	0.15 M	22	none
pepsin	1.0%	22	1.0%	22	0.10%
citrate	none		none		0.05%
malate	none		none		0.05%
lactic acid	none		none		0.5%
acetic acid	none		none		0.50%
soil:solution ratio	1:150	24	1:150	24	1:100
food added	yes	33	yes	33	no
		Intestinal Solution			
pH	5.5	24	5.5	24	7.0
pancreatin	0.35%	22	0.35%	22	0.018%
bile extract	0.035%	22	0.035%	22	0.05%
adsorbent (iron gel)	no		yes		no

[a] Reference *18*.

(2) In Vitro Gastrointestinal Method with Adsorption (IVG-AB). A second in vitro procedure was performed to determine if an intestinal absorption step could be simulated. This procedure is the same as the IVG method described above with the exception of adding freshly prepared amorphous iron hydroxide gel during the intestinal phase as an adsorbent. Iron hydroxide gel is prepared by making a 0.65 M $FeCl_3$ solution and then slowly adding a solution of 2.7 M NH_4OH until the pH is approximately 6 (*28*). The amorphous iron hydroxide gel is collected by centrifuging the solution at 10000 rpm for 15 min and then carefully pouring off the supernatant. Iron hydroxide (10 g) is placed onto a square (161 cm^2) of 8 μm nylon membrane filter. The nylon filter is tied with nylon string, similar to a tea bag, which is then allowed to suspend freely in the reactor vessel throughout the entire intestinal phase. At the end of the intestinal phase, the iron hydroxide bag is removed and placed into a 250-mL Erlenmeyer flask. Arsenate is desorbed by adding 200 mL of 0.2 M H_2SO_4 to the flask and shaking on a reciprocal shaker for 1 h. The resulting solution is filtered through a 0.45 μm pore size filter and analyzed for arsenic by ICP-HG.

(3) Physiologically Based Extraction Test (PBET). The PBET procedure was performed (*18*, *35*) with the following exceptions. To maintain anaerobic conditions, argon gas was diffused through the in vitro solutions continuously rather than utilizing closed reactor vessels, and the pH of the gastric solution was raised to 7.0 (to perform the intestinal phase step) by addition of a $NaHCO_3$ solution rather than using dialysis tubing packed with $NaHCO_3$ powder. The $NaHCO_3$ solution reacted much quicker (1.5−2 h faster) than using the $NaHCO_3$-packed dialysis tubing, which was subject to breaking by inadvertent contact with the mixing blade.

In Vivo Bioavailability Calculations. The amount of arsenic absorbed through the gastrointestinal tract (bioavailable arsenic) may be described in absolute or relative terms. Absolute bioavailability (ABA) is the ratio of the amount of arsenic absorbed compared to the amount ingested:

$$\text{ABA} = \frac{\text{absorbed dose}}{\text{ingested dose}} \quad (1)$$

Relative bioavailability (RBA) is the ratio of the absolute bioavailability of arsenic present in test material (study soil) compared to the absolute bioavailability of arsenic in an appropriate reference material:

$$\text{RBA} = \frac{\text{ABA (study soil)}}{\text{ABA (reference material)}} \quad (2)$$

In our study, we selected the $Na_2AsO_4{\cdot}7H_2O$ reference material as the control because it is a readily soluble form of arsenic that is easily absorbed. Arsenic excretion in urine was found to be a linear function of the administered dose and was approximately independent of time after 5 days of exposure during dosing trials. In most animals, including pigs, absorbed arsenic is excreted primarily in urine. Thus, the urinary excretion fraction (UEF), defined as the amount excreted in the urine divided by the amount dosed, is a reasonable approximation of the oral absorption fraction or ABA. However, this ratio will underestimate total absorption,

TABLE 4. Comparison of Methods Used To Measure Bioavailable Arsenic in Contaminated Soils and Solid Media[a]

	bioavailable arsenic method						
	IVG			PBET			
samples	stomach	intestinal	IVG-AB intestinal	stomach	intestine	in vivo	critical value[b]
all media ($n = 13$)	16.7 ab	14.8 b	15.3 b	11.8 bc	8.26 c	21.0 a	5.3
CV (%)[c]	75.2	74.6	73.0	95.2	76.4	65.6	
calcine ($n = 5$)	3.66 b	3.52 b	4.00 b	1.44 b	1.47 b	13.5 a	5.1
CV (%)	74.0	75.9	77.2	77.4	47.3	88.8	
iron slag ($n = 3$)	24.8 a	22.7 a	24.1 a	13.9 b	12.0 b	25.4 a	7.4
CV (%)	27.4	28.9	26.6	61.0	33.6	41.6	
all media except calcine ($n = 8$)	24.8 a	21.9 ab	23.0 ab	18.3 bc	12.5 c	25.9 a	6.6
CV (%)	41.3	42.4	38.7	58.9	40.7	49.1	

[a] Values reported are mean percent relative bioavailable arsenic for that group. Mean separation statistics were generated using Duncan's Multiple Range Test (*36*). Multiple comparison of mean values are made between bioavailable arsenic method (horizontally). Mean values with the same letter designation indicate no difference between groups at $P < 0.05$. [b] Quantitative difference between means necessary for methods significantly different at $P < 0.05$. [c] Coefficient of variation.

because some absorbed arsenic is excreted in the feces via the bile and some enters tissue compartments (e.g., liver, kidney, skin, hair, etc.) from which it is cleared very slowly or not at all. Thus, the urinary excretion fraction is not equated with the ABA. The UEF can be used, however, to compute the RBA as follows:

$$\text{RBA} = \frac{\text{UEF (study soil)}}{\text{UEF (reference material)}} \quad (3)$$

All in vivo bioavailabilities in this study are reported as RBAs.

In Vitro Bioavailability Calculations. The standard analysis for soil metal content, including arsenic, during the investigation of the nature and extent of contamination sites is by hot digestion with HNO_3 and H_2O_2, USEPA SW 846, Method 3050 (*33*). The resulting total metal concentration is then used for estimating risks to human health. The realization that probably not all (100%) of the total metal measured by complete digestion is bioavailable has led risk assessors to use a fraction (percentage) of total metal that better represents the bioavailable in the risk calculation. For our in vitro results, bioavailable arsenic is calculated by dividing the arsenic concentration measured in the in vitro stomach phase or the in vitro intestinal phase solutions by the total soil arsenic as described by the following equation:

$$\text{in vitro bioavailable As, \%} = \left[\frac{\text{in vitro As}}{\text{total As}}\right] \times 100 \quad (4)$$

For the in vitro method utilizing iron hydroxide adsorbing gel (IVG-AB), the intestinal phase solution arsenic and the arsenic dissolved from the iron hydroxide gel are summed to represent the total intestinal phase arsenic.

Statistical Methods. Analysis of variance using a randomized complete block design and subsequent separation of means by Duncan's Multiple Range Test (*36*) was used to compare results between in vitro chemical and in vivo methods. Linear regression was used to determine agreement between in vitro chemical and in vivo results. Linear regression parameters (slope equal to one and intercept equal to zero) were evaluated using t-tests to determine agreement between methods.

Results and Discussion

The length of time to perform the stomach phase and intestinal phase (reaction time) was not clearly described in the literature. Hence, an experiment was conducted using the PBET method (*18*, *35*) (on one calcine and one slag sample) to determine the dissolution of arsenic over time for each of the phases. The soluble concentration of arsenic remained relatively constant during the stomach phase with samples collected every 20 min. Likewise, samples were collected every 60 min over a 3-h intestinal phase, and, again, arsenic concentration in solution remained relatively constant. A 1-h duration was selected for reaction time of each phase.

Other studies have shown the type of food incorporated into the in vitro method can affect lead bioavailability (*35*). To replicate conditions of the in vivo experiment as closely as possible, an experiment was conducted to evaluate food added (using the soil dosing vehicle) versus without food added. An equivalent volume of soil dosing vehicle (which represented 200 g of vehicle) was added to the reactor vessel. One calcine sample and one slag sample were tested with and without food. For the slag sample, there was no difference in the soluble arsenic measured in either the stomach or intestinal phases of the vessels with-food as compared to the vessels without-food (21.1 mg kg^{-1} stomach phase and 24.4 mg kg^{-1} intestinal phase, without-food conditions versus 19.7 mg kg^{-1} stomach phase and 24.3 mg kg^{-1} intestinal phase, with-food conditions). However, for the calcine sample, more arsenic was solubilized in the with-food treatment as compared to the without-food treatment (2.65 mg kg^{-1} stomach phase and 6.92 mg kg^{-1} intestinal phase, without-food conditions versus 7.86 mg kg^{-1} stomach phase and 10.3 mg kg^{-1} intestinal phase, with-food conditions). Apparently, adding food would not inhibit arsenic solubilization and, in some cases, may increase arsenic solubilization. For the IVG and the IVG-AB in vitro experiments, 200 g of dough, used as the pig dosing vehicle, was added to represent the addition of food. Presence of food in the digestive tract can affect bioavailability of heavy metal (*37*). For example, milk may lower Pb bioavailability by coprecipitation of Pb with calcium phosphate (*38*). The effect of food on Pb bioavailabity in gastrointestinal in vitro tests affects Pb bioavailability in some contaminated media (*35*). However, the effect on Pb bioavailability was found to depend on type of contaminated material and food included in the in vitro test. Inclusion of food affected dissolution of arsenic in our in vitro method but the exact mechanism is unclear.

Comparison of bioavailable arsenic estimated by in vitro methods with in vivo arsenic in contaminated media was accomplished using mean separation by Duncan's Multiple Range Test following analysis of variance (*36*) (Table 4). Only the IVG stomach phase method was equivalent with the in vivo method ($P < 0.05$) across all media. Evaluating the media separately, the iron slag material tested by the IVG stomach phase method was statistically equivalent to the in vivo method, yet the calcine materials were different (at $P < 0.05$). Arsenic extracted by in vitro methods (% RBA) was much lower than in vivo arsenic RBA. When only the slags and soils were evaluated (all media except the calcines), the agreement

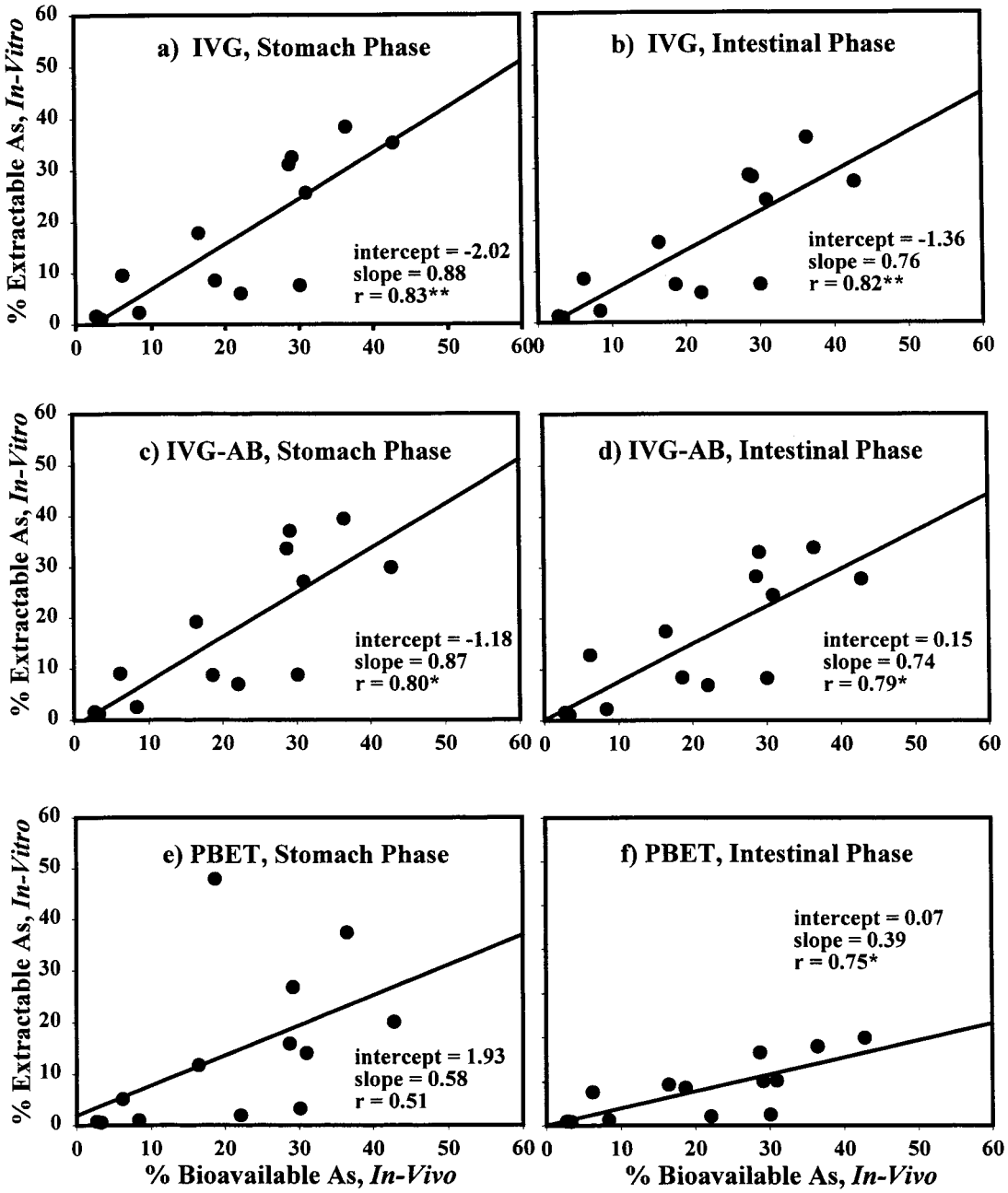

FIGURE 3. Comparisons of gastrointestinal in vitro stomach and intestinal phase bioavailable arsenic with in vivo bioavailable arsenic: (a) IVG, stomach phase; (b) IVG, intestinal phase; (c) IVG-AB, stomach phase; (d) IVG-AB, intestinal phase; (e) PBET, stomach phase; (f) PBET, intestinal phase. **Statistically significant at $P < 0.01$, *statistically significant at $P < 0.05$.

between percent RBA of the IVG stomach phase and in vivo methods improved. Similar results were demonstrated for the IVG-AB intestinal phase method. When only the slags and soils were evaluated, the IVG-AB intestinal method was statistically equivalent with the in vivo method (at $P < 0.05$). The calcine samples, as analyzed by any of the in vitro methods, were not statistically equivalent with the in vivo method.

Few statistical differences between the IVG stomach phase, IVG intestinal phase, and IVG-AB intestinal phase were found for most groups of material (Table 4). In other words, extending the in vitro method beyond the gastric phase did not improve the ability of the method to measure bioavailable As. To simulate absorption across the intestinal membrane, iron hyroxide gel was added to the IVG-AB intestinal phase solution. Intestinal absorption is a different process than adsorption of arsenic to iron hydroxide gel. However, both processes are sinks that remove arsenic from solution. To determine the quantity of iron hydroxide gel to use, an experiment was conducted using iron hydroxide gel to adsorb arsenic from the intestinal phase solution. Ten grams of Fe gel adsorbent in the IVG-AB method adsorbed 60 mg of arsenic from simulated gastric and intestinal solutions that contained 100 mg L^{-1} arsenic as sodium arsenate and 10 000 mg L^{-1} of sulfate. Arsenic concentrations in gastric and intestinal solutions of the IVG method for contaminated media ranged from 0.38 to 12.1 mg L^{-1} with a median value of 1.90 mg L^{-1}. Dissolved sulfate up to 3470 mg L^{-1} occurred in the in vitro solutions of contaminated media, which is below the sulfate concentration of the simulated IVG method solution. Therefore, the 10 g of Fe gel adsorbent was capable of adsorbing all of the arsenic from the gastric and intestinal solutions of the IVG method for contaminated media. However, the simulated absorption step of the IVG-AB did

not increase arsenic extracted from contaminated media compared to the IVG method (without adsorbent). Dissolution of arsenic solid phases of contaminated media by simulated gastrointestinal solutions appears to be the rate-limiting step rather than the subsequent Fe gel adsorption step in controlling dissolved arsenic in the IVG method.

Also of note is that neither the PBET stomach phase nor PBET intestinal phase in vitro methods were found to be statistically equivalent with the in vivo method for any group of contaminated materials tested (Table 4).

Coefficients of variation (CV) values, used to describe variability in bioavailability measurements, for in vivo and in vitro methods were determined (Table 4). The CV values for the IVG and IVG-AB methods for all media were similar ranging from 73.0 to 75.2%. The IVG and IVG-AB methods had a slightly lower degree of precision than the in vivo method (CV of 65.6%). The degree of precision for bioavailability measurements varied with test material. Determination of bioavailable arsenic by all methods for calcine was less precise than noncalcine materials (Table 4). The degree of precision of in vivo and in vitro methods for all media except calcine was very similar, with CV values ranging from 40.7 to 58.9%.

Linear regressions of percent RBA arsenic measured by in vitro tests with the swine in vivo method are presented in Figure 3. Although 15 soils were tested throughout all in vitro experiments, only 13 points are presented on each plot. Samples 5 and 6 (Table 2) had very low arsenic concentrations and were below in vivo bioavailability detection limits. Results of the IVG stomach phase were linearly correlated ($r = 0.83$) with in vivo bioavailable arsenic ($P < 0.01$) (Figure 3a). Statistical analysis of linear regression parameters showed the slope of 0.88 was not different than one, and the intercept of -2.02 was not different than zero; IVG stomach phase results were statistically the same as in vivo results. The IVG intestinal phase was also linearly correlated with in vivo arsenic with an r of 0.82 ($P < 0.05$) (Figure 3b). Statistical analysis of linear regression parameters showed the slope of 0.76 was not different than one, and intercept of -1.36 was not different than zero; IVG intestinal phase results were statistically the same as in vivo results. Comparison of stomach and intestinal phase results of the IVG methods (Table 4, Figure 3a,b) suggests arsenic bioavailability measured by the IVG method is controlled by dissolution of arsenic in gastric phase.

Figure 3c presents the results of the IVG-AB stomach phase. Because the IVG-AB stomach phase is the same procedure as the IVG stomach phase, the gastric solution arsenic for both methods is the same (Figure 3a,c). Slight differences were found between the IVG and the IVG-AB intestinal phases (Figure 3b,d). However, adding the adsorbing Fe gel to the in vitro solution of the IVG-AB method had little effect on the r values of the linear regressions of IVG methods vs in vivo (Figure 3b,d). Statistical analysis of linear regression parameters showed the slope of 0.79 was not different than one, and intercept of 0.15 was not different than zero (Figure 3d); IVG-AB intestinal phase results were statistically the same as in vivo results. Similar results between IVG or IVG-AB and in vivo methods were expected because the simulated absorption step of the IVG-AB did not increase arsenic extracted from contaminated media (Table 4).

Results from the PBET in vitro methods are shown in Figure 3e,f. The PBET stomach phase results are not linearly correlated with in vivo arsenic, while the intestinal phase is correlated with an r of 0.75 ($P < 0.05$). Slopes of the PBET vs in vivo method were different than one but intercepts were equivalent to zero (Figure 3e,f); results from the PBET method were not the same as in vivo results. The PBET method underestimated arsenic bioavailability in calcine materials (Table 4). Excluding calcine-derived contaminated media improved the PBET gastric phase vs in vivo linear regression. The PBET gastric phase arsenic was correlated ($r = 0.70$, $P < 0.10$) with in vivo arsenic when calcine was excluded producing a slope of 0.59 which was equivalant to one. Although both the PBET and IVG methods underestimated bioavailable arsenic for calcine materials (Table 4), parameters in the IVG method improved extraction of arsenic from contaminated media. One difference between the PBET and IVG methods is that the PBET method does not incorporate any type of food into the gastric solution. Food has been shown to have an affect on bioavailability (*35*). Another difference between these methods is the amount of pepsin used in the in vitro solutions. The PBET solution contains one-tenth the pepsin concentration of the IVG solutions. We selected the IVG pepsin concentrations from the human nutrition and medical literature (*22*, *24*). Pepsin is one of the most important of the digestive enzymes; it hydrolyzes peptide bonds in proteins and polypeptides with a low degree of specificity. Perhaps hydrolyzed products are enhancing arsenic solubilization.

It is unlikely that an in vitro method can be developed which will replicate in vivo bioavailability. The human digestive system is too complex and dynamic to simulate in the laboratory. A more reasonable approach may be to develop in vitro methods that are based upon human gastrophysiology and correlate well with in vivo method results. From this correlation, mathematical relationships can be developed that will be useful in making risk estimates. The discipline of soil science has used this concept successfully when early work was performed to find suitable chemical extractants to measure plant-available nutrients. Chemical extractants cannot extract plant nutrients in the same manner as a living plant under the conditions of the plant root environment. However, good correlation between soil extractants and plant uptake has allowed soil scientists to use that relationship to make reasonable predictions of plant available nutrients in soil and fertilizer recommendations (*39*). Similar relationships between in vitro and in vivo methods may lead to the development of mathematical relationships from which predictions can be made to derive bioavailable As concentrations in soils for risk estimates that have a lower degree of uncertainty and aid in the design and cost-effectiveness of remedial strategies at contaminated sites.

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