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Validation of the In Vitro Gastrointestinal (IVG) Method to Estimate Relative Bioavailable Lead in Contaminated Soils

J. L. Schroder, N. T. Basta,* S. W. Casteel, T. J. Evans, M. E. Payton, and J. Si

ABSTRACT

The effect of the dosing vehicle (e.g., dough) on the ability of an in vitro gastrointestinal (IVG) method to predict relative bioavailable Pb associated with soil ingestion was evaluated. Bioaccessible Pb determined by the IVG method was compared with relative bioavailable Pb measured from dosing trials using juvenile swine for 18 contaminated soils ranging from 1270 to 14 200 mg Pb kg^{-1}. Bioaccessible Pb was measured in the IVG gastric extraction (GE) and intestinal extraction (IE) solutions. Mean bioaccessible Pb values were 32.2% for GE without dough, 23.0% for GE with dough, 1.06% for IE without dough, and 0.56% for IE with dough. It is possible that phytic acid associated with the dough addition decreased bioaccessible Pb. In vivo relative bioavailable Pb ranges for different swine tissues were 1 to 87% for blood, 0 to 110% for liver, 1 to 124% for kidney, and 0.04 to 94% for bone. Strong linear relationships between IVG GE Pb with dough ($r > 0.76$, $P < 0.0002$), IVG IE Pb with dough ($r > 0.56$, $P < 0.015$), and IVG GE Pb without dough ($r > 0.81$, $P < 0.0001$) and in vivo bioavailable Pb as estimated with blood, kidney, liver, and bone were found. Inexpensive in vitro methods may be useful in providing an estimate of the variability in relative bioavailable Pb at a single study site. The IVG method can be used to estimate relative bioavailable Pb, As, and Cd in contaminated soil.

Lead is a naturally occurring, bluish-gray metal usually found as a mineral combined with other elements, such as sulfur (i.e., PbS, $PbSO_4$) or oxygen ($PbCO_3$), and ranges from 10 to 30 mg kg^{-1} in the earth's crust (United States Department of Health and Human Services, 1999). Typical mean Pb for surface soils worldwide averages 32 mg kg^{-1} and ranges from 10 to 67 mg kg^{-1} (Kabata-Pendias and Pendias, 1992, p. 187–198). Typical background Pb levels in surface soils of the United States range from 0.5 to 135 mg kg^{-1} with a median value of 11 mg kg^{-1} (Holmgren et al., 1993). Lead is used for a variety of industrial and consumer materials, including lead-acid batteries (63.0%), pigments and other compounds (12%), rolled and extruded products (7.7%), cable sheathing (4.5%), and gasoline additives (2.2%) (Adriano, 2001, p. 349–410; United States Department of Health and Human Services, 1999). Lead contamination of soil may result from mining and smelting activities, sewage sludge usage in agriculture, contamination from vehicle exhausts, manufacturing processes involving Pb, and recycling and disposal of Pb-containing products (Adriano, 2001, p. 349–410; Davies, 1990). Past uses of lead in the United States that have resulted in soil contamination include its addition to gasoline and its use in pesticides, batteries, firing ranges, and Pb-based paint chips (Adriano, 2001, p. 349–410; Davies, 1990).

Lead is considered a possible human carcinogen by the International Agency for Research on Cancer (2002) as well as a probable human carcinogen by the United States Environmental Protection Agency (USEPA, 1996b). Human exposure to Pb can occur through the consumption of contaminated foods or drinking water, incidental ingestion of soil or dust, inhalation of Pb-containing particles from ambient air, ingestion of paint chips from Pb-painted surfaces, use of medications in the form of folk remedies, inhalation of automobile emissions, or from working in occupations involving exposure to Pb fumes and dust (Adriano, 2001, p. 349–410; United States Department of Health and Human Services, 1999). Lead is a toxic element, and exposure results in a variety of effects in humans. In both adults and children, the main target of lead toxicity is the central nervous system (United States Department of Health and Human Services, 1999). Acute exposure to high levels of Pb may result in gastrointestinal symptoms (cramping, colicky abdominal pain, nausea, and vomiting), brain damage, kidney damage, lowered sperm production, miscarriages, and possibly death. Chronic exposure to Pb may result in effects on the blood (anemia), central nervous system (CNS), blood pressure, kidneys, and vitamin D metabolism (United States Department of Health and Human Services, 1999). Central nervous system effects on adults consist of subtle behavior changes, fatigue, and impaired concentration. Children are more susceptible to Pb exposure because they absorb and retain approximately 50% more in proportion to their body weight (Mushak et al., 1989). Exposure of children to Pb may result in impaired neurological development (both cognitive and behavioral) as evidenced by deficits in intelligence scores, speech and language processing, attention, and classroom performance (da la Burde and Choate, 1972; Grant and Davis, 1989; Needleman et al., 1979, 1990; Rummo et al., 1979; Winneke, 1995).

Lead is ubiquitous in the environment primarily as a result of anthropogenic activities; the United States Department of Health and Human Services (1999) estimates that 89.4% of the total environmental release of Pb in 1996 (including Pb going to landfills) was to soil. Lead ranks first on the priority list of hazardous substances found at Superfund sites (based on its frequency

J.L. Schroder and J. Si, Department of Plant and Soil Sciences, Oklahoma State University, Stillwater, OK 74078. N.T. Basta, School of Natural Resources, The Ohio State University, Columbus, OH 43210. S.W. Casteel and T.J. Evans, Veterinary Medicine Diagnostic Laboratory, University of Missouri-Columbia, Columbia, MO 65205. M.E. Payton, Department of Statistics, Oklahoma State University, Stillwater, OK 74078. This manuscript was published with the approval of the Director, Oklahoma Agricultural Experiment Station. Received 2 Feb. 2003. *Corresponding author (basta.4@osu.edu).

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677 S. Segoe Rd., Madison, WI 53711 USA

Abbreviations: GE, gastric extraction; IE, intestinal extraction; IVG, in vitro gastrointestinal; PBET, physiologically based extraction test; SRM, standard reference material.

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at sites, its toxicity, and its potential for human exposure) and has been identified in soils from 675 of the 1026 National Priorities List (NPL) hazardous waste sites (Adriano, 2001, p. 349–410; United States Department of Health and Human Services, 1999). Concentrations as high as 60 000 mg kg^{-1} have been reported in soils adjacent to a smelter in Missouri (Palmer and Kucera, 1980). Additionally, soils adjacent to Pb-painted houses may contain >10 000 mg kg^{-1} (USEPA, 1986). The incidental ingestion of soil by children is an important pathway in the assessment of public health risks due to exposure of metal-contaminated soils. Most risks from Pb in ingested soil or waste materials are associated with the fraction of the soil or waste material that is available for absorption from the gastrointestinal tract into the circulatory system. The amount of Pb absorbed through the gastrointestinal tract (bioavailable Pb) may be described in absolute or relative terms. Absolute bioavailability (ABA), also referred to as the oral absorption fraction, is equal to the absorbed dose/ingested dose as described by Eq. [1]:

$$\text{ABA} = \text{absorbed dose/ingested dose} \quad [1]$$

Relative bioavailability (RBA) is the ratio of the ABA of Pb present in some test material (study soil) compared with the ABA of Cd in an appropriate reference material (Eq. [2]):

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

Lead acetate, a readily soluble form of Pb and thus easily absorbed from the gastrointestinal tract, is used as the reference material in the critical toxicity study reported in the Integrated Risk Information System (IRIS; USEPA, 1996b). Relative bioavailability can be determined experimentally without specifically measuring absolute bioavailability. For example, the tissue concentration of Pb in animals dosed with study soil can be compared with tissue concentration of Pb in animals dosed with reference material. In this case, relative bioavailability is defined by Eq. [3]:

$$\text{RBA} = \text{tissue Pb (study soil)/tissue Pb (reference material)} \quad [3]$$

Often, baseline risk assessments used for contaminated sites assume that the relative bioavailability of Pb in soil is 60%, which is the default value used by the Integrated Exposure and Uptake Biokinetic (IEUBK) model for lead in children (USEPA, 1994). However, because of the different geochemical and physical forms of Pb present in contaminated soils and waste, the relative bioavailability of Pb may be different than the default IEUBK value.. Therefore, a more accurate estimation of the relative bioavailability of metal contaminants (e.g., Pb and As) in waste materials from hazardous waste sites has been assessed using in vivo animal dosing trials and used for risk assessment.

Less expensive in vitro chemical extraction methods that simulate gastrointestinal biochemistry have been developed to estimate relative bioavailable Pb (Ellickson et al., 2001; Hamel et al., 1998; Ruby et al., 1992, 1996), As (Rodriguez et al., 1999), and Cd (Schroder et al., 2003). The amount of contaminant dissolved in the gastrointestinal environment and available for absorption is termed "bioaccessible" (Ruby et al., 1999). Most in vitro methods are sequential extractions with two distinct extraction steps: (i) a gastric phase extraction that simulates the acidic biochemical stomach environment and (ii) a subsequent intestinal phase extraction that simulates the biochemical environment of the small intestine. The fraction of the contaminant dissolved by the in vitro procedure, the "bioaccessible" contaminant, has been used to estimate the relative bioavailability of the contaminant in soil (Ruby et al., 1999). While many different in vitro methods have been developed to estimate bioaccessible Pb, few have related their results to relative bioavailable Pb as measured by an animal model. The in vitro physiologically based extraction test (PBET), which does not use food in the extraction to mimic fasting conditions, has been correlated with relative bioavailable Pb as estimated by two animal models (weanling rats and swine) (Medlin, 1997; Ruby et al., 1996, 1999). The in vitro gastrointestinal (IVG) method developed by Rodriguez et al. (1999) is an accurate predictor of relative bioavailable As in contaminated soils and waste materials as estimated by a juvenile swine model while utilizing food in the extraction procedure. Recently, Schroder et al. (2003) showed that the IVG method was correlated with in vivo relative bioavailable Cd using a juvenile swine model. The objective of this study was to determine the ability of the IVG method of Rodriguez et al. (1999), with and without food, to predict relative bioavailable Pb in contaminated soil as measured by in vivo juvenile swine dosing trials.

MATERIALS AND METHODS

Contaminated Soils and Solid Media

Eighteen contaminated soils from eight different hazardous waste sites were evaluated using the IVG method of Rodriguez et al. (1999). Air-dried soil was sieved through nylon mesh (<250 μm) to obtain the soil fraction considered to adhere to fingers and likely to be ingested. Total metal content of soil was determined by acid digestion using USEPA Method 3050 (USEPA, 1996a) and total elemental analysis was conducted using a high-resolution Thermo Jarrell Ash inductively coupled plasma atomic emission spectrophotometer (ICP–AES; Thermo Elemental, Franklin, MA).

In Vivo Swine Dosing Study

In vivo relative bioavailable Pb in contaminated soil was determined by in vivo dosing trials using standard operating procedures (Casteel, 1995). Male swine (5–6 wk old) weighing 10 to 12 kg were dosed for 15 d with varying concentrations of Pb in substrates. Five swine were randomly assigned to treatment groups consisting of a dosing group, a negative control group (no substrate), and a positive control group that received oral lead acetate. All swine were individually housed in stainless steel cages and daily fed a powdered grower's diet (referred to as "dough" in this paper), which approximated 5% of body weight (Ziegler Bros., Gardner, PA). The diet was commercially formulated to have a protein content of

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approximately 19% and contained <0.2 mg Pb kg^{-1} diet. After a 7-d acclimation period, the swine were dosed with contaminated soil that was placed in a 5- to 10-g doughball of moistened grower diet. The swine were dosed twice daily to mimic childhood Pb ingestion, which is likely to occur between meals while children are in a fasted or semi-fasted state. A dose of 6.25 mg soil per kg body weight per day was used with half of the first dose being delivered at 0900 h after an overnight fast and the second half of the dose being delivered at 1500 h after a 4-h fast. All swine were fed 2 h after dosing.

Tissue Analyses

Blood (1.0 mL) was mixed with 9.0 mL of a matrix modifier consisting of 0.2% v/v trace metal nitric acid, 0.5% v/v Triton X-100, and 0.2% w/v ammonium phosphate in deionized distilled water before analyses. Kidney or liver (1.0 g) were digested overnight at 90°C in 2.0 mL of concentrated trace metal HNO_3 (i.e., extremely low levels of Pb) and diluted to a final volume of 10.0 mL with deionized distilled water (>18 ohm). Femurs were oven-dried overnight at 100°C and were ashed in a muffle furnace at 450°C for 48 h. Aliquots of ashed femurs (200 mg) were dissolved in 10.0 mL of a 1:1 mixture of trace metal HNO_3 (i.e., extremely low levels of Pb) and deionized distilled water. All samples were filtered through 0.45-mm membrane filters before analyses by graphite furnace atomic absorption spectroscopy (GFASS). Blanks, spikes and duplicate analyses were conducted every 20 samples to meet quality assurance–quality control (QA–QC) requirements. Relative Pb bioavailability was estimated using measured Pb concentrations in blood, liver, kidney, and bone.

Calculation of In Vivo Relative Bioavailability

Relative bioavailability was calculated from Eq. [3]. Lead acetate was selected as reference material in our study because it is a readily soluble form of Pb that was used in critical toxicity studies as reported in the Integrated Risk Information System. More specifically, for each study substrate, the amount of Pb bioaccumulated in tissue (e.g., μg Pb L^{-1} for blood and mg Pb kg^{-1} kidney, liver, or bone) was plotted as a function of Pb dosed (e.g., μg Pb kg^{-1} body weight d^{-1}) for both reference material and study substrate. The resulting best-fit straight lines (calculated by linear regression) for both the reference material and the study substrate were used to estimate the relative bioavailability. Relative bioavailability was calculated by dividing the slope for the study substrate by the slope for the reference material.

In Vitro Gastrointestinal Method

Bioaccessible Pb was estimated in our study using the IVG method developed by Rodriguez et al. (1999). The IVG method is a two-step sequential extraction: a gastric solution extraction followed by an intestinal solution extraction. An equivalent amount of the dosing vehicle (200 g of wet feed termed "dough") was added to the gastric solution to mimic the in vivo dosing of 100 mg soil to 5 g of dough. Gastric solution was 0.15 *M* NaCl and 1% porcine pepsin (Sigma Chemical Company, St. Louis, MO; Catalog no. P7000). The in vitro method was conducted using 1-L glass jars in a water bath at body temperature (37°C). Soil (4.0 g) was placed in 600 mL of gastric solution to which either 0 g (e.g., no dough) or 200 g of dough was added. The pH of the gastric solution was adjusted to pH 1.8 with trace-metal-grade HCl. Anaerobic conditions were maintained by constantly bubbling argon through the solution; pH was continuously monitored and adjusted to 1.8 throughout the 1-h procedure. Mixing (to simulate gastric mixing) was maintained during the procedure using individual paddle stirrers set at a speed of 100 rpm. After 1 h, 40 mL of gastric solution, removed for Pb analysis, was replaced with 40 mL of fresh gastric solution. Subsequently, the extraction solution was modified to simulate intestinal solution by adding saturated $NaHCO_3$ solution to adjust the pH to 5.5 followed by the addition of 2.10 g of porcine bile extract (Sigma Chemical Company; Catalog no. B8631) and 0.21 g of porcine pancreatin (Catalog no. P1500). A small amount of anti-foam agent (decanol) was added to each reaction vessel. After 1 h, 40 mL of intestinal solution was collected for Pb analysis. Gastric and intestinal solution samples were centrifuged (5211 × *g*) for 15 min and filtered through 0.45-μm membrane filters immediately after their collection. The samples were acidified to pH of 2 using trace metal HCl, and Pb was determined using ICP–AES.

In Vitro Bioaccessibility Calculations

Bioaccessible Pb was calculated by dividing the Pb concentration measured in the in vitro gastric or intestinal solutions by the total soil Pb content (e.g., USEPA Method 3050).

Statistical Analysis

Analysis of variance using PROC MIXED (SAS Institute, 2001) was performed to evaluate the effects of the extraction step (gastric or intestinal) and method (dough or no dough addition) on bioaccessible Pb. The data were analyzed as a split plot arrangement in a randomized complete block design. The combination of replicate and soil were used as blocks, method was the whole plot factor, and phase was the split plot factor. Simple effects of method given phase and phase given method were analyzed with a SLICE option in the LSMEANS statement. The relationship between mean in vitro bioaccessible Pb and mean in vivo relative bioavailable Pb in different tissues was determined using PROC REG (SAS Institute, 2001).

RESULTS AND DISCUSSION

Soil Lead Concentrations

The Pb content of the contaminated soils ranged from 1270 to 14 200 mg kg^{-1} (Table 1), which is well above the Pb content of 10 to 67 mg kg^{-1} reported for uncontaminated soils (Kabata-Pendias and Pendias, 1992, p. 187–198). The study soils were also contaminated with other heavy metals (e.g., Cd, Zn) and metalloids (As) (Table 1). The soils also contained significant amounts of elements known to affect Pb uptake and bioavailability including Fe, Ca, and Zn.

In Vivo Relative Bioavailable Lead

Ranges for percent relative bioavailable Pb estimated using the young swine model varied by tissue and were 1 to 87% blood, 0 to 110% for liver, 1 to 124% for kidney, and 0.04 to 94% for bone for the soils evaluated in our study (Table 2). In a review on the oral bioavailability of inorganics in soil, Ruby et al. (1999) reported that the bioavailability of Pb in an ingested soil depends on the chemistry, particle size distribution, mechanism of dissolution, and geochemistry of the soil. Our results are similar to those of Ruby et al. (1999) who reported that the relative bioavailability of Pb in contaminated

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Table 1. Elemental content of select metal contaminants, Ca, and Fe in study soils.

Soil	Predominant form of Pb†	Pb	As	Cd	Zn	Ca	Fe
		mg kg^{-1}			g kg^{-1}		
1	$PbPO_4$, $PbSO_4$	1 590	51	4.20	0.90	13.6	16.1
2	$PbSO_4$	8 600	240	43.0	1.20	16.0	50.0
3	PbS	11 200	4.9	0.80	0.11	2.65	10.0
4	$PbCO_3$	10 800	25	33.7	10.0	45.8	40.2
5	$PbCO_3$	4 050	11	188	50.0	81.8	18.0
6	$PbCO_3$	6 940	16	139	17.2	19.9	26.6
7	$PbCO_3$, $PbSO_4$, PbO	7 510	203	59.9	13.7	20.1	68.1
8	PbO	4 320	110	38.5	2.65	3.93	27.5
9	PbO	10 600	1 050	12.8	67.3	117	207
10	PbS	1 270	1 290	4.00	0.44	8.29	391
11	PbO	7 895	591	24.4	31.9	90.1	196
12	PbO	11 500	695	29.9	48.9	88.1	169
13	PbO, PbS	3 200	310	23.8	10.4	69.0	38.7
14	$PbCO_3$, PbO	8 350	5	4.00	1.88	11.8	8.89
15	PbO	3 230	110	195	6.50	1.16	25.9
16	PbO	2 150	134	319	19.1	2.48	26.7
17	$PbCO_3$, $PbSO_4$, PbO	14 200	67	41.9	6.58	37.2	33.7
18	$PbCO_3$, $PbSO_4$, PbO	3 870	17	47.4	4.11	17.3	23.0

† Predominant mineralogical form of Pb in soil determined by electron microprobe. Mineral forms listed in table may be amorphous forms of these minerals associated with Mn and/or Fe oxides.

soils ranged from 1 to 90% based on a combination of data from blood, bone, liver, and kidney using juvenile swine with blood data weighted more heavily.

In Vitro Bioaccessible Lead

The in vitro bioaccessible Pb measured by the IVG gastric solution extraction step (GE) using dough in the extraction ranged from 0.70 to 36.3% with an overall mean of 23.0% (Table 2). In vitro bioaccessible Pb measured by the IVG intestinal solution extraction step (IE) using dough in the procedure ranged from 0.02 to 1.16% and averaged 0.56% for the soils (Table 2). The IVG GE Pb without using dough in the extraction ranged from 1.4 to 64.4% with an overall mean of 32.2% (Table 2). Within the IE, the in vitro Pb without using dough in the extraction ranged from 0.03 to 3.23% with an overall mean of 1.06% (Table 2). Much literature has been published concerning the estimation of bioaccessible Pb in soil using in vitro procedures (Davis et al., 1992; Ellickson et al., 2001; Hamel et al., 1998; Ruby et al., 1992, 1993, 1996). Previous work has reported a range of values for bioaccessible Pb in soils with most values being <100%. Davis et al. (1992) used an in vitro procedure to compare the solubility of lead acetate with the solubility of a soil from the Butte, MT, mining district and found that Pb in lead acetate was 70 and 5 times

Table 2. Comparison of soil Pb and in vivo relative bioavailable Pb with bioaccessible Pb determined by the in vitro gastrointestinal (IVG) method with and without dough additive.

						Bioaccessible Pb			
		In vivo relative bioavailable Pb‡				IVG with dough		IVG without dough	
Soil	Soil Pb†	Blood	Liver	Kidney	Bone	GE§	IE¶	GE§	IE¶
	mg kg^{-1}	%							
1	1 590	33	33	21	21	19.7	0.54	21.1	2.79
2	8 600	22	9	13	13	5.90	0.17	6.81	0.48
3	11 200	1	0	1	1	0.70	0.02	1.40	0.32
4	10 800	56	92	50	55	27.8	1.16	55.2	1.66
5	4 050	78	110	77	70	31.6	1.06	64.4	0.49
6	6 940	82	66	50	94	34.3	0.95	58.8	2.22
7	7 510	71	92	91	62	26.4	0.47	41.0	1.93
8	4 320	87	96	124	84	35.0	0.80	53.0	1.95
9	10 600	20	11	10	18	8.24	0.04	7.50	0.09
10	1 270	6	5	4	0.04	4.74	0.18	6.71	0.18
11	7 895	20	8	8	9	13.8	0.06	6.85	0.03
12	11 500	55	37	44	61	22.3	0.57	24.7	0.05
13	3 200	67	87	102	63	31.6	0.32	51.9	0.07
14	8 350	82	85	70	63	29.6	0.84	36.9	1.01
15	3 230	74	50	42	47	31.1	0.59	32.2	0.75
16	2 150	58	54	34	39	36.3	0.87	36.3	0.36
17	14 200	56	86	68	72	23.3	0.66	37.7	1.43
18	3 870	58	74	74	68	31.0	0.73	36.2	3.23
Mean	6 740	51	55	49	47	23.0	0.56	32.2	1.06
Median	7 230	57	60	47	58	27.1	0.58	36.3	0.62
Minimum	1 270	1	0	1	0.04	0.70	0.02	1.40	0.03
Maximum	14 200	87	110	124	94	36.3	1.16	64.4	3.23

† SW 846, USEPA Method 3050 (USEPA, 1996a).
‡ Determined from juvenile swine dosing trial.
§ Gastric solution extraction step.
¶ Intestinal solution extraction step.

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more available than an equivalent mass of Pb found in the soil under simulated stomach and intestine conditions, respectively. Ruby et al. (1992, 1993) investigated mine-waste samples and found that bioaccessible Pb ranged from 0.5 to 6%. Hamel et al. (1998) used an in vitro extraction procedure composed of a gastric step at a pH of 1.1 to evaluate the bioaccessibility of Pb and other contaminants in a National Institute of Standards and Technology (NIST) standard reference material (SRM) (NIST soil SRM 2710). Their study investigated the effect of varying the liquid to solid ratio on the extractability of As, Cr, Ni, Cd, and Pb without using food in the extraction. Their results indicate that the solubility of Pb in SRM 2710 was affected only slightly by changing the liquid to solid ratio; they reported the bioaccessibility of Pb in SRM 2710 as 36% at a liquid to solid ratio of 100:1, 46% at a liquid to solid ratio of 200:1, and 35% at a liquid to solid ratio of 2000:1. The IVG extraction of Rodriguez et al. (1999) used a liquid to solid ratio of 150:1. In vitro bioaccessible Pb was measured in NIST soil SRM 2710 both with and without dough. In vitro gastrointestinal GE Pb (without dough) was 60%, while IVG IE Pb (without dough) was 4%. Measured IVG GE Pb (with dough) was 28%, and IVG IE Pb (with dough) was 0.4%. Ellickson et al. (2001) used a two step in vitro procedure (without food) composed of a saliva-gastric step (pH = 1.4) and an intestinal step (pH = 6.5) to evaluate the bioaccessibility of Pb and As in NIST soil SRM 2710. Using a liquid to solid ratio of approximately 3500:1, they reported the bioaccessibility of Pb in the saliva-gastric step as 76.1% and the bioaccessibility of Pb in the intestinal step as 10.7%. Ruby et al. (1996) used the PBET composed of a stomach step (pH = 2.5) and an intestinal step (pH = 7.0) at a liquid to solid ratio of 160:1 without food to estimate bioaccessible Pb in a set of seven soils. Percent bioaccessible Pb extracted by their stomach step ranged from 3.8 to 26%, while percent bioaccessible Pb for their intestinal step ranged from 0.6 to 29%.

Dough versus No Dough

Lead extracted by the IVG GE was greater than Pb extracted by the IVG IE for the 18 individual soils for both dough and no dough methods (Table 2). Mean IVG GE Pb was also greater than mean IVG IE Pb for the soils using both methods ($p < 0.001$; Table 2). In part, the reduction of measured Pb between IVG GE and IVG IE can be attributed to the reduced solubility of Pb in the higher solution pH of the IE as compared with the GE (pH 5.5 vs. 1.8). During our study, mean Pb in the IE without dough decreased by approximately 97% as compared with mean GE Pb without dough. Our results are similar to those of Ruby et al. (1996), who showed that solubilized Pb decreased by 74% upon entering the small intestine step during the PBET due to adsorption and precipitation reactions removing Pb from solution as the pH increased.

Comparison of the dough vs. no-dough methods shows that the mean Pb of 16.6% for the combined GE and IE without using dough in the extraction was greater than the mean Pb extracted of 11.8% for the combined GE and IE using dough in the extraction ($p = 0.003$; Table 2). There was a significant interaction between method and extraction phase (e.g., gastric vs. intestinal) ($p = 0.011$). This was evident in the comparison of the simple effects of extraction phase for the given method. Mean IVG GE Pb of 32.2% without dough in the extraction was significantly greater than mean IVG GE Pb of 23.0% using dough in the extraction ($p < 0.001$) (Table 2). However, mean IVG IE Pb of 1.06% without dough in the extraction was not significantly greater than mean IVG IE Pb of 0.56% using dough in the extraction ($p = 0.689$; Table 2). Our results are similar to those of Ruby et al. (1993) who reported that the addition of rabbit chow to an in vitro procedure reduced the mass solubilized Pb during the stomach phase by approximately 10.8%.

In a review on human bioavailability, Ragan (1983) reported that the solubility and absorption of Fe, Cd, and Pb may be lowered by dietary components such as oxalates, phosphates, and phytates. The presence of food reduces absorption of ingested water-soluble Pb (e.g., lead chloride, lead nitrate, lead acetate) by humans primarily due to the presence of calcium and phosphate (Blake and Mann, 1983; Blake et al., 1983; Heard and Chamberlain, 1982; Rabinowitz et al., 1980). Madalonni et al. (1998) dosed human volunteers with contaminated soil from Bunker Hill, ID, and reported that the absorption of Pb was greatly affected by the presence of food in the gastrointestinal systems of test subjects. Their study reported the absorption of Pb in fasted test subjects as 26% and the absorption of Pb in fed test subjects as 2.5%. Phytic acid (*myo*inositol hexaphosphate) or its salt, phytate, is an important plant constituent accounting for up to 90% of total phosphorus in cereals, legumes, and oilseeds (Reddy et al., 1982). Phytic acid is capable of forming strong complexes with various metal cations under physiological conditions (Nolan et al., 1987). Wise (1981, 1983) conducted both acute (8 d) and chronic studies (6 mo) involving the addition of calcium phytate to Pb-contaminated diets fed to mice and reported that calcium phytate reduced blood Pb levels. Rose and Quarterman (1984) fed rats a diet containing 200 mg Pb kg^{-1} supplemented with phytate (10 g kg^{-1}) or calcium (6 g kg^{-1}) and found that the addition of phytate or calcium separately reduced the accumulation of Pb in bone, blood, and liver. They also reported that the greatest reduction in tissue accumulation of Pb occurred when phytate and calcium were fed together. Bullock et al. (1995) investigated the effect of phytate on the in vitro solubility of Al, Ca, Hg, and Pb as a function of pH at 37°C. They varied the Pb to phytate ratio across the pH range of 3.0 to 7.0 and found that the solubility of Pb varied with both pH and the Pb to phytate molar ratio. Lead solubility in their study was greatly reduced by the formation of Pb–phytate precipitates. Maximum reduction in Pb solubilities occurred at a Pb to phytate ratio of approximately 3:1 with reductions ranging from 96% (pH = 3.0) to 88% (pH = 7.0). The calcium–phytate complex has a strong affinity for both Pb and Cd (Wise, 1983). Also, Wise and Gilburth

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(1981) reported that almost complete binding of both Cd and Pb occurred at Ca to phytate ratios that are common in stock diets of laboratory animals. It is possible that Pb–phytate complexes or insoluble complexes involving phytic acid and Ca with Pb coprecipitating the complex were formed during the in vitro extraction of soils using dough, which resulted in lower bioaccessible Pb as compared with the extractions that did not use dough.

The dough material has a phosphorus content of 7580 mg kg^{-1}. A considerable amount of P was dissolved in the IVG methods. Results show that the inclusion of dough increased soluble P in the IVG GE solution from 53.9 to 1900 mg L^{-1} without soil. Similarly, inclusion of dough increased soluble P in the IVG IE from 64.5 to 1740 mg L^{-1} without soil. Results show that the inclusion of dough increased soluble P in the IVG GE solution from 49.1 to 1810 mg L^{-1} with contaminated SRM 2710 soil. Similarly, inclusion of dough increased soluble P in the IVG IE from 56.7 to 1570 mg L^{-1} with contaminated SRM 2710 soil. The equilibrium geochemical speciation model MINTEQA2 (Version 4.0) was used to investigate the possibility that the addition of dough to the IVG procedure resulted in precipitation of inorganic Pb phosphorus compounds, thus lowering measured concentrations of in vitro Pb (USEPA, 1999). In vitro concentrations of Ca, Pb, Fe, P, Zn, Na, Cl, and solution pH were used as model inputs. Total dissolved P was assumed to be present as orthophosphate ion, which would be consistent with the most likely scenario to form Pb phosphate mineral precipitate. MINTEQA2 predicted that the IVG GE solutions, with and without dough, were oversaturated with respect to lead phosphate solid phases for the contaminated SRM 2710 soil. MINTEQA2 indicated that 24.6% of the total Pb could precipitate as pyromorphite without dough and that 79.6% of the total Pb could precipitate as pyromorphite with dough, which is consistent with decreased in vitro Pb associated with dough addition during the gastric step of the in vitro procedure. MINTEQA2 predicted that oversaturation occurred for the IVG IE solutions with and without dough. The model predicted that 99.98% of the total Pb could precipitate as pyromorphite without dough, while 99.96% of the total Pb could precipitate as pyromorphite with dough. This is consistent with the results for the soils in our study that show there was not a significant difference between mean IVG IE Pb with dough and mean IVG IE without dough.

Linear Regressions

Concentrations of Pb in blood are the most widely used biomarkers of lead exposure (United States Department of Health and Human Services, 1999). However, approximately 94% of the total body burden of Pb is found in bones with Pb cycling between blood and bone (United States Department of Health and Human Services, 1999). The relationship between blood Pb and gastrointestinal Pb exposure is nonlinear high exposure concentrations. Lead in bone is considered a more appropriate biomarker of cumulative Pb exposure than Pb in blood (United States Department of Health and Human Services, 1999; USEPA, 1986). Linear regression indicated there was a strong relationship between IVG GE Pb using dough in the extraction and in vivo relative bioavailable Pb estimated using blood data ($P < 0.0001$, $r = 0.93$) (Fig. 1A). Regression analysis showed there was a strong relationship ($P < 0.0001$, $r = 0.80$) between IVG IE Pb using dough in the extraction and in vivo relative bioavailable Pb estimated using blood data (Fig. 1B). A strong relationship was found between IVG GE Pb without using dough in the extraction and in vivo relative bioavailable Pb using blood data ($P < 0.0001$, $r = 0.89$) (Fig. 1C). However, a significant relationship between IVG IE Pb (no dough) and in vivo relative bioavailable Pb using blood data was not found ($P = 0.121$, $r = 0.38$) (Fig. 1D). Strong relationships also existed between IVG GE Pb using dough in the extraction and estimated in vivo relative bioavailable Pb using other tissues (e.g., liver, kidney, and bone) with regression coefficients ranging from 0.76 to 0.85 (Table 3). Strong relationships were also found between IVG IE Pb using dough in the extraction and in vivo relative bioavailable Pb as estimated by the other tissues with regression coefficients ranging from 0.56 to 0.80 (Table 3). Significant relationships were also found between IVG GE (no dough) and in vivo relative bioavailable Pb using the other tissues, and regression coefficients ranged from 0.81 to 0.93 (Table 3). Conversely, within the IE without dough in the extraction, only the relationship between IVG Pb and in vivo relative bioavailable Pb using bone data was significant ($P = 0.049$, $r = 0.47$) (Table 3). Ruby et al. (1996) showed that the stomach phase of the PBET at pH values of 1.3 and 2.5 was highly correlated with in vivo relative bioavailable Pb as measured by a weanling rat model with blood as the target organ ($r^2 = 0.93$ for both pH values, $n = 7$). Their study reported a weaker relationship ($r^2 = 0.76$) between intestinal bioaccessible Pb and in vivo relative bioavailable Pb. In a review article on bioavailability of inorganics in soils, Ruby et al. (1999) cited a study by Medlin (1997) and indicated that the stomach phase of the PBET was strongly correlated ($r^2 = 0.79$, $n = 15$) with a "weighted estimate" (i.e., blood weighted 3:1 over each tissues) of in vivo relative bioavailable Pb from a young swine model. However, correlations between the intestinal phase of the PBET and in vivo relative bioavailable Pb were not reported in the review. In general, the results of our study are very similar to those reported by Ruby et al. (1996, 1999) in that the GE without dough was highly correlated with in vivo relative bioavailable Pb as estimated with all tissues.

Many interactions in the gastrointestinal systems of various animals affect Pb absorption. Gastrointestinal absorption of Pb is a complex and dynamic process involving dissolution, absorption, and interactions with other dietary components. Iron deficiencies in children have been associated with elevated blood Pb concentrations, and several animal studies have shown that Fe deficiencies increase gastrointestinal absorption of Pb

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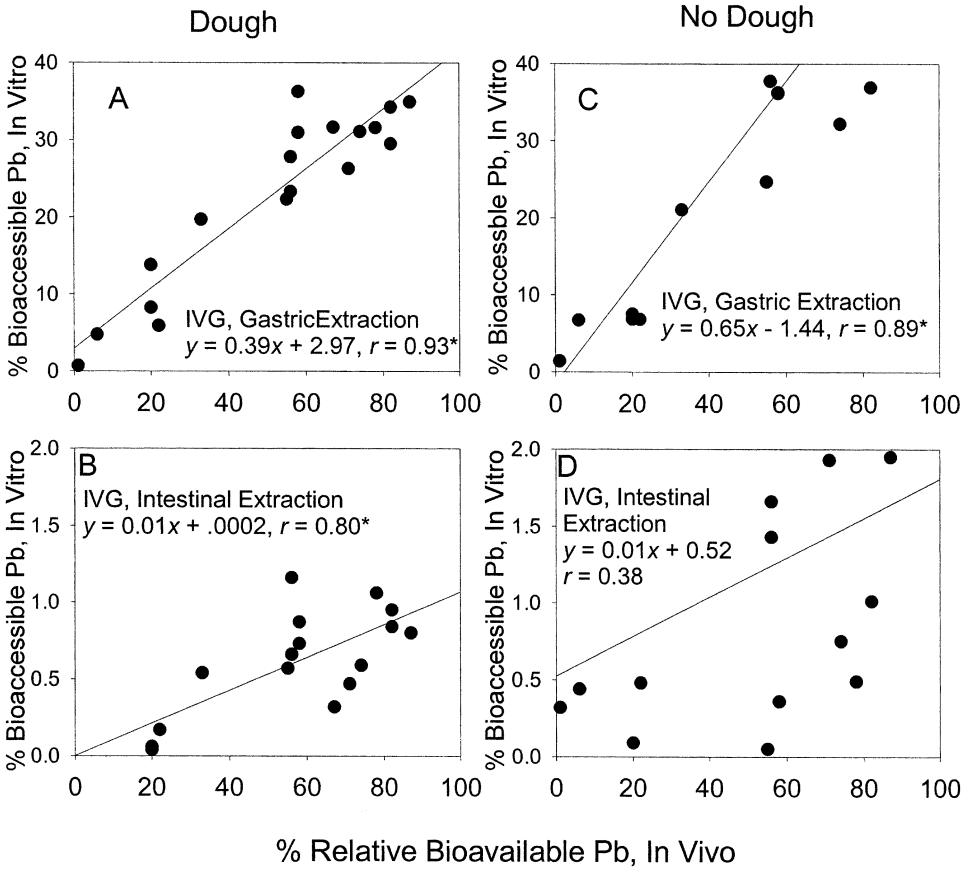


Fig. 1. Linear regression of in vitro bioaccessible Pb extracted by (A) gastric extraction step with dough; (B) intestinal extraction step with dough; (C) gastric extraction step without dough; or (D) intestinal extraction step without dough vs. relative bioavailable Pb, in vivo estimated with blood data. *Significant at the 0.001 probability level.

(Barton et al., 1978; Flanagan et al., 1979; Mahaffey and Annest, 1986; Morrison and Quarterman, 1987; Sullivan and Ruemmler, 1987). Dietary deficiencies of Ca, Zn, and P enhance absorption of Pb in humans, rats, mice, and pigs (Heard and Chamberlain, 1982; Hsu et al., 1975; Johnson and Tenuta, 1979; United States Department of Health and Human Services, 1999; Van Barneveld and Van Den Hamer, 1985; World Health Organization, 1996). The ratios of total elemental content for Zn to Pb ranged from 0.01 to 12 with a median value of 2, Ca to Pb ranged from 0.24 to 22 with a median value of 4, and Fe to Pb ranged from 0.89 to 308 with a median value of 8. It is possible large amounts of Ca, Fe, and/or Zn decreased Pb absorption in some soils more than others.

CONCLUSIONS

Three of the solution extraction steps (GE with dough, IE with dough, and GE without dough) of the IVG method were able to predict bioavailable Pb in contaminated soils as measured by in vivo pig dosing trials. The combination of the complex biochemistry and biological processes in the gastrointestinal system makes it difficult to estimate bioavailable Pb by in vitro methods. However, the ability of the IVG method to estimate bioavailable Pb is promising. Additional studies that compare in vitro results with in vivo bioavailable Pb should be conducted on more soils from a wide range of matrices (soil, slag, etc.). It is unlikely that an in vitro method can be developed to replace animal models in the estimation of in vivo bioavailability; however, in vitro meth-

Table 3. Regression coefficients (r) and regression equations between percent bioaccessible Pb (in vitro) gastric and intestinal steps and percent relative bioavailable Pb (in vivo) as determined in different tissues of juvenile swine.

	Dough				No dough			
Tissue	GE†	Regression equation	IE‡	Regression equation	GE	Regression equation	IE‡	Regression equation
Blood	0.93*	$y = 0.39x + 2.97$	0.80*	$y = 0.01x + 0.0001$	0.89*	$y = 0.65x - 1.44$	0.38	$y = 0.01x + 0.52$
Liver	0.84*	$y = 0.26x + 8.65$	0.80*	$y = 0.01x + 1.13$	0.93*	$y = 0.50x + 4.22$	0.43	$y = 0.01x + 0.41$
Kidney	0.76*	$y = 0.24x + 11.1$	0.56*	$y = 0.01x + 0.29$	0.81*	$y = 0.45x + 10.0$	0.39	$y = 0.01x + 0.53$
Bone	0.85*	$y = 0.33x + 7.40$	0.78*	$y = 0.01x + 0.12$	0.89*	$y = 0.61x + 3.53$	0.47*	$y = 0.02x + 0.30$

* Significant at the 0.05 probability level.
† Gastric solution extraction step.
‡ Intestinal solution extraction step.

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ods (i.e., the IVG method) may be useful as rapid screening tools in assessing bioavailability of Pb on contaminated sites. Because in vitro methods are inexpensive, they can be used to analyze large numbers of soil samples and provide an estimate of the variability in bioavailable Pb at a single study site. The GE step of the IVG method has the ability to provide an estimate of bioavailable As (Rodriguez et al., 1999) and Pb in contaminated soil. The GE can be used to estimate relative bioavailable Pb, As, and Cd (Schroder et al., 2003) in contaminated soil.

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