Evaluation of SBRC-Gastric and SBRC-Intestinal Methods for the Prediction of In Vivo Relative Lead Bioavailability in Contaminated Soils

ALBERT L. JUHASZ,*,†,‡ JOHN WEBER,†,‡ EUAN SMITH,†,‡ RAVI NAIDU,†,‡ BERND MARSCHNER,§ MATTHEW REES,∥ ALLAN ROFE,∥ TIM KUCHEL,∥ AND LLOYD SANSOM⊥

Centre for Environmental Risk Assessment and Remediation, Division of Information Technology, Engineering and the Environment, University of South Australia, Mawson Lakes Campus, Mawson Lakes, SA 5095, Australia, Cooperative Research Centre for Contamination Assessment and Remediation of the Environment, Mawson Lakes, SA 5095, Australia, Department of Soil Science and Soil Ecology, Geographical Institute, Ruhr-University Bochum, 44780, Germany, Institute for Medical and Veterinary Science, Frome Road, SA 5000, Australia, and Sansom Institute, School of Pharmacy and Medical Sciences, Division of Health Science, University of South Australia, City East Campus, SA 5001, Australia

Received November 16, 2008. Revised manuscript received April 14, 2009. Accepted April 27, 2009.

In this study, lead (Pb) bioaccessibility in contaminated soils was assessed using an in vitro method (SBRC) encompassing gastric (SBRC-G) and intestinal (SBRC-I) phases. Initially, bioaccessibility studies were performed with a Pb reference material (Pb acetate, 1−10 mg L^{-1}) in order to determine the influence of pH on Pb solubility. In the gastric phase (pH 1.5), Pb solubility was 100% (100 ± 2.9%, $n = 16$) irrespective of the Pb concentration added, however, when the pH of the intestinal phase was increased to near neutral, Pb solubility decreased to 14.3 ± 7.2%. In contaminated soils, Pb bioaccessibility varied from 35.7 to 64.1% and 1.2 to 2.7% for SBRC-G and SBRC-I phases, respectively. When relative bioaccessibility (Rel-SBRC-I) was calculated by adjusting the dissolution of Pb from contaminated soils by the solubility of Pb acetate at pH 6.5 (intestinal phase pH), Rel-SBRC-I values ranged from 11.7−26.1%. A stepwise regression model based on Pearson correlation factors was used to determine the suitability of in vitro assays for predicting in vivo (swine assay) relative Pb bioavailability. Rel-SBRC-I provided the best estimate of in vivo relative Pb bioavailability for soils used in this study (in vivo relative Pb bioavailability [%] = Rel-SBRC-I [pH 6.5%] × 0.58 + 1.98, $r^2 = 0.53$). The versatility of Rel-SBRC-I was demonstrated by accurately predicting relative Pb bioavailability from other reported in vivo studies.

* Corresponding Author phone: +618 8302 5045; fax: +618 8302 3057; e-mail: Albert.Juhasz@unisa.edu.au.
† University of South Australia, Mawson Lakes Campus.
‡ Cooperative Research Centre for Contamination Assessment and Remediation of the Environment.
§ Ruhr-University Bochum.
∥ Institute for Medical and Veterinary Science.
⊥ University of South Australia, City East Campus.

Introduction

For young children, incidental ingestion of soil and dust, via hand-to-mouth activity, is considered an important pathway for Pb exposure. However, the Pb dose that is absorbed into systemic circulation (the bioavailable fraction) may vary depending on the nature and solubility of Pb present, the physicochemical properties of the ingested material as well as the child's nutritional status (*1, 2*). When estimating soil Pb exposure for children, the integrated exposure uptake biokinetic model (IEUBK), developed by the U.S. Environmental Protection Agency (USEPA) (*3*), assumes that 50% of an oral Pb dose from food or water will be absorbed into systemic circulation while only 30% of a soil Pb dose will be absorbed (i.e., 60% relative bioavailability). However, due to the dependence of Pb bioavailability on physical and chemical form, application of the IEUBK Pb bioavailability value may not be appropriate for all Pb-contaminated soils (*1*).

In order to adjust the default Pb bioavailability value, an assay is required that is rapid and reliable and which can quantitatively measure site specific Pb bioavailability. Currently, in vivo bioassays (e.g., juvenile swine) are the method of choice (*4*), however, in vitro assays have been developed in order to estimate Pb bioavailability. In vitro methods offer the advantage of simplicity, speed, and affordability over the laborious, time-consuming, and expensive in vivo assays (*5*), however, correlation of these methods against a suitable animal model across a range of soil types and chemical forms is essential for their validation (*6, 7*).

Research undertaken as part of a USEPA study (*8, 9*) determined that dissolution of Pb phases following gastric phase extraction provided a good prediction of relative Pb bioavailability determined using juvenile swine. Similar results were obtained by Ruby et al. (*1*) (PBET method and a Sprague−Dawley rat model) and Schroder et al. (*10*) (IVG method and a swine model). Poor in vivo−in vitro correlations were obtained for intestinal phase data and relative Pb bioavailability which was attributed to the complex non-equilibrium chemical system for Pb in the small intestines (*1*). It was suggested, however, that the use of the small intestinal phase data would be preferable as a measure of Pb bioaccessibility (*1*).

While a good relationship was observed between gastric phase Pb dissolution and in vivo relative Pb bioavailability, the USEPA (*8*) cautioned that the majority of samples used were derived from similar sources (mining and milling activities) and that some forms of Pb that were absent in these soils may not follow the observed correlation (*8*). This was highlighted in the study of Marschner et al. (*11*) who reported that the absolute and relative bioavailability of Pb in five urban and industrial soils, determined using liver, kidney, bone, and urine data from soil dosed minipigs, was not related to Pb bioaccessibility determined using the standardized German in vitro assay (*12*).

The aforementioned studies highlight the conflicting and limited information regarding in vitro−in vivo correlations, the differences in in vitro and in vivo methodologies employed and the dearth of bioavailability data available for Pb-contaminated soils originating from sources other than mining-impacted locations. In order to address this limitation, the aim of this study was to assess gastric and intestinal in vitro methods for their ability to predict in vivo swine relative Pb bioavailability in Pb-contaminated soils from a former domestic incinerator site and urban residential land.

10.1021/es803238u CCC: $40.75 © 2009 American Chemical Society
Published on Web 05/11/2009

TABLE 1. Selected Properties of Soils Used in This Study

		soil properties[a,b]					
soil	source[c]	Pb (mg kg^{-1})	P (g kg^{-1})	Fe (g kg^{-1})	Al (g kg^{-1})	OC (%)	pH
A	urban residential	646	7.9	36.8	7.6	10.6	7.0
B	urban residential	765	4.6	62.6	4.9	9.5	6.4
C	domestic incinerator	2885	1.3	41.6	23.4	0.2	6.9
D	domestic incinerator	2980	1.5	44.8	17.3	0.2	7.7
E	domestic incinerator	3905	1.8	57.9	22.2	0.2	6.8

[a] The <250 μm particle size fraction was used for all analysis. [b] Data represents the mean of duplicate analysis. Values varied by less than 5%. [c] Source of Pb-contaminated soil.

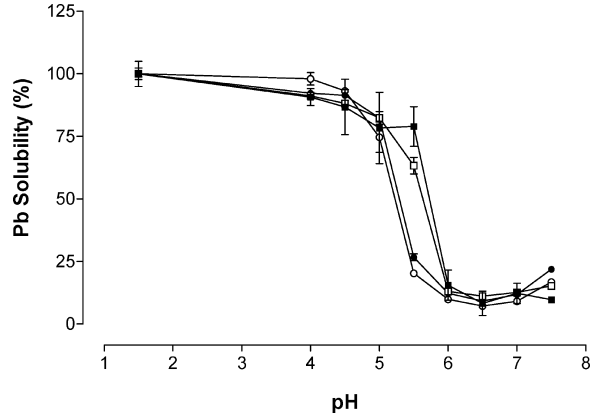


FIGURE 1. Solubility of Pb acetate in in vitro solutions (gastric and intestinal phases) as a function of pH. Lead acetate was supplied to achieve initial concentrations of 1 (■), 2.5 (□), 5 (●) and 10 mg Pb L^{-1} (○). Data points represent the mean and standard deviation of quadruplicate samples.

Materials and Methods

Lead-Contaminated Soil. Soils used in this study were collected from a former domestic incinerator site and residential land developed on quarry fill material (Table 1). Soils were air-dried then sieved with the <250 μm fraction retained for bioavailability/bioaccessibility assessment. Soil physicochemical properties were determined in duplicate for each soil. Soil pH was determined using 1:5 soil:water extracts, and organic carbon content was determined by oxidation/combustion (*13*). Total metal concentration of the soil was determined using USEPA 3015H dissolution procedure (*14*) with a CEM MarsX microwave. Total metals in digest solutions were determined by Inductively coupled plasma atomic emission spectroscopy (ICP-AES). A certified reference material (GBW 07411) was included in the analysis to ensure internal quality assurance/quality control (QA/QC) practices (see the Supporting Information (SI)). Table 1 outlines selected properties for soils used in this study.

Determination of Lead Bioaccessibility. The Solubility Bioavailability Research Consortium (SBRC) in vitro assay (*15*) was utilized for the assessment of Pb bioaccessibility. In this study, both gastric and intestinal phases were used with some modifications. Initially, Pb acetate or contaminated soil was combined with gastric phase solution (30.03 g L^{-1} glycine adjusted to pH 1.5 with concentrated HCl) in polyethylene screw cap flasks. Lead acetate was added to to achieve a final concentration of 1−10 mg Pb L^{-1} while soil was added at a soil:solution ratio of 1:100. The pH was noted then the flasks were incubated at 37 °C, 40 rpm on a Ratek suspension mixer. After 1 h, the pH was determined and gastric phase samples (10 mL) were collected, filtered through 0.2 μm filters for analysis by ICP-AES.

Following gastric phase dissolution, the gastric solution was modified to the intestinal phase by adjusting the pH (up to 7.5) with either 5 or 50% NaOH and by the addition of bovine bile (1750 mg L^{-1}) and porcine pancreatin (500 mg L^{-1}). The influence of pH on Pb bioaccessibility was assessed by conducting the intestinal phase extraction at a range of pH values (4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, and 7.5). After 4 h, intestinal phase samples (10 mL) were collected and filtered through 0.2 μm filters for analysis by ICP-AES or ICP-MS. All gastric and intestinal phase extractions were performed in quadruplicate for Pb acetate and in triplicate for each soil sample. Absolute Pb bioaccessibility was calculated by dividing the gastric phase extractable Pb (termed SBRC-G) and intestinal phase extractable Pb (termed SBRC-I) by the total Pb acetate or soil Pb concentration (eq 1). Relative Pb bioaccessibility (termed Rel-SBRC-I) was determined by adjusting the dissolution of Pb from contaminated soils by the solubility of Pb acetate at the corresponding pH value (eq 2).

$$\text{absolute Pb bioaccessibility, \%} = \left(\frac{\text{in vitro Pb}}{\text{total Pb}}\right) \times 100 \quad (1)$$

Where in vitro Pb = Pb (μg) extracted from soil following gastric phase (SBRC-G) or intestinal phase (SBRC-I) treatment, total Pb = Pb (μg) present in contaminated soil or Pb acetate added to the in vitro assay prior to treatment (*16*).

$$\text{relative Pb bioaccessibility, \%} = \left[\frac{\left(\frac{\text{SBRC-I}_{\text{soil Pb}}}{\text{total soil PB}}\right)}{\left(\frac{\text{SBRC-I}_{\text{Pbacetate}}}{\text{total Pb acetate}}\right)}\right] * 100 \quad (2)$$

Where SBRC-I $_{\text{soil Pb}}$ = Pb (μg) extracted from soil following intestinal phase (SBRC-I) treatment of Pb-contaminated soil at an intestinal pH of 6.5, total Soil Pb = Pb (μg) present in contaminated soil prior to treatment. SBRC-I $_{\text{Pb acetate}}$ = Pb (μg) solubilized following intestinal phase (SBRC-I) treatment of Pb acetate at an intestinal pH of 6.5, and total Pb acetate = Pb acetate (μg) added to the in vitro assay prior to treatment.

Assessment of Pb Bioavailability: In Vivo Swine Assays. Lead bioavailability studies were performed according to Juhasz et al. (*6, 17*) with minor modifications. Female Large White swine (6−8 weeks old), weighing 20−25 kg, were used for in vivo bioavailability assays. Following acclimation for 12 days to animal house conditions, swine were fasted for 24 h prior to surgery for the insertion of jugular catheters using the method of Bain et al. (*18*). During bioavailability assays, animals were housed in metabolic cages. Swine were fed twice daily (500 g of low Pb pig pellets), initially two hours following Pb dosage, while water was supplied ad libitum. Prior to Pb dosage, blood samples were taken to determine baseline blood Pb concentrations. Lead-contaminated soils (<250 μm particle size fraction) were administered as soil slurries via gavage (5−10 g of soil in 150 mL of Pb free water), whereas Pb acetate (100 μg Pb kg^{-1}) was administered as the reference dose. An intravenous Pb dosage (25 μg Pb

TABLE 2. Assessment of Pb Bioaccessibility in Contaminated Soils Using SBRC-G, SBRC-I and Rel-SBRC-I Methods

		Pb bioaccessibility (%)		
soil	Pb (mg kg^{-1})	SBRC-G[a]	SBRC-I[b]	Rel-SBRC-I[c]
A	646	61.0 ± 4.6 (394 ± 30)[d]	2.7 ± 0.4 (17.2 ± 2.6)	26.1 ± 6.5 (168 ± 42)
B	765	35.7 ± 1.3 (373 ± 10)	2.1 ± 0.1 (15.8 ± 0.7)	20.0 ± 4.0 (153 ± 31)
C	2885	64.1 ± 0.2 (2115 ± 7)	1.5 ± 0.1 (43.0 ± 3.4)	14.4 ± 2.6 (416 ± 76)
D	2980	64.1 ± 0.8 (1910 ± 25)	2.3 ± 0.5 (69.5 ± 14.3)	22.5 ± 5.0 (669 ± 150)
E	3905	60.9 ± 0.6 (2378 ± 24)	1.2 ± 0.3 (47.3 ± 11.0)	11.7 ± 2.8 (455 ± 109)

[a] Lead bioaccessibility determined using the SBRC gastric phase (SBRC-G) at pH 1.5. [b] Lead bioaccessibility determined using the SBRC intestinal phase (SBRC-I). Following gastric phase dissolution, Pb bioaccessibility was determined in the intestinal phase at pH 6.5 in the presence of bile and pancreatin. [c] Relative lead bioaccessibility determined using Rel-SBRC-I. Relative Pb bioaccessibility was calculated by adjusting the dissolution of Pb from contaminated soils by the solubility of Pb acetate at the corresponding pH value (pH 6.5). [d] Values in parentheses represent the concentration of soil-borne Pb (mg kg^{-1}) that was bioaccessibility following SBRC-G, SBRC-I or Rel-SBRC-I analysis.

kg^{-1}) was administered using a catheter separate from the blood sampling catheter. Blood samples were routinely taken over a 5 day period following dosage and collected in 7.5 mL EDTA collection tubes. Blood was stored at −20 °C prior to Pb analysis. For each in vivo treatment (intravenous dose, oral dose, or Pb-contaminated soil dose), three separate animals were used.

Lead bioavailability was assessed using pharmacokinetic analysis encompassing areas under the blood concentration (AUC) time curves following zero correction and dose normalization. Absolute and relative Pb bioavailability was calculated according to eqs 3 and 4, respectively. When calculating relative Pb bioavailability, the AUC for the Pb acetate oral treatment was used for comparison.

$$\text{absolute Pb bioavailability, \%} = \left(\frac{\text{AUC}_{\text{oral-Pb}}}{\text{AUC}_{\text{IV-Pb}}} \times \frac{\text{DR}_{\text{IV-Pb}}}{\text{DR}_{\text{oral-Pb}}}\right) \times 100 \quad (3)$$

Where AUC $_{\text{oral-Pb}}$ = area under the Pb blood concentration versus time curve for an oral Pb dose, AUC $_{\text{IV-Pb}}$ = area under the Pb blood concentration versus time curve for an intravenous Pb dose, DR $_{\text{IV-Pb}}$ = dose of intravenously administered Pb (mg kg^{-1}), and DR $_{\text{oral-Pb}}$ = dose of orally administered Pb (mg kg^{-1}).

$$\text{relative Pb bioavailability, \%} = \left(\frac{\text{AUC}_{\text{oral-soil}}}{\text{AUC}_{\text{oral-Pbacetate}}} \times \frac{\text{DR}_{\text{oral-Pbacetate}}}{\text{DR}_{\text{oral-soil}}}\right) \times 100 \quad (4)$$

Where AUC $_{\text{oral-soil}}$ = area under the Pb blood concentration versus time curve for an oral Pb-contaminated soil dose, AUC $_{\text{oral-Pb}}$ = area under the Pb blood concentration versus time curve for an oral dose of lead acetate, DR $_{\text{oral-soil}}$ = dose of orally administered soil (mg kg^{-1}), and DR $_{\text{oral-Pb}}$ = dose of orally administered lead acetate (mg kg^{-1}).

Lead Determination in Blood. Blood was diluted 10-fold in diluent solution containing 1-butanol (2% w/v), EDTA (0.05% w/v), Triton X-100 (0.05% w/v), and ammonium hydroxide (1% w/v) in Milli-Q water (*19*) prior to analysis. All samples were analyzed by ICP-MS with the appropriate number of duplicate samples, duplicate analysis, spiked sample recoveries and check values included for quality assurance and quality control purposes (see SI).

Results and Discussion

Soil Properties. Lead concentrations in the five contaminated soils ranged from 646 to 3905 mg Pb kg^{-1}. These values exceed the Australian National Environmental Protection Measure for the Assessment of Site Contamination (NEPM-ASC) Pb health investigation level for "standard" residential garden/accessible soil and children's day care centers, kindergartens, preschools, and primary schools (HIL-A) by 2−12 fold (*20*). Other soil properties including P, Fe, Al, OC, and pH are presented in Table 1.

Lead Acetate Solubility. Initial bioaccessibility studies were performed with Pb acetate to determine the influence of solution pH on Pb solubility. Lead acetate was chosen as it is a highly soluble form of Pb that has been used as a reference dose for in vivo Pb bioavailability assessment (*1, 10, 11*) as well as in toxicity studies as reported in the Integrated Risk Information System (*21*). Lead acetate (1−10 mg Pb L^{-1}) was added to in vitro solutions and the solubility of Pb determined following gastric and intestinal phase incubations. The influence of intestinal phase pH was also assessed by adjusting the pH of this phase from 4 to 7.5 in 0.5 pH increments (see Materials and Methods).

Figure 1 illustrates the effect of pH on percentage Pb solubility in gastric and intestinal phases. In the gastric phase (pH 1.5), Pb solubility was approximately 100% (100 ± 2.9%; $n = 16$) irrespective of the concentration of Pb added. When the in vitro solution was modified to the intestinal phase, through bile and pancreatin addition and an adjustment in pH, Pb solubility varied depending on the pH of the intestinal phase. Increasing the intestinal phase pH from 1.5 to 5.0 had a minor effect on Pb solubility (solubility decreased to 80 ± 8.0%), however, the greatest variability in Pb solubility was observed at pH 5.5 (Figure 1). At Pb concentrations of 1 and 2.5 mg L^{-1}, 79 ± 11% and 63 ± 6.6% of the initial Pb added remained in solution at pH 5.5. In contrast to this, at initial Pb concentrations of 5 and 10 mg Pb L^{-1}, significantly less Pb remained in solution (27 ± 2.7% and 20 ± 1.4% respectively) when the intestinal phase solution was adjusted to pH 5.5. The concentration dependent decrease in Pb solubility at this pH was not unexpected as pH has been shown to be one of the predominant factors controlling the solubility of metals, including Pb, in aqueous solutions (*22*).

When the pH of the intestinal phase was increased to near neutral, Pb solubility decreased to 14.3 ± 7.2%. No significant difference in Pb solubility was observed at pH values of 6.0, 6.5, and 7.0. Increasing the intestinal phase pH to 7.5 resulted in a significant increase ($p < 0.05$, $n = 8$) in Pb solubility for 5 and 10 mg Pb L^{-1} additions (compared to Pb solubility at pH 7.0), however, this was not observed for the lower (1 and 2.5 mg L^{-1}) Pb concentrations (Figure 1). Presumably, the increased solubility of Pb (5 and 10 mg Pb L^{-1}) in the intestinal phase at pH 7.5 occurred as a result of organo (bile/pancreatin)-Pb complexes.

Some in vitro Pb bioaccessibility assays (*23, 24*) utilize an intestinal phase pH of 5.5. The Pb acetate results from this study indicate that small variations in pH when transitioning from gastric to intestinal phases may result in large variations in Pb solubility and therefore Pb bioaccessibility. Conversely, due to the stability of Pb solubility at pH values between 6.0 and 7.0, small variations in intestinal phase pH between these

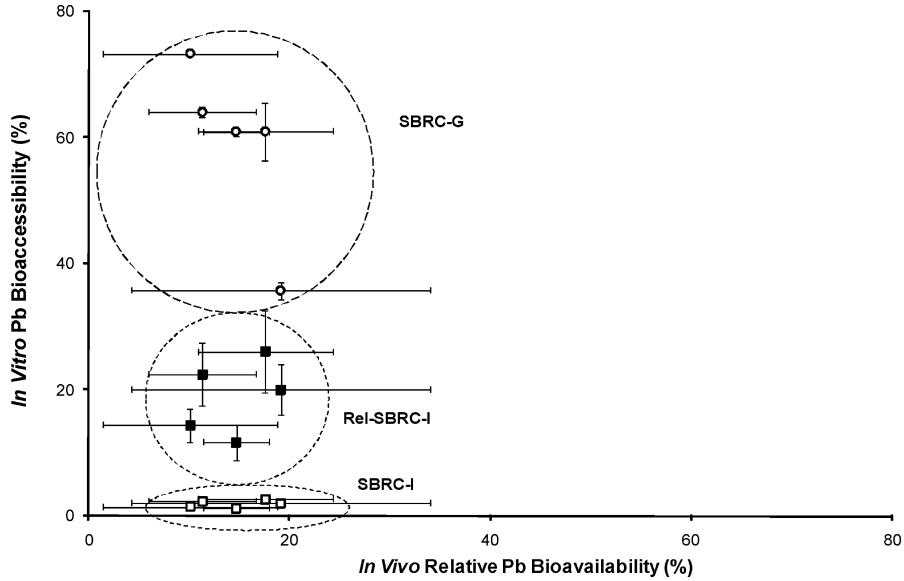


FIGURE 2. Relationship between Pb bioaccessibility, determined using SBRC-G (○), SBRC-I (□), and Rel-SBRC-I (■) methods and relative Pb bioavailability, determined using the in vivo swine assay.

TABLE 3. Relationship between in Vivo Relative Pb Bioavailability (Swine Assay) and in Vitro Pb Bioaccessibility (SBRC-G, SBRC-I and Rel-SBRC-I)

model variables	model	r^2	pearson correlation	durbin-watson factor
in vivo, SBRC-G	Pb bioavailability[a] = SBRC-G[a] × −0.18 + 25.26	0.12	−0.34	2.81
in vivo, SBRC-I	Pb bioavailability = SBRC-I[a] × 4.07 + 6.84	0.14	0.38	2.50
in vivo, Rel-SBRC-I[b]	Pb bioavailability = Rel-SBRC-I[a] × 0.58 + 1.98	0.53	0.73	2.05

[a] Pb bioavailability and bioaccessibility values expressed in percentage terms. [b] Relative SBRC-I determined at an intestinal phase pH of 6.5.

values will not significantly affect Pb solubility. As a result, in vitro assays utilizing an intestinal phase pH between 6.0 and 7.0 will provide a more robust system for the determination of Pb bioaccessibility.

With the exception of the study of Ruby et al. (*1*), the impact of gastrointestinal conditions on the solubility of Pb reference materials (e.g., Pb acetate) has not been reported for bioaccessibility studies. This is surprising as soluble Pb salts such as Pb acetate are commonly utilized as reference materials for the determination of relative Pb bioavailability while undertaking in vivo studies. While Ruby et al. (*1*) reported the relative bioaccessibility of the Pb spike (5 mg Pb L^{-1}) to be 48 and 41% based on the initial solubility in the stomach phase (pH 1.3 and 2.5, respectively), our data indicates that Pb solubility is significantly less at the pH conditions encountered in the intestinal phase (pH 7.0). In this study, at an equivalent dose of 5 mg L^{-1}, 11.75 ± 1.64% ($n = 4$) of the added Pb acetate was soluble at pH 7.0. As a result, when calculating relative bioaccessibility based on small intestine data, the solubility of the reference material at the intestinal phase pH will have a significant impact on the derived relative bioaccessibility values.

Lead Bioaccessibility in Contaminated Soils. Following solubility studies with Pb acetate, the bioaccessibility of Pb in contaminated soils was determined in both gastric and intestinal phases. In addition, the pH of the intestinal phase was varied to determine the influence of pH on Pb bioaccessibility. In four of the five soils, Pb bioaccessibility was >60% following gastric phase dissolution: the exception being soil B where 35.7 ± 1.3% of the total soil-bound Pb was bioaccessible at pH 1.5 (Table 2). When solution conditions were modified to reflect intestinal phase conditions, a similar decrease in soluble Pb with increasing pH was observed for contaminated soils as was seen for Pb acetate. A representative graph illustrating the change in Pb bioaccessibility in contaminated soil E with increasing pH is shown in SI Figure S1. For soil E, transition from the gastric (pH 1.5) to the intestinal phase (at pH 4.0) resulted in a significant ($p < 0.05$) decrease in soluble Pb: correspondingly, Pb bioaccessibility decreased from 60.9 ± 0.6% (2378 ± 24 mg Pb kg^{-1}) to 13.3 ± 2.1% (676 ± 83 mg Pb kg^{-1}). Increasing the intestinal phase pH from 4 to 7.5 resulted in further decreases in soluble Pb, however, the concentration of Pb in solution stabilized at pH values between 6.5 (47.3 ± 11 mg kg^{-1}) and 7.5. A similar trend in gastric and intestinal Pb bioaccessibility was observed for the other contaminated soils as outlined in Table 2.

Numerous in vitro assays (*1, 12, 23*−*29*) have been utilized for the assessment of Pb bioaccessibility with a number of researchers reporting Pb bioaccessibility in contaminated soils to range from 50 to 100% when gastric phase dissolution has been performed (*27, 29, 30*). As Pb dissolution in the gastric phase is influenced by parameters such as Pb mineralogy, the source of Pb contamination will significantly influence Pb bioaccessibility. The dissolution of Pb minerals such as galena, anglesite and Pb jarosite at gastric phase pH values has been reported to be low compared to the highly soluble Pb oxides, cerussite, manganese−Pb oxides, and metal−Pb oxides (*1*). Lead mineralogy in the soils used in this study was investigated using SEM-EDX and TOF SIMS, but distinct (discrete) Pb mineral phases were not observed (data not shown).

Following transition from the gastric phase to the intestinal phase, a significant proportion of solubilized Pb will be readsorbed onto soil particles or precipitated at the neutral intestinal phase pH (*11, 27*). Lead may remain solubilized if

TABLE 4. Comparison of Pb Bioaccessibility (SBRC-G, SBRC-I, Rel-SBRC-I) in Contaminated Soils from Marschner et al. (*11*) and Relative Pb Bioavailability Using an in Vivo Swine Assay

soil	Pb (mg kg^{-1})[a]	IVG − PM (%)[a,b]	IVG + PM (%)[a,c]	in vivo RBA (%)[a,d]	SBRC-G (%)[e]	SBRC-I (%)[f]	Rel-SBRC-I (%)[g]
Bruchsal	237	14	52	40	42.4 ± 7.0	2.7 ± 0.1	30.7 ± 6.1
Carl-1	786	6	26	63	35.0 ± 0.1	4.7 ± 1.3	52.7 ± 10.4
Hamburg	578	3	30	36	70.7 ± 1.6	2.8 ± 0.1	31.6 ± 6.2
Oker-11	6330	20	30	55	66.8 ± 2.3	6.8 ± 0.2	62.9 ± 11.0

[a] Data from Marschner et al. (*11*). Standard deviations not reported. [b] Lead bioaccessibility determined using the method of Hack and Selenka (*12*) in the absence of powdered milk (PM). [c] Lead bioaccessibility determined using the method of Hack and Selenka (*12*) in the presence of powdered milk (PM). [d] Relative Pb bioavailability in contaminated soils following administration of soil to minipigs for 28 days. The amount of Pb in selected organs, bones and urinary excretions was determined and relative bioavailability calculated with reference to Pb acetate absorption. [e] Lead bioaccessibility determined using the SBRC gastric phase (SBRC-G, this study). [f] Lead bioaccessibility determined using the SBRC intestinal phase following gastric phase dissolution (SBRC-I, this study). [g] Relative lead bioaccessibility determined using Rel-SBRC-I (this study).

the respective Pb solubility constants have not been exceeded or if Pb complexes with organic or inorganic ligands (*11*). Correspondingly, Pb bioaccessibility values derived from gastrointestinal assays will be significantly lower than bioaccessibility values derived from gastric phase dissolution alone. In fact Pb bioaccessibility values, determined using intestinal phase data, has been reported to be 2- to 45-times lower than Pb bioaccessibility values determined using gastric phase data (*1, 10, 27, 31*).

Relative Pb Bioaccessibility. Due to the observed effect of pH on Pb solubility, relative Pb bioaccessibility was calculated for each of the contaminated soils, i.e., the dissolution of Pb from contaminated soils was adjusted for Pb acetate solubility at the corresponding pH. SI Figure S1 shows the comparison of "absolute" and relative Pb bioaccessibility at each pH value for soil E, whereas Table 2 presents relative Pb bioaccessibility values (intestinal phase at pH 6.5) for all soils used in the study. Absolute and relative Pb bioaccessibility were comparable for all soils studied at pH values ≤5.0; however, increasing the intestinal phase pH above 5.0 resulted in significantly ($p < 0.05$) higher relative Pb bioaccessibility values compared to absolute Pb bioaccessibility (Table 2). Relative Pb bioaccessibility ranged from 11.7 ± 2.8% to 26.1 ± 6.5% compared to 1.2 ± 0.3% to 2.7 ± 0.4% for absolute Pb bioaccessibility values. Table 2 highlights the large variability in Pb bioaccessibility data when different in vitro approaches are employed. If SBRC-G is only considered, Pb bioaccessibility values will be elevated as the gastric phase is strictly a dissolution process. In contrast, low Pb bioaccessibility values will be obtained using SBRC-I as Pb solubility is controlled by the pH of the intestinal phase. Calculating relative Pb bioaccessibility (Rel-SBRC-I) by adjusting the dissolution of Pb from contaminated soils by the solubility of Pb acetate at the corresponding pH value will yield values approximately 10-fold greater than SBRC-I bioaccessibility values at an intestinal phase pH of 6.5.

In Vivo Pb Bioavailability. In vivo swine experiments were performed to determine the bioavailability of Pb in contaminated soils. Lead doses were administered under fasting conditions to represent a worst-case scenario for Pb exposure. Feed was administered to swine 2 h after Pb dosage to ensure the lowest gastric conditions at the time of Pb exposure. The relative bioavailability of Pb in contaminated soils was determined by comparing the area under the blood−Pb concentration time curve for orally administered contaminated soil and a Pb acetate reference dose (refer to eqs 3 and 4). Determination of Pb bioavailability based on time course blood−Pb data is more statistically robust compared to Pb bioavailability values calculated using single tissue measurements (*32*).

Mean relative Pb bioavailability for contaminated soils ranged from 10.1 ± 8.7% to 19.1 ± 14.9%. Variability in relative Pb bioavailability was observed between triplicate soil analysis due to physiological intraspecies differences including genetic factors, disparity in stomach clearance times, stomach pH, and rate of Pb absorption (*6*). Previous Pb bioavailability assays utilizing swine have reported relative Pb bioavailability in contaminated soils to range from 1 to 87% (*10, 11*), although in the study of Marschner et al. (*11*), relative Pb bioavailability (17−63%) was calculated following the determination of Pb in selected organs, bone and urinary excretions but not blood analysis.

The determination of relative Pb bioavailability using in vivo assays is time-consuming, laborious, and expensive; however, in vitro assays may provide a surrogate method for predicting Pb bioavailability that is rapid and inexpensive. To date, a limited number of studies have investigated the correlation between in vitro and in vivo assays for the determination of relative Pb bioavailability (*1, 8*−*11*). Figure 2 shows the relationship between Pb bioaccessibility determined using either the SBRC-G, SBRC-I, and Rel-SBRC-I methods and relative Pb bioavailability, determined using the in vivo swine assay for the soils used in this study. When relative Pb bioavailability is plotted against Pb bioaccessibility, three distinct data groupings are evident. Comparison of in vitro and in vivo results indicated that the correlation between Pb bioaccessibility and relative Pb bioavailability varied depending on the in vitro methodology used (Table 3).

Regression models (SPSS, Release 15.0.1, 2006) determined that Rel-SBRC-I provided the best estimate of in vivo relative Pb bioavailability for soils used in this study (Table 3). Although research undertaken as part of a USEPA study (*8, 9*) determined that gastric phase extraction provided a good estimate of relative Pb bioavailability, the relationship between SBRC-G and in vivo relative Pb bioavailability was poor. However, if in vitro-in vivo relationships were calculated using bioaccessibility/bioavailability data expressed as mg available Pb kg^{-1}, SBRC-G provided a good estimate of relative Pb bioavailability (SI Table S1). This was not surprising as the amount of Pb in the intestinal phase is dependent on gastric phase dissolution. A poor relationship was observed between in vivo relative Pb bioavailability and SBRC-I values as previously reported by Ruby et al. (*1*), Schroder et al. (*10*), and Marschner et al. (*11*).

Validation of rel-SBRC-I. One of the major challenges for the validation of bioaccessibility methods is the use of independent scientific data. In a recent publication by Marschner et al. (*11*), a comparison of soil Pb bioaccessibility (determined using the in vitro methodology of Hack and Selenka (*12*)) and in vivo Pb bioavailability (using a minipig dosing study) was determined for soils collected from various urban and industrial locations in Germany. The study determined that absolute or relative Pb bioavailability was not related to Pb bioaccessibility for either of the test methods utilized. Soils used in the study of Marschner et al. (*11*) were kindly provided and Pb bioaccessibility determined using

SBRC-G, SBRC-I, and Rel-SBRC-I methodologies. Data from these assays were compared to relative Pb bioavailability data obtained from minipig dosing studies. Table 4 provides a comparison of relative Pb bioavailability data from in vivo studies (*11*) and Pb bioaccessibility data (SBRC-G, SBRC-I, and Rel-SBRC-I) for Bruchsal, Carl-1, Hamburg and Oker-11 soils (this study). As previously observed, Pb bioaccessibility varied depending on the in vitro methodology used. Lead bioaccessibility was greater following gastric phase extraction compared to intestinal phase extraction. Data from these in vitro methodologies correlated poorly to in vivo relative bioavailability values obtained from minipig dosing experiments. In contrast, when rel-SBRC-I was calculated using Pb acetate solubility at an intestinal phase pH of 6.5, values obtained were comparable to relative Pb bioavailability data. From the data presented in Table 4, statistical comparison of the in vitro SBRC-G and Rel-SBRC-I methodologies and the reported in vivo results (In vivo RBA) was conducted. Analysis showed that SBRC-G and Rel-SBRC-I methods were correlated (R^2 values of 0.199 and 0.466, respectively) with minipig data from Marschner et al. (*11*), with individual Pearson correlation values of 0.446 and 0.683, respectively. Regression data modeling produced trend equations of in vivo Pb RBA = 29.5 + (0.421) × Rel-SBRC-I for the SBRC-I variable, while the SBRC-G bioaccessibility data resulted in the equation in vivo Pb RBA = 65.5 + (−0.316) × SBRC-G indicating a negative relationship between the in vivo Pb RBA and the SBRC-G variable. While it is acknowledged that SBRC-G is a good predictor of in vivo relative Pb bioavailability (*8, 9*) for soils where Pb mineralogy limits Pb dissolution (e.g., mine site soils), applying this method to soils containing more soluble forms of Pb may overpredict relative Pb bioavailability. The application of the Rel-SBRC-I method for the estimation of relative Pb bioavailability in (nonmine site) contaminated soils improves on both gastric and intestinal phase estimates of Pb exposure for the incidental soil ingestion pathway.

Acknowledgments

In vivo bioavailability research was funded through the Australian Research Council Linkage Grant Scheme (grant number LP0347301). Swine assays were approved and conducted according to application 1702 of the Institute of Medical and Veterinary Science Animal Ethic Committee. In vitro bioaccessibility research was funded by the Cooperative research Centre for Contamination Assessment and Remediation of the Environment (Grant number 1-3-01-05/6). We acknowledge the support of the Centre for Environmental Risk Assessment and Remediation (University of South Australia), Sansom Institute (University of South Australia), and the Institute of Medical and Veterinary Science for this research.

Supporting Information Available

Details of analytical quality assurance/quality control protocols, a representative graph illustrating the change in Pb bioaccessibility in contaminated soil with increasing pH (Figure S1), and regression models for estimating Pb bioavailability (Table S1) is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Ruby, M. V.; Davis, A.; Schoof, R.; Eberle, S.; Sellstone, C. M. Estimation of lead and arsenic bioavailability using a physiologically based extraction test. *Environ. Sci. Technol.* **1996**, *30*, 422–430.

(2) Ryan, J. A.; Scheckel, K. G.; Berti, W. R.; Brown, S. L.; Casteel, S. W.; Chaney, R. L.; Hallfrisch, J.; Doolan, M.; Grevatt, P.; Maddaloni, M.; Mosby, D. Reducing children's risk from lead in soil. *Environ. Sci. Technol.* **2004**, *38*, 19A–24A.

(3) *Guidance Manual for the Integrated Exposure Uptake Biokinetic Model for Lead in Children*; U.S. Environmental Protection Agency: Washington, DC, 1994.

(4) Weis, C. P.; LaVelle, J. M. Characteristics to consider when choosing an animal model for the study of lead bioavailability. *Chem. Speciation Bioavailability* **1991**, *3*, 113–119.

(5) Juhasz, A. L.; Smith, E.; Weber, J.; Rees, M.; Rofe, A.; Kuchel, T.; Sansom, L.; Naidu, R. In vitro assessment of arsenic bioaccessibility in contaminated (anthropogenic and geogenic) soils. *Chemosphere* **2007**, *69*, 69–78.

(6) Juhasz, A. L.; Smith, E.; Weber, J.; Rees, M.; Rofe, A.; Kuchel, T.; Sansom, L.; Naidu, R. Comparison of in vivo and in vitro methodologies for the assessment of arsenic bioavailability in contaminated soil. *Chemosphere* **2007**, *69*, 961–966.

(7) Zagury, G. J. Comments on "Effect of soil properties on arsenic fractionation and bioaccessibility in cattle and sheep dipping vat sites". *Environ. Int.* **2007**, *33*, 164–169, 2007.

(8) *Estimation of Relative Bioavailability of Lead in Soil and Soil-Like Material Using in Vivo and in Vitro Methods*; U.S. Environmental Protection Agency: Washington, DC, 2007.

(9) Drexler, J. W.; Brattin, W. J. An in vitro procedure for the estimation of lead relative bioavailability: with validation. *Human Ecol. Risk Assess.* **2007**, *13*, 383–401.

(10) Schroder, J. L.; Basta, N. T.; Casteel, S. W.; Evans, T. J.; Payton, M. E.; Si, J. Validation of the in vitro gastrointestinal (IVG) method to estimate relative bioavailable lead in contaminated soils. *J. Environ. Qual.* **2004**, *33*, 513–521.

(11) Marschner, B.; Welge, P.; Hack, A.; Wittsiepe, J.; Wilhelm, M. Comparison of soil Pb in vitro bioaccessibility and in vivo bioavailability with Pb pools from a sequential soil extraction. *Environ. Sci. Technol.* **2006**, *40*, 2812–2818.

(12) Hack, A.; Selenka, F. Mobilisation of PAH and PCB from contaminated soil using a digestive track model. *Toxicol. Lett.* **1996**, *88*, 199–210.

(13) Nelson, D. W.; Sommers, L. E. Total carbon, organic carbon, and organic matter. In *Methods of Soil Analysis, Part 2*, Agronomy. 9:961-1010, 2nd ed.; Page, A. L., Ed.; American Society of Agronomy, Inc.: Madison, WI, 1996.

(14) Method 3015A, Microwave assisted acid digestion of aqueous samples and extracts. In *USEPA Methods*; U.S. Environmental Protection Agency: Washington, DC, 1998; pp 3015A/1−18.

(15) Kelley, M. E.; Brauning, S. E.; Schoof, R. A.; Ruby, M. A. *Assessing Oral Bioavailability of Metals in Soil.* Battelle Press: OH, 2002.

(16) Van de Wiele, T. R.; Oomen, A. G.; Wragg, J.; Cave, M.; Minekus, M.; Hack, A.; Cornelis, C.; Rompelberg, C. J. M.; de Zwart, L. L.; Klinck, B.; van Wijen, J.; Verstraete, W.; Sips, A. J. A. M. Comparison of five in vitro digestion models to in vivo experimental results: Lead bioaccessibility in the human gastrointestinal tract. *J. Environ. Sci. Health, Part A: Toxicol/Hazard. Subst.* **2007**, *42*, 1203–1211.

(17) Juhasz, A. L.; Smith, E.; Weber, J.; Rees, M.; Rofe, A.; Kuchel, T.; Sansom, L.; Naidu, R. In vivo assessment of arsenic bioavailability in rice and its significance for human health risk assessment. *Environ. Health Perspect.* **2006**, *114*, 1826–1831.

(18) Bain, S. A. F.; Ting, J.; Simeonovic, C. J.; Wilson, J. D. Technique of venous catheterization for sequential blood sampling from the pig. *J. Invest. Surg.* **1991**, *4*, 103–107.

(19) *Determination of Heavy Metals in Whole Blood by ICP-MS*, Agilent Technologies publication number 5988-0533EN; Agilent Technologies: Santa Clara, CA, 2006.

(20) *National Environmental Protection Measure for the Assessment of Site Contamination*, Schedule B1; National Environmental Protection Council Service Corporation: Adelaide, Australia, 1999, pp 1−12.

(21) *Integrated Risk Information System (IRIS)*; U.S. Environmental Protection Agency: Cincinnati, OH, 1996.

(22) Stumn, W.; Morgan, J. J. *Aquatic Chemistry, Chemical Equilibria and Rates in Natural Waters*; John Wiley and Sons: New York 1996.

(23) Rodriguez, R.; Basta, N. T.; Casteel, S. W.; Pace, L. W. An in vitro gastrointestinal method to estimate bioavailable arsenic in contaminated soil and solid media. *Environ. Sci. Technol.* **1999**, *33*, 642–649.

(24) Oomen, A. G.; Tolls, J.; Sips, A. J. A. M.; Groten, J. P. *Arch. Environ. Contam. Toxicol.* **2003**, *44*, 116–124.

(25) Hamel, S.; Ellickson, K. M.; Lioy, P. J. The estimation of the bioaccessibility of heavy metals in soils using artificial biofluids by two novel methods: Mass-balance and soil recapture. *Sci. Total Environ.* **1999**, *243*, 273–283.

(26) Helrich, K. *Official Methods of Analysis for the Association of Official Analytical Chemists*, 15th ed.; Association of Official Analytical Chemists: Arlington, VA, 1999.

(27) Yu, C. H.; Yin, L.-M.; Lioy, P. J. The bioaccessibility of lead (Pb) from vacuumed house dust on carpets in urban residences. *Risk Anal.* **2006**, *26*, 125–134.
(28) Carrizales, L.; Razo, I.; Téllez-Hernández, J. I.; Torres-Nerio, R.; Torres, A.; Batres, L. E.; Cubillas, A.-C.; Díaz-Barriga, F. Exposure to arsenic and lead of children living near a copper-smelter in San Luis Potosi, Mexico: Importance of soil contamination for exposure to children. *Environ. Res.* **2006**, *101*, 1–10.
(29) Fendorf, S.; La Force, M. J.; Li, G. Temporal changes in soil partitioning and bioaccessibility of arsenic, chromium and lead. *J. Environ. Qual.* **2004**, *33*, 2049–2055.
(30) Turner, A.; Simmonds, L. Elemental concentrations and metal bioaccessibility in UK household dust. *Sci. Total Environ.* **2006**, *371*, 74–81.
(31) Navarro, M. C.; Pérez-Sirvent, C.; Martínez-Sánchez, M. J.; Vidal, J.; Marimón, J. Lead, cadmium and arsenic bioavailability in the abandoned mine site of Cabezo Rajao (Murcia, SE Spain). *Chemosphere* **2006**, *63*, 484–489.
(32) Casteel, S. W.; Cowart, R. P.; Weis, C. P.; Henningsen, G. M.; Hoffman, E.; Brattin, W.; Guzman, R. E.; Starost, M. F.; Payne, J. T.; Stockman, S. L.; Becker, S. V.; Drexler, J. W.; Turk, J. R. Bioavailability of lead to juvenile swine dosed with soil from the Smuggler Mountain NPL site of Aspen, Colorado. *Fundam. Appl. Toxicol.* **1997**, *36*, 177–187.

ES803238U