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Assessment of *in Vitro* Lead Bioaccessibility in House Dust and Its Relationship to *in Vivo* Lead Relative Bioavailability

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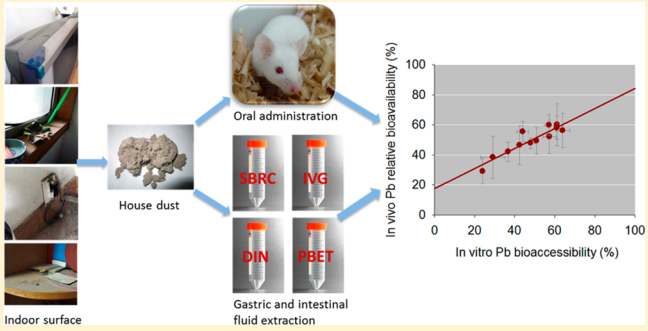
Supporting Information

ABSTRACT: House dust samples containing 25−738 mg of Pb kg^{-1} from 15 cities in China were assessed for *in vitro* Pb bioaccessibility and *in vivo* Pb relative bioavailability. On the basis of stable Pb isotope ratios, the Pb in dust samples mainly originated from coal combustion. Lead bioaccessibility was determined using gastric (GP) and intestinal phase (IP) of solubility bioaccessibility research consortium (SBRC), *in vitro* gastrointestinal (IVG), Deutsches Institut für Normunge.V. (DIN), and physiologically based extraction test methods (PBET), while Pb relative bioavailability (RBA) was determined using a mouse blood model. Lead bioaccessibility in 24 house dust samples varied significantly (23−99%) depending on the methods. Values from the IP were considerably lower than those from the GP because of the co-precipitation of Pb with iron and re-adsorption onto the dust matrix. The SBRC assay with lower GP pH produced higher Pb bioaccessibility because of enhanced Pb dissolution. When compared to mouse blood data using 12 dust samples (29−60%), SBRC−GP and DIN−GP data were correlated with Pb RBA with r^2 values of 0.68 and 0.85 and intercepts 3.15 and 17.4, respectively. Overall, SBRC−GP had potential to predict Pb RBA in dust samples. However, our data suggested that more research is needed to develop a valid *in vitro* method for predicting Pb RBA in house dust.



INTRODUCTION

Childhood exposure to lead (Pb) is a major public concern because of its well-established link to adverse health effects.[1,2] Recent research shows that there is no safe Pb exposure level for children as even low blood Pb concentrations (<10 μg dL^{-1}) show subtle neurotoxic effects.[3] Following phasing out of leaded paint and gasoline, incidental ingestion of house dust has become a significant pathway of Pb exposure for children.[4−6] It has been estimated that children ingest between 20 and 200 mg of dust per day via hand-to-mouth activity.[7] Elevated Pb concentrations in house dust may result from leaded paint,[8,9] tracked-in soil and street dust, and windblown air particles.[10] As detailed by Rasmussen,[11] Pb concentration in house dust may be considerably higher than that in soil from urban environments because of Pb enrichment in smaller particles.

In the assessment of Pb exposure via incidental dust ingestion, using total Pb concentrations often provides a conservative estimate of exposure as not all ingested Pb in dust may be absorbed into the bloodstream because of bioavailability constraints. Reliable estimates of Pb exposure depend on accurate quantification of the Pb fraction in house dusts that is absorbed into the systemic circulation, i.e., bioavailable Pb.[12,13] *In vivo* studies have used different animal models (e.g., swine and rodents) to assess Pb relative bioavailability (RBA) in contaminated soils.[14−19] Lead RBA is determined by comparing Pb absorption from the contaminated matrix to Pb acetate, the compound used to derive Pb toxicity reference value.

However, given the time required, expense, and ethical issues associated with *in vivo* assays, simple, rapid, and inexpensive *in vitro* methods have been developed as a surrogate measure of Pb RBA. Bioaccessibility assays determine the amount of Pb solubilized from the solid matrix in simulated human digestive fluids and represents the amount of Pb that is potentially available for absorption into the systemic circulation. Commonly used bioaccessibility assays include the solubility bioaccessibility research consortium (SBRC),[20] *in vitro* gastrointestinal (IVG),[21] Deutsches Institut für Normunge.V. (DIN),[22] physiologically based extraction test (PBET),[23] and unified BARGE method (UBM) assays.[24] However, because of differences in assay parameters (e.g., gastric pH, extraction time, and soil/solution ratio), Pb bioaccessibility may vary depending on the assay utilized. While those parameters have been

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Table 1. Locations, Total Pb, Fe, and Organic Carbon (TOC) Contents, and *in Vivo* Pb Relative Bioavailabilities (RBA) in House Dust Samples

sample	location (city, province)	setting	Pb (mg kg^{-1})	Fe (g kg^{-1})	TOC (%)	Pb RBA (%)
1	Jieshou, Anhui	urban	738 ± 103	27.0 ± 0.8	10.9 ± 0.6	55.5 ± 6.7
2	Nanjing, Jiangsu	urban	440 ± 12.0	36.9 ± 0.3	5.7 ± 0.5	42.2 ± 6.4
3	Jining, Shandong	rural	306 ± 7.7	48.7 ± 3.8	4.2 ± 0.0	29.1 ± 8.4
4	Wuhan, Hubei	urban	235 ± 3.3	32.8 ± 0.4	3.7 ± 0.0	47.9 ± 3.7
5	Nanjing, Jiangsu	urban	229 ± 7.9	25.6 ± 0.7	6.4 ± 1.0	52.1 ± 11.1
6	Taian, Shandong	rural	200 ± 5.5	32.6 ± 0.5	9.6 ± 0.8	46.5 ± 12.9
7	Shenyang, Liaoning	urban	150 ± 3.2	25.6 ± 0.4	24.1 ± 3.2	59.9 ± 6.7
8	Nantong, Jiangsu	urban	142 ± 2.4	33.0 ± 0.6	4.6 ± 0.5	38.4 ± 14.1
9	Liaocheng, Shandong	rural	141 ± 0.2	27.2 ± 1.2	9.1 ± 0.3	60.1 ± 14.0
10	Jining, Shandong	rural	105 ± 1.8	21.0 ± 0.8	10.7 ± 0.3	56.3 ± 11.4
11	Nantong, Jiangsu	rural	75 ± 1.2	24.1 ± 1.3	4.5 ± 0.5	49.4 ± 8.8
12	Nanjing, Jiangsu	urban	63 ± 1.0	13.5 ± 0.6	5.5 ± 0.6	58.0 ± 7.7
13	Ningbo, Zhejiang	rural	255 ± 2.6	32.4 ± 1.0	2.7 ± 0.1	
14	Ningbo, Zhejiang	rural	204 ± 2.0	19.9 ± 0.8	11.3 ± 0.0	
15	Taizhou, Jiangsu	urban	145 ± 4.9	17.5 ± 0.2	9.6 ± 0.7	
16	Yancheng, Jiangsu	urban	125 ± 0.9	22.3 ± 0.3	10.5 ± 0.2	
17	Nanjing, Jiangsu	urban	80 ± 1.3	24.5 ± 0.4	6.4 ± 0.9	
18	Chuzhou, Anhui	rural	79 ± 1.5	13.2 ± 0.2	11.0 ± 0.9	
19	Yancheng, Jiangsu	urban	77 ± 0.7	15.5 ± 0.3	22.4 ± 3.4	
20	Nanjing, Jiangsu	urban	63 ± 1.8	17.5 ± 0.4	18.2 ± 1.4	
21	Nantong, Jiangsu	rural	51 ± 0.6	15.9 ± 0.3	5.0 ± 0.0	
22	Nantong, Jiangsu	rural	45 ± 1.1	16.2 ± 0.5	4.6 ± 0.7	
23	Baoding, Hebei	rural	28 ± 0.5	12.8 ± 0.6	2.8 ± 0.0	
24	Luoyang, Henan	rural	25 ± 0.3	21.3 ± 0.5	3.4 ± 1.4	
NIST2711a			1,173 ± 15.4	22.1 ± 0.7		37.0 ± 5.4

investigated for Pb-contaminated soils,[25−27] little information about Pb bioaccessibility in house dust is available.

However, before a bioaccessibility assay can be used as a surrogate measure to predict Pb RBA, Pb bioaccessibility data need to be correlated against Pb RBA data across different samples. Several studies have established the correlation between Pb bioaccessibility and Pb RBA in contaminated soils. For example, Ruby et al.[23] showed that Pb bioaccessibility determined using the PBET was correlated (r^2 = 0.93) with Pb RBA via a rat model in seven Pb-contaminated soils. Similarly, for different Pb-contaminated soils, Pb bioaccessibility is well correlated with Pb RBA using swine models in Schroder et al.[28] (IVG; r^2 > 0.74), Denys et al.[29] (UBM; r^2 > 0.7), and Drexler and Brattin[30] (RBALP; r^2 > 0.92). More recently, because of their lower cost and ease of care, mice have been used as an animal model to determine Pb RBA in contaminated soils. Smith et al.[15] demonstrated that Pb RBA was correlated with the gastric phase (GP) of the SBRC assay (r^2 = 0.78) and the intestinal phase (IP) (r^2 = 0.88) in 12 Pb-contaminated soils. Although *in vitro* assays have been correlated against animal models in contaminated soils, it is unclear if such a relationship exists for Pb in house dust. As house dust differs from contaminated soils (i.e., elevated organic matter content of 18−37% and a smaller particle size),[31] conceivably these differences may influence the relationship between Pb bioaccessibility and Pb RBA in dust.

To address this research shortfall, we collected 24 house dust samples from 15 cities in eight provinces in China and compared the ability of different *in vitro* assays to assess Pb bioaccessibility in house dusts. The specific objectives were (1) to measure Pb bioaccessibility in 24 house dust samples using four *in vitro* assays, (2) to determine Pb RBA in 12 house dust samples using an *in vivo* mouse model, and (3) to correlate *in vitro* assays to assess Pb RBA in house dust in China.

MATERIALS AND METHODS

House Dust Samples. Twenty-four house dust samples were collected from 15 cities in eight provinces in China between February and March 2013. Detailed information regarding the sampling locations is provided in Table 1. In each house, dust was brushed into polyethylene bags from surfaces in the family room (floor, interior window sills, and tables) that were most accessible to children. Dusts were freeze-dried, sieved through a 150 μm nylon sieve to remove debris, and stored at −20 °C in sealed polyethylene bags until the samples were analyzed. Total Pb and iron (Fe) contents in dusts were determined by inductively coupled plasma mass spectrometry (ICP-MS) (NexION300X, PerkinElmer) and flame atomic absorption spectrophotometry (FAAS) (PinAAcle 900T, PerkinElmer) following acid digestion using U.S. Environmental Protection Agency (EPA) Method 3050B. The total organic carbon (TOC) in dusts was measured using an element analyzer (vario TOC select, Elementar) after removing carbonate carbon with HCl.

Pb Bioaccessibility Using Four *in Vitro* Assays. The SBRC, IVG, DIN, and PBET methods were used to assess Pb bioaccessibility in dust samples. They are commonly used to assess the bioaccessibility of heavy metals in soils.[32] The compositions and analytical parameters for all methods are listed in Table S1 of the Supporting Information. For each soil, assays were performed in triplicate. Briefly, 0.1 g of dust was extracted using 5 mL (DIN), 10 mL (SBRC and PBET), or 15 mL (IVG) of gastric fluid at 37 °C for 1 h (SBRC, IVG, and PBET) or 2 h (DIN) while the samples were being shaken horizontally at 100 rpm. During extraction of the gastric phase

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(GP), the solution pH was monitored every 15 min and maintained at 1.5 (SBRC), 1.8 (IVG), 2.0 (DIN), or 2.5 (PBET) by the addition of concentrated HCl when required. At the end of the GP extraction, dust suspensions were centrifuged (4000 rpm for 10 min) and aliquot supernatant samples (0.5 mL for DIN, 1 mL for SBRC and PBET, and 1.5 mL for IVG) were collected for analysis by ICP-MS and FAAS.

After GP extraction, assays were extended to the intestinal phase (IP) by adjusting the solution pH to 5.5 for IVG or 7.0 for SBRC and PBET with either NaOH (SBRC) or Na_2CO_3 (IVG and PBET) and through the addition of bile and pancreatin. For the DIN method, 5 mL of intestinal fluid was added and the pH adjusted to 7.5 using $NaHCO_3$. After IP extraction at 37 °C and 100 rpm for 1 h (IVG), 4 h (SBRC and PBET), or 6 h (DIN), samples were centrifuged and supernatants analyzed by ICP-MS and FAAS. Lead bioaccessibility in dust samples was calculated by dividing extractable Pb from GP or IP by the total Pb concentration in dust (eq S1 and Table S2).

The National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) Montana Soil 2711a was included in bioaccessibility assays for quality assurance (QA) and quality control (QC). Its Pb bioaccessibility was 91 ± 1.3% using the SBRC−GP method (Table S2), which was in good agreement with the U.S. EPA recommended value of 86%.[33] During ICP-MS measurement, external Pb standard solutions were determined every 20 samples. The average recovery was 103 ± 1.5%.

Pb Relative Bioavailability Using an *in Vivo* Mouse Model. Dust samples 1−12 were assessed for Pb relative bioavailability (RBA) using Balb/c mice with body weights (BWs) of 18−20 g. Following acclimation for 1 week in a 12 h/12 h light/dark photocycle at 25 °C and 50% humidity, mice were randomly grouped (three mice per cage) and fasted for 24 h prior to exposure to dust. Initially, a single dose (0.5 mL) of a Pb acetate (PbAc) solution was administered to mice via gavage, which resulted in PbAc dose levels of 5 and 10 mg of Pb (kg of BW)$^{-1}$ (Figure S1). Mice were sacrificed at 4, 8, 16, 24, and 48 h (n = 3 per time point) with blood samples collected in tubes containing lithium heparin. Blood from control mice not exposed to PbAc was also collected to determine the baseline blood Pb concentration. Blood was acid-digested using U.S. EPA Method 3050B and analyzed for Pb by ICP-MS with an appropriate number (5% of samples) of duplicate analysis and check values (recovery of 99.7 ± 2.6%). Blood Pb concentrations (micrograms per liter) versus time curves following zero correction were established, and the area under the curve (AUC) was calculated for Pb exposure. To assess the Pb RBA, dust was administered as a single dose (0.1−0.3 g of dust in 0.5 mL of Milli-Q water), and the blood Pb concentration versus time was determined (Table S3). Because dust dose levels ranged from 0.34 to 6.22 mg of Pb (kg of BW)$^{-1}$, the 5 mg (kg of BW)$^{-1}$ PbAc AUC was used to calculate the Pb RBA in dust samples (eq S2). The calculated Pb RBA varied by <2% when a value of 10 mg (kg of BW)$^{-1}$ was used. For QA and QC, *in vivo* assessment was also performed on SRM NIST 2711a with a Pb RBA of 37 ± 5.4%.

Stable Isotope Ratios in Total and Bioaccessible Pb in House Dust. Stable isotope ratios of Pb ($^{207}Pb/^{206}Pb$ and $^{208}Pb/^{206}Pb$) in total Pb extracted by acid digestion and bioaccessible Pb extracted in the GP of SBRC, IVG, DIN, and PBET assays were determined by ICP-MS. Prior to being analyzed, digested samples or GP extracts were diluted with 0.1 M high-purity HNO_3 to achieve a Pb concentration of ∼15 μg L^{-1}. Instrument parameters were set as 190 sweeps/reading, 1 reading/replicate, and 10 replicates/sample solution. A dwell time of 40 ms was used for ^{204}Pb and 25 ms for ^{206}Pb, ^{207}Pb, and ^{208}Pb. The procedure control was measured and corrected for the final intensity of each isotopic Pb in the sample. During sample analysis, an SRM NIST 981 Common Pb Isotopic Standard solution (15 μg of Pb L^{-1}) was measured every five samples to obtain ratio correction factors (RCFs) to compensate for mass discrimination, which were applied to samples. An indium isotope (^{114}In) was used as an internal standard. Measured $^{204}Pb/^{206}Pb$ (0.0590 ± 0.0002), $^{207}Pb/^{206}Pb$ (0.9152 ± 0.0012), and $^{208}Pb/^{206}Pb$ (2.1685 ± 0.0037) ratios of NIST 981 were in agreement with the certified values of 0.0590, 0.9146, and 2.1681, respectively. The relative standard deviation of 10 replicates for samples was generally <0.5% for $^{207}Pb/^{206}Pb$ and $^{208}Pb/^{206}Pb$ ratios.

Statistics. SAS (version 9.1.3 for Windows) was used for performing analysis of variance to determine differences in Pb bioaccessibility among the four assays and the linear correlation between Pb RBA and Pb bioaccessibility. AUC for dust and PbAc exposure was calculated using SigmaPlot (version 10.0, Systat Software Inc., San Jose, CA).

RESULTS AND DISCUSSION

Coal Combustion Ash Was a Major Source of Pb in House Dust. Table 1 shows the sampling locations and the properties of 24 house dust samples. The total Pb concentrations ranged from 25 mg kg^{-1} (rural Luoyang, Henan) to 738 mg kg^{-1} (urban Jieshou, Anhui). Mean and median Pb concentrations were significantly higher in samples from urban areas (144 and 207 mg kg^{-1}) than those from rural areas (92 and 126 mg kg^{-1}). This reflected the fact that anthropogenic activities are more concentrated in cities than in the countryside, resulting in higher Pb dry atmospheric deposition in urban (19.4 mg m^{-2} a^{-1}) than rural areas (5.31 mg m^{-2} a^{-1}),[34] which are main sources of house dust. Our results are comparable with the results of the Canadian House Dust Study. The mean and median total Pb concentrations in their dust samples from 1025 urban homes are 100 and 210 mg kg^{-1}, respectively.[35] Han et al.[36] reported that the mean Pb concentration in 122 house dust samples from rural areas of eight provinces in China is 208 mg kg^{-1}. The geometric mean Pb concentration in 32 house dust samples from the United Kingdom is 150 mg kg^{-1}.[37] In addition to total Pb, dust properties in our samples also varied with locations, with Fe and TOC ranging from 12.8 g kg^{-1} (rural Baoding, Hebei) to 48.7 g kg^{-1} (rural Jining, Shandong), and from 2.7% (rural Ningbo, Zhejiang) to 24.1% (urban Shenyang, Liaoning). This is consistent with previous studies showing enrichment of organic matter (10−30%) in house dust compared to soils.[31,37,38]

Unlike soils whose sources of Pb contamination are more easily known, the source of Pb in house dust is more diffuse and harder to trace. Therefore, fingerprinting based on stable isotope ratios was used to identify the Pb source in house dust. Stable isotope $^{207}Pb/^{206}Pb$ and $^{208}Pb/^{206}Pb$ ratios were 0.844−0.882 and 2.079−2.133, respectively, in total Pb after acid digestion (Figure S2). The total Pb in dust samples consists of both natural and anthropogenic Pb, such as fine soil particles and coal combustion ash. In general, natural sources of Pb have lower $^{207}Pb/^{206}Pb$ and $^{208}Pb/^{206}Pb$ ratios than anthropogenic sources.[39,40] The isotopic composition ($^{208}Pb/^{206}Pb$ vs

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$^{207}Pb/^{206}Pb$) in total Pb in dust samples partially overlapped with that of coal combustion ash in China. Except for dust sample 9, with a composition being closer to that of leaded gasoline, the rest were distant from that of leaded gasoline, paint, and metallurgy dust (Figure 1).[39,40] Coal combustion has

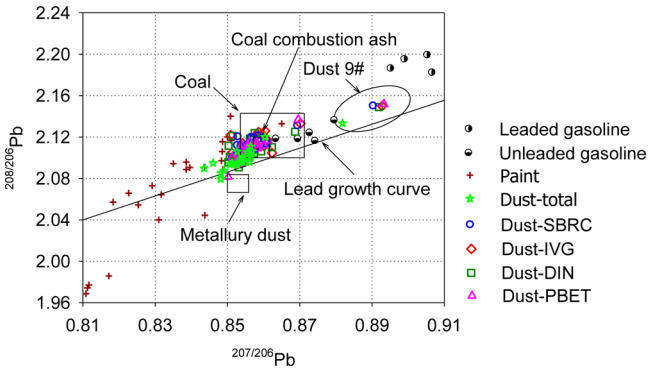

Figure 1. Biplot of $^{208}Pb/^{206}Pb$ vs $^{207}Pb/^{206}Pb$ ratios in total Pb and gastric phase bioaccessible Pb extracted using SBRC, IVG, DIN, and PBET assays of 24 house dust samples. Ranges or individual isotope ratios of possible anthropogenic Pb sources, i.e., coal, coal combustion dust, leaded and unleaded gasoline, metallurgy dust,[39] and paint,[40] are shown. The Pb growth curve is also presented.[48]

been identified as the most important Pb source in China.[41] Our data are different from those of European and North American studies, showing that paint is an important contributor to Pb in indoor dust.[8,9] Dust sample 9 was collected from a house close to a busy freeway. Conceivably, the Pb probably came from leaded gasoline emissions from traffic even though the use of leaded fuel was discontinued in China in 2000.[39]

To determine the sources of Pb in the bioavailable fraction, the stable Pb isotope ratios in solutions after GP fraction were also determined. The $^{208}Pb/^{206}Pb$ and $^{207}Pb/^{206}Pb$ ratios in GP bioaccessible Pb in all four assays were similar, being 0.850−0.893 and 2.081−2.152, respectively (Figure S2). Compared to total Pb, the $^{207}Pb/^{206}Pb$ and $^{208}Pb/^{206}Pb$ ratios in bioaccessible Pb were higher. The results confirmed that total Pb contained a greater portion of Pb from natural sources than bioaccessible Pb. A previous study also reported the difference in stable isotope ratios between the total and 1 M HCl extractable Pb in soils.[41] Being higher than that of total Pb, the isotopic composition ($^{208}Pb/^{206}Pb$ vs $^{207}Pb/^{206}Pb$) of GP bioaccessible Pb in dust samples better overlapped with that of coal combustion than that of total Pb, with that of sample 9 being closer to that of leaded gasoline (Figure 1), further confirming that dust Pb mainly came from coal combustion.

The Lower Gastric Phase pH Led to Higher Pb Bioaccessibility. Significant ($p < 0.05$) differences in Pb bioaccessibility in GP were observed among the four methods. Lead bioaccessibility in 24 dust samples was 46−99, 41−90, 23−63, and 22−60% based on SBRC, IVG, DIN, and PBET assays, with SBRC > IVG > DIN > PBET (Figure 2A). These values were similar to those of dust samples analyzed using HCl extraction (17−65%),[36] pepsin in diluted HCl (~80%),[37] and PBET (12−36%) methods.[38] The difference in Pb bioaccessibility based on the four assays was mainly attributed to different GP pH values (1.5, 1.8, 2.0, and 2.5) as dissolution of Pb in the GP is sensitive to pH.[13] The SBRC method with the lowest GP pH produced the highest Pb bioaccessibility, whereas the PBET

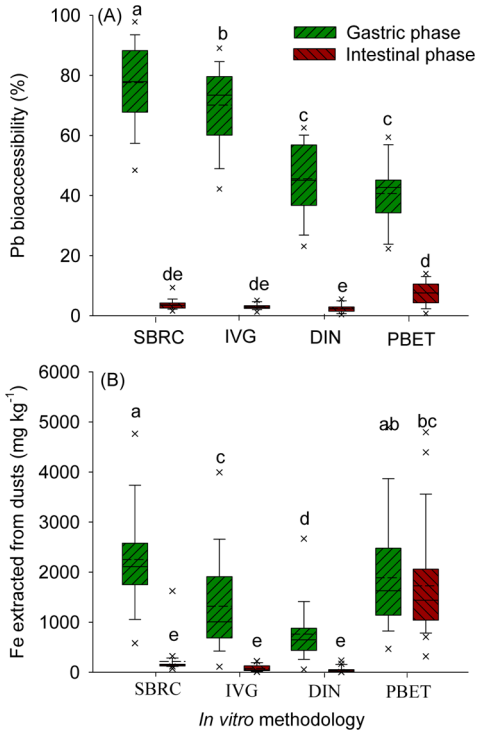

Figure 2. *In vitro* Pb bioaccessibility (A) and concentration of Fe extracted in 24 house dust samples (B) in gastric and intestinal phases of SBRC, IVG, DIN, and PBET assays. Boxes represent the 25th to 75th percentiles. Solid and dashed lines in boxes denote the median and mean values, respectively. Error bars represent the 5th and 95th percentiles, and times signs represent the 1st and 99th percentiles, respectively. Means marked with different letters indicate significant ($p < 0.05$) differences.

assay with the highest GP pH had the lowest Pb bioaccessibility. Hence, increasing the GP pH from 1.5 to 2.5 decreased Pb bioaccessibility by a factor of up to ~2 (Figure 2A). Oomen et al.[25] compared five assays to assess Pb bioaccessibility in soils. They conclude that the GP pH is the major source of variability among methods, lowering the GP pH results in a higher Pb bioaccessibility.

X-ray absorption near-edge structure (XANES) analysis showed that Pb in house dust exists as Pb carbonate and oxide and/or is adsorbed onto Fe oxyhydroxides.[42] Analysis of the XANES spectra of contaminated soil before and after GP extraction showed that Pb associated with Fe oxyhydroxides is solubilized.[15] In this study, the average concentration of Fe solubilized in the GP decreased significantly ($p < 0.05$) from 2250 mg kg^{-1} to 1318 and 761 mg kg^{-1} as the GP pH increased from 1.5 (SBRC) to 1.8 (IVG) and 2.0 (DIN), respectively (Figure 2B), explaining the significant decrease in Pb bioaccessibility. As a result, we hypothesized that the pH-controlled dissolution of Fe oxyhydroxides in the GP played an important role in controlling Pb bioaccessibility in dust samples. However, this was not the case for the PBET assay, as a significantly ($p < 0.05$) higher Fe concentration was solubilized in its GP than that in the IVG and DIN assays, yet it had the lowest Pb bioaccessibility (Figure 2B). We hypothesized that its GP components, including lactic acid, acetic acid, and sodium citrate, which are unique for PBET (Table S1), may have contributed to the increased level of Fe solubilization compared to that in IVG and DIN assays.

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To test this hypothesis, we modified the PBET assay by removing lactic and acetic acid or sodium citrate in the GP and tested four dust samples (3, 10, 12, and 14) to determine their influence on bioaccessible Pb and Fe. Removing lactic and acetic acid decreased both bioaccessible Fe and Pb compared to the values with the PBET (Figure 3A,C), suggesting that Pb in

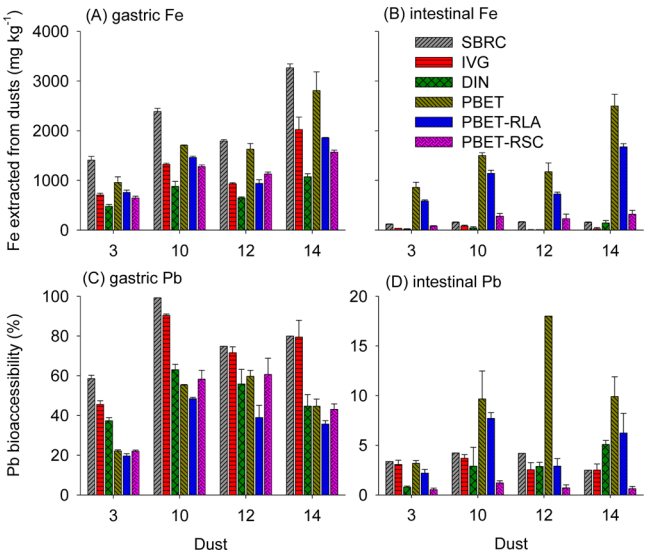

Figure 3. Lead bioaccessibility and concentration of Fe extracted in house dust samples 3, 10, 12, and 14 in gastric and intestinal phases of SBRC, IVG, DIN, PBET, and modified PBET assays after the removal of lactic and acetic acid (PBET−RLA) or sodium citrate (PBET−RSC) from gastric components. Bars represent the means and standard deviations of triplicate analyses.

dust was probably sorbed onto Fe oxyhydroxides. In contrast, removing sodium citrate significantly decreased the level of Fe extraction but had little effect on bioaccessible Pb. It was possible that Pb was not sorbed onto Fe extracted by citrate, which was due to its effective complexation with iron.[43] Hence, citrate was the key component that contributed to the increased level of Fe solubilization but lower bioaccessible Pb in the PBET than IVG and DIN assays. Although Pb dissolution from dusts was controlled and accompanied by Fe dissolution, the differences in bioaccessible Pb with *in vitro* methods cannot be fully explained by the differences in bioaccessible Fe, especially for the PBET method. Differential dissolution of Fe and Pb solid phases in different methods might explain the difference in bioaccessible Pb. A follow-up XANES study is needed to investigate the change in the Fe and Pb solid phase in dusts before and after GP extraction.

Co-precipitation with Fe Reduced Pb Bioaccessibility in the Intestinal Phase. Compared to that in the GP, bioaccessible Pb in the IP was significantly ($p < 0.05$) lower. The values were 1.4−10, 1.1−5.1, 0.3−5.1, and 0.5−14 fold lower for SBRC, IVG, DIN, and PBET assays, respectively (Figure 2A). A similar decrease has been reported in bioaccessible Pb from the GP to IP in soils.[12,15,25] Among the four assays, significantly ($p < 0.05$) higher bioaccessible Pb in the IP was observed for PBET compared to the other assays.

Following the adjustment from acidic GP solutions to neutral IP solutions, solubilized Fe in the GP may re-precipitate in the IP because of oversaturation of hydrolyzed Fe and/or co-precipitate with Pb.[15] The Fe−Pb co-precipitation in the IP was supported by a concurrent decrease in solubilized Pb and Fe in SBRC, IVG, and DIN assays (Figure 2A,B). To test this hypothesis, after SBRC-GP extraction of dust samples 10 and 14 (105 and 204 mg of Pb kg^{-1}), the residues were removed by 0.45 μm filters. The solution without dust was subjected to IP extraction, and the newly formed precipitates were collected by centrifugation. The precipitates contained 2.39 and 2.87% Fe and 543 and 1083 mg of Pb kg^{-1}, respectively, ascertaining Fe−Pb co-precipitation probably occurred during IP extraction. Mass balance calculation showed that 80 and 88% of dissolved Pb from the two dust samples from GP extraction was in the precipitates.

In contrast to SBRC, IVG, and DIN assays, when the PBET assay was extended from the GP to IP, its dissolved Fe did not decrease significantly. Considerably higher Fe concentrations were observed in the PBET−IP extractions (1733 mg kg^{-1}) than in SBRC (213 mg kg^{-1}), IVG (77 mg kg^{-1}), and DIN (45 mg kg^{-1}) assays (Figure 2B), which may explain the higher bioaccessible Pb in the PBET−IP assay. However, Fe precipitation was observed in the modified PBET assays when sodium citrate was removed from the GP; i.e., extractable Fe declined sharply from 858−2496 to 80−319 mg kg^{-1} in four dust samples (Figure 3B). This indicated that Fe precipitation in the PBET−IP assay was probably inhibited by citrate-complexed Fe, which has a high stability constant (log k of 32.7).[44] Citrate can chelate Fe to form a monoiron dicitrate complex, $[Fe(Cit)_2]^{5-}$, the predominant species under neutral-pH conditions. Following removal of sodium citrate, bioaccessible Pb by the modified PBET−IP method declined sharply to levels lower than those determined by SBRC, IVG, and DIN assays (Figure 3D), indicating that the inhibition of Fe precipitation in the presence of citrate increased bioaccessible Pb in PBET−IP assays. These results showed that dissolved Fe in the GP played an important role in controlling bioaccessible Pb in the IP.

In addition to the co-precipitation with Fe during the IP extraction, some soluble Pb probably precipitated with OH^-, reducing bioaccessible Pb.[13,38] Lead solubility may also be influenced by intestinal components such as bile and pancreatin[14] and/or re-adsorption of Pb onto the dust matrix. Employing XANES analysis to investigate Pb speciation in soil before and after *in vitro* assessment, Smith et al.[15] showed that the Pb distribution changed from 100% in association with Fe oxyhydroxides before SBRC−GP extraction to 70% with Fe oxyhydroxides and 30% with montmorillonite after the IP extraction, suggesting the re-adsorption of Pb onto soil particles during the IP extraction. We tested this hypothesis on one dust sample to determine whether dissolved Pb was re-adsorbed onto the dust matrix during the IP extraction. After the GP extraction using the four assays, the residue was retrieved via centrifugation of the suspensions. The residue was then used to adsorb spiked Pb [6 mg of Pb L^{-1} as $Pb(NO_3)_2$] in the presence of corresponding IP solutions (10 mL at 37 °C) for up to 4 h (SBRC, IVG, and PBET) or 6 h (DIN). When the IP solutions were incubated with GP residue, Pb concentrations sharply decreased from 2.10−5.43 to 0.23−1.35 mg L^{-1} within 1 h, with little variation during the next 3−5 h (Figure S3). The decrease in Pb concentration was greater upon incubation with dust compared to controls without dust, indicating that the dust matrix probably adsorbed dissolved Pb in the IP.

A Higher Level of Fe Resulted in Lower Pb Relative Bioavailability. Twelve dust samples (1−12) with total Pb concentrations of 63−738 mg kg^{-1} were assessed for Pb RBA. Lead RBA ranged from 29.1 ± 8.4% (sample 3) to 60.1 ±

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14.0% (sample 9) (Table 1). This was the first study of Pb RBA in house dust samples, although Pb RBA in contaminated soils has been reported.[14,15,17−19,23,28,29,45] The variability in Pb RBA in contaminated soils has been attributed to differences in Pb contamination source, Pb speciation, and soil properties. Soil with a high Fe concentration usually has low Pb RBA. Similar results were found in house dust samples, with dust sample 3 having the highest Fe and the lowest Pb RBA. The narrow range (29−60%) in the Pb RBA for the dust samples could probably be attributed to coal combustion as the major Pb source (Figure 1). On the basis of XANES analysis, Pb in coal combustion ash consists largely of Pb sulfate ($PbSO_4$) and Pb chloride ($PbCl_2$).[46] These Pb species are moderately soluble under gastrointestinal conditions compared to the highly soluble Pb oxide (PbO), which is the main combustion product of leaded gasoline.[9]

Pb Bioaccessibility Was Correlated with Pb Relative Bioavailability. Several benchmark criteria have been suggested to validate an assay as a surrogate measure for the RBA.[29]The first is that the relative standard deviation (RSD) of the RBA replicate measurements should be <20%. In this study, the RSD values for the Pb RBA were below the benchmark at 4−14% (Table 1). The second benchmark is that within-laboratory and between-laboratory variability of bioaccessible Pb should be <10 and 20%, respectively. In this study, only within-laboratory data were available. The median RSDs of bioaccessible Pb in triplicate measurements for dust samples were 1.9−3.8% for the GP and 8.3−17.6% for the IP for the four assays.

The additional benchmark includes statistical parameters (e.g., r^2, intercept, and slope) of the linear regression relationship between bioavailable Pb and bioaccessible Pb. Wragg et al.[47] suggested the following: (1) $r^2 > 0.6$; (2) slope being between 0.8 and 1.2; and (3) intercept being not significantly different from 0. In our study, bioaccessible Pb in 12 house dust samples based on the four assays was correlated with Pb RBA. Relationships between *in vivo* and *in vitro* Pb varied considerably with *in vitro* methods (Figure 4 and Table 2). For the GP, a strong correlation was established for SBRC and DIN assays with r^2 values of 0.68 and 0.85 and a weak correlation for IVG and PBET assays with r^2 values of 0.56 and 0.51, respectively. Our data were similar to those of Smith et al.,[15] who found that the SBRC−GP assay provides a good prediction of the Pb RBA by the mouse model in soils with an r^2 of 0.78. Among the four assays, only the SBRC−GP assay showed an intercept (3.15) close to 0, while the intercepts for the IVG, DIN, and PBET−GP assays were much higher (14.3, 17.4, and 20.2). The slopes of the regressions were 0.61, 0.48, 0.67, and 0.69 for the GP of SBRC, IVG, DIN, and PBET assays, respectively. Though a stronger *in vivo−in vitro* correlation (r^2 = 0.85) was found for the DIN−GP assay, it could not predict low Pb RBA because of the the large intercept of 17.4. Though a slope between 0.8 and 1.2 does not meet the strict criterion, the acceptable correlation coefficient (r^2 = 0.68) and the low intercept (3.15) for SBRC−GP assay suggested that the assay has potential to predict the Pb RBA in dust samples.

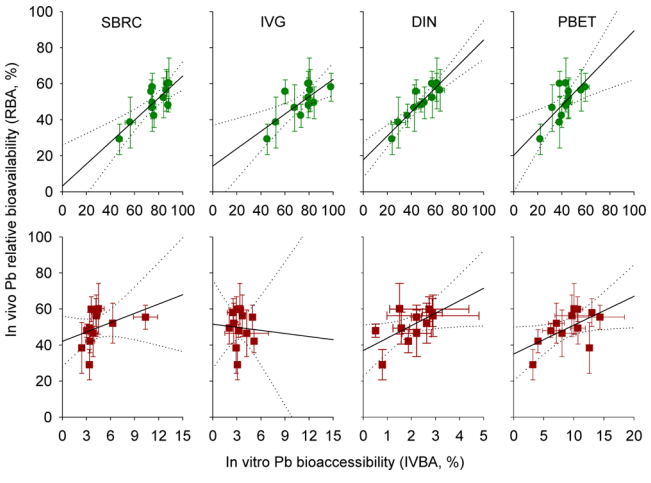

Figure 4. Comparison of *in vitro* Pb bioaccessibility determined in gastric (●) and intestinal (■) phases of SBRC, IVG, DIN, and PBET assays and *in vivo* Pb relative bioavailability determined using a mouse assay for 12 house dust samples. Values represent the means and standard deviations of triplicate analyses.

Table 2. Linear Relationships between *in Vivo* Relative Pb Bioavailability (RBA) and *in Vitro* Pb Bioaccessibility (IBA) in House Dust Samples Based on SBRC, IVG, DIN, and PBET Assays

in vitro assay	phase	*in vivo−in vitro* predictive model	correlation coefficient (r^2)
SBRC	gastric	RBA = 3.15 + 0.61 (IBA)	0.68
	intestinal	RBA = 42.0 + 1.72 (IBA)	0.15
IVG	gastric	RBA = 14.3 + 0.48 (IBA)	0.56
	intestinal	RBA = 51.6 − 0.57 (IBA)	0.01
DIN	gastric	RBA = 17.4 + 0.67 (IBA)	0.85
	intestinal	RBA = 36.9 + 6.90 (IBA)	0.38
PBET	gastric	RBA = 20.2 + 0.69 (IBA)	0.52
	intestinal	RBA = 35.0 + 1.60 (IBA)	0.35

Unlike that in the GP, Pb bioaccessibility in the IP based on the four assays was poorly correlated with Pb RBA (r^2 = 0.003−0.35) (Figure 4). This was probably attributed to low bioaccessible Pb following IP extraction (<15%) as a result of re-adsorption of Pb to the Fe phase/solid matrix and/or Pb precipitation. Juhasz et al.[45] and Smith et al.[15] reported that the correlation between bioaccessible Pb based on the SBRC−IP assay and Pb RBA can be improved by adjusting the dissolution of Pb in soils by PbAc solubility. However, in this study, applying this approach did not improve *in vitro−in vivo* correlations. Our data suggested that more research is needed to develop a better *in vitro* method to predict Pb RBA in dust samples.

The difference in correlation data between Smith et al.[15] (r^2 = 0.78 and 0.88) and this study (r^2 = 0.68 and 0.15) may be attributed to the low Pb concentrations in house dusts and differences in Pb sources. Total Pb concentrations in 12 soils used by Smith et al.[15] ranged from 536 to 3450 mg kg^{-1} versus a range from 63 to 738 mg kg^{-1} in the 12 house dust samples in this study. In addition, the soils of Smith et al.[15] were contaminated by different sources (shooting range, former gas works, incinerator waste, landfill, smelting, and mining activities), whereas the house dust samples were contaminated primarily via coal combustion ash. The diverse contamination sources led to a larger range of Pb RBA values (13−89%) in soils[15] than in house dust (29−60%). For more robust correlation data, house dust samples contaminated by other sources (i.e., paint, smelting, and mining) with higher Pb concentrations should be included.

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By comparing results obtained by four assays, this study highlighted the difference in Pb bioaccessibility in house dust depending on the assays utilized. Assays with a lower GP pH produced higher bioaccessible Pb. In the IP, bioaccessible Pb declined significantly due to co-precipitation of Pb with Fe. Inhibition of re-precipitation of Fe by sodium citrate explained why bioaccessible Pb in the PBET−IP assay was higher than those determined by other methods. Our results provided preliminary evidence that the SBRC−GP assay may have the potential to predict Pb RBA, with acceptable r^2 (0.68) and intercept (3.15) values. Further development of a better *in vitro* assay for predicting the Pb RBA in dust samples based on different Pb sources with higher concentrations is needed.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Equations for calculating the bioaccessible Pb and Pb RBA and graphs and tables detailing *in vitro* methods used, bioaccessible Pb, *in vivo* moue dose and AUC values, mouse blood Pb concentration versus time curves for Pb acetate exposure, stable isotope ratios of total Pb and gastric phase bioaccessible Pb in dust samples, and the kinetics of re-adsorption of spiked Pb to the dust matrix in the IP. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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