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Measuring Pb Bioavailability from Household Dusts Using an in Vitro Model

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Despite an extensive cleanup program in the Port Pirie region, South Australia, the levels of lead (Pb) in blood of children have been found to exceed the "level of concern" (10 μg/dL). The ingestion of household dust is a major pathway for elevated blood lead by children in the community. Significant differences in levels of Pb in blood in children were observed in various localities around the smelter. In this study an in vitro test was assessed as one method for determining the bioavailability of Pb in household dust and for predicting levels of Pb in blood of children. The solubility of Pb in the dust decreased significantly as pH of the in vitro mixture increased. Correlation studies with average blood Pb levels of children in the corresponding area and in vitro measures of Pb bioavailability found that the best relationship was with total dust Pb ($r^2 = 0.92$, **). A significant positive relationship was also found with Pb concentrations determined in the in vitro test at pH 3.0 ($r^2 = 0.82$, **). This suggested that for these dust samples, which all had a similar environmental matrix, the use of the in vitro test was not a better indicator of blood Pb levels in children compared with a total Pb analysis.

Introduction

The ingestion of lead (Pb) by young children as a result of hand-to-mouth and object-to-mouth behavior is recognized as an important pathway of Pb exposure to children (*1*). This pathway will become increasingly the dominant source of Pb for children as atmospheric sources (e.g., Pb in motor vehicle emissions) are progressively decreased.

It is well recognized that the biological availability of Pb varies with the nature and different forms of Pb matrices present in contaminated soils and dusts (*2*). Assessment of the bioavailability of Pb in different materials is of interest from both risk assessment and remediation viewpoints. It is generally acknowledged that total Pb values overestimate the fraction of Pb available for absorption so other measures of determining bioavailability are used. Bioavailability is generally assessed using animal feeding studies, but such studies are time-consuming and expensive. The U.S. Environmental Protection Agency (EPA) has developed an Uptake Biokinetic Model for estimating risks due to exposure to soil Pb. In this model soil Pb exposure to children is estimated assuming 30% of ingested soil Pb is bioavailable (*3*). This assumption neither considers the nature of material containing Pb in the environmental sample nor its effect on bioavailability. Consequently bioavailability of Pb from some environmental samples may be overestimated, while from others it may be underestimated. In this study an in vitro test was used to estimate the bioavailability of Pb from household dust samples. The validity of the in vitro test was assessed by correlating the in vitro data with Pb bioavailability, namely blood Pb values in children. Blood Pb data were also correlated with total dust Pb.

Study Site. Port Pirie is situated 230 km north of Adelaide, South Australia. For the last 100 years Pb smelting and refining has been the major industry in the town. Concentrated Pb ore is refined to produce Pb. Historically the township had been contaminated with Pb and other metals from the use of slag and coal ash by residents prior to the 1940s to establish gardens and by dust fallout over the area. Levels of blood Pb in some children in the area were found to exceed the "level of concern" (10 μg/dL). In 1984 a Pb implementation program began which involved a process of decontamination of houses (*4*). Despite this extensive cleanup program the blood Pb levels of some children in the area were still exceeding the level of concern. For this reason various techniques to determine the source of the contamination and assess the efficacy of a remediation program have been evaluated. In this study an in vitro technique developed previously (*5*) was used to assess whether geographic differences in levels of blood Pb in the Port Pirie region may be explained on the basis of Pb bioavailability in household dusts.

Materials and Methods

Dust Collection and Pretreatment. Seven dusts, collected from November 1995 to January 1996, were used in this study. Five of the dusts (C2, C3, C5, C6, and C7) were collected as composites from several houses in each geographic region corresponding to those areas with the highest, intermediate, and lowest area mean blood Pb levels. Dust C1 was collected from the floors of a public school, and dust C4 was a composite from a large number of houses which was assembled as a bulk reference material. Homeowners using domestic vacuum cleaners collected two composites (C4 and C6), while the other five were collected by commercial vacuum cleaners with high efficiency filtration of particles above 1 μm diameter. Houses that had been undergoing renovation in the previous 6 months or were inhabited by Pb smelter workers were excluded.

The samples were separated into two size fractions by passing dust through stainless steel sieves with mesh sizes of 250 and 53 μm. Detailed particle size fractionation was determined on the <53 μm fraction (*6*). The in vitro experiments were performed on the <53 μm fraction, as this was regarded as the fraction most likely to adhere to children's hands and therefore contribute to dust ingestion through hand to mouth activities (*7*).

Dust Characterization. *Dust pH, Surface Area, and Carbonate Content.* Sample pH was determined following end-over-end shaking of a 5-g sample with 25 mL of 0.01 M $CaCl_2$ solution for 1 h. The pH of the supernatant solution was measured using a combination glass electrode. Surface area was determined by a nitrogen technique using a Quantachrome "quantasorb surface area analyzer" (*8*). Total C was determined by LECO CR12 total carbon analyzer (*9*). Carbonate content was determined using a modified Collins calcimeter (*10*).

Total Elemental Analysis. Concentrations of elements in the dusts were determined in duplicate by aqua regia digests

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of the materials. Elemental analysis (excluding Pb) of the digest samples was by an inductively coupled plasma atomic emission spectrometer. Total Pb concentrations were determined using a flame atomic absorption spectrometer (FASS) fitted with deuterium background correction, and the enrichment of Pb in the <53 μm fraction (total Pb <53 μm/total Pb < 250 μm) was calculated.

In Vitro Bioavailability Model. *Experiment 1. Maintaining the Stomach Phase at a Stable pH of 1.3.* The in vitro test system used for estimating bioavailability of Pb from dusts was similar to that described earlier (*5*). In this study, however, the gastric solution consisted of 80 mL of 0.1 M HCl to which 1.0 g of monohydrate citric acid, 1.0 g of DL-malic acid, 1.0 mL of glacial acetic acid, 1.0 mL of DL-lactic acid, and 0.1 g pepsin (activity of 800−2500 units/mg) were added. The pH of the solution was adjusted to 1.30 ± 0.03 with 1 M $NaHCO_3$. The solution was equilibrated to 37 °C in a temperature-controlled shaking waterbath. Following the addition of the dust (8 g) to the flask, concentrated HCl was added to maintain the pH at 1.30 ± 0.03. This pH was representative of the stomach conditions of a fasting child and provided the extreme conditions under which maximum Pb dissolution would occur (*11*). The equilibrated suspensions were sampled (3 mL) at 30, 60, and 120 min intervals after the pH had been measured and adjusted to 1.3 when necessary. The sampled extracts were passed through a 0.45 μm filter to remove any particulate matter.

After 2 h in the acidic (stomach) phase the mixture was titrated to approximately pH 7.0 by adding dialysis tubing (8000 MWCO, cellulose ester tubing) containing 3 mL of deionized water and adequate $NaHCO_3$ (solid) to neutralize the total acidity in the flask. Initially the dialysis tubing was removed when the pH reached 7.00 ± 0.20, but after much difficulty trying to maintain the pH at approximately 7.0 the tubing was removed as soon as the pH was between 6.5 and 7.0. After the removal of the dialysis tubing pancreatin (0.04 g, activity equivalent to 4 × U.S. Pharmacopia specifications) and bile extract (0.14 g) were added.

Aliquots (3 mL) were taken for analysis at the following time intervals following the removal of the dialysis tubing: 60, 120, and 180 min and approximately 16 h. Samples were acidified with five drops of concentrated HCl for storage prior to analysis. Lead concentrations in the solution were determined by FAAS. The dusts were analyzed in triplicate, and a blank containing no dust was also carried through the procedure with each experimental run.

Experiment 2. Variable pH in the Stomach Phase. In this study, following the addition of the dust, pH of the suspension was allowed to drift. For most dust samples the suspension pH rose from 1.3 to approximately 3.0 and remained stable. Pancreatin and pepsin were not added to these samples. The reproducibility of Pb dissolution of experimental runs was tested on different days. The effect of pepsin, pancreatin, and bile salts on Pb dissolution was determined in a separate experiment using the bulk dust sample C4, following the procedure described for experiment 2.

Experiment 3. Dissolution of Pb from Pb Acetate in the in Vitro System. The dissolution of Pb from Pb acetate ($Pb(CH_3CO_2)_2 \cdot 3H_2O$) was studied using conditions outlined in experiment 2, with a similar amount of Pb as the mean value for the dusts. This was done to evaluate Pb availability from a soluble salt in the absence of a complex matrix present.

Determination of Blood Pb Values. Children's blood Pb levels vary substantially across Port Pirie (fifth percentile = 7 μg/dL, 95th percentile = 29 μg/dL) (*12*). Measurements of blood Pb for the participating children were collected as part of the routine screening program of blood Pb administered by the Port Pirie Environmental Health Centre. The blood Pb data used in this study were collected from February to March 1996 since these data are more closely related in time to the collection of dust samples. All children in the survey were aged between 12 and 59 months. The protocol for the capillary sample collection is based on the recommendations contained in "Australian Standard 2636−1983 Sampling of Venous and Capillary Blood for the Determination of Lead Content". Capillary blood samples were analyzed for Pb using a graphite furnace AAS. Quality control was assured by participation in an international reference program. After every 10 capillary samples, a spike sample using a reference pig's blood and a control blank of deionized water were included. Approximately 10% of the samples were also analyzed as a split sample at a separate "reference" laboratory. At the beginning and end of each testing week 300 μL of reference pig's blood was also sent to the reference laboratory. After every 20 capillary samples a venous sample was drawn from a volunteer adult and analyzed to validate the capillary sampling method.

Statistical Analysis. Lead concentrations in the in vitro test were analyzed by ANOVA. The mean Pb concentrations in dust (Table 5) in the acidic phase were determined using all the values at either pH 1.3 (experiment 1) or 3.0 (experiment 2). In the alkaline phase, however, it was not possible to determine a mean value at pH 7.0 since the pH range was much greater. Therefore a regression line was fitted to all the data in the alkaline stage, and from the fitted line a Pb concentration (mg/kg) at pH 7.0 was interpolated.

The effect of different sample sizes for the values of blood Pb on the regression analysis was assessed by weighting the

TABLE 1. Particle Size Classes for the <53 μm Fraction of the Dusts

	%				%		
sample ID	clay[a]	silt[a]	sand[a]	sample ID	clay[a]	silt[a]	sand[a]
C1	38.8	36.9	24.3	C6	35.3	43.2	21.5
C2	34.8	46.4	18.8	C7	33.5	51.4	15.1
C3	36.6	47.8	15.6	mean	34.8	44.6	20.6
C4	32.6	45.8	21.6	SD[b]	2.4	4.7	4.4
C5	31.8	41.0	27.2				

[a] Clay <2 μm, silt 2−20 μm, sand >20 μm. [b] SD = standard deviation.

TABLE 2. Sample pH, Total C, and Carbonate-C Concentrations

			%	
sample ID	pH[a] <53 μm	pH 53−250 μm	total C	$CaCO_3$−C
C1	5.3	5.3	9.8	10.7
C2	6.6	6.8	17.6	11.2
C3	7.1	6.6	11.3	11.2
C4	8.0	8.1	17.5	11.4
C5	7.8	7.8	16.7	12.1
C6	7.6	7.6	16.7	11.4
C7	7.6	7.4	16.8	12.1

[a] 1:5, 0.01 M $CaCl_2$.

TABLE 3. Mean Elemental Composition of Dust (<53 μM)[a]

sample ID	Ca (%)	Cd (mg/kg)	Cu (mg/kg)	Fe (%)	Mg (%)	P (%)	S (%)	Zn (mg/kg)
C1	5.51	10.7	402	1.58	0.81	1.47	0.84	2546
C2	3.58	9.7	216	1.11	0.50	0.13	0.86	2730
C3	6.55	32.1	527	2.26	0.88	0.15	2.41	10947
C4	4.69	14.5	315	1.33	0.56	0.16	1.16	3619
C5	4.38	23.9	286	1.3	0.67	0.14	1.36	7370
C6	3.59	13.6	247	1.18	0.48	0.15	1.39	3728
C7	4.75	13.0	214	1.14	0.62	0.17	1.13	4113

[a] The coefficient of variation was below 5% in most cases. $n = 2$.

TABLE 4. Total Mean Pb Concentrations for Dusts[a]

sample ID	Pb (mg/kg) <53 μm	Pb (mg/kg) <250 μm	enrichment ratio
C1	975	481	2.0
C2	1693	1407	1.2
C3	6799	4590	1.5
C4	4187	2317	1.8
C5	4734	ND[b]	ND[b]
C6	2750	1604	1.7
C7	2621	1872	1.4

[a] The coefficient of variation was below 4% in all cases. $n = 2$. [b] ND = not determined.

TABLE 5. The Mean Concentration of Pb ± SE (mg/kg) in Solution for Each Dust under the Specified Conditions

sample ID	experiment 1		experiment 2	
	pH 1.3[a]	pH 7.0[b]	pH 3.0[c]	pH 7.0[c]
C1	258.9 ± 50.5	96.5 ± 15.2	220.2 ± 85.1	19.7 ± 4.4
C2	777.1 ± 70.4	178.1 ± 16.5	497.9 ± 19.9	233.0 ± 15.3
C3	3055.6 ± 171.2	505.0 ± 13.6	1616.0 ± 192.1	443.9 ± 21.1
C4	1705.1 ± 64.7	331.3 ± 36.8	1255.9 ± 183.1	272.0 ± 16.5
C5	1481.4 ± 52.1	250.0 ± 11.6	924.5 ± 129.7	348.0 ± 18.7
C6	1227.2 ± 87.6	317.0 ± 15.4	779.3 ± 32.4	343.0 ± 18.5
C7	1013.7 ± 79.9	152.1 ± 11.0	727.0 ± 23.9	182.0 ± 13.5

[a, c] Means have been determined from all data at each respective pH ($n = 9$ or 12). [b] Means have been interpolated from a fitted line calculated using all data in the alkaline stage.

dust Pb data on the basis of relative variability (coefficient of variation). The regression of blood Pb with dust Pb was compared using weighted data and unweighted data. There was no significant difference between the regression estimates ($P > 0.05$) obtained by both calculations; therefore, the relationships between blood Pb values and Pb concentrations determined by the in vitro technique, and total Pb concentrations in dust were determined by simple linear regression analysis.

Results and Discussion

Dust Characterization. Particle size analysis of the <53 μm fraction indicated all dusts to be similar in textural content (Table 1). The dusts ranged from slightly acid (C1) to alkaline (C4 and C5), and the total carbonate contents were high, in line with the measured pH values (Table 2). Surface areas were similar for all the dusts and were low (approximately 3 m^2/g). Total organic C (as determined by total C minus $CaCO_3$−C) indicated the presence of appreciable amounts of organic material (Table 2).

There was a wide variation between the dusts in their elemental composition (Table 3). Calcium concentrations were high, which combined with the carbonate data suggests that a large proportion of the carbonate in the materials was present as $CaCO_3$. Concentrations of Cd, Cu, and particularly Zn in the dusts were high, averaging 16.8, 315, and 5007 mg/kg, respectively (Table 3). By comparison, earlier studies found the Cd, Cu, and Zn concentration in soils (0−10 cm) collected 4−7 km from the smelter ranged from 1 to 2.6, 5.5−7.3, and 43−90 mg/kg, respectively (13). Most of the dusts (<53 μm) were highly contaminated with Pb, with approximately a 7-fold difference between the lowest (C1−975 mg/kg) and highest (C3−6799 mg/kg) Pb concentration (Table 4). The concentration of Pb in soils (0−10 cm) taken 4−7 km away from the smelter ranged from 140 to 390 mg/kg (13). On average the Pb concentration was 1.6 times greater in the <53 μm fraction (Table 4). Elevated concentrations of Pb have similarly been found in the (<53 μm − >38 μm) fraction of vacuum cleaner dust compared with the bulk sample (14). It was not possible in this study to assess the impact of different dust collection methods.

Solubility of Pb from Dusts Using the in Vitro Model. Experiment 1. In all cases the addition of dust to the solution increased the pH from 1.3 to approximately 3.0. This reflects the high carbonate content in the dusts (Table 2). Concentrated HCl (approximately 4.0 mL) was added to decrease the pH to 1.3. The mean concentration of Pb (mg/kg) from the dusts in solution at pH 1.3 ranged from 258.9 ± 50.5 for dust C1 to 3055.6 ± 171.2 for dust C3 (Table 5). When the amount of dissolved Pb was expressed as a percentage of total Pb in the dust, the amounts ranged from 26% (C1) to 46% (C2). Others have extracted vacuum cleaner dusts (53−38 μm fraction) collected from households in Broken Hill with 0.1 M HCl and found 13−55% of total Pb was extracted after 2 h (14).

Using a similar in vitro method to that described in this paper, others found the percentage of Pb solubilized from four mine waste samples after 2 h at pH 1.3 ranged from 0.5 to 6.0% (5). Others have similarly studied Pb dissolution from mine waste using the in vitro method and found the maximum amount of Pb from soil (<250 μm) after 2 h at pH 1.3 was 4% (15). The higher percentage Pb solubilized from dusts in this study compared with the findings of others is most likely due to the different environmental matrices and the differences in particle size of samples used in the two studies. The earlier studies cited (refs 5 and 15) used the <250 μm fraction, while in this study the <53 μm fraction was used. Lead bioavailability in the human gastrointestinal (GI) tract is strongly influenced by particle size, particularly for diameters <100 μm (2). Studies on dissolution kinetics of $PbSO_4$, however, found that particle size did not affect dissolution rates of $PbSO_4$ except at mean diameters of <10 μm (16).

The kinetics of Pb dissolution from $PbSO_4$ and a test soil (53% anglesite and 24% galena) was studied, and it was found that after 2 h at pH 1.3 and 2.0 only 20% and 40% of total Pb, respectively, from the test soil had solubilized (15). Although the dusts in the present study were only monitored for 2 h in the acidic stage, there was negligible change in Pb dissolution over time as indicated by the clustering of points at pH 1.3 or 3.0. A representative example of the response shown with all the dusts is in Figure 1. This suggests that the smaller particles in dust, which have a larger surface area, dissolve more rapidly. The dissolution rates of $PbSO_4$ have similarly been found to be dependent upon particle size (15). In addition, soils and mine waste samples are more likely to have a higher percentage of encapsulated particles than that found in dust, which would be expected to solubilize more slowly.

As expected, the solution concentration of Pb decreased when the pH was raised to approximately 7.0. As mentioned in the Methods section, the pH continued to rise beyond 7.0, even after the removal of the dialysis tubing containing $NaHCO_3$. The effect of increasing pH on the solubility of Pb, however, varied among the dust samples. There was no significant change in Pb (mg/kg) concentrations with increasing pH from 7.0 to 8.0 for C1, C3, C4, C5, and C7 (data not shown). However, increasing the pH significantly ($P < 0.05$) decreased the concentration of solubilized Pb (mg/kg) from approximately 180 at pH 7.0 to 80 at pH 8.0 for C2 (data not shown) and from 300 at pH 7.0 to 200 at pH 8.0 for C6 (data not shown). The amount of Pb in solution (mg/kg) at pH 7.0 ranged from 96.5 ± 15.2 for C1 to 505.0 ± 13.6 for C3 (Table 5). The decrease in the amount of Pb solubilized as pH was increased to 7.0 is consistent with adsorption and precipitation reactions removing Pb from solution (17).

Experiment 2. In all cases the addition of dust to the solution increased the pH from 1.3 to approximately 3.0 and

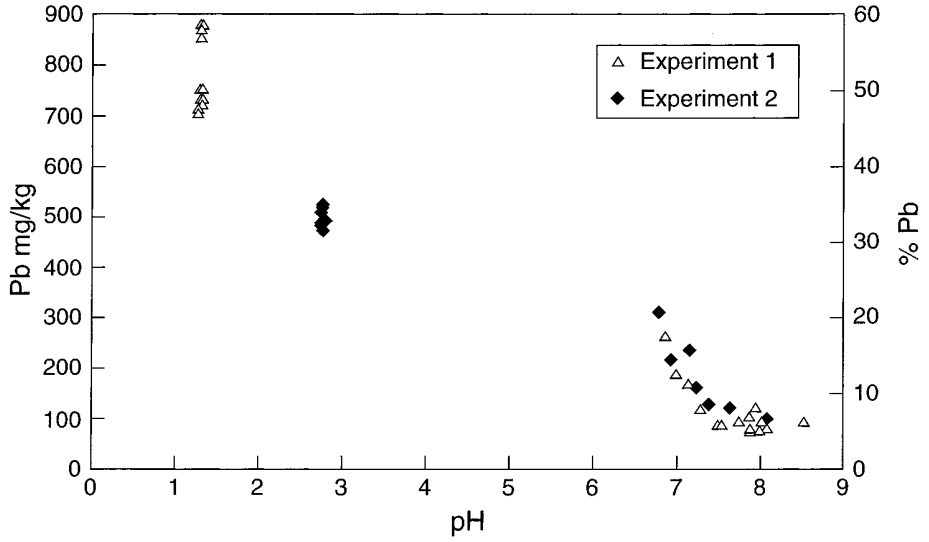

FIGURE 1. Solubilization of Pb from dust C2 with pH.

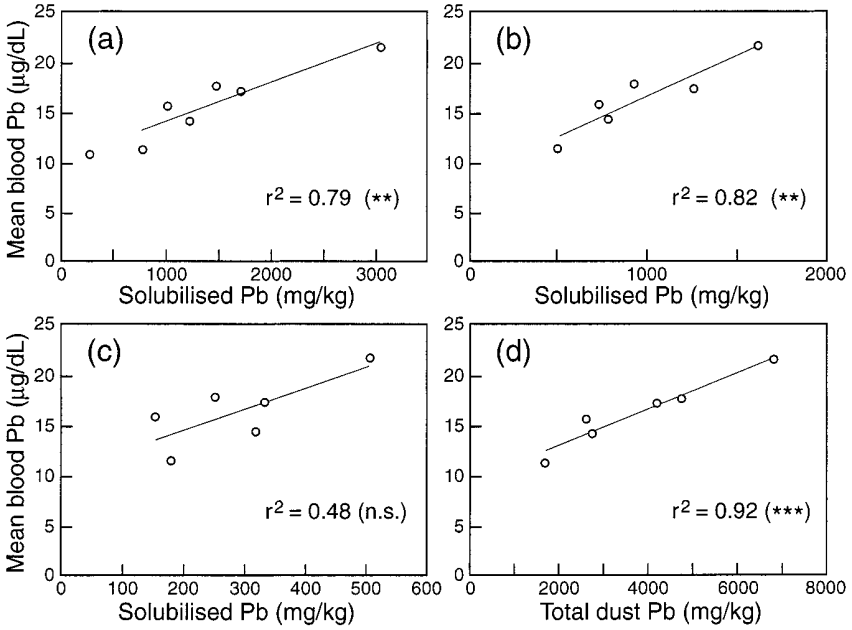

FIGURE 2. Relationship between mean blood Pb concentrations (µg/dL) from children at each location and mean Pb solubilized from all dusts in the in vitro procedure at different pH values: (a) experiment 1, pH 1.3 (stomach phase); (b) experiment 2, pH 3.0 (stomach phase); (c) experiment 1, pH 7.0 (small intestine); and (d) total dust Pb (mg/kg).

maintained this pH for the duration of the "acidic" (stomach) phase. The mean concentration of Pb (mg/kg) in solution with dust ranged from approximately 220.2 ± 85.1 for C1 to 1616.0 ± 192.1 for C3 (Table 5). When the amount of dissolved Pb was expressed as a percentage of total Pb in the dust, the mean amounts solubilized at pH 3.0 were similar for all dusts. The percentages ranged from 20% for C5 to 30% for C2, C4, and C6, which is approximately 10−20% lower than the amount solubilized at pH 1.3. However, for C1 the percent Pb solubilized at pH 1.3 and 3.0 was almost the same. This reflects the greater range of Pb concentrations in experiment 1 for C1 compared with the other dusts. The reproducibility between the experimental runs at pH 3.0 was consistent (data not shown). For example, for duplicate runs using C4 the mean Pb in solution (mg/L) at pH 3.0 was 85.1 ± 5.8 and 84.7 ± 3.7 and for C7 the mean Pb in solution (mg/L) at pH 3.0 was 72.7 ± 2.4 and 75.8 ± 4.2. There was negligible Pb in the blank.

In the intestinal phase, after the removal of the dialysis bag, there were no significant changes in the concentrations of Pb in solution (mg/kg) with increasing pH from 7.0 to 8.0 for C1, C3, and C4. For dust C2, however, the amount of Pb in solution continued to decrease as the pH increased (Figure 1). A similar trend was seen for dusts C5, C6, and C7 (data not shown) until the pH reached approximately 7.2−7.5. At pH values above this, Pb in solution remained fairly constant. This suggests that maintenance of pH at 7.0 is not critical for Pb dissolution in this in vitro test, as was expected on the basis of gut physiology. There was good reproducibility between experimental runs in the "alkaline" stage, and an example is shown for dust C2 (Figure 1).

At pH 7.0 the percent Pb still in solution was similar to that found in experiment 1 and ranged from 2% for C1 (where the concentrations were similar to the blank) to approximately 10% for dusts C2 and C6.

Dissolution of Pb Acetate in the in Vitro System. Although there was a decrease in the concentration of Pb in solution from $Pb(OAc)_2$ when the pH was raised to approximately 7.0, the decrease was much less than that observed with the dusts in the in vitro system. The concentration of Pb in the acidic phase ranged from 292 to 319 mg/L and in the alkaline phase from 229 to 276 mg/L (data not shown). Also the pH in the acidic phase did not increase with the addition of Pb acetate as it did when the dusts were added to the system but remained at approximately 1.4.

When the concentration was expressed as percentage of total Pb added (3393 mg/kg), the amount of Pb in the acidic phase ranged from 86% to 94% and in the alkaline phase from 68% to 81%. It has been reported that after 2 h in the acidic phase (pH maintained at 1.3) 68% of Pb from $Pb(OAc)_2$ was bioavailable in vitro in the presence of a mine-waste matrix, while 76% was available when no matrix was present (5). Other studies reported that after 2 h at pH 1.3 all the Pb from $Pb(OAc)_2$ was bioavailable (15). The significantly ($P < 0.005$) lower concentration of Pb in solution at pH 1.3 and pH 7.0 for the dusts (approximately 26−46 and 5−10%, respectively) compared with $Pb(OAc)_2$ suggests that Pb adsorption to particle surfaces or entrapment within the dust may decrease Pb solubility in the small intestine. The dust samples had a high organic content, which would provide possible sites for entrapment of particles containing Pb. This difference in Pb solubility between a highly soluble Pb compound and Pb in dust demonstrates the need for any tests that are assessing bioavailability to consider the nature of the chemical species and the environmental matrix of the sample.

Effect of Enzymes on Pb Dissolution. In the stomach stage (acidic stage) the addition of pepsin had no effect on the blanks. However, pepsin significantly ($P < 0.05$) decreased the Pb dissolved from dust C4 (<250 μm) by 28% with Pb in the extracting solution decreasing from a mean of 76 ± 3 to 55 ± 6 mg/L (data not shown). In the small intestinal stage (alkaline stage) there was no significant difference in the amount of Pb solubilized in the presence or absence of pancreatin. This is contrary to an earlier study where the amount of Pb solubilized significantly increased (20%) when enzymes were added (5), and the gastrointestinal tract enzymes in the pH 7.0 environment of the small intestine were necessary to retain Pb in solution in this stage. This was not found in our study using house dust.

Effect of Bile Salts on Pb Dissolution. The results for Pb (mg/kg) in the acidic phase are more variable with bile salts than when bile salts were excluded, ranging from 720 to 920 mg/kg and 800−900 mg/kg, respectively. Despite the wider range of Pb concentrations in the acidic phase the concentrations in both the acid and alkaline phases are similar with or without bile salts (data not shown). This indicates that when dusts or soils are being investigated, the exclusion of bile salts does not affect the amount of Pb remaining in solution when pH is raised. Since bile salts act as a fat emulsifier (18), it would be expected that when no food or source of fat was included in the in vitro study that the exclusion of bile salts would not affect the measure of Pb concentrations in solution.

Correlations with Blood Pb Concentrations. Since no feeding tests were conducted in this study, the validity of the in vitro was determined by correlating average blood Pb concentrations from children in the area where the dusts were collected with the Pb concentration in both phases of the in vitro test—the stomach (acidic) at pH 1.3 and 3.0 and intestinal phase at pH 7.0 (alkaline) stage. Blood Pb concentrations were also correlated with total Pb concentrations in the dusts (Figure 2a−d). The mean blood Pb data corresponding with each dust collection site is given in Table 6.

TABLE 6. Mean ± SD of Blood Pb (μg/dL) Collected from February to March 1996 from Children (12−59 Months) Living in Areas Corresponding to the Sites from Which Dust Was Collected[a]

site	mean	SD[b]	*n*	site	mean	SD[b]	*n*
2	11.25	5.94	88	5	17.67	8.06	69
3	21.48	2.29	6	6	14.19	5.44	80
4	17.13	5.77	54	7	15.67	4.49	46

[a] Data corresponding to site C1 has been excluded because it was a school. [b] SD = standard deviation.

There was a positive relationship between blood Pb levels and all four measures of Pb bioavailability. The best correlation was found with total dust Pb ($r^2 = 0.92$, **) and Pb concentrations determined in the in vitro test at pH 3.0 ($r^2 = 0.82$, **). Significant ($P < 0.05$) positive relationships were also found with Pb concentrations determined by the in vitro tests at pH 1.3 ($r^2 = 0.79$, **). No significant relationship was found between blood Pb and Pb solubilized in the alkaline stage in either experiment 1 or 2 ($r^2 = 0.48$ and 0.34, respectively). These correlations, however, are limited by only having six dust samples for the analyses (samples from site C1 were excluded from the analysis because this site was a school). However, these regressions would suggest that for these samples total Pb analysis of dusts would be adequate for estimating blood Pb levels in children. However, all the dusts in this study were contaminated by Pb from the same source. If samples from different sources with different environmental matrices were studied the correlation between total Pb and blood Pb may not have been as good.

Difficulties with the in Vitro Test. The two main problems with the in vitro test were the time taken for the pH to increase from the acidic stage (pH either 1.3 or 3.0) to pH 7.0 and the lack of pH stability at 7.0. In this study the time taken for the pH to rise to 7.0 ranged from 3 to 6 h for the blank solutions containing no dust and from 2 to 4 h for the test solutions. By comparison others have reported that the pH was raised from 1.3 to 7.0 in 30 min (refs 5 and 15). Within the small intestine the pH is rapidly increased to 7.0. The greater time required in this study to increase the pH to 7.0 compared with other studies may affect the kinetics of Pb complexation with particles in the dust, which may in turn affect the estimation of bioavailability.

In this study stabilization of the pH at 7.0 was difficult despite adding the exact amount of $NaHCO_3$ to neutralize the total acidity in the flask. Earlier studies reported no such problems with the maintenance of pH (5 and 19). Titrations with 1 M NaOH showed that the inflection point of the titration curve occurred at pH 6.8−7.0 (data not shown). Therefore around this pH value small changes in acidity or base added will have large effects on pH. For this reason the dialysis bag was removed when the pH was between 6.5 and 6.8, the pH was monitored, and aliquots were taken when the pH reached approximately 7.0.

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