Supporting Information

Dietary Lead and Phosphate Interactions Affect Oral Bioavailability of Soil Lead in the Mouse

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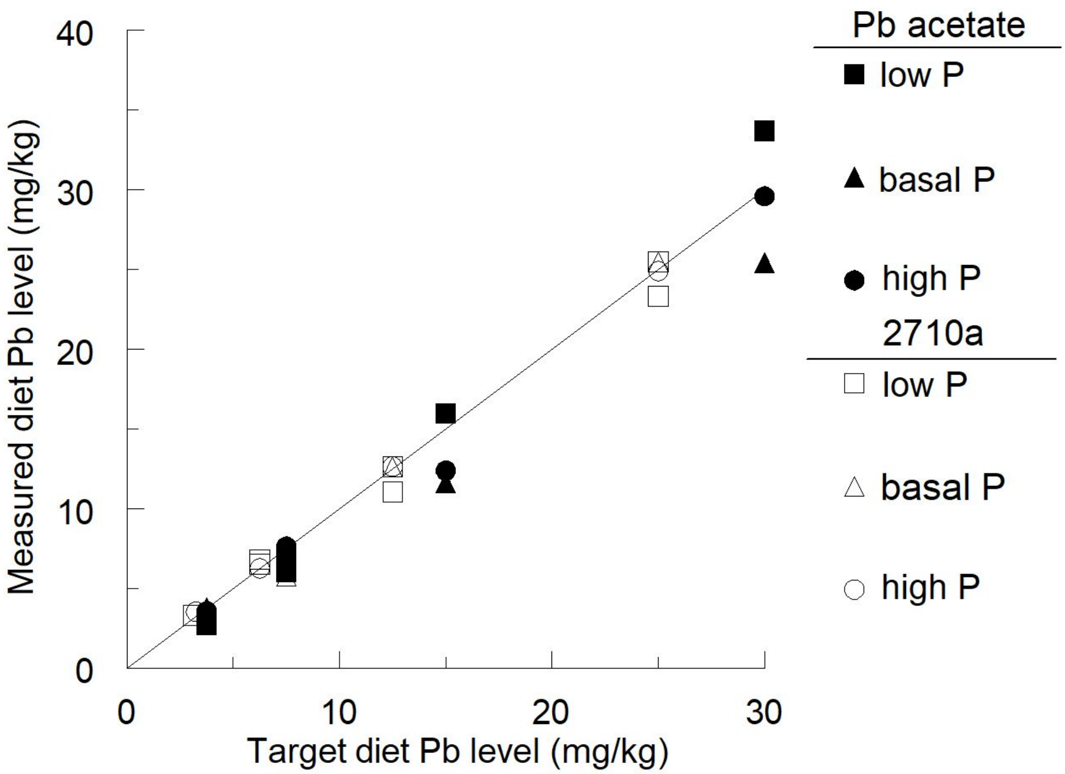
Table of contents

Figure S1. Dietary Pb levels



Relation between target and measured Pb levels in diets. Line of identity shown.

Lead Speciation Analysis

XAS data were collected at the DuPont-Northwestern-Dow Collaborative Access Team (DND-CAT) Sector 5, beam line 5BM-D, at the Advanced Photon Source (APS) of the Argonne National Laboratory (ANL), U.S. The storage ring operated at 7 GeV in top-up mode. A liquid N_2 cooled double crystal Si(111) monochromator was used to select the incident photon energies and a platinum-coated mirror was used for harmonic rejection. Calibration was performed by assigning the first derivative inflection point of the absorption L_{III}-edge of Pb metal (13035 eV), and each sample scan was collected simultaneously with a Pb metal foil. The samples were ground and pressed into pellets, affixed to a multiport sample holder, and mounted for analysis without any further modifications. Data collection was conducted in fluorescence mode with two Vortex-ME4 four-element silicon drift detectors for the samples. Various Pb standards were used as reference spectra, including mineral sorbed Pb [Pb-ferrihydrite, Pb-kaolinite, Pb-goethite, Pbgibbsite, Pb-birnessite, and Pb-montmorillonite in which each mineral was equilibrated with $Pb(NO_3)_2$ at pH 6 for a target surface loading of 2500 mg kg^{-1} after dialysis], organic bound Pb [Pb-fulvic acid and Pb-humic acid as reagent grade organic acids equilibrated with $Pb(NO_3)_2$ at pH 6 for a target loading of 1500 mg kg^{-1} after dialysis, and reagent grade Pb acetate, Pb cysteine, and Pb citrate], Pb carbonate [Smithsonian Natural History Minerals Collection specimens of cerussite, hydrocerussite, and plumbonacrite with X-ray diffraction verification], PbO [massicot and litharge], Pb-phosphates [chloropyromorphite, hydroxypyromorphite, lead phosphate ($Pb_3(PO_4)_2$), $PbHPO_4$, and Pb sorbed to apatite at pH 6 and surface loading of 2000 mg kg^{-1}], and other lead minerals [leadhillite, magnetoplumbite, plumboferrite, plumbogummite, plumbojarosite, anglesite, and galena from the Smithsonian Natural History Minerals Collection with X-ray diffraction verification]. All reference spectra were collected in transmission mode with dilution calculations determined by XAFSMass[1] mixed in binder and pressed into a pellet.

All sample and standard spectra were calibrated to a Pb foil on the same energy grid, averaged, and normalized, and the background was removed by spline fitting using IFEFFIT.[2] Principal components analyses were performed in Sixpack[3] on the normalized scans, and target factor analyses of each Pb standard were performed to determine the most appropriate standards to be used for linear combination fitting (LCF) analyses. Pb standards with SPOIL values <3.0 were used in the LCF analyses, which included mineral sorbed Pb [sum of Pb-ferrihydrite, Pb-goethite, and Pb-birnessite], organic bound Pb [sum of Pb-fulvic acid and Pb-humic acid], Pb carbonate [sum of cerussite and hydrocerussite], PbO [sum of massicot and litharge], Pb-phosphates [chloropyromorphite, hydroxypyromorphite, $Pb_3(PO_4)_2$, and Pb sorbed to apatite], and other lead minerals [leadhillite, plumboferrite, plumboyarosite, anglesite, and galena]. The k–space functions of the standards and samples were used for all linear combination fitting. Levenberg–Marquardt least squares algorithm was applied to a fit range of 0.6 to 9.0 $Å^{-1}$. Best-fit scenarios, defined as having the smallest residual error, also had sums of all fractions close to 1. To fully describe any particular sample within 1% reproducible error, a minimum of two components was necessary, and results have a ±10% accuracy.

Data analysis

Table S1 - Formulas for calculation of experimental variables

Variable	Formula
Cumulative food intake (g)	Sum of daily food intake
Cumulative Pb intake (mg)	Cumulative food intake*dietary Pb level
Cumulative P intake (mg)	Cumulative food intake*dietary P level
% Body weight change	((Final body weight – Initial body weight)/Initial body weight)*100
% Feed efficiency	((Final body weight – Initial body weight)/ Cumulative food intake)*100

Table S2 - Effect of dietary P concentration on food intake, relative growth, and feed efficiency in mice consuming unamended AIN-93G rodent diet[a]

Dietary P level	N	Cumulative food intake (g)	% Body weight change	% Feed efficiency
Low	4	81.8 ± 7.4[a]	6.04 ± 3.58[a]	3.71 ± 1.97[a]
Basal	5	73.9 ± 2.4[a]	7.85 ± 3.75[a]	5.38 ± 2.38[a]
High	4	70.9 ± 2.1[b]	4.52 ± 3.67[a]	3.16 ± 2.58[a]

a. Mean ± SD shown. Means denoted with different letters are significantly different ($p \leq 0.05$, ANOVA, Tukey HSD).

Table S3 – Effect of dietary P concentration on food intake, relative growth, and feed efficiency in mice consuming either unamended or Pb-amended AIN-93G rodent diet[a]

Dietary P level	N	Cumulative food intake (g)	% Body weight change	% Feed efficiency
Low	43	77.8 ± 4.36[a]	7.23 ± 3.04[a]	4.81 ± 1.96[a]
Basal	29	81.6 ± 5.86[b]	7.27 ± 2.81[a]	4.76 ± 1.72[a]
High	28	72.5 ± 4.54[c]	1.93 ± 3.58[b]	1.35± 2.52[b]

a. Mean ± SD shown. Means denoted with different letters are significantly different ($p \leq 0.05$, ANOVA, Tukey HSD).

Table S4 – Effect of dietary amendment on food intake, relative growth, and feed efficiency in mice consuming either unamended or Pb-amended AIN-93G rodent diet[a]

Dietary amendment	N	Cumulative food intake (g)	% Body weight change	% Feed efficiency
Unamended	13	75.4 ± 6.45[a]	6.27 ± 3.65[a]	4.18 ± 2.36[a]
PbAc	42	77.8 ± 6.18[a]	5.41 ± 4.50[a]	3.57 ± 2.97[a]

2710a	45	77.7 ± 5.63^{a}	5.94 ± 3.40^{a}	3.96 ± 2.24^{a}

a. Mean ± SD shown. Means denoted with different letters are significantly different ($p \leq 0.05$, ANOVA, Tukey HSD).

Table S5 – Effect of dietary amendment and dietary P level on food intake, relative growth, and feed efficiency in mice consuming either high P unamended or Pb-amended AIN-93G rodent diet[a]

Dietary amendment – diet P level	N	Cumulative food intake (g)	% Body weight change	% Feed efficiency
Unamended-high P	4	70.9 ± 2.1^{a}	4.52 ± 3.67^{a}	3.16 ± 2.58^{a}
PbAc-high P	12	72.6 ± 5.3^{a}	-0.42 ± 2.46^{b}	-0.30 ± 1.84^{b}
2710a-high P	12	72.9 ± 4.4^{a}	3.42 ± 3.28^{a}	2.39± 2.24^{a}

a. Mean ± SD shown. Means denoted with different letters are significantly different ($p \leq 0.05$, ANOVA, Tukey HSD).

Regression analysis of dietary Pb source and dietary P levels on percent body weight change and percent feed efficiency

Stepwise linear regression evaluated dietary Pb level, dietary P level, dietary Pb source, cumulative Pb intake, cumulative P intake, cumulative food intake, and two interaction terms, (cumulative food intake*dietary P level) and (dietary P*Pb source) to explain variance in percent body weight change and feed efficiency. In constructing the regression models, the entry p-value for dependent variables was 0.15.

Among these variables, cumulative food intake, dietary Pb source, (cumulative food intake*dietary P level), and (dietary P level*dietary Pb source) remained in the final model as significant predictors of percent body weight change (adjusted r^2 =0.50). Results of this analysis is shown in Table S6.

Table S6 – Identification of variables retained as significant predictors of percent body weight change in stepwise linear regression

Dependent Variable: Percent body weight change			
Independent Variable	Mean	Partial r^2	P
Intercept	-18.12 ± 3.89		<0.0001
Cumulative food intake	0.38 ± 0.05	0.27	<0.0001
Cumulative food intake*dietary P level	-0.044 ± 0.006	0.15	<0.0001
Dietary Pb source	-3.31 ± 1.05	0.02	0.0021
Dietary P level*dietary Pb source	2.12 ± 0.50	0.09	<0.0001
Adjusted r^2 =0.50			

Table S7 shows results of stepwise linear regression analysis of these variables in relation to % feed efficiency. Here, cumulative food intake, dietary Pb source, cumulative food intake*dietary P level, and dietary P level*dietary Pb source remained in the final model as significant predictors of feed efficiency (adjusted r^2 =0.48).

Table S7 - Identification of variables retained as significant predictors of percent feed efficiency in stepwise linear regression

Dependent Variable: Percent feed efficiency			
Independent Variable	Mean	Partial r^2	p
Intercept	-10.00 ± 2.61		0.0002
Cumulative food intake	0.23 ± 0.03	0.22	<0.0001
Cumulative food intake*dietary P level	-0.030± 0.004	0.16	<0.0001
Dietary Pb source	-2.38 ± 0.70	0.02	0.0010
Dietary P level*dietary Pb source	1.51 ± 0.34	0.10	<0.0001
Adjusted r^2 =0.48			

Table S8. Effect of dietary P level and dietary Pb source on relative growth predicted from regression models

	Dietary Pb source			
	Pb Acetate		NIST SRM 2710a	
Dietary P level	% Body weight change (at mean cumulative food intake)	% Body weight change across cumulative food intake range (min, max)	% Body weight change (mean cumulative food intake)	% Body weight change across cumulative food intake range (min, max)
Low	8.3	3.9, 14.4	7.1	2.7, 13.2
Basal	4.9	1.0, 10.1	5.8	2.0, 11.1
High	1.5	-1.8, 6.0	4.5	1.2, 9.0

Table S9. Effect of dietary P level and Pb source on relative growth predicted from regression models

Dietary Pb source	Dietary P level		
	Low	Basal	High
PbAc	8.3 (3.9, 14.4)	4.9 (1.0, 10.1)	1.5 (-1.8, 6.0)
2710a	7.1 (2.7, 13.2)	5.8 (2.0, 11.1)	4.5 (1.2, 9.0)

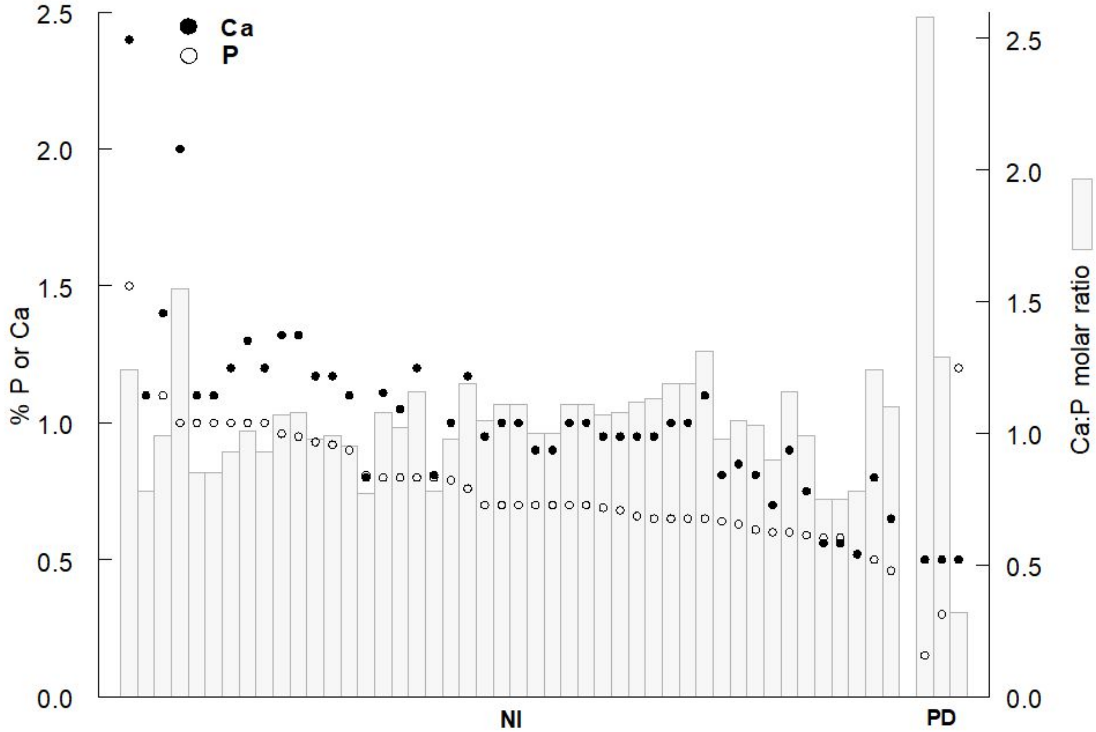
Estimates of % body weight change at mean cumulative food intake and across food intake range (min, max)

Table S10. RBA estimates for SRM 2710a

Dietary Pb	RBA - Bone	RBA - Blood	RBA – Kidney	RBA – Point Estimate
Low	0.28 (0.23, 0.33)	0.42 (0.31, 0.55)	0.40 (0.32, 0.49)	0.37 (0.32, 0.42)
Basal	0.24 (0.06, 0.42)	0.47 (0.37, 0.58)	0.52 (0.43, 0.65)	0.41 (0.33, 0.48)
High	0.72 (0.45, 1.08)	0.49 (0.34, 0.63)	0.29 (0.21, 0.37)	0.50 (0.39, 0.61)

Values shown in parentheses are 95% confidence limits.

Figure S2. Dietary P and Ca levels



Levels of phosphate and calcium and their molar ratio in rodent diets. Levels of phosphate (P) and calcium (Ca) expressed as % of total diet mass and on a molar ratio basis in 46 natural ingredient (NI) diets and in AIN-93G purified diet (PD).

References

(1) Klementiev, K. V. 2012. XAFSmass, freeware: www.cells.es/Beamlines/CLAESS/software/xafsmass.html

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(3) Webb, S. M. SIXpack: A graphical user interface for XAS analysis using IFEFFIT. Phys Scr. 2005, T115, 1011−1014.