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ESTIMATION OF RELATIVE BIOAVAILABILITY OF LEAD IN SOIL AND SOIL-LIKE MATERIALS USING *IN VIVO* AND *IN VITRO* METHODS

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Washington, DC 20460

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IN VIVO STUDIES

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IN VITRO STUDIES

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REVIEWERS

A draft of this report was provided to three independent experts for external peer review and comment. This satisfies the Agency's requirements for peer review. These reviewers were:

Paul Mushak, PB Associates, Durham, NC

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Rosalind Schoof, Integral Consulting, Inc., Mercer Island, WA

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EXECUTIVE SUMMARY

1.0 INTRODUCTION

Reliable analysis of the potential hazard to children from ingestion of lead in environmental media depends on accurate information on a number of key parameters, including the rate and extent of lead absorption from each medium ("bioavailability"). Bioavailability of lead in a particular medium may be expressed either in absolute terms (absolute bioavailability, ABA) or in relative terms (relative bioavailability, RBA). For example, if 100 micrograms (μg) of lead dissolved in drinking water were ingested and a total of 50 μg were absorbed into the body, the ABA would be 0.50 (50%). Likewise, if 100 μg of lead contained in soil were ingested and 30 μg were absorbed into the body, the ABA for soil would be 0.30 (30%). If the lead dissolved in water was used as the frame of reference for describing the relative amount of lead absorbed from soil, the RBA would be 0.30/0.50, or 0.60 (60%).

When reliable data are available on the absolute or relative bioavailability of lead in soil, dust, or other soil-like waste material at a site, this information can be used to improve the accuracy of exposure and risk calculations at that site. Based on available information in the literature on lead absorption in humans, the U.S. Environmental Protection Agency (U.S. EPA) estimates that relative bioavailability of lead in soil compared to water and food is about 60%. Thus, when the measured RBA in soil or dust at a site is found to be less than 60%, it may be concluded that exposures to and hazards from lead in these media at that site are probably lower than typical default assumptions. Conversely, if the measured RBA is higher than 60%, absorption of and hazards from lead in these media may be higher than usually assumed.

This report summarizes the results of a series of studies performed by scientists in U.S. EPA Region 8 to measure the RBA of lead in a variety of soil and soil-like test materials using both *in vivo* and *in vitro* techniques.

2.0 *IN VIVO* STUDIES

Basic Approach for Measuring RBA *In Vivo*

The *in vivo* method used to estimate the RBA of lead in a particular test material compared to lead in a reference material (lead acetate) is based on the principle that equal absorbed doses of lead will produce equal increases in lead concentration in the tissues of exposed animals. Stated another way, RBA is the ratio of oral doses that produce equal increases in tissue burden of lead.

Based on this, the technique for estimating lead RBA in a test material is to administer a series of oral doses of reference material (lead acetate) and test material (site soil) to groups of experimental animals, and to measure the increase in lead concentration in one or more tissues in the animals. For each tissue, the RBA is calculated by fitting an appropriate dose-response model to the data, and then solving the equations to find the ratio of doses that produce equal responses. The final estimate of RBA for the test material then combines the RBA estimates across the different tissues.

Animal Exposure and Sample Collection

All animals used in this program were intact male swine approximately 5 to 6 weeks of age. In general, exposure occurred twice a day for 15 days. Most groups were exposed by oral administration, with one group usually exposed to lead acetate by intravenous injection.

Lead concentrations were measured in four different tissues: blood, liver, kidney, and bone. For blood, samples were collected from each animal at multiple times during the course of the study (*e.g.*, days 0, 1, 2, 3, 4, 6, 9, 12, and 15), and the blood concentration integrated over time (commonly referred to as "area under the curve" or AUC) was used as the measure of blood lead response. For liver, kidney, and bone, the measure of response was the concentration of lead in these tissues on day 15.

Calculation of RBA

Based on testing several different types of dose-response models to the data, it was concluded that most dose-response curves for liver, kidney, and bone lead were well described by a linear model, and that most blood lead AUC data sets were well described by an exponential model:

Liver, Kidney, Bone

$$C_{tissue} = a + b \cdot Dose$$

Blood AUC

$$AUC = a + b \cdot \left[1 - \exp(-c \cdot Dose)\right]$$

where C_{tissue} is the concentration of lead in a given tissue; a, b, and c are the terms of the mathematic equation used to describe the shape of the curve; and *Dose* is the total daily administered dose of lead (µg/kg-day).

Based on these models, RBA is calculated from the best model fits as follows:

$$RBA_{liver,\,kidney,\,bone} = \frac{b_{test\ material}}{b_{reference\ material}}$$

$$RBA_{blood\ AUC} = \frac{b_{test\ material}}{b_{reference\ material}}$$

Results and Discussion

RBA Values for Various Test Materials

Table ES-1 lists the 19 different materials tested in this program and shows the RBA values estimated using each of the four alternative endpoints (blood AUC, liver, kidney, bone).

Based on an analysis that indicated that each endpoint has approximately equal reliability, the point estimate for each test material is the mean of the four endpoint-specific values.

Inspection of these RBA point estimates for the different test materials reveals that there is a wide range of values across different samples, both within and across sites. For example, at the California Gulch site in Colorado, RBA estimates for different types of material range from about 6% (Oregon Gulch tailings) to 105% (Fe/Mn lead oxide sample). This wide variability highlights the importance of obtaining and applying reliable RBA data in order help to improve risk assessments for lead exposure.

Correlation of RBA with Mineral Phase

Available data are not yet sufficient to establish reliable quantitative estimates of RBA for each of the different mineral phases of lead that are observed to occur in the test materials. However, multivariate regression analysis between point estimate RBA values and mineral phase content of the different test materials allows a tentative rank ordering of the phases into three semi-quantitative tiers (low, medium, or high RBA), as follows:

Low Bioavailability	Medium Bioavailability	High Bioavailability
Fe(M) Sulfate Anglesite Galena Pb(M) Oxide Fe(M) Oxide	Lead Phosphate Lead Oxide	Cerussite Mn(M) Oxide

(M) = Metal

3.0 *IN VITRO* STUDIES

Measurement of lead RBA in animals has a number of potential benefits, but is also rather slow and costly and may not be feasible in all cases. It is mainly for this reason that a number of scientists have been working to develop alternative *in vitro* procedures that may provide a faster and less costly alternative for estimating the RBA of lead in soil or soil-like samples. These methods are based on the concept that the rate and/or extent of lead solubilization in gastrointestinal fluid is likely to be an important determinant of lead bioavailability *in vivo*, and most *in vitro* tests are aimed at measuring the rate or extent of lead solubilization in an extraction solvent that resembles gastric fluid. The fraction of lead which solubilizes in an *in vitro* system is referred to as *in vitro* bioaccessibility (IVBA).

Description of the Method

The IVBA extraction procedure is begun by placing 1.0 g of test substrate into a bottle and adding 100 mL of extraction fluid (0.4 M glycine, pH 1.5). This pH is selected because it is similar to the pH in the stomach of a fasting human. Each bottle is placed into a water bath adjusted to 37°C, and samples are extracted by rotating the samples end-over-end for 1 hour. After 1 hour, the bottles are removed, dried, and placed upright on the bench top to allow the soil

to settle to the bottom. A sample of supernatant fluid is removed directly from the extraction bottle into a disposable syringe and is filtered to remove any particulate matter. This filtered sample of extraction fluid is then analyzed for lead.

Results

Table ES-2 summarizes the *in vitro* bioaccessibility results for the set of 19 different test materials evaluated under the Phase II program. As seen, IVBA values span a considerable range (min of 4.5%, max of 87%), with a mean of about 55%. This variability among test materials indicates that the rate and extent of solubilization of lead from the solid test material into the extraction fluid do depend on the attributes of the test material, and that IVBA may be a useful indication of absorption *in vivo* (see below).

Comparison of *In Vivo* and *In Vitro* Results

In order for an *in vitro* bioaccessibility test system to be useful in predicting the *in vivo* RBA of a test material, it is necessary to establish empirically that a strong correlation exists between the *in vivo* and the *in vitro* results across many different samples. Figure ES-1 shows the best fit weighted linear regression correlation between the *in vivo* lead RBA estimates and the *in vitro* lead bioaccessibility estimates for each of the 19 test materials investigated during this program. The equation of the line is:

$$RBA = 0.878 \cdot IVBA - 0.028 \quad (r^2 = 0.924)$$

These results indicate that the *in vivo* RBA of lead in soil-like materials can be estimated by measuring the IVBA and using the equation above to calculate the expected *in vivo* RBA. Actual RBA values may be either higher or lower than the expected value, as indicated by the 95% prediction interval shown in Figure ES-1.

At present, it appears that this equation is likely to be widely applicable, having been found to hold true for a wide range of different soil types and lead phases from a variety of different sites. However, most of the samples tested have been collected from mining and milling sites, and it is plausible that some forms of lead that do not occur at this type of site might not follow the observed correlation. Thus, whenever a sample that contains an unusual and/or untested lead phase is evaluated by the *in vitro* bioaccessibility protocol, this should be identified as a potential source of uncertainty. In the future, as additional samples with a variety of new and different lead forms are tested by both *in vivo* and *in vitro* methods, the applicability of the method will be more clearly defined.

4.0 CONCLUSIONS

The data from the investigations performed under this program support the following main conclusions:

1. Juvenile swine are believed to be a useful model for the evaluation of lead absorption in children and provide a reliable system for measuring the RBA of lead in a variety of soil and soil-like materials.

2. Each of the four different endpoints employed in these studies (blood AUC, liver, kidney, bone) to estimate RBA *in vivo* yield reasonable data, and the best estimate of the RBA value for any particular sample is the average across all four endpoint-specific RBA values.

3. There are clear differences in the *in vivo* RBA of lead between different types of test material, ranging from near zero to close to 100%. Thus, knowledge of the RBA value for different types of materials at a site can be very important in improving lead risk assessments at a site.

4. Available data support the view that certain types of lead minerals are well-absorbed (*e.g.*, cerussite, manganese lead oxide), while other forms are poorly absorbed (*e.g.*, galena, anglesite). However, the data are not yet sufficient to allow reliable quantitative calculation or prediction of the RBA for a test material based on knowledge of the lead mineral content alone.

5. *In vitro* measurements of bioaccessibility performed using the protocol described in this report correlate well with *in vivo* measurements of RBA, at least for 19 materials tested under this program. At present, the results appear to be broadly applicable, although further testing of a variety of different lead forms is required to determine if there are exceptions to the apparent correlation.

TABLE ES-1. SUMMARY OF ESTIMATED RBA VALUES FOR TEST MATERIALS

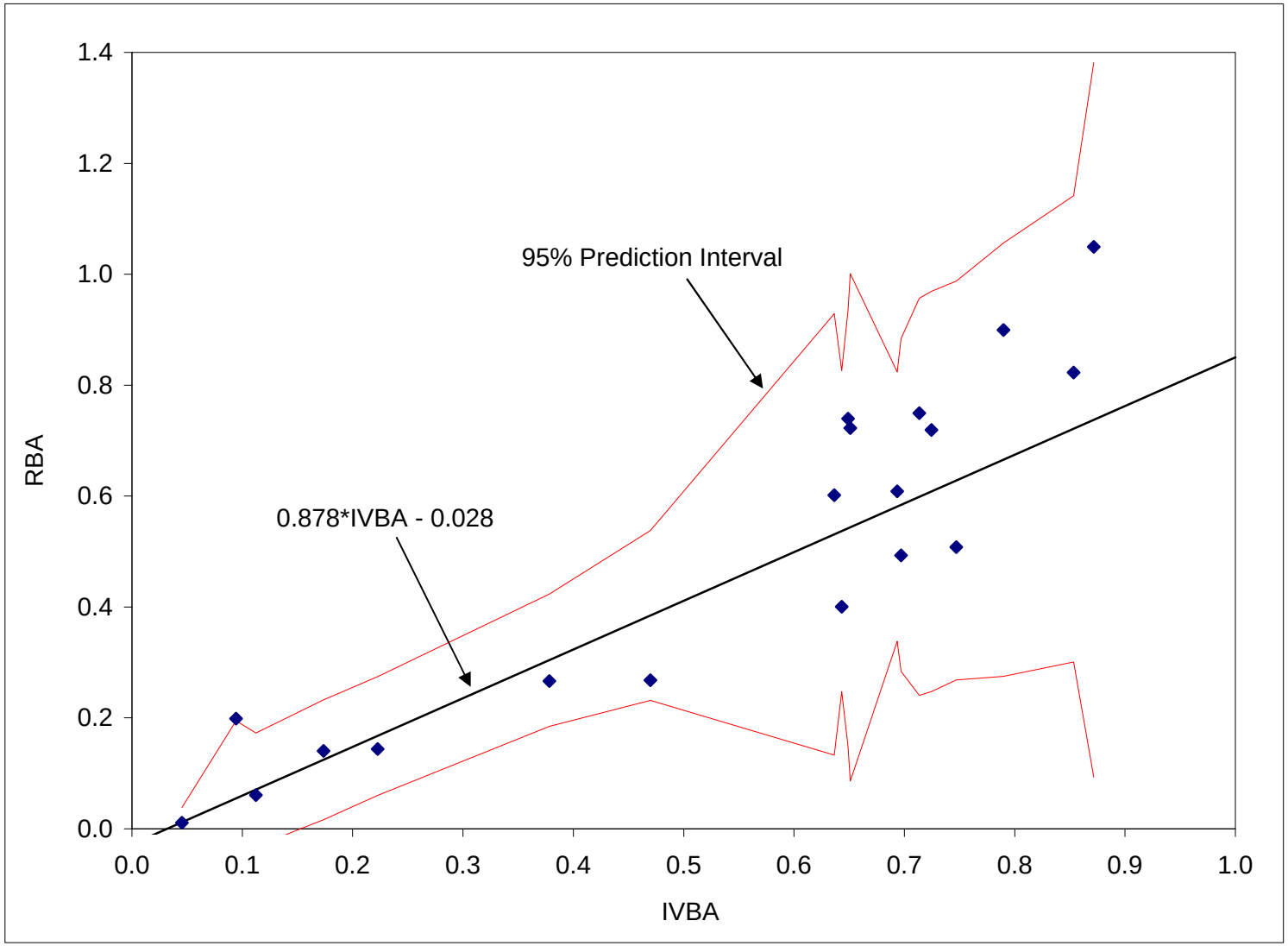
Experiment	Test Material	Blood AUC			Liver			Kidney			Femur			Point Estimate		
		RBA	LB	UB	RBA	LB	UB	RBA	LB	UB	RBA	LB	UB	RBA	LB	UB
2	Bingham Creek Residential	0.34	0.23	0.50	0.28	0.20	0.39	0.22	0.15	0.31	0.24	0.19	0.29	0.27	0.17	0.40
	Bingham Creek Channel Soil	0.30	0.20	0.45	0.24	0.17	0.34	0.27	0.19	0.37	0.26	0.21	0.31	0.27	0.19	0.36
3	Jasper County High Lead Smelter	0.65	0.47	0.89	0.56	0.42	0.75	0.58	0.43	0.79	0.65	0.52	0.82	0.61	0.43	0.79
	Jasper County Low Lead Yard	0.94	0.66	1.30	1.00	0.75	1.34	0.91	0.68	1.24	0.75	0.60	0.95	0.90	0.63	1.20
4	Murray Smelter Slag	0.47	0.33	0.67	0.51	0.33	0.88	0.31	0.22	0.46	0.31	0.23	0.41	0.40	0.23	0.64
	Jasper County High Lead Mill	0.84	0.58	1.21	0.86	0.54	1.47	0.70	0.50	1.02	0.89	0.69	1.18	0.82	0.51	1.14
5	Aspen Berm	0.69	0.54	0.87	0.87	0.58	1.39	0.73	0.46	1.26	0.67	0.51	0.89	0.74	0.48	1.08
	Aspen Residential	0.72	0.56	0.91	0.77	0.50	1.21	0.78	0.49	1.33	0.73	0.56	0.97	0.75	0.50	1.04
6	Midvale Slag	0.21	0.15	0.31	0.13	0.09	0.17	0.12	0.08	0.18	0.11	0.06	0.18	0.14	0.07	0.24
	Butte Soil	0.19	0.14	0.29	0.13	0.09	0.19	0.15	0.09	0.22	0.10	0.04	0.19	0.14	0.06	0.23
7	California Gulch Phase I Residential Soil	0.88	0.62	1.34	0.75	0.53	1.12	0.73	0.50	1.12	0.53	0.33	0.93	0.72	0.38	1.07
	California Gulch Fe/Mn PbO	1.16	0.83	1.76	0.99	0.69	1.46	1.25	0.88	1.91	0.80	0.51	1.40	1.05	0.57	1.56
8	California Gulch AV Slag	0.26	0.19	0.36	0.19	0.11	0.32	0.14	0.08	0.25	0.20	0.13	0.30	0.20	0.09	0.31
9	Palmerton Location 2	0.82	0.61	1.05	0.60	0.41	0.91	0.51	0.30	0.91	0.47	0.37	0.60	0.60	0.34	0.93
	Palmerton Location 4	0.62	0.47	0.80	0.53	0.37	0.79	0.41	0.25	0.72	0.40	0.32	0.52	0.49	0.29	0.72
11	Murray Smelter Soil	0.70	0.54	0.89	0.58	0.42	0.80	0.36	0.25	0.52	0.39	0.31	0.49	0.51	0.29	0.79
	NIST Paint	0.86	0.66	1.09	0.73	0.52	1.03	0.55	0.38	0.78	0.74	0.59	0.93	0.72	0.44	0.98
12	Galena-enriched Soil	0.01	0.00	0.02	0.02	0.00	0.04	0.01	0.00	0.02	0.01	-0.01	0.03	0.01	0.00	0.03
	California Gulch Oregon Gulch Tailings	0.07	0.04	0.13	0.11	0.04	0.21	0.05	0.02	0.09	0.01	-0.04	0.06	0.06	-0.01	0.15

LB = 5% Lower Confidence Bound
UB = 95% Upper Confidence Bound

TABLE ES-2 *IN VITRO* BIOACCESSIBILITY VALUES

Experiment	Test Material	Sample	In Vitro Bioaccessibility (%) (Mean ± Standard Deviation)
2	1	Bingham Creek Residential	47.0 ± 1.2
2	2	Bingham Creek Channel Soil	37.8 ± 0.7
3	1	Jasper County High Lead Smelter	69.3 ± 5.5
3	2	Jasper County Low Lead Yard	79.0 ± 5.6
4	1	Murray Smelter Slag	64.3 ± 7.3
4	2	Jasper County High Lead Mill	85.3 ± 0.2
5	1	Aspen Berm	64.9 ± 1.6
5	2	Aspen Residential	71.4 ± 2.0
6	1	Midvale Slag	17.4 ± 0.9
6	2	Butte Soil	22.3 ± 0.6
7	1	California Gulch Phase I Residential Soil	65.1 ± 1.5
7	2	California Gulch Fe/Mn PbO	87.2 ± 0.5
8	1	California Gulch AV Slag	9.4 ± 1.6
9	1	Palmerton Location 2	63.6 ± 0.4
9	2	Palmerton Location 4	69.7 ± 2.7
11	1	Murray Smelter Soil	74.7 ± 6.8
11	2	NIST Paint	72.5 ± 2.0
12	1	Galena-enriched Soil	4.5 ± 1.2
12	3	California Gulch Oregon Gulch Tailings	11.2 ± 0.9

FIGURE ES-1. RELATION BETWEEN RBA AND IVBA



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ACRONYMS AND ABBREVIATIONS

°C	Degrees Celsius
µg	Microgram
µm	Micrometer
ABA	Absolute bioavailability
AF_o	Oral absorption fraction
AIC	Akaike's Information Criterion
AUC	Area under the curve
cc	Cubic centimeter
CDC	Centers for Disease Control and Prevention
dL	Deciliter
g	Gram
GLP	Good Laboratory Practices
HCl	Hydrochloric acid
HDPE	High density polyethylene
ICP-AES	Inductively Coupled Plasma-Atomic Emission Spectrometry
ICP-MS	Inductively Coupled Plasma-Mass Spectrometry
IV	Intravenous
IVBA	*In vitro* bioaccessibility
kg	Kilogram
L	Liter
M	Molar
(M)	Metal
MDL	Method detection limit
mg	Milligram
mL	Milliliter
mm	Millimeter
NIST	National Institute of Standards and Testing
Pb	Lead
PbAc	Lead acetate
ppm	Parts per million
RBA	Relative bioavailability
RLM	Relative lead mass

ACRONYMS AND ABBREVIATIONS
(CONTINUED)

rpm	Revolutions per minute
SOP	Standard operating procedure
SRM	Standard Reference Material
TAL	Target Analyte List
TCLP	Toxicity Characteristic Leaching Procedure
U.S. EPA	U.S. Environmental Protection Agency

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ESTIMATION OF RELATIVE BIOAVAILABILITY OF LEAD IN SOIL AND SOIL-LIKE MATERIALS USING *IN VIVO* AND *IN VITRO* METHODS

1.0 INTRODUCTION

1.1 Overview

Reliable analysis of the potential hazard to children from ingestion of lead in the environment depends on accurate information on a number of key parameters, including 1) lead concentration in environmental media (*e.g.*, soil, dust, water, food, air, paint), 2) childhood intake rates of each medium, and 3) the rate and extent of lead absorption from each medium ("bioavailability"). Knowledge of lead bioavailability is important because the amount of lead which actually enters the body from an ingested medium depends on the physical-chemical properties of the lead and of the medium. For example, lead in soil may exist, at least in part, as poorly water-soluble minerals, and may also exist inside particles of inert matrix such as rock or slag of variable size, shape, and association. These chemical and physical properties may tend to influence (usually decrease) the absorption (bioavailability) of lead when ingested. Thus, equal ingested doses of different forms of lead in different media may not be of equal health concern.

Bioavailability of lead in a particular medium may be expressed either in absolute terms (absolute bioavailability) or in relative terms (relative bioavailability).

Absolute Bioavailability (ABA) is the ratio of the amount of lead absorbed compared to the amount ingested:

$$ABA = \frac{Absorbed\ Dose}{Ingested\ Dose}$$

This ratio is also referred to as the oral absorption fraction (AF_o).

Relative Bioavailability (RBA) is the ratio of the absolute bioavailability of lead present in some test material compared to the absolute bioavailability of lead in some appropriate reference material:

$$RBA = \frac{ABA_{test\ material}}{ABA_{reference\ material}}$$

Usually the form of lead used as reference material is a soluble compound such as lead acetate that is expected to completely dissolve when ingested.

For example, if 100 micrograms (μg) of lead dissolved in drinking water were ingested and a total of 50 μg entered the body, the ABA would be 50/100, or 0.50 (50%). Likewise, if

100 μg of lead contained in soil were ingested and 30 μg entered the body, the ABA for soil would be 30/100, or 0.30 (30%). If the lead dissolved in water were used as the frame of reference for describing the relative amount of lead absorbed from soil, the RBA would be 0.30/0.50, or 0.60 (60%).

For additional discussion about the concept and application of bioavailability, see Gibaldi and Perrier (1982), Goodman *et al*. (1990), Mushak (1991), and/or Klaassen *et al*. (1996).

1.2 Using Bioavailability Data to Improve Exposure Calculations for Lead

When reliable data are available on the bioavailability of lead in soil, dust, or other soil-like waste material at a site, this information can be used to improve the accuracy of exposure and risk calculations at that site. For example, the basic equation for estimating the site-specific ABA of a test soil is as follows:

$$ABA_{soil} = ABA_{soluble} \cdot RBA_{soil}$$

where:

ABA_{soil} = Absolute bioavailability of lead in soil ingested by a child

$ABA_{soluble}$ = Absolute bioavailability in children of some dissolved or fully soluble form of lead

RBA_{soil} = Relative bioavailability of lead in soil

Based on available information in the literature on lead absorption in humans, the U.S. EPA estimates that the absolute bioavailability of lead from water and the diet is usually about 50% in children (U.S. EPA, 1994). Thus, when a reliable site-specific RBA value for soil is available, it may be used to estimate a site-specific absolute bioavailability in that soil, as follows:

$$ABA_{soil} = 50\% \cdot RBA_{soil}$$

In the absence of site-specific data, the absolute absorption of lead from soil, dust, and other similar media is estimated by U.S. EPA to be about 30% (U.S. EPA, 1994). Thus, the default RBA used by U.S. EPA for lead in soil and dust compared to lead in water is 30%/50%, or 60%. When the measured RBA in soil or dust at a site is found to be less than 60% compared to some fully soluble form of lead, it may be concluded that exposures to and hazards from lead in these media at that site are probably lower than typical default assumptions. If the measured RBA is higher than 60%, absorption of and hazards from lead in these media may be higher than usually assumed.

1.3 Overview of U.S. EPA's Program to Study Lead Bioavailability in Animals

Scientists in U.S. EPA Region 8 have been engaged in a multi-year investigation of lead absorption from a variety of different environmental media, especially soils and solid wastes associated with mining, milling, and smelting sites. All studies in this program employed juvenile swine as the animal model. Juvenile swine were selected for use in these studies because they are considered to be a good physiological model for gastrointestinal absorption in children (see Appendix A).

Initial studies in the program (referred to as "Phase I") were performed by Dr. Robert Poppenga and Dr. Brad Thacker at Michigan State University (Weis *et al*., 1995). The Phase I study designs and protocols were refined and standardized by Dr. Stan Casteel and his colleagues at the University of Missouri, Columbia, and this group has performed a large number of studies (collectively referred to as "Phase II") designed to further characterize the swine model and to quantify lead absorption from a variety of different test materials. Section 2 of this report summarizes the Phase II work performed at the University of Missouri.

1.4 Overview of Methods for Estimating Lead RBA *In Vitro*

Measurement of lead RBA in animals has a number of potential benefits, but is also rather slow and costly and may not be a feasible option in all cases. It is mainly for these reasons that a number of scientists have been working to develop *in vitro* procedures that may provide faster and less costly alternatives for estimating the RBA of lead in soil or soil-like samples (Miller and Schricker, 1982; Imber, 1993; Ruby *et al*., 1993, 1996; Medlin, 1997; Rodriguez *et al*., 1999). These methods are based on the concept that the rate and/or extent of lead solubilization in the gastrointestinal fluid are likely to be important determinants of lead bioavailability *in vivo*, and most *in vitro* tests are aimed at measuring the rate or extent of lead solubilization from soil into an extraction solvent that resembles gastric fluid. To help avoid confusion in nomenclature, the fraction of lead which solubilizes in an *in vitro* system is referred to as **bioaccessibility**, while the fraction that is absorbed *in vivo* is referred to as **bioavailability**.

More recently, development and testing of a simplified *in vitro* method for estimating lead bioaccessibility has been performed by Dr. John Drexler at the University of Colorado. Section 3 of this report describes this *in vitro* method and presents the results.

2.0 *IN VIVO* STUDIES

2.1 Basic Approach for Measuring RBA *In Vivo*

The basic approach for measuring lead absorption *in vivo* is to administer an oral dose of lead to test animals and measure the increase in lead level in one or more body compartments (blood, soft tissue, bone). In order to calculate the RBA value of a test material, the increase in lead in a body compartment is measured both for that test material and a reference material (lead acetate). Equal absorbed doses of lead (as Pb^{+2}) are expected to produce approximately equal increases in concentration in tissues regardless of the source or nature of the ingested lead, so the RBA of a test material is calculated as the ratio of doses (test material and reference material) that produce equal increases in lead concentration in the body compartment. Note that this approach is general and yields reliable results for both non-linear and linear responses.

2.2 Animal Exposure and Sample Collection

All *in vivo* studies carried out during this program were performed as nearly as possible within the spirit and guidelines of Good Laboratory Practices (GLP: 40 CFR 792). Standard Operating Procedures (SOPs) for all of the methods are documented in a project notebook that is available through the administrative record.

Experimental Animals

All animals used in this program were intact male swine approximately 5 to 6 weeks of age. All animals were monitored to ensure they were in good health throughout the study.

Diet

In order to minimize lead exposure from the diet, animals were fed a special low-lead diet purchased from Zeigler Brothers, Inc. (Gardners, PA). The amount of feed provided was equal to 5% of the average body weight of animals on study. The feed was nutritionally complete and met all requirements of the National Institutes of Health–National Research Council (NRC, 1988). The typical nutritional components and chemical analysis of the feed are presented in Table 2-1. Periodic analysis of feed samples during this program indicated the mean lead level was less than 50 μg/kg, corresponding to a daily intake of less than 2.5 μg/kg-day.

Drinking water was provided *ad libitum* via self-activated watering nozzles within each cage. Periodic analysis of samples from randomly selected drinking water nozzles indicated the mean lead concentration was less than 2 μg/L, corresponding to a daily intake of less than 0.2 μg/kg-day.

Exposure

Appendix B provides the details of animal exposure, including the design (number of dose groups, number of animals, dosing material, and dose levels) for all of the Phase II studies. A typical study design is summarized in Table 2-2. In general, groups of animals were exposed to a series of doses of either lead acetate or test material. For convenience, in this report, lead acetate is abbreviated as "PbAc." Exposure occurred twice a day for 15 days. Most groups were exposed by oral administration, with one group usually exposed to lead acetate by intravenous (IV) injection via an indwelling venous catheter.

2.3 Preparation of Biological Samples for Analysis

Samples of blood were collected from each animal at multiple times during the course of a study (*e.g.*, days 0, 1, 2, 3, 4, 6, 9, 12, and 15). On day 15, the animals were sacrificed and samples of liver, kidney, and bone (femur) were collected.

Appendix C presents details of biological sample collection, preparation, and analysis. In brief, samples of blood were diluted in "matrix modifier," a solution recommended by the Centers for Disease Control and Prevention (CDC) for analysis of blood samples for lead (CDC, 2001). Samples of soft tissue (kidney, liver) were digested in hot acid, while samples of bone were ashed and then dissolved in acid.

Prepared samples were analyzed for lead using a Perkin Elmer Model 5100 graphite furnace atomic absorption spectrophotometer. All results from the analytical laboratory were reported in units of μg Pb/L of prepared sample. The detection limit was defined as three-times the standard deviation of a set of seven replicates of a low-lead sample (typically about 2 to 5 μg/L).

2.4 Data Reduction

The basic data reduction task required to calculate an RBA for a test material is to fit mathematical equations to the dose-response data for both the test material and the reference material, and then solve the equations to find the ratio of doses that would be expected to yield equal responses. After testing a variety of different equations, it was found that nearly all blood lead AUC data sets could be well-fit using an exponential equation, while most data sets for liver, kidney, and bone lead could be well-fit using a linear equation:

Linear: $$Response = a + b \cdot Dose \quad (1)$$

Exponential: $$Response = a + b \cdot \left[1 - \exp(-c \cdot Dose)\right] \quad (2)$$

where *a*, *b*, and *c* are the parameters of the models, and *Dose* is the total daily administered dose of lead (μg/kg-day).

Appendix D presents a detailed description of the curve-fitting methods and rationale, along with the methods used to quantify uncertainty in the RBA estimates for each test material. Detailed dose-response data and curve-fitting results are presented in Appendix E.

2.5 Results and Discussion

2.5.1 Effect of Dosing on Animal Health and Weight

Lead exposure levels employed in this program are substantially below those which cause clinical symptoms in swine, and no evidence of treatment-related toxicity was observed in any dose group. All animals exposed to lead by the oral route remained in good health throughout each study, and the only clinical signs observed were characteristic of normal swine. However, animals implanted with indwelling venous catheters (used for intravenous injections) were subject to infection, and a few animals became quite ill. This was a problem mainly at the start of the program and tended to diminish as experience was gained on the best surgical and prophylactic techniques for catheter implantation. When an animal became ill, if good health could not be restored by administration of antibiotics, the animal was promptly removed from the study.

All animals were weighed every three days during the course of each study. The rate of weight gain (kg/day) averaged across all Phase II studies is illustrated in Figure 2-1. As shown, animals typically gained about 0.3 to 0.5 kg/day, and the rate of weight gain was generally comparable in all groups.

2.5.2 Time Course of Blood Lead Response

The time course of the blood lead response to oral or intravenous exposure may be thought of on two different time scales: the short-term "spike" that occurs immediately following an exposure, and the longer-term trend toward "steady-state" blood lead following repeated exposures.

Initial studies performed during Phase I of this program revealed that a single oral dose of lead acetate causes blood lead levels rise to a peak about two hours post-ingestion, and then decrease over the course of 12 to 24 hours to a near steady-state value (Weis *et al*., 1993). Although knowledge of these rapid kinetics is important in fully understanding the toxicokinetics of lead, investigations in Phase II of this program focused mainly on quantifying the slower rise in "steady-state" blood lead following repeated exposures. To achieve this goal, all blood lead samples were collected 17 hours after lead exposure, at a time when the rate of change in blood lead due to the preceding dose is minimal.

Figure 2-2 presents an example graph of the time course of "steady-state" blood lead levels following repeated oral and intravenous exposure to lead acetate. As seen, blood lead levels begin below the detection limit (usually about 1 μg/dL) and stay very low in control animals throughout the course of the study. In animals exposed to lead acetate, blood lead values begin to rise within 1 to 2 days and tend to flatten out to a near steady-state in about 7 to 10 days.

2.5.3 Dose-Response Patterns

Figures 2-3 to 2-6 present the dose response patterns observed for blood, liver, kidney, and bone (femur) following repeated oral or intravenous exposure to lead acetate. For blood, the endpoint is the area under the blood lead vs time curve (AUC). For femur, kidney, and liver, the endpoint is the concentration in the tissue at the time of sacrifice. The data for intravenous exposure are based on a single study[1], while the patterns for oral exposure are based on the combined results across all studies performed during Phase II.

As seen, there is substantial variability in response between individuals (both within and between studies), and this variability tends to increase as dose (and response) increases. This pattern of increasing variance in response is referred to as heteroscedasticity, and is accounted for in the model-fitting procedure through the use of weighted least squares regression (see Appendix D). Despite the variability in response, the regression analyses indicate that the dose response pattern is typically non-linear for blood lead AUC following both oral and intravenous exposure, but is approximately linear in both cases for liver, kidney, and bone lead (see Table D1). This pattern of dose-response relationships suggests that, at least over the dose range tested in this program, absorption of lead from the gastrointestinal tract of swine is linear, and that the non-linearity observed in blood lead AUC response is due to some sort of saturable binding in the blood.

2.5.4 Estimation of ABA for Lead Acetate

Inspection of Figures 2-3 to 2-6 reveals that each of the measured responses to ingested lead acetate is smaller than the response for intravenously injected lead acetate. These data were used to calculate the absolute bioavailability of ingested lead acetate using the data reduction approach described in Section 2.4. The results are summarized below:

Measurement Endpoint	Estimated ABA of PbAc
Blood AUC	0.10 ± 0.02
Liver	0.16 ± 0.05
Kidney	0.19 ± 0.05
Femur	0.14 ± 0.03

Although the four different measurement endpoints do not agree precisely, it seems clear that the absolute bioavailability of lead acetate in juvenile swine is about 15% ± 4%. Although data are limited, results from balance studies in infants and young children (age 2 weeks to 8 years) suggest that lead absorption is probably about 42% to 53% (Alexander *et al*., 1974; Ziegler *et al*., 1978). If so, lead absorption in juvenile swine is apparently lower than for young

[1] Most studies in Phase II utilized only one intravenous dose level (100 μg/kg-day) and, hence, do not provide dose-response data. Study 8 included three intravenous exposure levels (25, 50, and 100 μg/kg-day); the data from this study are shown in Figures 2-3 to 2-6.

humans. Although the reason for this apparent difference is not known, it is important to note that even if swine do absorb less lead than children under similar dosing conditions, this does not invalidate the swine as an animal model for estimating relative bioavailability of lead in different test materials.

2.5.5 Estimation of RBA for Lead in Test Materials

Characterization of Test Materials

Table 2-3 describes the Phase II test materials for which RBA was measured in this program and provides the analytical results for lead. Data on other Target Analyte List (TAL) metals, if available, are provided in Appendix F. As seen, 17 different samples from eight different sites were investigated, along with one sample of paint flakes mixed with clean soil and one sample of finely-ground native galena mixed with clean soil. Prior to analysis and dosing, all samples were dried (<40°C) and sieved, and only materials which passed through a 60-mesh screen (corresponding to particles smaller than about 250 μm) were used. This range of particle sizes was selected because the U.S. EPA considers particles less than about 250 μm to be the most likely to adhere to the hands and be ingested by hand-to-mouth contact, especially in young children (U.S. EPA, 2000).

Each sample of test material that was evaluated in the swine bioassay program was thoroughly characterized with regard to mineral phase, particle size distribution, and matrix association using electron microprobe analysis. Detailed results for each test material are presented in Appendix F, and the results are summarized in Tables 2-4 to 2-6.

Table 2-4 lists the different lead phases observed in the test materials, and gives the relative lead mass (RLM) for each phase in each test material. The RLM is the estimated percentage of the total lead in a sample that is present in a particular phase. Of the 22 different phases detected in one or more samples, 9 are very minor, with RLM values no higher than 2% in any sample. However, 13 of the phases occur at concentrations that could contribute significantly to the overall bioavailability of the sample (RLM >10%). It should be noted that a particle is classified as “slag” only if the particle is glassy or vitreous in nature. Inclusions or other non-vitreous grains of lead-bearing material are classified according to their mineral content and are not classified as slag particles (even if they are observed in bulk samples that are referred to as “slag”).

Table 2-5 summarizes information on the degree to which lead-bearing grains in each sample are partially or entirely liberated (i.e., exposed to gastric fluids when ingested) or included (i.e., fully enclosed or encased in mineral or vitreous matrices). Data are presented both on a particle frequency basis and on the basis of relative lead mass. As seen, the majority of lead-bearing particles in most samples are partially or entirely liberated, although the tailings sample from Oregon Gulch is a clear exception.

Table 2-6 summarizes data on the distribution (frequency) of particle sizes (measured as the longest dimension) in each sample. For convenience, the data presented are for liberated particles only (Appendix F contains the data for all particles). As seen, most samples contain a

range of particle sizes, often with the majority of the particles being less than 50 μm. (Remember that all samples were sieved to isolate particles less than 250 μm before analysis.)

RBA Results for Test Materials

Detailed model fitting results and RBA calculations for each test material are presented in Appendix E and are summarized in Table 2-7.

As shown in Table 2-7, there are four independent estimates of RBA (based on blood AUC, liver, kidney, and bone) for each test material. Conceptually, each of these four values is an independent estimate of the RBA for the test material, so the estimates from all four endpoints need to be combined to yield a final point estimate for each test material. As discussed in Appendix D (Section 4.7), an analysis of the relative statistical reliability of each endpoint (as reflected in the average coefficient of variation in RBA values derived from each endpoint) suggests that the four endpoint-specific RBA values are all approximately equally reliable. Based on this, the point estimate for a test material is the simple average across the four endpoint-specific RBA values. The resulting point estimate values are presented in the far right portion of Table 2-7. Uncertainty bounds around the point estimates were derived as described in Appendix D (Section 4.7).

Inspection of these point estimates for the different test materials reveals that there is a wide range of values across different samples, both within and across sites. For example, at the California Gulch site in Colorado, RBA estimates for different types of material range from about 6% (Oregon Gulch tailings) to about 105% (Fe/Mn lead oxide sample). This wide variability highlights the importance of obtaining and applying reliable RBA data to site-specific samples in order help to improve risk assessments for lead exposure.

2.5.6 Effect of Food

Studies in humans indicate that lead absorption is reduced by the presence of food in the stomach (Garber and Wei, 1974; U.S. EPA, 1996). The mechanism by which the presence of food leads to decreased absorption is not certain, but may be related to competition between lead and calcium for active and/or passive uptake sites in the gastrointestinal epithelium (Diamond, 2000). Because of the potential inhibitory effects of food, all of the studies performed during this program were designed to estimate the RBA of lead associated with a fasting state, each dose being administered to animals no less than six hours after the last feeding. In order to investigate how the presence of food in the stomach might influence absorption, a study was performed to measure the absorption of lead acetate given two hours before feeding and compare that to the absorption of lead acetate given either at the time of feeding or two hours after feeding. The results, expressed using the absorption two hours before feeding as the frame of reference, are summarized below:

Measurement Endpoint	Ratio of PbAc Absorption Given With Food Compared to PbAc Given Without Food	Ratio of PbAc Absorption Given 2 Hours After Feeding Compared to PbAc Given Without Food
Blood Lead AUC	0.39 ± 0.05	0.40 ± 0.06
Liver Lead	0.86 ± 0.24	0.58 ± 0.16
Kidney Lead	0.72 ± 0.26	0.73 ± 0.27
Bone Lead	0.35 ± 0.05	0.33 ± 0.05
Point Estimate	0.58 ± 0.28	0.51 ± 0.22

These findings indicate that uptake of lead is reduced by close to half (RBA point estimates are 51% and 58%) when the lead is administered to animals along with food compared to when it is administered on an empty stomach. This effect appears to endure for at least two hours after feeding, which is consistent with the results of a gastric emptying time study in juvenile swine that indicated that food is held in the stomach for up to four hours after eating (Casteel *et al*., 1998).

This study, which utilized lead acetate only, does not provide information about the effect of food on the absorption of lead ingested in a solid form such as soil. However, it is suspected that the magnitude of the decrease in absorption caused by food is likely to be at least as large as that observed for lead acetate, and perhaps even larger. This is because food may influence not only the absorption of soluble lead ions, but might also tend to decrease the rate and extent of lead solubilization from soil by tending to increase the pH of gastric fluids.

2.5.7 Correlation of RBA with Mineral Phase

In principle, each unique combination of phase, size, and matrix association constitutes a unique mineralogical form of lead, and each unique form could be associated with a unique RBA that is the inherent value for that "type" of lead. If so, then the concentrated-weighted average RBA value for a sample containing a mixture of different types of lead is given by:

$$RBA_{sample} = \sum_{i=1}^{n} \sum_{j=1}^{s} \sum_{k=1}^{m} C_{i,j,k} \cdot RBA_{i,j,k} \qquad (3)$$

where:

RBA_{sample} = Observed RBA of lead in a sample
$C_{i,j,k}$ = Fraction of total lead in phase i of size j and matrix association k
$RBA_{i,j,k}$ = RBA of lead in phase i of size j and matrix association k
n = Number of different lead phase categories
s = Number of different size categories
m = Number of different matrix association categories

If the number of different lead phases which can exist in the environment is on the order of 20, the number of size categories is on the order of five, and the number of matrix association categories is two (included and liberated), then the total number of different "types" of lead is on the order of 200. Because measured RBA data are available from this study for only 19 different samples, it is clearly impossible (with the present data set) to estimate type-specific RBA values for each combination of phase, size, and matrix association. Therefore, in order to simplify the analysis process, it was assumed that the measured RBA value for a sample was dominated by the liberated mineral phases present, and the effect of included materials or of particle size were not considered. That is, the data were analyzed according to the following model:

$$RBA_{sample} = \sum_{i=1}^{n} C_{i,liberated} \cdot RBA_{i,liberated} \quad (4)$$

Because 22 different phases were identified and only 19 different samples were analyzed, it was necessary to reduce the number of phases to a smaller number so that regression analysis could be performed. Therefore, the different phases were grouped into ten categories as shown in Table 2-8. These groups were based on professional judgment regarding the expected degree of similarity between members of a group, along with information on the relative abundance of each phase (see Table 2-4).

The total lead mass in each group was calculated by summing the relative lead mass for each individual component in the group. As noted above, only the lead mass in partially or entirely liberated particles was included in the sum.

Group-specific RBA values were estimated by fitting the grouped data to the model (equation 4) using minimization of squared errors. Two different options were employed. In the first option, each parameter (group-specific RBA) was fully constrained to be between zero and one, inclusive. In the second option, each parameter was partially constrained to be greater than or equal to zero. Because Group 10 contains only phases which are present in relatively low levels, an arbitrary coefficient of 0.5 was assumed for this group and the coefficient was not treated as a fitting parameter.

The resulting estimates of the group-specific RBA values are shown in Figure 2-7. As seen, there is a wide range of group-specific RBA values, with equal results being obtained by both methods of constraint. It is important to stress that these group-specific RBA estimates are derived from a very limited data set (nine independent parameter estimates based on only 19 different measurements), so the group-specific RBA estimates are inherently uncertain. In addition, both the measured sample RBA values and the relative lead mass in each phase are subject to additional uncertainty. Therefore, the group-specific RBA estimates should not be considered to be highly precise, and calculation of a quantitative sample-specific RBA value from these estimates is <u>not</u> appropriate. Rather, it is more appropriate to consider the results of this study as sufficient to support only semi-quantitative rank-order classification of phase-specific RBA values, as follows:

Low Bioavailability (RBA <0.25)	Medium Bioavailability (RBA = 0.25-0.75)	High Bioavailability (RBA >0.75)
Fe(M) Sulfate Anglesite Galena Fe(M) Oxide Pb(M) Oxide	Lead Oxide Lead Phosphate	Cerussite Mn(M) Oxide

(M) = Metal

As noted above, the estimates apply only to particles that are liberated, not those that are included.

2.5.8 Quality Assurance

A number of steps were taken throughout each of the studies in this program to assess and document the quality of the data that were collected. These steps are summarized below.

Duplicates

A randomly selected set of about 5% of all blood and tissue samples generated during each study were submitted to the laboratory in a blind fashion for duplicate analysis. Figure 2-8 plots the results for blood (Panel A) and for liver, kidney, and bone (Panel B). As seen, there was good intra-laboratory reproducibility between duplicate samples for both blood and tissues, with both linear regression lines having a slope near 1.0, an intercept near zero, and an R^2 value near 1.00.

Standards

The CDC provides blood lead "check samples" that may be used for use in quality assurance programs for blood lead studies. Three types of check samples (nominal concentrations of 1.7 μg/dL, 4.8 μg/dL, and 14.9 μg/dL) were used in these studies. Each day that blood samples were collected from experimental animals, several check samples of different concentrations were also prepared and submitted for analysis in random order and in a blind fashion. The results (averaged across all studies) are plotted in Figure 2-9. As seen, the analytical results obtained for the check samples were generally in good agreement with the expected value at all three concentrations, with an overall mean of 1.4 μg/L for the low standards (nominal concentration of 1.7 μg/L), 4.3 μg/L for the middle standard (nominal concentration of 4.8 μg/L), and 14.5 μg/L for the high standards (nominal concentration of 14.9 μg/L).

Interlaboratory Comparison

In each study, an interlaboratory comparison of blood lead analytical results was performed by sending a set of about 15 to 20 randomly selected whole blood samples to CDC for blind independent preparation and analysis. The results are plotted in Figure 2-10. As seen, the results of analyses by U.S. EPA's laboratory are generally similar to those of CDC, with a mean inter-sample difference (U.S. EPA minus CDC) of 0.07 μg/dL. The slope of the best-fit straight line through the data is 0.84, indicating that the concentration values estimated by the U.S. EPA

laboratories tended to be about 15% lower than those estimated by CDC. The reason for this apparent discrepancy between the U.S. EPA laboratory and the CDC laboratory is not clear, but might be related to differences in sample preparation techniques. Regardless of the reason, the differences are sufficiently small that they are likely to have no significant effect on calculated RBA values. In particular, it is important to realize that if both the lead acetate and test material dose-response curves are biased by the same factor, then the biases cancel in the calculation of the ratio.

Reproducibility of RBA Estimates

As with any study involving animals, there may be substantial variability between animals within each dose group, and there may also be variability in observed responses to exposure across different studies. Because each study involved administration of a standard series of doses of lead acetate, the data for lead acetate can be used to assess the stability and reproducibility of the swine model. Table 2-9 lists the best-fit parameters for the best-fit curves for oral lead acetate dose responses for blood AUC, liver, kidney, and bone in each study, and for all studies combined. As seen, the variability (expressed as the between-study coefficient of variation) is generally on the order of 25 to 50% for the *b* and *c* parameters, with somewhat higher variability in the intercept parameter (*a*). This degree of between-study variability is not unexpected for a study in animals and emphasizes the need for generating the dose-response curve for the reference material within each study. The source of the between-study variation is likely to be mainly a consequence of variation in animals between different groups (different dams, different ages, different weights), although a possible contribution from other variables (time of year, laboratory personnel, etc.) cannot be excluded.

Because RBA calculations are based on the within-study ratio of responses between a test material and reference material, the variability in response between studies may be at least partly cancelled in the calculation of the RBA. The most direct way to test this hypothesis is to compare RBA estimates for the same material that has been tested in two different studies. To date, only two test materials have been tested more than once. The results are shown in Table 2-10 and are summarized below.

For the Palmerton Location 2 sample (tested twice in Phase II), agreement is moderately good between the two studies for the blood AUC and kidney endpoints and for the point estimate, although there is relatively low agreement for the liver and bone endpoints. For the Residential Soil Composite from the California Gulch Superfund site (tested once by the University of Michigan during Phase I and again by the University of Missouri during Phase II), agreement is good for all four endpoints, with between-study differences of less than 20%. These differences are generally similar to the within-study confidence bounds, which are typically in the 10% to 20% range. Taken together, these studies support the view that the *in vivo* RBA assay has acceptable inter-study and inter-laboratory reproducibility.

3.0 *IN VITRO* STUDIES

3.1 Introduction

Measurement of lead RBA in animals using the approach described above has a number of potential benefits, but is also rather slow and costly and may not be feasible in all cases. It is mainly for this reason that a number of scientists have been working to develop alternative *in vitro* procedures that may provide a faster and less costly alternative for estimating the RBA of lead in soil or soil-like samples. These methods are based on the concept that the rate and/or extent of lead solubilization in gastrointestinal fluid is likely to be an important determinant of lead bioavailability *in vivo*, and most *in vitro* tests are aimed at measurement of the rate or extent of lead solubilization in an extraction solvent that resembles gastric fluid. The fraction of lead which solubilizes in an *in vitro* system is referred to as *in vitro* bioaccessibility (IVBA), which may then be used as an indicator of *in vivo* RBA.

Background on the development and validation of *in vitro* methods for estimating lead bioaccessibility can be found in Imber (1993), Ruby *et al*. (1993, 1996), and Medlin (1997).

3.2 *In Vitro* Method

The method described in this report represents a simplification from most preceding approaches. The method was designed to be fast, easy, and reproducible, and some test conditions were adjusted to yield results that best correlated with *in vivo* measurements of lead bioavailability. The detailed standard operating procedure (SOP) is presented below; additional information on this procedure may be obtained from http://www.colorado.edu/geolsci/legs.

3.2.1 Sample Preparation

All test materials tested in the bioaccessibility protocol were identical to the test materials administered to swine in the *in vivo* studies described above. As noted previously, soils were prepared by drying (<40°C) and sieving to <250 μm. The <250-μm size fraction was used because this particle size is representative of that which adheres to children's hands. Samples were thoroughly mixed prior to use to ensure homogenization. All samples were archived after the study completion and retained for further analysis for a period of six months.

3.2.2 Apparatus

The main piece of equipment used for this procedure is the extraction device shown in Figure 3-1. An electric motor (the same motor as is used in the Toxicity Characteristic Leaching Procedure, or TCLP) drives a flywheel, which in turn drives a Plexiglass block situated inside a temperature-controlled water bath. The Plexiglass block contains ten 5-centimeter holes with stainless steel screw clamps, each of which is designed to hold a 125-mL wide-mouth high density polyethylene (HDPE) bottle. The water bath was filled such that the extraction bottles were completely immersed. Temperature in the water bath was maintained at 37±2 °C using an immersion circulator heater. The 125-mL HDPE bottles had air-tight screw-cap seals, and care

was taken to ensure that the bottles did not leak during the extraction procedure. All equipment was properly cleaned, acid washed, and rinsed with deionized water prior to use.

3.2.3 Selection of IVBA Test Conditions

The dissolution of lead from a test material into the extraction fluid depends on a number of variables including extraction fluid composition, temperature, time, agitation, solid/fluid ratio, and pH. These parameters were evaluated to determine the optimum values for maximizing sensitivity, stability, and the correlation between *in vitro* and *in vivo* values.

All reagents were free of lead and the final fluid was tested to confirm that lead concentrations were less than one-fourth the project required detection limit (PRDL) of 10 μg/L (i.e., less than 2 μg/L lead in the final fluid). Cleanliness of all materials used to prepare and/or store the extraction fluid and buffer is essential; all glassware and equipment used to prepare standards and reagents were properly cleaned, acid washed, and triple-rinsed with deionized water prior to use.

Extraction Fluid: The extraction fluid selected for this procedure was 0.4 M glycine (free base, reagent grade glycine in deionized water), adjusted to a pH of 1.50±0.05 at 37°C using trace metal grade concentrated hydrochloric acid (HCl). Most previous *in vitro* test systems have employed a more complex fluid intended to simulate gastric fluid. For example, Medlin (1997) used a fluid that contained pepsin and a mixture of citric, malic, lactic, acetic, and hydrochloric acids. When the bioaccessibility of a series of test substances were compared using 0.4 M glycine buffer (pH 1.5) with and without the inclusion of these enzymes and metabolic acids, no significant difference was observed ($p=0.196$). This indicates that the simplified buffer employed in the procedure is appropriate, even though it lacks some constituents known to be present in gastric fluid.

Temperature: In order to evaluate the effect of the extraction temperature, seventeen substrates were analyzed (generally in triplicate) at both 37°C and 20°C. The results are shown in Figure 3-2 (Panel A). In some cases, temperature had little effect, but in three cases the amount of lead solubilized was more than 20% greater at 37°C than at 20°C, and in two cases it was more than 20% less. Because the results appeared to depend on temperature in at least some cases, a temperature of 37°C was selected because this is approximately the temperature of gastric fluid *in vivo*.

Extraction Time: The time that ingested material is present in the stomach (i.e., stomach-emptying time) is about one hour for a child, particularly when a fasted state is assumed (see Appendix A). To investigate the effect of extraction time on lead solubilization, 11 substrates were extracted for periods of 1, 2, or 4 hours. The results are shown in Figure 3-2 (Panel B). As seen, in most cases, the amount of lead solubilized was approximately constant over time, with only one substrate (test material 6) showing a variation that exceeded the method precision. Therefore, an extraction time of one hour was selected for the final method. In a subsequent test (data not shown), it was found that allowing the bottles to stand at room temperature for up to 4 hours after rotation at 37°C caused no significant variation (<10%) in lead concentration.

pH: Human gastric pH values tend to range from about 1 to 4 during fasting (see Appendix A). Previous studies have used stomach phase pH values between 1.0 and 2.5 for their *in vitro* experiments (Ruby *et al*., 1993; CBR, 1993; Gasser *et al*., 1996; Buckley, 1997; Medlin, 1997; Rodriguez *et al*., 1999; Mercier *et al*., 2000). To evaluate the effect of pH on lead bioaccessibility, 24 substrates were analyzed at pH values of 1.5, 2.5, or 3.5. As shown in Figure 3-2 (Panel C), the amount of lead solubilized is strongly pH-dependent, with the highest extraction at pH 1.5. For the subset of test materials for which *in vivo* RBA had been estimated at that time (N = 13), the empiric correlation between IVBA and *in vivo* RBA was slightly better at pH 1.5 (rho = 0.919) than at pH 2.5 (rho = 0.881). Thus, a pH of 1.5 was selected for use in the final protocol.

Agitation: If the test material is allowed to accumulate at the bottom of the extraction apparatus, the effective surface area of contact between the extraction fluid and the test material may be reduced, and this may influence the extent of lead solubilization. Depending on which theory of dissolution is relevant (Nernst and Brunner, 1904, or Dankwerts, 1951), agitation will greatly affect either the diffusion layer thickness or the rate of production of fresh surface. Previous workers have noted problems associated with both stirring and argon bubbling methods (Medlin and Drexler, 1995; Drexler, 1997). Although no systematic comparison of agitation methods was performed, an end-over-end method of agitation was chosen to best simulate the complex peristaltic motion of the gastrointestinal system.

Solid/Fluid Ratio and Mass of Test Material: A solid to fluid ratio of 1/100 (mass per unit volume) was chosen to reduce the effects of metal dissolution that were noted by Sorenson *et al*. (1971) when lower ratios (1/5 and 1/25) were used. Tests using Standard Reference Materials showed no significant variation (within ± 1% of control means) in the fraction of lead extracted with soil masses as low as 0.2 gram (g) per 100 mL. However, use of low masses of test material could introduce variability due to small scale heterogeneity in the sample and/or to weighing errors. Therefore, the final method employs 1.0 g of test material in 100 mL of extraction fluid.

In special cases, the mass of test material may need to be less than 1.0 g to avoid the potential for saturation of the extraction solution. Tests performed using lead acetate, lead oxide, and lead carbonate indicate that if the bulk concentration of a test material containing these relatively soluble forms of lead exceeds approximately 50,000 ppm, the extraction fluid becomes saturated at 37°C and, upon cooling to room temperature and below, lead chloride crystals will precipitate. To prevent this from occurring, the concentration of lead in the test material should not exceed 50,000 ppm, or the mass of the test material should be reduced to 0.50±0.01g.

3.2.4 Summary of Final Leaching Protocol

The extraction procedure began by placing 1.00±0.05 g of sieved test material and 100±0.5 mL of the buffered extraction fluid (0.4 M glycine, pH 1.5) into a 125-mL wide-mouth HDPE bottle. Care was taken to ensure that static electricity did not cause soil particles to adhere to the lip or outside threads of the bottle; if necessary, an antistatic brush was used to eliminate static electricity prior to adding the test substrate. The bottle was tightly sealed and then shaken or inverted to ensure that there was no leakage and that no soil was caked on the bottom of the bottle.

Each bottle was placed into the modified TCLP extractor (water temperature 37±2°C). Samples were extracted by rotating the samples end-over-end at 30 ± 2 rpm for 1 hour. After 1 hour, the bottles were removed, dried, and placed upright on the bench top to allow the soil to settle to the bottom. A 15-mL sample of supernatant fluid is removed directly from the extraction bottle into a disposable 20-cc syringe. After withdrawal of the sample into the syringe, a Luer-Lok attachment fitted with a 0.45-μm cellulose acetate disk filter (25 mm diameter) is attached, and the 15 mL aliquot of fluid is filtered through the attachment to remove any particulate matter. This filtered sample of extraction fluid is then analyzed for lead, as described below. If the total time elapsed for the extraction process exceeds 90 minutes, the test must be repeated.

As noted above, in some cases (mainly slags), the test material can increase the pH of the extraction buffer, and this could influence the results of the bioaccessibility measurement. To guard against this, the pH of the fluid was measured at the end of the extraction step (just after a sample was withdrawn for filtration and analysis). If the pH was not within 0.5 pH units of the starting pH (1.5), the sample was re-analyzed. If the second test also resulted in an increase in pH of greater than 0.5 units, it was apparent that the test material was buffering the solution. In these cases, the test was repeated using manual pH adjustment during the extraction process, stopping the extraction at 5, 10, 15, and 30 minutes and manually adjusting the pH down to pH 1.5 at each interval by drop-wise addition of HCl.

3.2.5 Analysis of Extraction Fluid for Lead

The filtered samples of extraction fluid were stored in a refrigerator at 4°C until they were analyzed (within 1 week of extraction). Once received by the laboratory, all media were maintained under standard chain-of-custody. The samples were analyzed for lead by ICP-AES or ICP-MS (U.S. EPA Method 6010 or 6020, U.S. EPA 1986). The method detection limit (MDL) in extraction fluid was calculated to be around 19 μg/L for Method 6010 and typically 0.1-0.3 μg/L for Method 6020.

3.2.6 Quality Control/Quality Assurance

Quality assurance for the extraction procedure consisted of the following quality control samples:

- Reagent Blank — extraction fluid analyzed once per batch.
- Bottle Blank — extraction fluid only (no test soil) run through the complete procedure at a frequency of 1 in 20 samples (minimum of 1 per batch).
- Blank Spike — extraction fluid spiked at 10 mg/L lead, and run through the complete procedure at a frequency of 1 in 20 samples (minimum of 1 per batch).
- Matrix Spikes — a subsample of each material used for duplicate analyses was used as a matrix spike. The spike was prepared at 10 mg/L lead and run through the extraction procedure at a frequency of 1 in 10 samples (minimum of 1 per batch).

- Duplicate Sample — duplicate sample extractions were performed on 1 in 10 samples (minimum of 1 per batch).

- Control Soil — National Institute of Standards and Testing (NIST) Standard Reference Material (SRM) 2711 (Montana Soil) was used as a control soil. The SRM was analyzed at a frequency of 1 in 20 samples (minimum 1 per batch).

Control limits for these quality control samples were as follows:

Analysis	Frequency	Control Limits
Reagent blank	once per batch	<25 µg/L lead
Bottle blank	5%*	<50 µg/L lead
Blank spike (10 mg/L)	5%*	85-115% recovery
Matrix spike (10 mg/L)	10%*	75-125% recovery
Duplicate sample	10%*	±20% RPD
Control soil (NIST 2711)	5%*	±10% RPD

RPD = Relative percent difference
*Minimum of once per batch

To evaluate the precision of the *in vitro* bioaccessibility extraction protocol, approximately 67 replicate analyses of both NIST SRM 2710 and 2711 were conducted over a period of several months. Results are shown in Figure 3-3. As seen, both standards yield highly reproducible results, with a mean coefficient of variation of about 6%.

3.3 Results and Discussion

3.3.1 IVBA Values

Table 3-1 summarizes the *in vitro* bioaccessibility results for the set of 19 different test materials evaluated under the Phase II program. Each value is the mean and standard deviation of three independent measurements performed at the University of Colorado at Boulder.

Figure 3-4 shows the results of an inter-laboratory comparison of results for these test materials. The participating laboratories included ACZ Laboratories Inc.; University of Colorado at Boulder; U.S. Bureau of Reclamation Environmental Research Chemistry Laboratory; and National Exposure Research Laboratory. As seen in the figure, within-laboratory variability (as shown by the error bars) is quite small (average ≤2%) and there is very good agreement between laboratories (average difference of 2 to 3%, range of difference from 1 to 9%).

3.3.2 Comparison with In Vivo Results

In order for an *in vitro* bioaccessibility test system to be useful in predicting the *in vivo* RBA of a test material, it is necessary to establish empirically that a strong correlation exists between the *in vivo* and the *in vitro* results across many different samples. A scatter plot of the *in vivo* RBA and *in vitro* bioaccessibility data from this program is shown in Figure 3-5. The Spearman rank order correlation coefficient between the paired RBA and IVBA point estimates is 0.896 ($p < 0.001$) and the Pearson product moment correlation coefficient is 0.917 ($p < 0.001$), indicating that there is a statistically significant positive correlation between IVBA and RBA.

Several different mathematical models were tested to describe the relation between RBA and IVBA, including linear, power, and exponential. All fitting was done using weighted least square regression, as detailed in Appendix D. The results are summarized below:

Model	R^2	AIC
Linear: $RBA = a + b \cdot IVBA$	0.924	-30.46
Power: $RBA = a + b \cdot IVBA^c$	0.931	-29.92
2-Parameter Exponential: $RBA = a + b \cdot \exp(IVBA)$	0.936	-33.02
3-Parameter Exponential: $RBA = a + b \cdot \exp(c \cdot IVBA)$	0.936	-31.11

As seen, all of the models fit the data reasonably well, with the two exponential models fitting slightly better than the linear model. However, as discussed in Appendix D, the difference in quality of fit between linear and exponential models is not judged to be meaningful, and the linear model is selected as the preferred model at present. As more data become available in the future, the relationship between IVBA and RBA will be reassessed and the best-fit model form will be reconsidered and revised if needed.

Because there is measurement error not only in RBA but also in IVBA, linear fitting was also performed taking the error in both RBA and IVBA into account. There was nearly no difference in fit, so the results of the weighted linear regression were selected for simplicity. This decision may be revisited as more data become available. Based on this decision, the currently preferred model is:

$$RBA = 0.878 \cdot IVBA - 0.028$$

It is important to recognize that use of this equation to calculate RBA from a given IVBA measurement will yield the "typical" RBA value expected for a test material with that IVBA, and the true RBA may be somewhat different (either higher or lower). The best fit line and the 95% prediction interval for this data set are shown in Figure 3-6.

Applicability of the IVBA-RBA Model

At present, it appears that the equation relating IVBA to RBA should be widely applicable, having been found to hold true for a wide range of different soil types and lead

phases from a variety of different sites. However, most of the samples tested have been collected from mining and milling sites, and it is plausible that some forms of lead that do not occur at this type of site might not follow the observed correlation. Thus, whenever a sample containing an unusual and/or untested lead phase is evaluated by the IVBA protocol, this should be identified as a potential source of uncertainty. In the future, as additional samples with a variety of new and different lead forms are tested by both *in vivo* and *in vitro* methods, the applicability of the method will be more clearly defined.

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TABLES

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TABLE 2-1. TYPICAL FEED COMPOSITION

Nutrient Name	Amount
Protein	20.1021%
Arginine	1.2070%
Lysine	1.4690%
Methionine	0.8370%
Met+Cys	0.5876%
Tryptophan	0.2770%
Histidine	0.5580%
Leucine	1.8160%
Isoleucine	1.1310%
Phenylalanine	1.1050%
Phe+Tyr	2.0500%
Threonine	0.8200%
Valine	1.1910%
Fat	4.4440%
Saturated Fat	0.5590%
Unsaturated Fat	3.7410%
Linoleic 18:2:6	1.9350%
Linoleic 18:3:3	0.0430%
Crude Fiber	3.8035%
Ash	4.3347%
Calcium	0.8675%
Phos Total	0.7736%
Available Phosphorous	0.7005%
Sodium	0.2448%
Potassium	0.3733%

Nutrient Name	Amount
Chlorine	0.1911%
Magnesium	0.0533%
Sulfur	0.0339%
Manganese	20.4719 ppm
Zinc	118.0608 ppm
Iron	135.3710 ppm
Copper	8.1062 ppm
Cobalt	0.0110 ppm
Iodine	0.2075 ppm
Selenium	0.3196 ppm
Nitrogen Free Extract	60.2340%
Vitamin A	5.1892 kIU/kg
Vitamin D3	0.6486 kIU/kg
Vitamin E	87.2080 IU/kg
Vitamin K	0.9089 ppm
Thiamine	9.1681 ppm
Riboflavin	10.2290 ppm
Niacin	30.1147 ppm
Pantothenic Acid	19.1250 ppm
Choline	1019.8600 ppm
Pyridoxine	8.2302 ppm
Folacin	2.0476 ppm
Biotin	0.2038 ppm
Vitamin B12	23.4416 ppm

Feed obtained from and nutritional values provided by Zeigler Bros., Inc

TABLE 2-2. TYPICAL *IN VIVO* STUDY DESIGN

Dose Group	Dose Material	Exposure Route	Target Dose µg Pb/kg-day	Number of Animals
1	None	Oral	--	2-5
2	Lead Acetate	Oral	25	5
3			75	5
4			225	5
5	Test Material 1	Oral	75	5
6			225	5
7			625	5
8	Test Material 2	Oral	75	5
9			225	5
10			625	5
11	Lead Acetate	Intravenous	100	5-8

TABLE 2-3. DESCRIPTION OF PHASE II TEST MATERIALS

Experiment	Sample Designation	Site	Sample Description	Lead Concentration (ppm) [1]
2	Bingham Creek Residential	Kennecott NPL Site, Salt Lake City, Utah	Soil composite of samples containing less than 2500 ppm lead; collected from a residential area (Jordan View Estates) located along Bingham Creek in the community of West Jordan, Utah.	1,590
	Bingham Creek Channel Soil	Kennecott NPL Site, Salt Lake City, Utah	Soil composite of samples containing 3000 ppm or greater of lead; collected from a residential area (Jordan View Estates) located along Bingham Creek in the community of West Jordan, Utah.	6,330
3	Jasper County High Lead Smelter	Jasper County, Missouri Superfund Site	Soil composite collected from an on-site location.	10,800
	Jasper County Low Lead Yard	Jasper County, Missouri Superfund Site	Soil composite collected from an on-site location.	4,050
4	Murray Smelter Slag	Murray Smelter Superfund Site, Murray City, Utah	Composite of samples collected from areas where exposed slag existed on site.	11,700
	Jasper County High Lead Mill	Jasper County, Missouri Superfund Site	Soil composite collected from an on-site location.	6,940
5	Aspen Berm	Smuggler Mountain NPL Site, Aspen, Colorado	Composite of samples collected from the Racquet Club property (including a parking lot and a vacant lot).	14,200
	Aspen Residential	Smuggler Mountain NPL Site, Aspen, Colorado	Composite of samples collected from residential properties within the study area.	3,870
6	Midvale Slag	Midvale Slag NPL Site, Midvale, Utah	Composite of samples collected from a water-quenched slag pile in Midvale Slag Operable Unit 2.	8,170
	Butte Soil	Silver Bow Creek/Butte Area NPL Site, Butte, Montana	Soil composite collected from waste rock dumps in Butte Priority Soils Operable Unit (BPSOU).	8,530
7	California Gulch Phase I Residential Soil	California Gulch NPL Site, Leadville, Colorado	Soil composite collected from residential properties within Leadville.	7,510
	California Gulch Fe/Mn PbO	California Gulch NPL Site, Leadville, Colorado	Soil composite collected from near the Lake Fork Trailer Park located southwest of Leadville near the Arkansas River.	4,320
8	California Gulch AV Slag	California Gulch NPL Site, Leadville, Colorado	Sample collected from a water-quenched slag pile on the property of the former Arkansas Valley (AV) Smelter, located just west of Leadville.	10,600
9	Palmerton Location 2	New Jersey Zinc NPL Site, Palmerton, Pennsylvania	Soil composite collected from on-site.	3,230
	Palmerton Location 4	New Jersey Zinc NPL Site, Palmerton, Pennsylvania	Soil composite collected from on-site.	2,150
11	Murray Smelter Soil	Murray Smelter Superfund Site, Murray City, Utah	Soil composite collected from on-site.	3,200
	NIST Paint	--	A mixture of approximately 5.8% NIST Standard Reference Material (SRM) 2589 and 94.2% low lead soil (< 50 ppm) collected in Leadville, Colorado. NIST SRM 2589, composed of paint collected from the interior surfaces of houses in the US, contains a nominal lead concentration of 10% (100,000 ppm); the material is powdered with more than 99% of the material being less than 100 um in size.	8,350
12	Galena-enriched Soil	--	A mixture of approximately 1.2% galena and 98.8% low lead soil (< 50 ppm) that was collected in Leadville, Colorado. The added galena consisted of a mineralogical (i.e., native) crystal of pure galena that was ground and sieved to obtain fine particles smaller than about 65 um.	11,200
	California Gulch Oregon Gulch Tailings	California Gulch NPL Site, Leadville, Colorado	A composite of tailings samples collected from the Oregon Gulch tailings impoundment.	1,270

[1] Samples were analyzed for lead by inductively coupled plasma-atomic emission spectrometry (ICP-AES) in accord with USEPA Method 200.7.

TABLE 2-4. RELATIVE LEAD MASS OF MINERAL PHASES OBSERVED IN TEST MATERIALS

Experiment:	2		3		4		5		6		7		8	9		11		12	
Phase	Bingham Creek Residential	Bingham Creek Channel Soil	Jasper County High Lead Smelter	Jasper County Low Lead Yard	Murray Smelter Slag	Jasper County High Lead Mill	Aspen Berm	Aspen Residential	Midvale Slag	Butte Soil	Cal. Gulch Phase I Residential Soil	Cal. Gulch Fe/Mn PbO	Cal. Gulch AV Slag	Palmerton Location 2	Palmerton Location 4	Murray Smelter Soil	NIST Paint	Galena-enriched Soil	Cal. Gulch Oregon Gulch Tailings
Anglesite		28%	1%	0.5%	1.0%	2%	7%	1%		36%	10%		2%	6%	4%		1%		
As(M)O																0.003%			
Calcite			0.2%			0.1%													
Cerussite	2%	0.3%	32%	81%	1.1%	57%	62%	64%	4%	0.3%	20%		1%			14%	55%		
Clay			0.018%	0.003%		0.017%	0.1%			0.1%		0.01%		0.03%	0.13%				
Fe-Pb Oxide	6%	3%	14%	2%	2%	10%	9%	7%	0.3%	7%	6%	8%	51%	2%	2%	0.13%			
Fe-Pb Sulfate	22%	30%	3%	1%	0.3%	1%	5%	5%	0.1%	20%	6%	3%	0.3%	1%		0.6%			
Galena		9%		8%	9%	3%	12%	17%	6%	12%	2%		3%			20%		100%	100%
Lead Barite		0.04%				0.01%	0.06%			0.007%	0.15%	0.14%		1%	0.1%				
Lead Organic		0.3%					0.03%	0.03%			0.11%	0.11%	1%						
Lead Oxide			0.09%		69%	7%										27%	44%		
Lead Phosphate	50%	26%	21%	6%		7%	1%	1%		3.6%	30%	15%		24%	1%				
Lead Silicate				0.04%		0.5%					1.9%	0.8%			1.4%				
Lead Vanidate											0.1%	0.4%			18%				
Mn-Pb Oxide	18%	2%	2%	2%	0.8%	9%	4%	5%		20.2%	22%	72%		66%	66%				
Native Lead			22%		0.7%	2%			15%										
Pb(M)O					4%				26%						7%	3%			
Pb-As Oxide	2%	1%		0.15%	6%				33%		0.1%		31%			29%			
PbO-Cerussite											1%								
Slag			4%		7%	1%			16%		1%		10%			6%			
Sulfosalts									0.4%										
Zn-Pb Silicate					0.03%										2%				

(M) = Metal

TABLE 2-5. MATRIX ASSOCIATIONS FOR TEST MATERIALS

Experiment	Test Material	Particle Frequency		Relative Lead Mass	
		Liberated	Included	Liberated	Included
2	Bingham Creek Residential	100%	0%	100%	0%
	Bingham Creek Channel Soil	100%	0%	100%	0%
3	Jasper County High Lead Smelter	81%	19%	76%	24%
	Jasper County Low Lead Yard	100%	0%	94%	6%
4	Murray Smelter Slag	87%	13%	77%	23%
	Jasper County High Lead Mill	96%	4%	93%	7%
5	Aspen Berm	86%	14%	93%	8%
	Aspen Residential	98%	2%	94%	6%
6	Midvale Slag	91%	9%	77%	23%
	Butte Soil	91%	9%	91%	9%
7	California Gulch Phase I Residential Soil	79%	21%	65%	35%
	California Gulch Fe/Mn PbO	98%	2%	100%	0%
8	California Gulch AV Slag	78%	22%	80%	20%
9	Palmerton Location 2	100%	0%	100%	0%
	Palmerton Location 4	79%	21%	89%	11%
11	Murray Smelter Soil	80%	20%	70%	30%
	NIST Paint	100%	0%	100%	0%
12	Galena-enriched Soil	100%	0%	100%	0%
	California Gulch Oregon Gulch Tailings	2%	98%	5%	95%

TABLE 2-6. PARTICLE SIZE DISTRIBUTIONS FOR TEST MATERIALS

Experiment	Test Material	Particle Size (µm)								
		<5	5-9	10-19	20-49	50-99	100-149	150-199	200-249	>250
2	Bingham Creek Residential	38%	22%	19%	16%	4%	2%	0%	0%	0%
	Bingham Creek Channel Soil	66%	13.6%	10%	6.1%	3%	1%	0%	0%	0%
3	Jasper County High Lead Smelter	44%	19%	8%	8%	9%	9%	2%	1%	1%
	Jasper County Low Lead Yard	29%	20%	21%	20%	8%	3%	0%	0%	0%
4	Murray Smelter Slag	14%	13%	15%	6%	20%	24%	4%	3%	0%
	Jasper County High Lead Mill	23%	21%	22%	19%	9%	6%	1%	1%	0%
5	Aspen Berm	27%	19%	22%	17%	8%	6%	1%	1%	0%
	Aspen Residential	38%	35%	12%	8%	4%	2%	0%	0%	0%
6	Midvale Slag	6%	1%	3%	4%	20%	29%	18%	13%	5%
	Butte Soil	23%	15%	14%	23%	14%	9%	2%	1%	0%
7	California Gulch Phase I Residential Soil	24%	9%	18%	22%	15%	9%	1%	1%	1%
	California Gulch Fe/Mn PbO	26%	19%	24%	17%	10%	4%	0%	0%	0%
8	California Gulch AV Slag	19%	8%	8%	5%	9%	19%	10%	13%	9%
9	Palmerton Location 2	26%	23%	25%	18%	6%	1%	0%	0%	0%
	Palmerton Location 4	25%	15%	21%	25%	13%	2%	0%	0%	0%
11	Murray Smelter Soil	23%	10%	29%	17%	6%	8%	3%	3%	1%
	NIST Paint	76%	4%	6%	8%	6%	0%	0%	0%	0%
12	Galena-enriched Soil	48%	2%	4%	41%	4%	0%	0%	0%	0%
	California Gulch Oregon Gulch Tailings	85%	8%	6%	0%	0%	0%	0%	0%	0%

TABLE 2-7. ESTIMATED RBA VALUES FOR TEST MATERIALS

Experiment	Test Material	Blood AUC			Liver			Kidney			Femur			Point Estimate		
		RBA	LB	UB	RBA	LB	UB	RBA	LB	UB	RBA	LB	UB	RBA	LB	UB
2	Bingham Creek Residential	0.34	0.23	0.50	0.28	0.20	0.39	0.22	0.15	0.31	0.24	0.19	0.29	0.27	0.17	0.40
	Bingham Creek Channel Soil	0.30	0.20	0.45	0.24	0.17	0.34	0.27	0.19	0.37	0.26	0.21	0.31	0.27	0.19	0.36
3	Jasper County High Lead Smelter	0.65	0.47	0.89	0.56	0.42	0.75	0.58	0.43	0.79	0.65	0.52	0.82	0.61	0.43	0.79
	Jasper County Low Lead Yard	0.94	0.66	1.30	1.00	0.75	1.34	0.91	0.68	1.24	0.75	0.60	0.95	0.90	0.63	1.20
4	Murray Smelter Slag	0.47	0.33	0.67	0.51	0.33	0.88	0.31	0.22	0.46	0.31	0.23	0.41	0.40	0.23	0.64
	Jasper County High Lead Mill	0.84	0.58	1.21	0.86	0.54	1.47	0.70	0.50	1.02	0.89	0.69	1.18	0.82	0.51	1.14
5	Aspen Berm	0.69	0.54	0.87	0.87	0.58	1.39	0.73	0.46	1.26	0.67	0.51	0.89	0.74	0.48	1.08
	Aspen Residential	0.72	0.56	0.91	0.77	0.50	1.21	0.78	0.49	1.33	0.73	0.56	0.97	0.75	0.50	1.04
6	Midvale Slag	0.21	0.15	0.31	0.13	0.09	0.17	0.12	0.08	0.18	0.11	0.06	0.18	0.14	0.07	0.24
	Butte Soil	0.19	0.14	0.29	0.13	0.09	0.19	0.15	0.09	0.22	0.10	0.04	0.19	0.14	0.06	0.23
7	California Gulch Phase I Residential Soil	0.88	0.62	1.34	0.75	0.53	1.12	0.73	0.50	1.12	0.53	0.33	0.93	0.72	0.38	1.07
	California Gulch Fe/Mn PbO	1.16	0.83	1.76	0.99	0.69	1.46	1.25	0.88	1.91	0.80	0.51	1.40	1.05	0.57	1.56
8	California Gulch AV Slag	0.26	0.19	0.36	0.19	0.11	0.32	0.14	0.08	0.25	0.20	0.13	0.30	0.20	0.09	0.31
9	Palmerton Location 2	0.82	0.61	1.05	0.60	0.41	0.91	0.51	0.30	0.91	0.47	0.37	0.60	0.60	0.34	0.93
	Palmerton Location 4	0.62	0.47	0.80	0.53	0.37	0.79	0.41	0.25	0.72	0.40	0.32	0.52	0.49	0.29	0.72
11	Murray Smelter Soil	0.70	0.54	0.89	0.58	0.42	0.80	0.36	0.25	0.52	0.39	0.31	0.49	0.51	0.29	0.79
	NIST Paint	0.86	0.66	1.09	0.73	0.52	1.03	0.55	0.38	0.78	0.74	0.59	0.93	0.72	0.44	0.98
12	Galena-enriched Soil	0.01	0.00	0.02	0.02	0.00	0.04	0.01	0.00	0.02	0.01	-0.01	0.03	0.01	0.00	0.03
	California Gulch Oregon Gulch Tailings	0.07	0.04	0.13	0.11	0.04	0.21	0.05	0.02	0.09	0.01	-0.04	0.06	0.06	-0.01	0.15

LB = 5% Lower Confidence Bound
UB = 95% Upper Confidence Bound

TABLE 2-8. GROUPED LEAD PHASES

Group	Group Name	Phase Constituents
1	Galena	Galena (PbS)
2	Cerussite	Cerussite
3	Mn(M) Oxide	Mn-Pb Oxide
4	Lead Oxide	Lead Oxide
5	Fe(M) Oxide	Fe-Pb Oxide (including Fe-Pb Silicate) Zn-Pb Silicate
6	Lead Phosphate	Lead Phosphate
7	Anglesite	Anglesite
8	Pb(M) Oxide	As(M)O Lead Silicate Lead Vanidate Pb(M)O Pb-As Oxide
9	Fe(M) Sulfate	Fe-Pb Sulfate Sulfosalts
10	Minor Constituents	Calcite Clay Lead Barite Lead Organic Native Lead PbO-Cerussite Slag

(M) = Metal

TABLE 2-9. CURVE FITTING PARAMETERS FOR ORAL LEAD ACETATE DOSE-RESPONSE CURVES

Experiment	Blood AUC			Liver Lead		Kidney Lead		Bone Lead	
	a	b	c	a	b	a	b	a	b
2	13.6	116	0.0084	63	2.0	44	2.4	0.7	0.084
3	8.3	163	0.0040	10	2.3	10	2.2	1.8	0.062
4	8.5	144	0.0064	57	1.7	68	2.8	0.5	0.076
5	8.0	163	0.0038	62	2.0	60	1.8	0.5	0.062
6	8.4	85	0.0101	23	2.0	15	2.1	0.4	0.043
7	--[a]	--[a]	--[a]	10	1.7	10	1.4	0.8	0.059
8	8.0	159	0.0032	11	2.1	17	2.4	0.8	0.065
9	7.5	96	0.0087	11	2.3	14	2.3	0.6	0.071
11	7.2	160	0.0035	14	1.3	20	1.7	0.7	0.053
12	7.6	169	0.0040	9	0.7	8	1.1	0.6	0.032
Mean	8.6	140	0.0058	27	1.8	27	2.0	0.7	0.061
Standard Deviation	1.9	32	0.0026	24	0.5	22	0.5	0.4	0.015
Coefficient of Variation	23%	23%	46%	88%	27%	84%	26%	55%	25%

Basic Equations:

Blood AUC = a + b*(1-exp(-c*Dose))

a = baseline blood lead value in unexposed animals

b = maximum increase in steady-state blood lead cause by exposure

c = "shape" parameter that determines how steeply the response increases as dose increases

Tissue concentration (bone, liver, kidney) = a + b*Dose

a = baseline blood lead value in unexposed animals

b = slope of the increase in tissue content per unit increase in dose

Coefficient of Variation = Standard Deviation / Mean

[a] Experiment 7 Blood AUC: No stable solution was obtained using the exponential model.

TABLE 2-10. REPRODUCIBILITY OF RBA MEASUREMENTS

RBA Estimate	Palmerton Location 2		California Gulch Phase I Residential Soil	
	Test 1 (Phase 2 Study 9)	Test 2 (Phase 2 Study 12)	Test 1* (Phase 1 Study 2)	Test 2 (Phase 2 Study 7)
Blood AUC	0.82 ± 0.12	0.71 ± 0.09	0.69	0.88 ± 0.19
Liver	0.60 ± 0.14	1.25 ± 0.32	0.58	0.75 ± 0.16
Kidney	0.51 ± 0.16	0.54 ± 0.13	0.62	0.73 ± 0.17
Bone	0.47 ± 0.07	0.95 ± 0.18	0.50	0.53 ± 0.15
Point Estimate	0.60 ± 0.18	0.86 ± 0.33	0.60	0.72 ± 0.21

*Calculated using ordinary least squares.

TABLE 3-1. *IN VITRO* BIOACCESSIBILITY VALUES

Experiment	Test Material	Sample	In Vitro Bioaccessibility (%) (Mean ± Standard Deviation)
2	1	Bingham Creek Residential	47.0 ± 1.2
2	2	Bingham Creek Channel Soil	37.8 ± 0.7
3	1	Jasper County High Lead Smelter	69.3 ± 5.5
3	2	Jasper County Low Lead Yard	79.0 ± 5.6
4	1	Murray Smelter Slag	64.3 ± 7.3
4	2	Jasper County High Lead Mill	85.3 ± 0.2
5	1	Aspen Berm	64.9 ± 1.6
5	2	Aspen Residential	71.4 ± 2.0
6	1	Midvale Slag	17.4 ± 0.9
6	2	Butte Soil	22.3 ± 0.6
7	1	California Gulch Phase I Residential Soil	65.1 ± 1.5
7	2	California Gulch Fe/Mn PbO	87.2 ± 0.5
8	1	California Gulch AV Slag	9.4 ± 1.6
9	1	Palmerton Location 2	63.6 ± 0.4
9	2	Palmerton Location 4	69.7 ± 2.7
11	1	Murray Smelter Soil	74.7 ± 6.8
11	2	NIST Paint	72.5 ± 2.0
12	1	Galena-enriched Soil	4.5 ± 1.2
12	3	California Gulch Oregon Gulch Tailings	11.2 ± 0.9

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FIGURES

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FIGURE 2-1. AVERAGE RATE OF BODY WEIGHT GAIN IN TEST ANIMALS

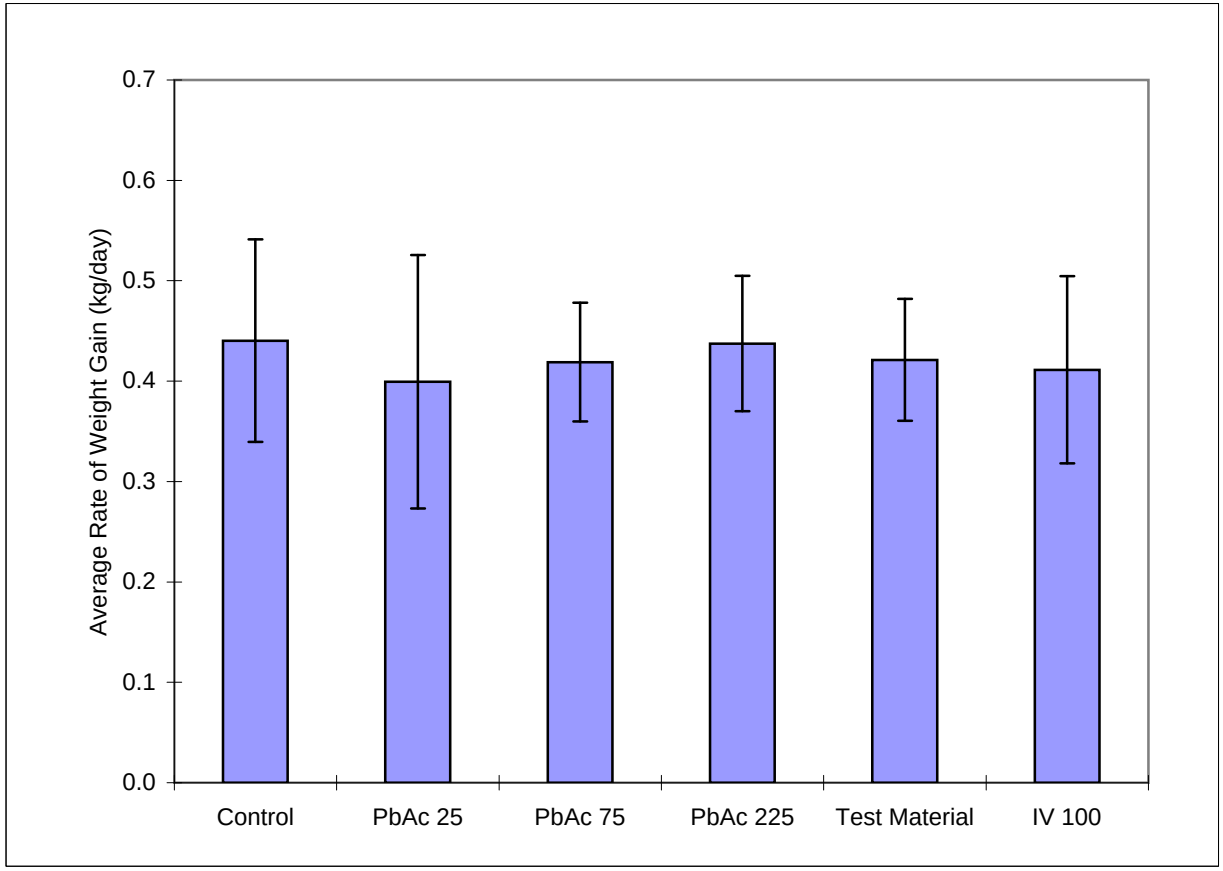


FIGURE 2-2. EXAMPLE TIME COURSE OF BLOOD LEAD RESPONSE

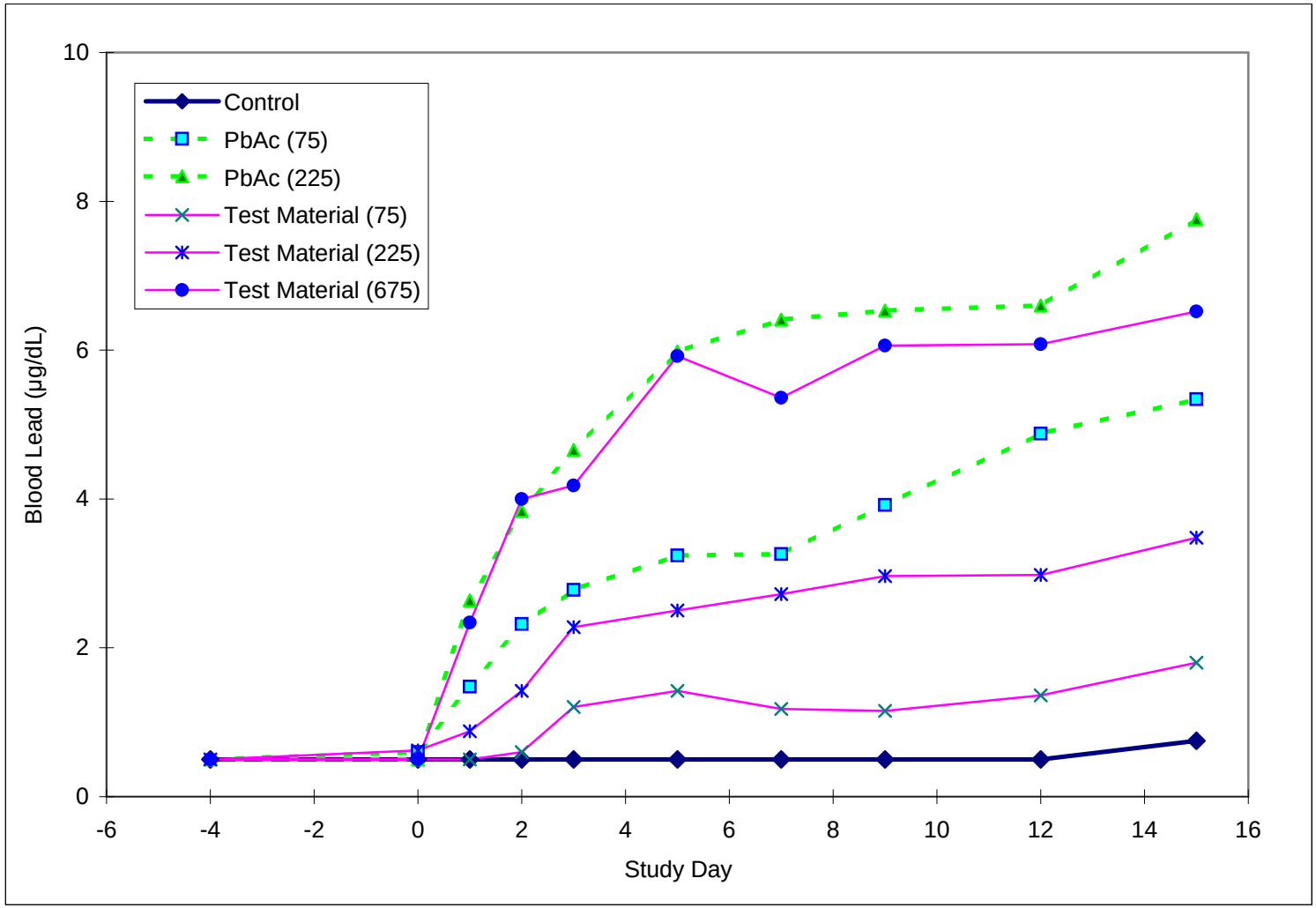


Fig 2-2 to 2-6 Dose Resp (outliers excl).xls

FIGURE 2-3. DOSE RESPONSE CURVE FOR BLOOD LEAD AUC

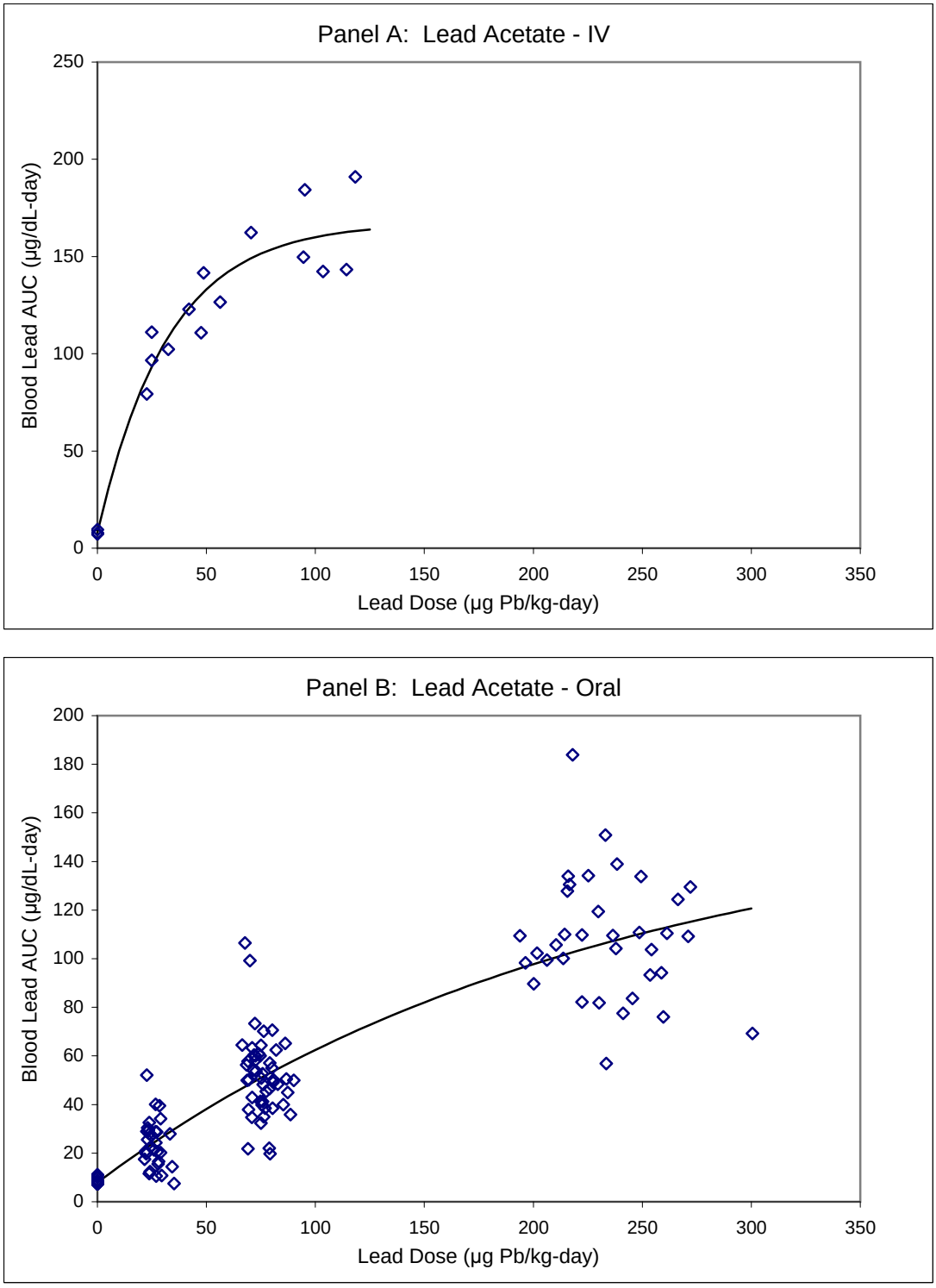
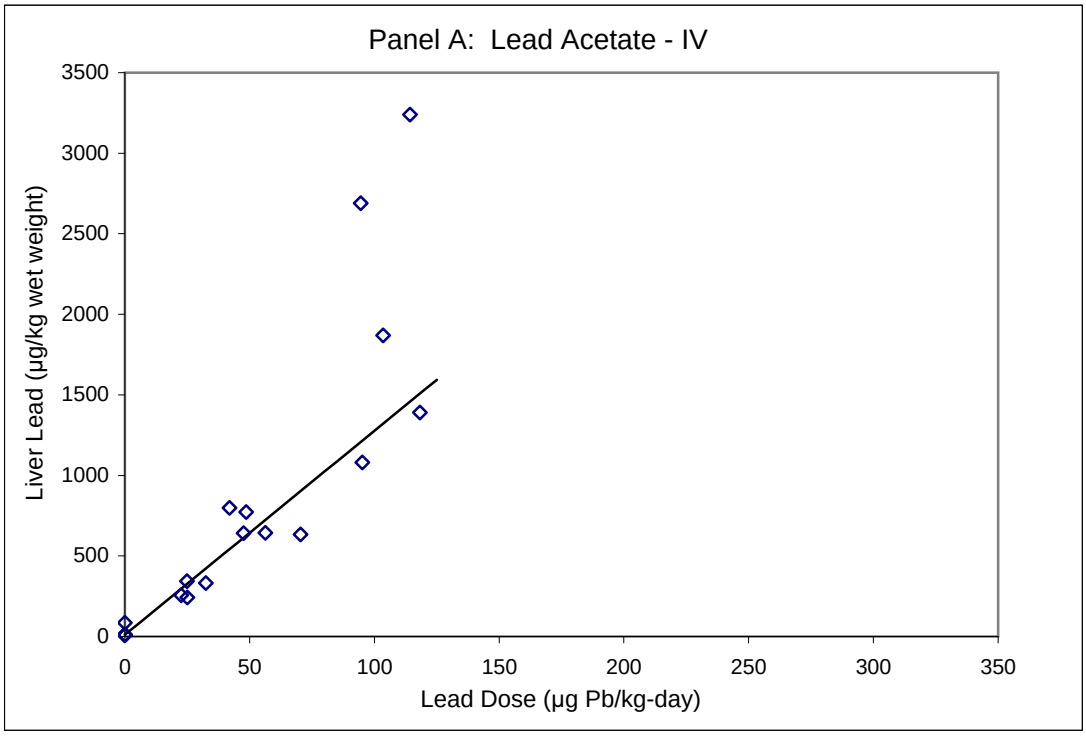


Fig 2-2 to 2-6 Dose Resp (outliers excl).xls (Fig 2-3_AUC)

FIGURE 2-4. DOSE RESPONSE CURVE FOR LIVER LEAD CONCENTRATION



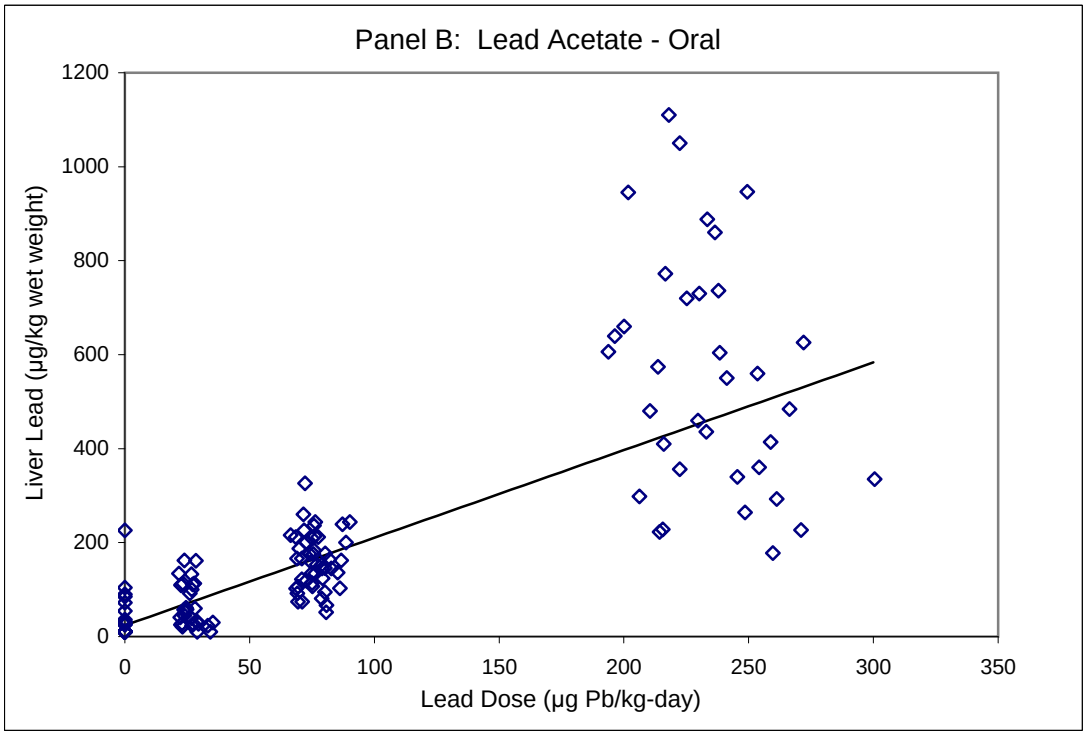
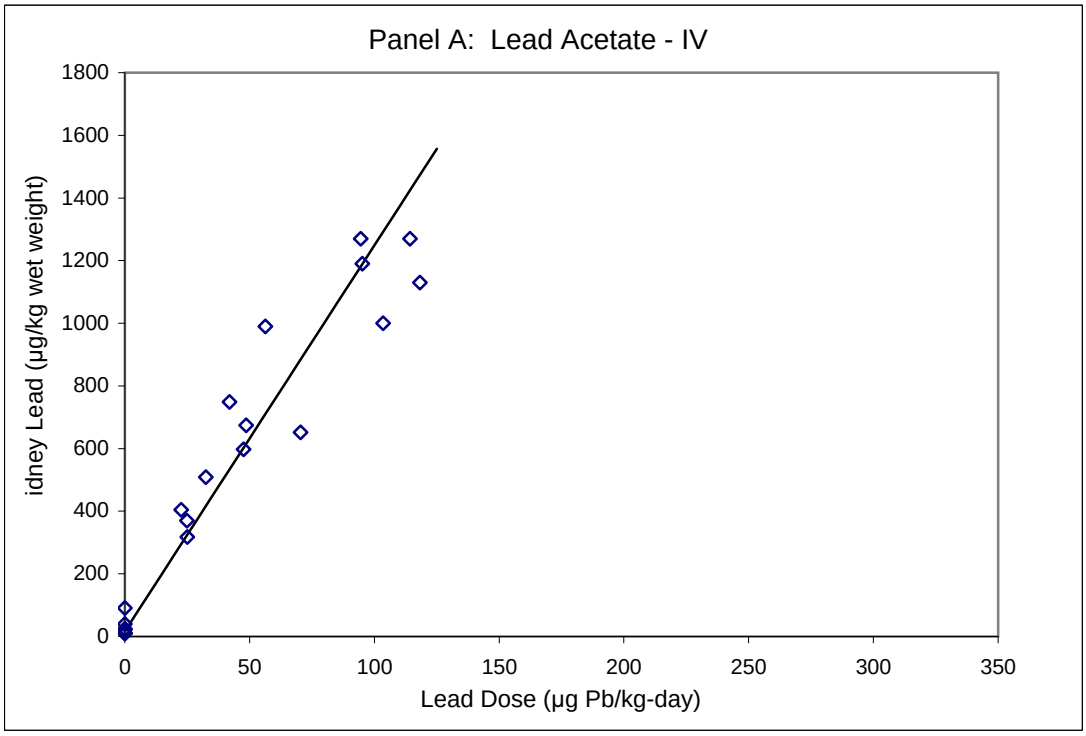


Fig 2-2 to 2-6 Dose Resp (outliers excl).xls (Fig 2-4_Liver)

FIGURE 2-5. DOSE RESPONSE CURVE FOR KIDNEY LEAD CONCENTRATION



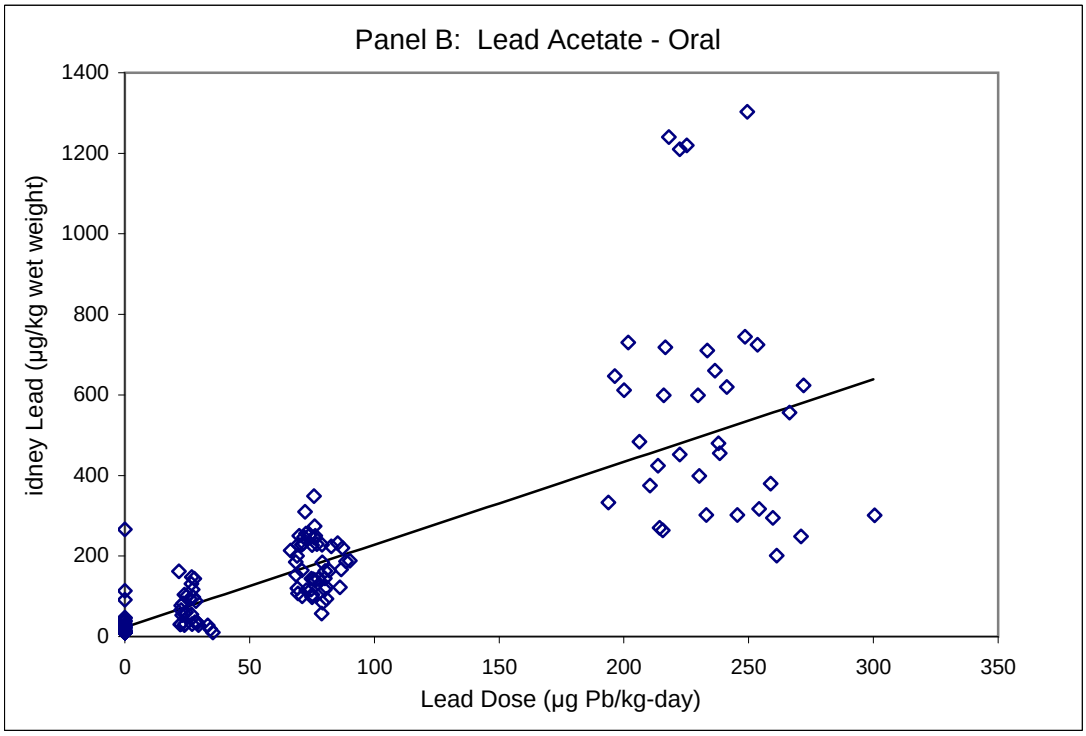


Fig 2-2 to 2-6 Dose Resp (outliers excl).xls (Fig 2-5_ idney)

FIGURE 2-6. DOSE RESPONSE CURVE FOR FEMUR LEAD CONCENTRATION

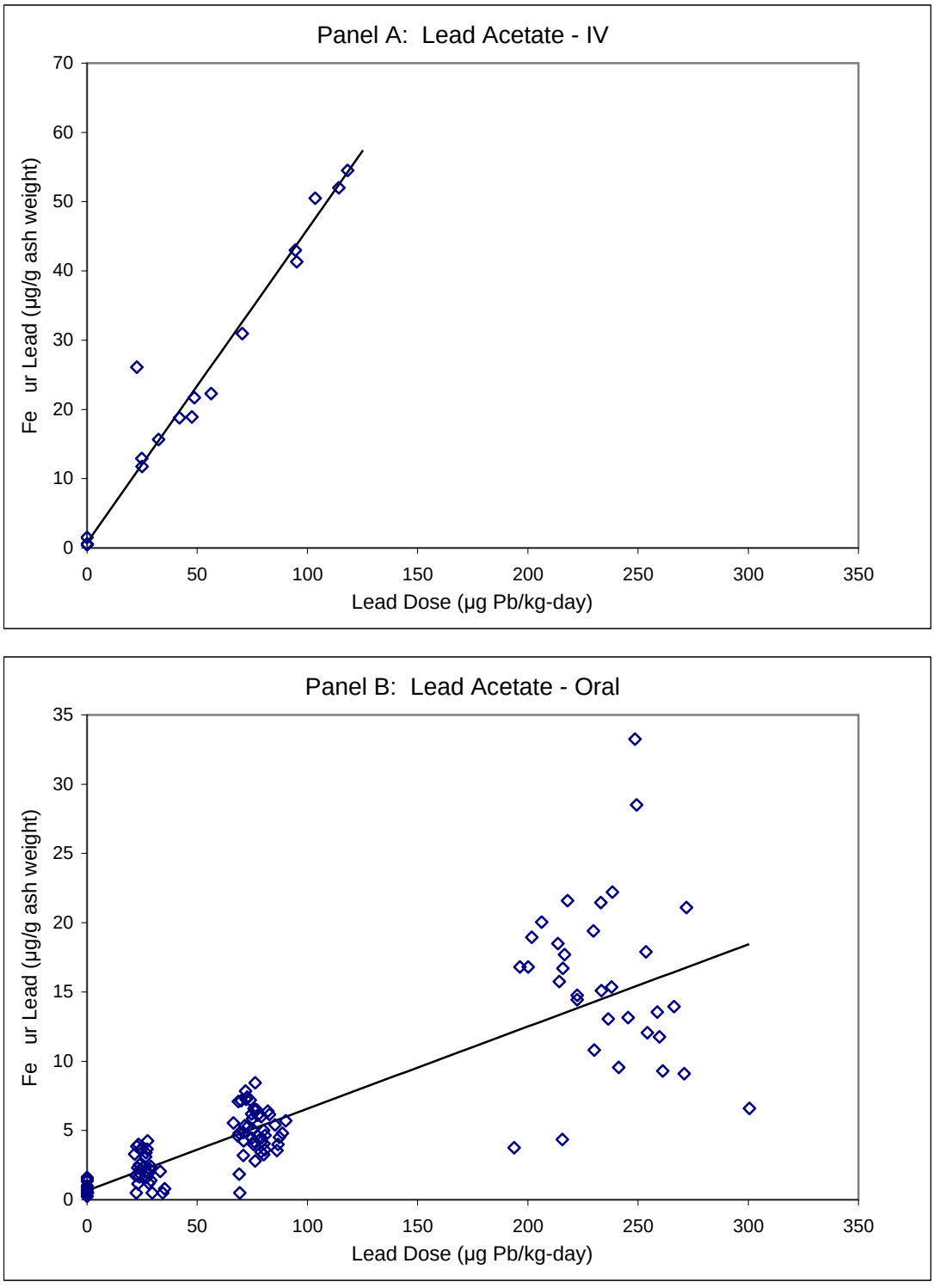


Fig 2-2 to 2-6 Dose Resp (outliers excl).xls (Fig 2-6_Fe ur)

FIGURE 2-7. ESTIMATED GROUP-SPECIFIC RBA VALUES

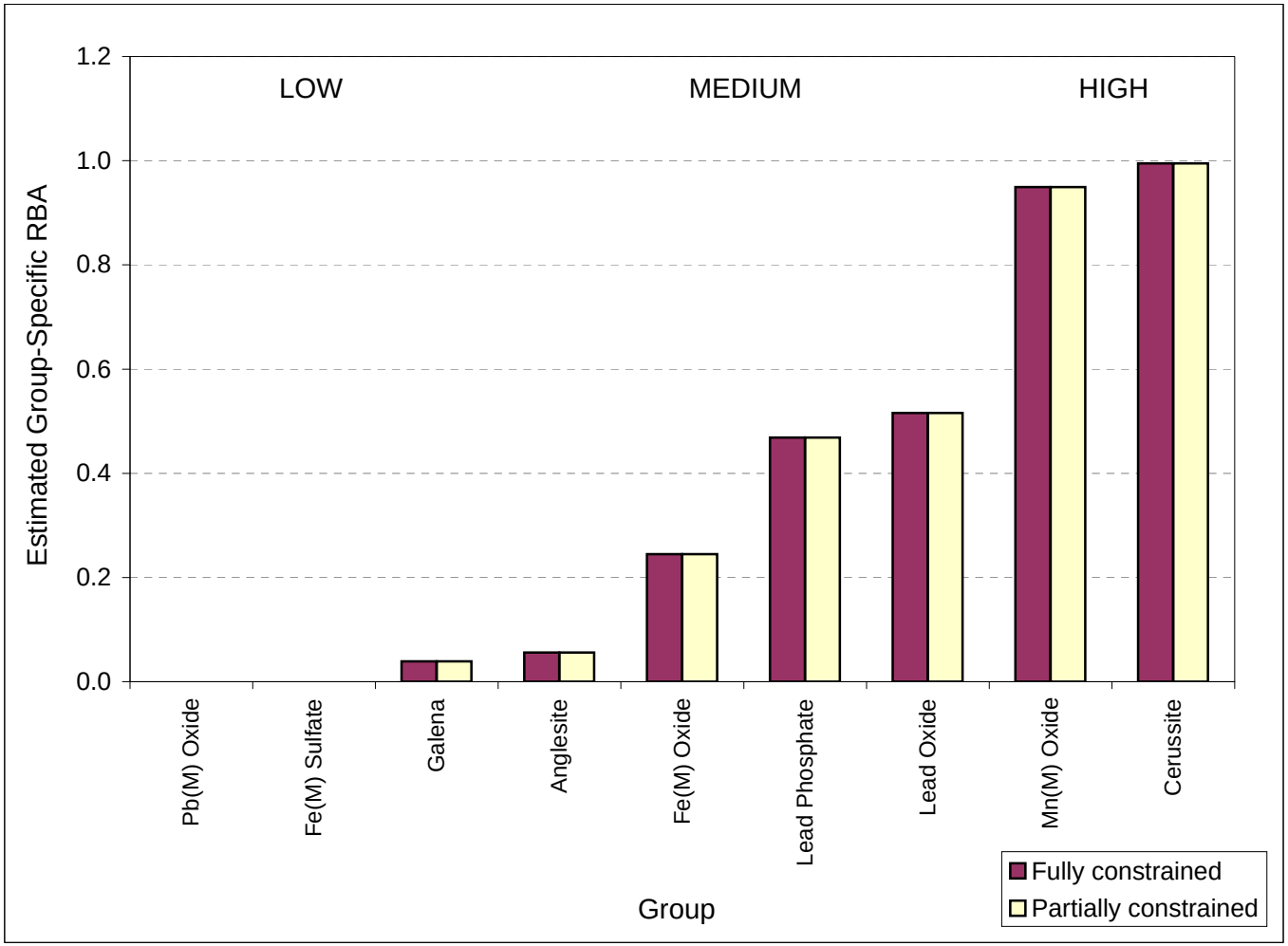


Fig 2-7_Phase RBAs.xls (Fig 2-7)

FIGURE 2-8. CORRELATION OF DUPLICATE ANALYSES

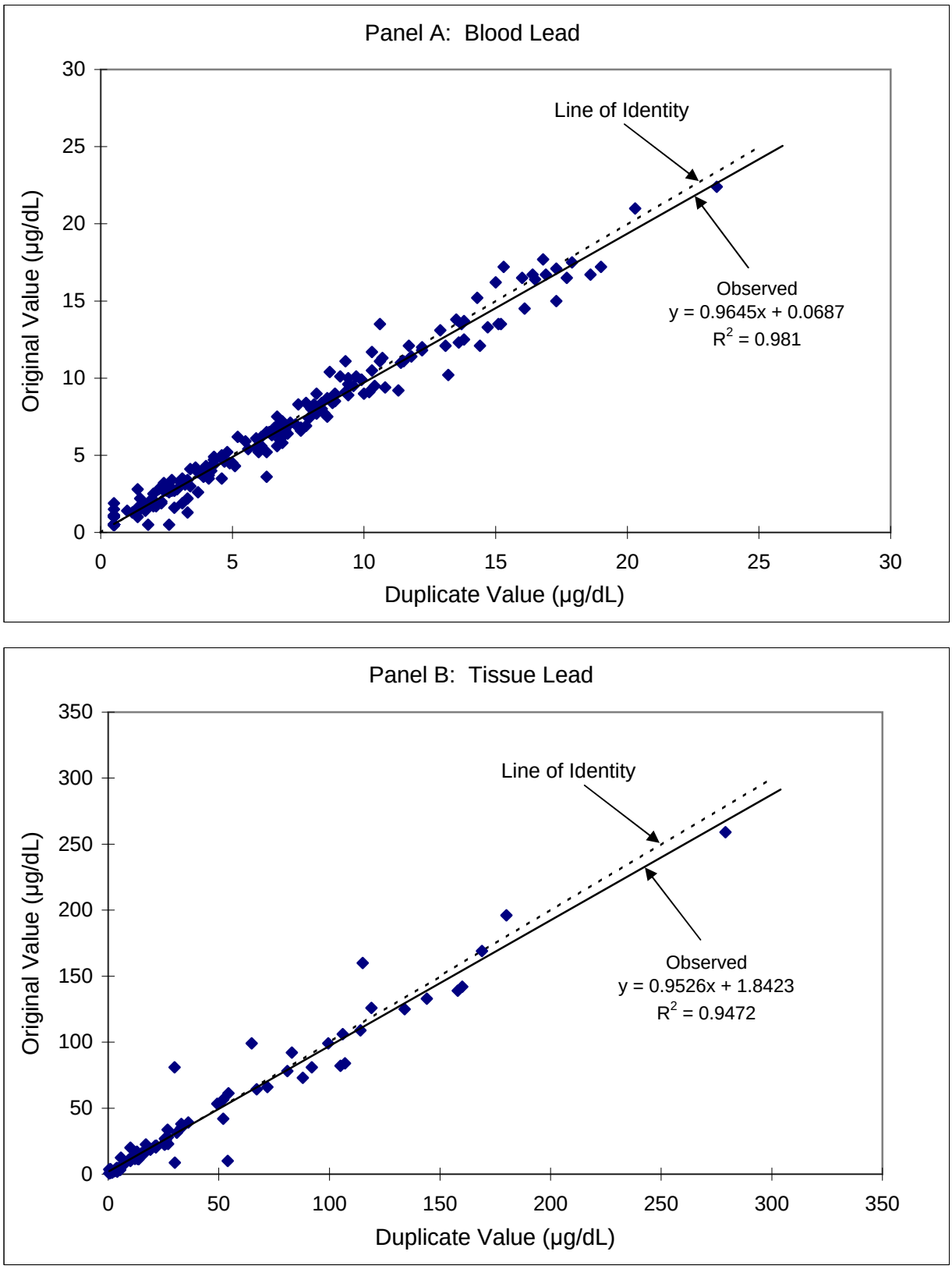


FIGURE 2-9. RESULTS FOR CDC BLOOD LEAD CHECK SAMPLES

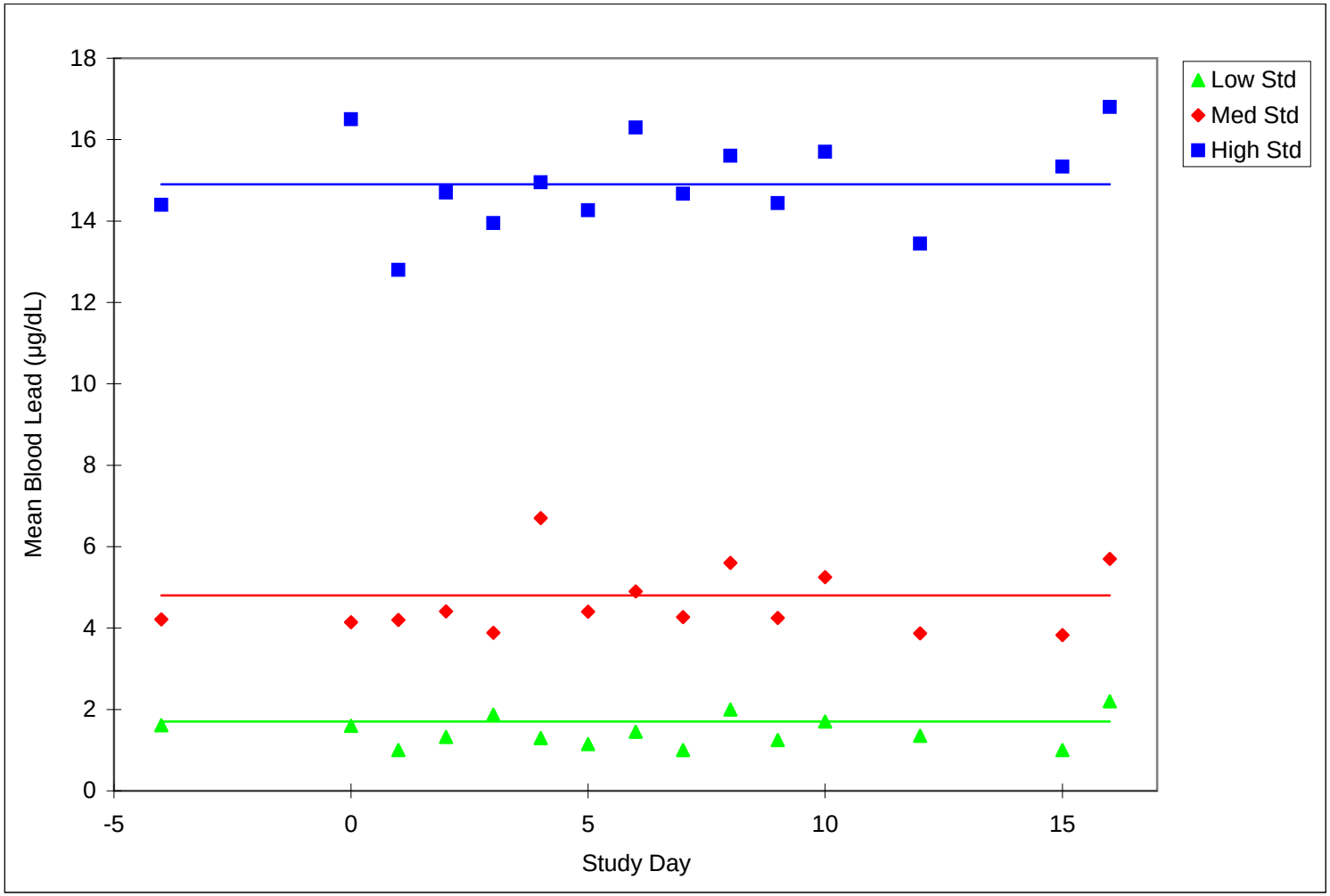
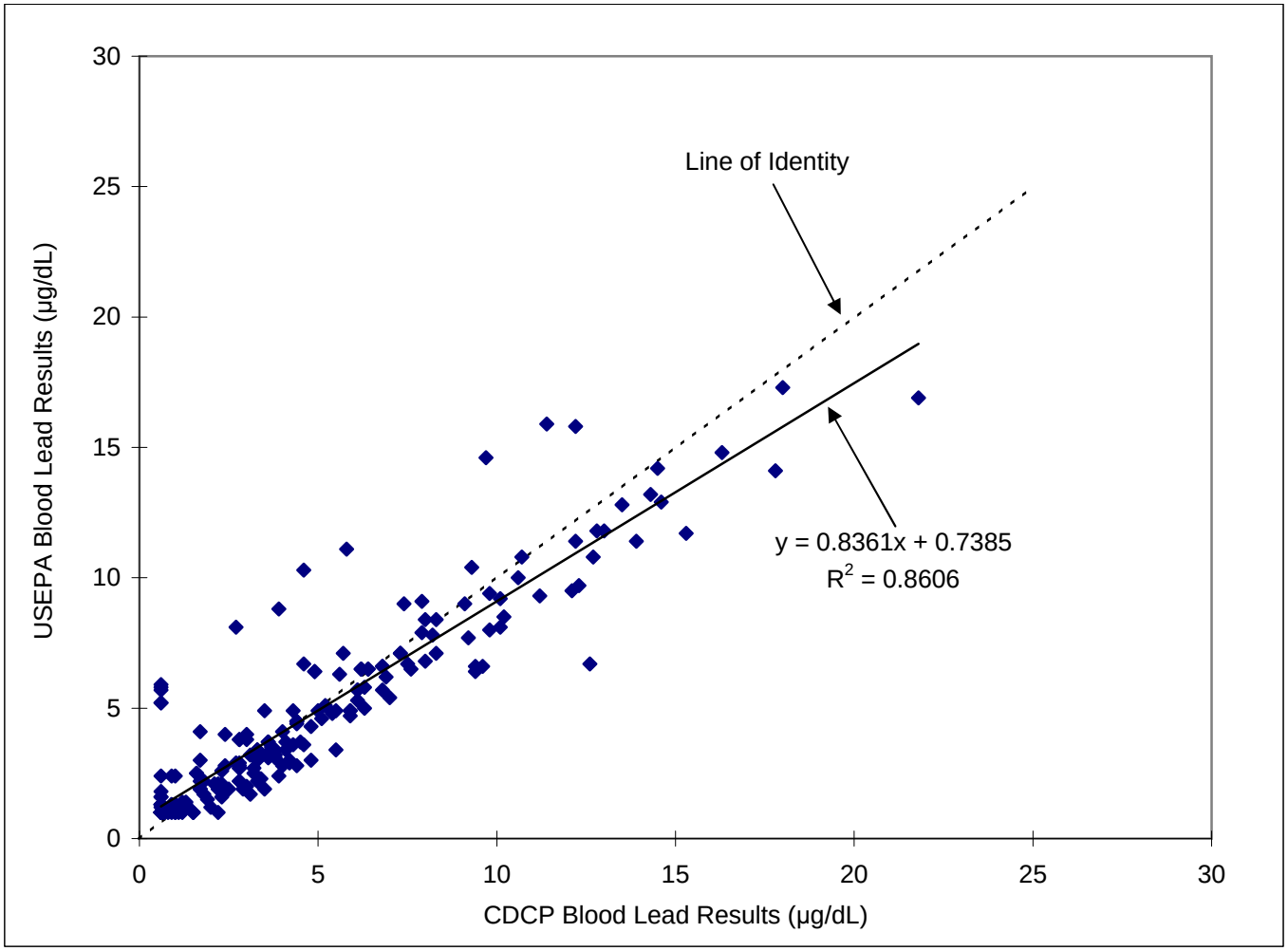


FIGURE 2-10. INTERLABORATORY COMPARISON OF BLOOD LEAD RESULTS



Figures.xls (2-10_Interlab)

FIGURE 3-1. *IN VITRO* BIOACCESSIBILITY EXTRACTION APPARATUS

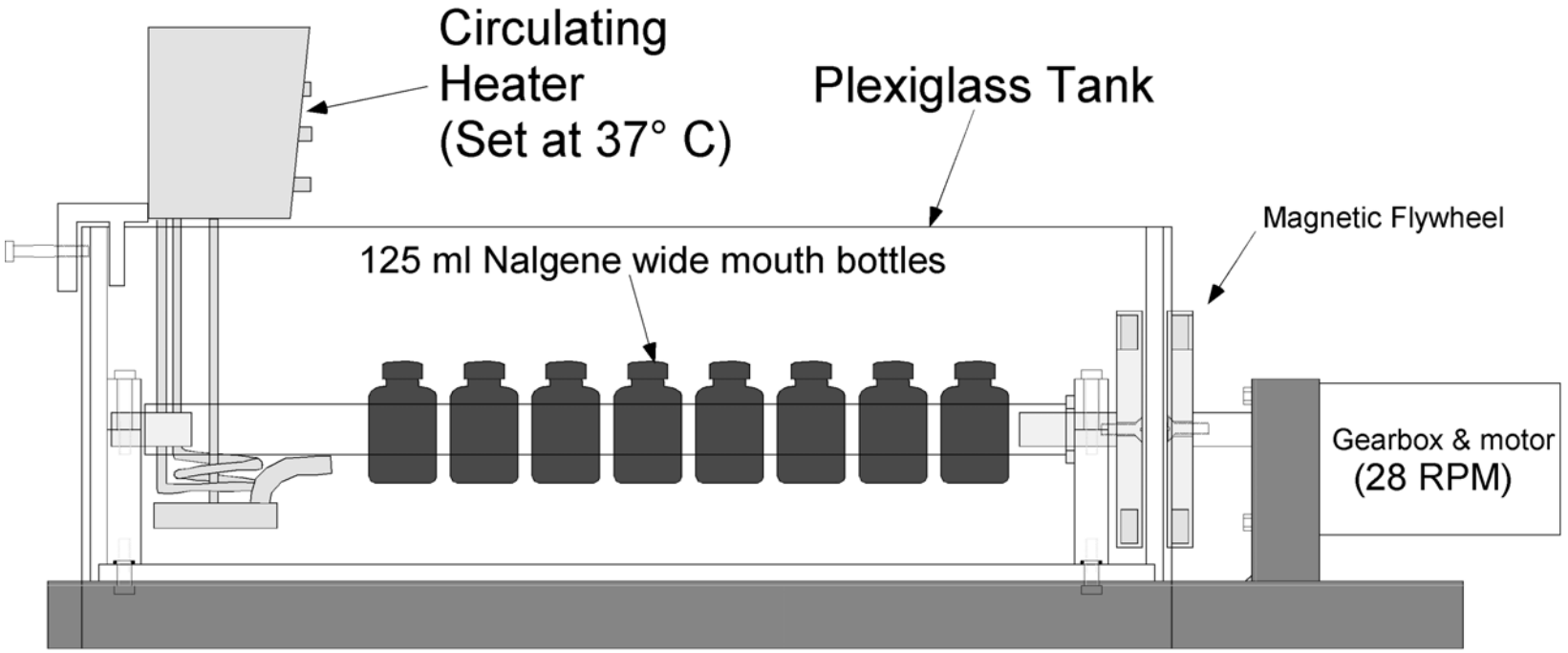


FIGURE 3-2. EFFECT OF TEMPERATURE, TIME, AND pH ON IVBA

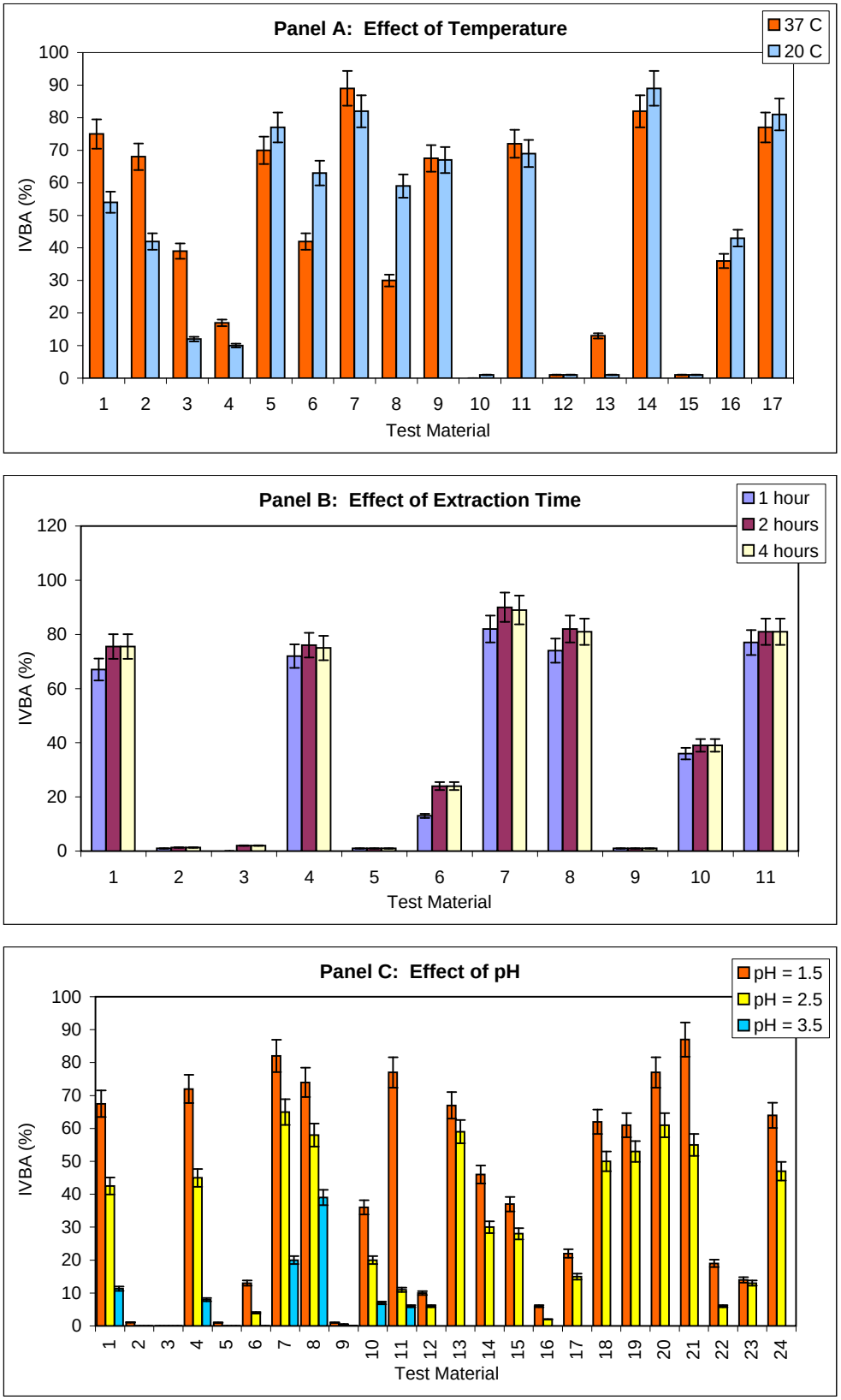


Fig 3-2_Effects on IVBA.xls (Fig 3-2_Effects)

FIGURE 3-3. PRECISION OF *IN VITRO* BIOACCESSIBILITY MEASUREMENTS

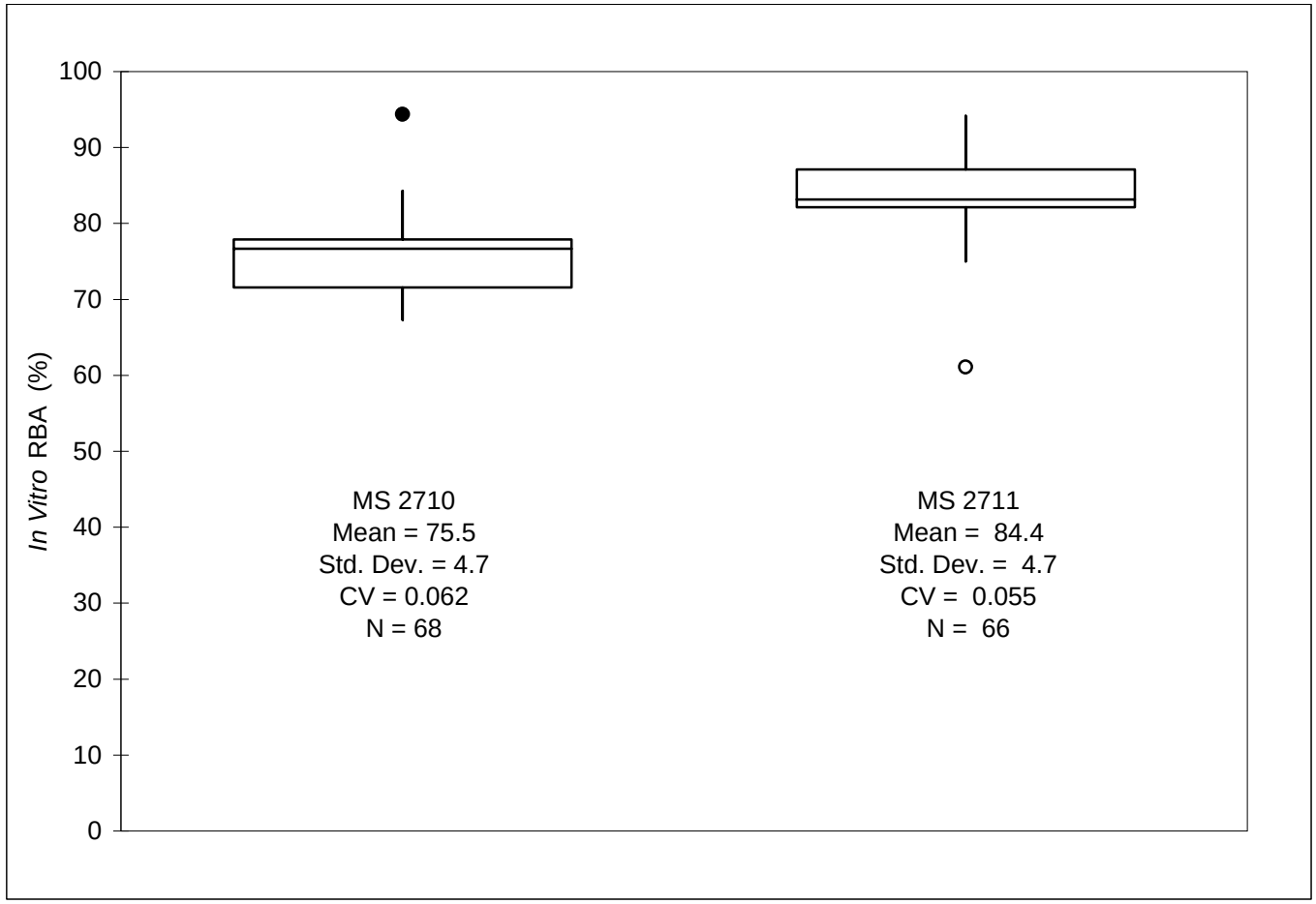
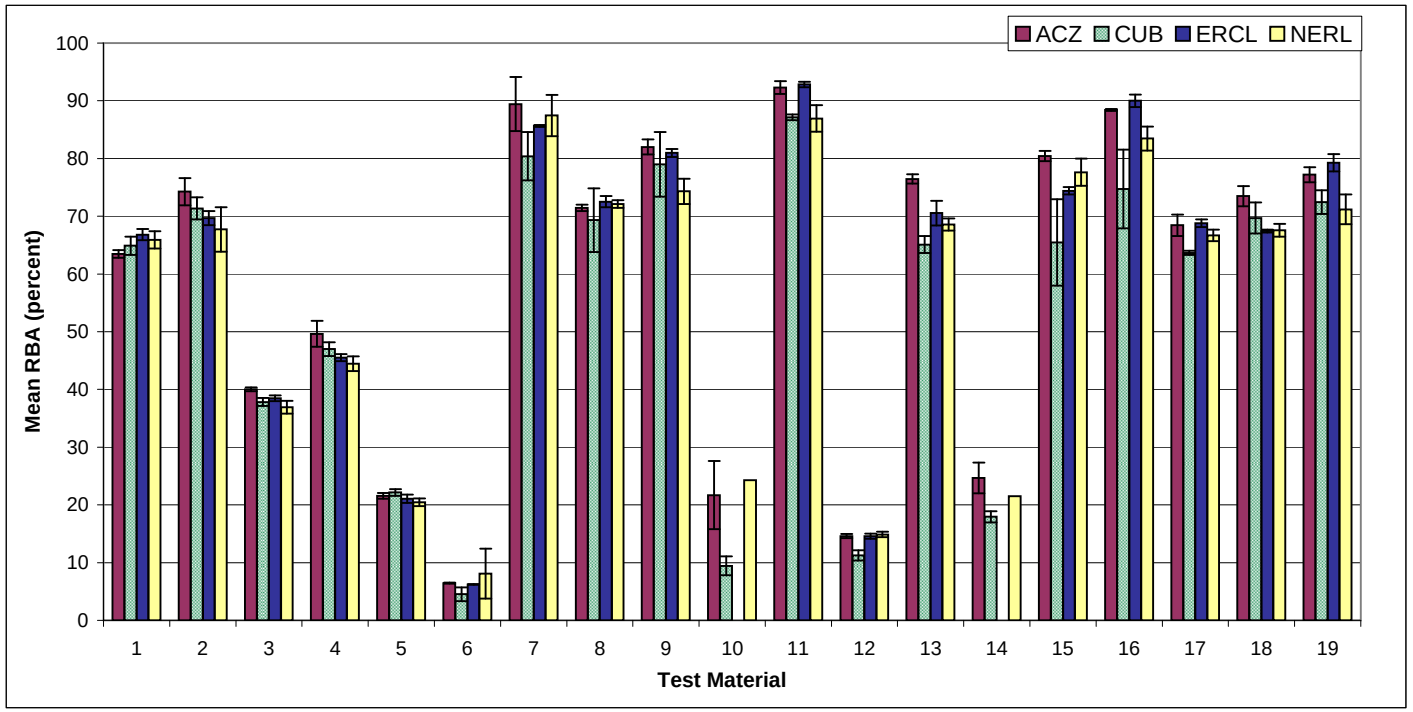


FIGURE 3-4. REPRODUCIBILITY OF *IN VITRO* BIOACCESSIBILITY MEASUREMENTS



Test Materials

1 = Aspen Berm	8 = Jasper County High Lead Smelter	14 = Midvale Slag
2 = Aspen Residential	9 = Jasper County Low Lead Yard	15 = Murray Smelter Slag
3 = Bingham Creek Channel Soil	10 = California Gulch AV Slag	16 = Murray Smelter Soil
4 = Bingham Creek Residential	11 = California Gulch Fe/Mn PbO	17 = Palmerton Location 2
5 = Butte Soil	12 = California Gulch Oregon Gulch Tailings	18 = Palmerton Location 4
6 = Galena-enriched Soil	13 = California Gulch Phase I Residential Soil	19 = NIST Paint
7 = Jasper County High Lead Mill		

Laboratories

ACZ = ACZ Laboratories, Inc.
CUB = University of Colorado at Boulder
ERCL = Environmental Research Chemistry Laboratory, U.S. Bureau of Reclamation
NERL = National Exposure Research Laboratory

FIGURE 3-5. RBA vs. IVBA

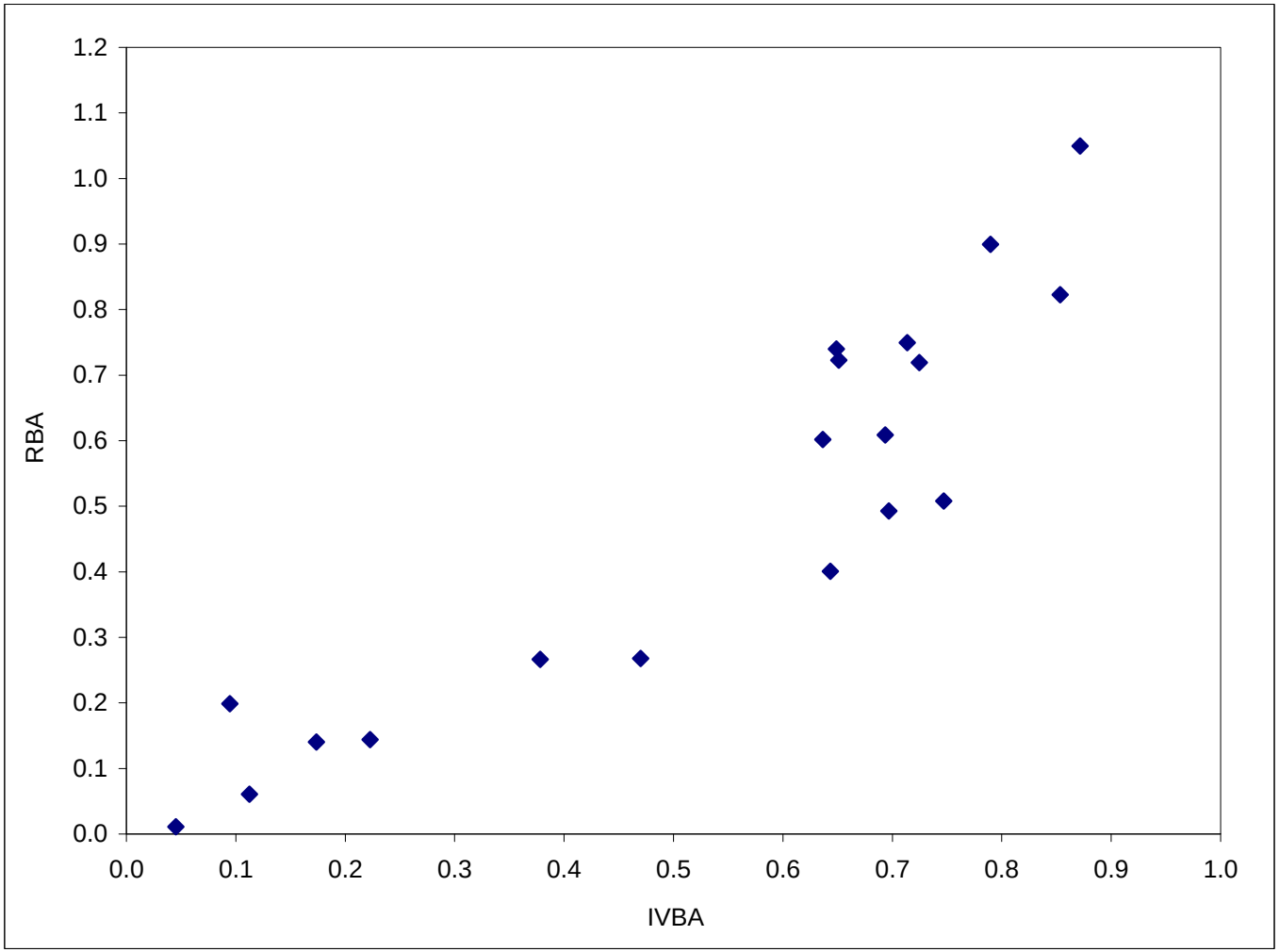


Fig 3-5_IVBA-RBA.xls (Fig 3-5)

FIGURE 3-6. PREDICTION INTERVAL FOR RBA BASED ON MEASURED IVBA

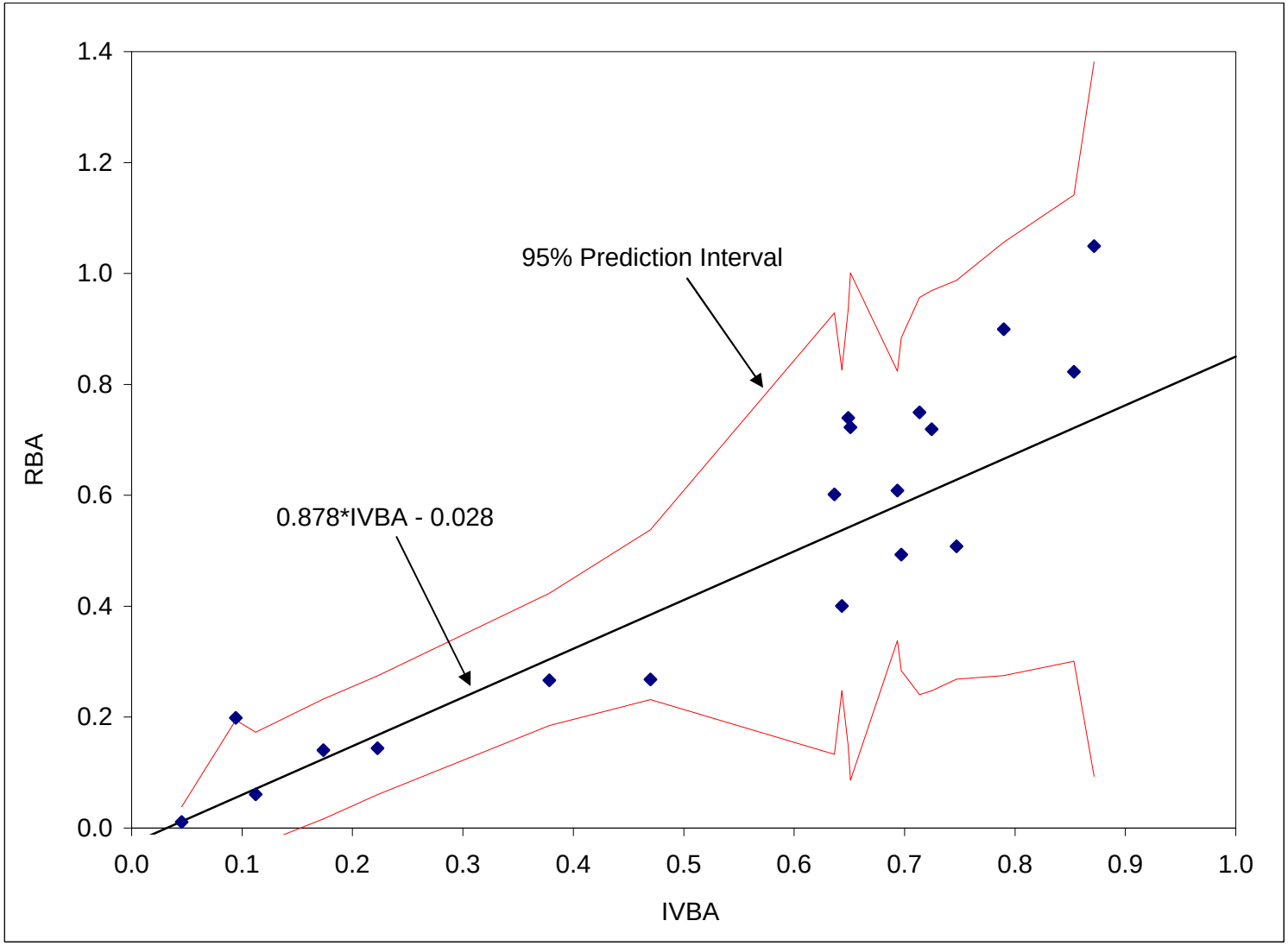


Fig 3-6, D-7_Prediction Intervals_NEW2.xls (Fig 3-6)