

FINAL REPORT

Bioavailability of Lead in Small Arms Range Soils

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14. ABSTRACT

Ammunition manufacture is the second largest consumer of lead in the United States, after batteries, and represents approximately 80,000 metric tonnes per year. About 3000 small arms ranges exist, the berms of which act as reservoirs for spent ammunition, and the DoD has a vested interest in monitoring the status of these sites so that military personnel may continue to be trained in a sustainable environment. Risk management of lead at small arms ranges depends on the site end use, whether the range is open or closed, and whether the risk drivers are human or ecologic. EPA risk assessment guidelines allows for applications of site specific bioavailability for lead, where this bioavailability differs from the assumed default of 60%. Assessing the site specific bioavailability of lead has historically been carried out using the *in vivo* juvenile swine model, but significant reduction in cost and time could be achieved by using less expensive, less technical, and less time consuming *in vitro* models. This study compared bioavailability of lead from small arms range soils from eight different sites, using both an established *in vivo* and *in vitro* method. The *in vivo* method was based on the measured absorption of soil-lead (compared to lead acetate) by swine dosed daily for 14 days, using the ratio of the blood dose-response slopes for each compound. For the *in vitro* method lead was extracted from an aliquot of soil for one hour at 37°C using glycine-HCl buffer at pH 1.5. The extractable lead was expressed as a percentage of the total lead in the sample. The initial aims of the study were to compare a projected range of bioavailability in a range of soils, creating a linear comparison between both methods. However, all eight soils carefully selected for testing were determined to have high bioavailability, regardless of source, pH, CEC, or organic matter. The mean *in vivo* and *in vitro* bioavailability results were $102 \pm 15\%$ and $95 \pm 6\%$, respectively, indicating a high degree of concordance, in spite of the widely different methods. This indicates that the *in vitro* method is a good predictor of the *in vivo* results, further strengthening the existing data for correlation between these two methods. Furthermore, speciation analysis showed that lead in these eight soils existed predominantly as lead carbonate or oxide, compounds with known high bioavailability. An additional 20 small arms range soils from across the US, screened using only the *in vitro* method also had high bioavailability ($91 \pm 11\%$), leading to the overall conclusion that lead at the majority of small arms ranges has high bioavailability.

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EXECUTIVE SUMMARY

Ammunition manufacture is the second largest consumer of lead in the United States, after batteries, and represents approximately 80,000 metric tonnes per year. About 3000 small arms ranges exist, the berms of which act as reservoirs for spent ammunition, and the DoD has a vested interest in monitoring the status of these sites so that military personnel may continue to be trained in a sustainable environment. Risk management of lead at small arms ranges depends on the site end use, whether the range is open or closed, and whether the risk drivers are human or ecologic. EPA risk assessment guidelines allows for applications of site specific bioavailability for lead, where this bioavailability differs from the assumed default of 60%. Assessing the site specific bioavailability of lead has historically been carried out using the *in vivo* juvenile swine model, but significant reduction in cost and time could be achieved by using less expensive, less technical, and less time consuming *in vitro* models. This study compared bioavailability of lead from small arms range soils from eight different sites, using both an established *in vivo* and *in vitro* method. The *in vivo* method was based on the measured absorption of soil-lead (compared to lead acetate) by swine dosed daily for 14 days, using the ratio of the blood dose-response slopes for each compound. For the *in vitro* method lead was extracted from an aliquot of soil for one hour at 37°C using glycine-HCl buffer at pH 1.5. The extractable lead was expressed as a percentage of the total lead in the sample. The initial aims of the study were to compare a projected range of bioavailability in a range of soils, creating a linear comparison between both methods. However, all eight soils carefully selected for testing were determined to have high bioavailability, regardless of source, pH, CEC, or organic matter. The mean *in vivo* and *in vitro* bioavailability results were $102 \pm 15\%$ and $95 \pm 6\%$, respectively, indicating a high degree of concordance, in spite of the widely different methods. This indicates that the *in vitro* method is a good predictor of the *in vivo* results, further strengthening the existing data for correlation between these two methods. Furthermore, speciation analysis showed that lead in these eight soils existed predominantly as lead carbonate or oxide, compounds with known high bioavailability. An additional 20 small arms range soils from across the US, screened using only the *in vitro* method also had high bioavailability ($91 \pm 11\%$), leading to the overall conclusion that lead at the majority of small arms ranges has high bioavailability.

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1. INTRODUCTION

1.1 Background

The annual consumption of lead (Pb) in the United States varies between 1.5 and 2 million metric tons, which represents approximately 15 pounds of lead per person per year. Storage batteries consume 80% of this total, while ammunition accounts for about 4%, or 80,000 metric tons per year (Smith 1998). Whereas lead in storage batteries can be directed to recycling, lead in spent bullets ultimately ends up in soil at the 3000 or so small arms ranges used by the Department of Defense or in the 9000 non-military ranges thought to be in use (EPA 2005). For the Department of Defense, Federal Agencies, and State bodies, these small arms range soils represent significant efforts in stewardship, environmental risk assessment, and remediation, so that military personnel and civilian police can continue to be trained in a sustainable environment.

Lead in ammunition can be in the form of lead shot, copper jacketed bullets (80% lead), or to a lesser extent in lead compounds used as primers. Three general phases of Pb can be identified at firing ranges; the first is when spent copper-jacketed bullets on the soil remain relatively intact and filled with metallic Pb, the second when bullets have broken up upon impact into smaller Pb particles, and the third where physical and chemical weathering over time has generated oxidized forms of Pb, such as lead carbonate or lead oxide, which are then bound to, or sequestered by, soil particles. All three phases of Pb exist at small arms range soils, though with time there is a gradual oxidation from metallic lead to chemical forms that are available to biota, a process that has been estimated to take hundreds of years (Jorgensen 1987). At pH values around 7.0, compounds of lead in soils are adsorbed onto mineral phases or in the case of high concentrations precipitated out as carbonates or oxides (Cao, Ma et al. 2003).

Risk assessment at DoD sites that include small arms ranges is carried out on a case-by-case basis, depending on the intended final use of the site (residential, brown field, continuing range) and whether human or ecological receptors are affected. Chemical forms of lead have been shown to be relatively immobile in soils; assuming that pH values are not low. Furthermore, the absence of plumes around small arms range sites or and the low levels of lead in pore water measured by lysimeters further strengthen this assumption. Because of the assumed immobility of lead, there is no removal of the primary source (lead bullets) during use or at range closure. Unless specific sensitive species can be identified, human rather than ecological risk assessment drives the process, using tools such as the Integrated Exposure Uptake Biokinetic (IEUBK) model, which predicts population blood leads as a function of diet, soil, and water. When management is required, EPA residential standards for lead in soil are preferred by DoD risk assessors for cleanup, regardless of the end use. The IEUBK model, which is currently undergoing revision so that it can be used for all ages, rather than just children, contains a relative bioavailability term that estimates the fraction of lead that will enter systematic circulation. However, it has been recognized by the EPA that site specific bioavailability can be applied when there is evidence that bioavailability significantly differs from the default.

Knowledge of lead bioavailability is important because the amount of lead that 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 influence 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 is normally described as the fraction or percentage of a chemical that is absorbed by the body following an exposure of some specified amount, duration, and route (usually oral). 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:

$$\text{ABA} = (\text{Absorbed Dose}) / (\text{Ingested Dose})$$

This ratio is also referred to as the oral absorption fraction (AFo). Relative bioavailability (RBA) is the ratio of the absolute bioavailability of lead present in some test material compared the absolute bioavailability of lead in some appropriate reference material:

$$\text{RBA} = \text{ABA}(\text{test}) / \text{ABA}(\text{reference})$$

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 0.6

The Risk Assessment Guidelines (RAG) for Superfund sites states “if the medium of exposure [at] the site...differs from the medium of exposure assumed by the toxicity value...an absorption adjustment may...be appropriate.” Thus in several instances, juvenile bioavailability studies for specific sites have been used to adjust assumed RBA value of the model. However, these tests are expensive, time-consuming, and require specialized facilities and expertise. Currently, there is no cost-effective method to assess the bioavailability of lead at small-arms ranges; either the soils would have to be tested by costly studies in swine, as described above, or the universal default value of 60% RBA would be used. This demonstration conducted parallel studies on small arms range soils to compare the results of *in vivo* swine RBA studies with those of a rapid and low cost *in vitro* method to assess the RBA of lead at ranges (EPA 2004). The comparison was intended a validation of the *in vitro* method for use as a standalone method. With comparison of the results of these two techniques we intended to obtain regulatory acceptance for the use of the *in vitro* model for risk assessment at small arms ranges.

1.2 Objectives of the Demonstration

The original objectives of this demonstration were twofold;

1. To demonstrate that the *in vitro* method can be used as a standalone method for risk assessment. This was carried out by correlation of the *in vitro* and *in vivo* two methods over a selected concentration range of lead in small arms ranges (SARS). A correlation coefficient (r) of 0.8 or greater was considered a success.
2. To gain regulatory approval of the *in vitro* method by the EPA. This would be achieved by liaising with EPA personnel as the study progresses. Since EPA buy-in is critical to the ultimate success of this study, contact with critical EPA personnel would be initiated at the start of the study and continued throughout the study.

However, as the study progressed, it became evident that the range of bioavailability in small arms range soils would not extend from low to high, but would remain consistently high. Therefore the objectives of the demonstration were supplanted by the emerging picture of high bioavailability at all small arms ranges tested (see results). Nonetheless, the ability of the *in vitro* method to consistently register the same high bioavailability as the *in vivo* method continued to be a consideration. This data can still be used to support the use of the *in vitro* method as a viable stand alone method.

1.3 Regulatory Drivers

SARS, which number in the thousands, are mostly confined to Pb as the major pollutant. Lead levels are generally localized to the firing end, the impact berms, and the target areas. In addition, SARS are generally small in size compared to Superfund sites. These combined factors make small arms ranges unique from the perspective of risk assessment and remediation.

Although paired *in vivo/in vitro* comparisons of firing range soils were used to validate the *in vitro* test in our study, this comparison will not be used to “calibrate” the *in vitro* test, in the sense that no adjustment will be made to the *in vitro* results. Instead, our goal was to refine the assumed default relative bioavailability of 60%, a term common to both Adult Lead Model (ALM) and Integrated Exposure Uptake Biokinetic Model (IEUBK) (US 1994) models as shown below, by using a measured *in vitro* value that is more accurate, site-specific, and cost effective. The *in vivo* comparison was intended to be used as a comparative validation of the new method and it is intended that the *in vitro* method, if validated, be used as a standalone method for prediction of *in vivo* bioavailability.

An Absorption Factor (AF) term is used in both the ALM and IEUBK models, but calculated differently in each. The ALM assumes 20 % absorption of soluble lead and a 60% relative bioavailability of lead in soil whereas the IEUBK assumes 50% absorption of soluble lead and 60% relative bioavailability of lead in soil. Using these default assumptions, the AF is calculated as follows:

$$\text{ALM: } 0.2 \times 0.6 = 0.12$$

$$\text{IEUBK: } 0.5 \times 0.6 = 0.30$$

Because the default assumption for relative bioavailability (0.6) is identical in both calculations the driving factor in the calculation of the AF therefore becomes the assumed 2.5 fold increase (50% vs 20%) in absorption for children over adults, a distinction with which we agree, since children absorb more lead than adults, and are also more vulnerable to its neurotoxic effects. Since we are refining a default value that is common to both ALM and IEUBK models, correlation with the juvenile pig model is an appropriate validation for the *in vitro* test. In addition using the juvenile pig model provides continuity with previous work on bioavailability

According to the EPA "...60% is a plausible default point estimate for the relative bioavailability of lead when site specific data are not available. Such data are highly desirable as variation in relative bioavailability is expected for different species of lead and different particle sizes..." Our study intended to provide validation of a rapid, cost-effective, and conservative relative bioavailability term for small arms range soils that can be used to calculate the AF in the ALM and IEUBK models.

1.4 Stakeholder/End-User Issues

The main stakeholders or end-users of the results of this project are;

1. Department of Defense: The Army, Navy, and Air Force all have small arms ranges which will require risk assessment and eventual remediation.
2. Other organizations such as the National Guard, local police forces both state and municipal, or any civil or military body which uses small arms range and needs an estimate of the risk posed by lead contamination.

2. TECHNOLOGY DESCRIPTION

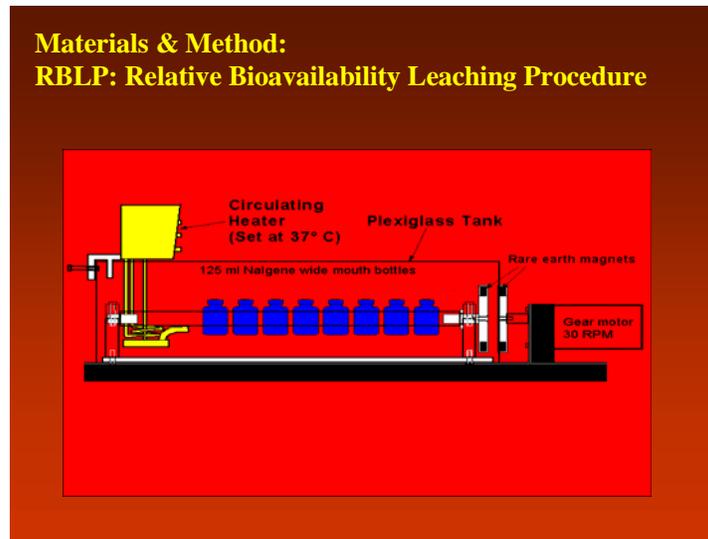
2.1 Technology Description and Application

In Vitro Method.

The Relative Bioaccessibility Leaching Procedure (RBLP) method of Drexler et al., (Drexler 1997; Drexler 2003) incorporates much of what has been learned over the past twenty years in developing bioequivalent *in vitro* methods. Components to the method have been added or dropped based on numerous years of study. These studies have always been evaluated based on their overall importance to a good *in vivo in vitro* correlation (IVIVC). This approach has developed a simple, rapid, and inexpensive method to determine bioaccessability of lead and arsenic in a wide range of media. This method is the first to follow the single (gastric) solution model. The authors use a simple, pH 1.5, 0.4 M glycine-buffered solution for the extraction. For the test, 100 ml of solution is placed in a 125 mL Nalgene® bottle along with 1.0 g of (<250 µm) material and sealed. The bottles are placed in the extraction device (Figure 1) and rotated end-over-end for 1 hour at 37 °C. After one hour a 10 ml aliquot is removed, filtered (0.45 µm) and analyzed. The SOP for the method is included in the Appendix.

This method has a very good IVIVC for both lead and arsenic based on USEPA Region VIII's swine studies (Casteel, Cowart et al. 1997) (this is the same data set used in Medlin, 1995). The correlation for lead, on 19 substrates having a broad RBA range and a

Figure 2.1 *In Vitro* Mixing System of Drexler (2002), using End-on-End Rotation.



diverse lead mineralogy, has an r^2 value of 0.93. Arsenic, at $r^2 = 0.86$, is currently based on a set of 11 substrates. This method contains data on both inter and intra-lab validation, 4% and 6% coefficient of variation, respectively. The standard operating procedure (SOP) provides for a complete evaluation of QA/QC, including; blanks, spikes (matrix and blank), duplicates, and traceability along with criteria for their frequency, and acceptability. As previously stated, this study has conducted extensive tests on the

sensitivity of each component to the method including; solid/liquid ratios, pH, temperature, extraction time, post extraction stability and others

In Vivo Method

Two representative site soil samples were selected for inclusion in each swine study. These samples were administered to juvenile swine using a daily dosing protocol. Blood samples were collected from the animals according to a defined schedule, and bone, kidney and liver tissue were collected upon sacrifice. These samples were analyzed for lead in order to determine the amount of lead absorbed from the soil. These amounts were compared to those obtained from a control group of animals which was dosed with lead acetate (PbAc).

The study was performed using young swine as the test species because the gastrointestinal system of swine is more nearly similar to humans than most other animal models. A general description is provided here but more detail is found in the Appendix. The animals were housed individually in metabolic cages. Groups of randomly selected animals (N= 5) were given oral doses of test material or lead acetate (abbreviated here as PbAc) for a total of 15 days (Table 2.1), with the dose for each day being administered in two equal portions given at 9:00 AM and 3:00 PM (two hours before feeding). Typical feed composition is in the detail for each in vivo study in the Appendix. Control animals (N=3) were given a dose consisting of vehicle material only. Doses were based on measured group mean body weights, and were adjusted every three days to account for animal growth. The dose material was placed in the center of a small portion (about 5 grams) of moistened feed (referred to as a "doughball"), and administered to the animals by hand. All missed doses were recorded and the time-weighted average dose calculation for each animal was adjusted downward accordingly.

One blood sample (6-8 mls) were drawn from each animal on days 0, 1, 2, 3, 5, 7, 9, 12, and 15, into into a new plastic lead-free syringe by venipuncture of the anterior vena cava. The blood was immediately transferred into lead-free Vacutainer^R tubes containing EDTA. In each case, blood samples were drawn 17 hours after the second dosing of the previous day. Animal weights were recorded and doses and feed adjusted on days -1, 2 and every third day thereafter until study termination. Animals were fed according to the regular daily schedule outlined in the Project Notebook. On study day #15, pigs were humanely sacrificed and representative samples of liver, kidney, and bone were collected and prepared for analysis. Detailed logbook notes recorded information pertinent to each sample collection. These notes were indexed and made available for review following sample collection.

The RBA of lead in site materials was estimated using the following approach:

1. Plot the biological responses of individual animals exposed to a series of oral doses of soluble lead (e.g., lead acetate). Fit an equation which gives a smooth line through the observed data points.
2. Plot the biological responses of individual animals exposed to a series of doses of test material. Fit an equation which gives a smooth line through the observed data.
3. Using the best fit equations for reference material and test material, calculate RBA as the ratios of doses of test material and reference material which yield equal biological responses. Depending on the relative shape of the best-fit lines

through the lead acetate and test material dose response curves, RBA may either be constant (dose-independent) or variable (dose-dependent).

Table 2.1 General Design for *In Vivo* Bioavailability Study.

Group	N	Treatment	Acetate/Soil mg/day	Lead Intake mg/kg/day
1	5	Pb(Ac) ₂ ·3H ₂ O	weight adjusted	25
2	5	Pb(Ac) ₂ ·3H ₂ O	weight adjusted	75
3	5	Pb(Ac) ₂ ·3H ₂ O	weight adjusted	225
4	5	Soil ₁	mass & weight adjusted	75
5	5	Soil ₁	mass & weight adjusted	225
6	5	Soil ₁	mass & weight adjusted	675
7	5	Soil ₂	mass & weight adjusted	75
8	5	Soil ₂	mass & weight adjusted	225
9	5	Soil ₂	mass & weight adjusted	675
10	3	Negative Control	oral vehicle	0

An RBA value of 1.0 means that lead in the test soil is just as well absorbed as lead acetate. An RBA value of 0.5 means that lead in the test soil is absorbed 50% as well as lead acetate.

2.2 Previous Testing of the Technology

This technology has been in use since 1997 (Drexler 1997) and has been tested with regard to precision. A published value of 6% relative standard deviation indicates good reproducibility. Since the *in vitro* method measures relative bioavailability, accuracy cannot be assessed in the same way that National Institute of Standards and Technology standard reference materials are used for total lead analysis. Therefore, the linear correlation of the *in vitro* and *in vivo* methods across a range of concentrations relevant to small arms ranges was used to indicate the accuracy of the method. The method has previously been compared with the swine model giving $r=0.989$ and slope 0.978. Although from diverse sources none of the soils tested were from small arms ranges, which in themselves have a unique spectrum of pollutants. Therefore, even though some previous testing of the technology has been carried out, comprehensive testing of the method in the context of small arms ranges is important if this technology is to be used at these sites.

2.3 Factors Affecting Cost and Performance

The *in vitro* method costs about \$100.00 per sample, making it vastly cheaper than the current swine model. Samples were analyzed in triplicate making the total cost \$600 per soil sample. The cost of the *in vivo* analysis was \$36,000 per soil sample, with a minimum of two soil samples required (total \$74,000). The cost of setting up analysis would therefore involve purchase of an end-on-end heating bath/end on end rotator. Because CHPPM already has a Directorate of Laboratory Science that has atomic absorption (flame and furnace), inductively coupled plasma (optical and mass spec) costs would be limited to the heating bath, rotator, and supplies. The performance of the method is affected by particle size and soil chemistry. Samples were sieved to <250 micron, which is the particle size that is thought to adhere to the hands in inadvertent transfer of lead to the mouth.

2.4 Advantages and Limitations of the Technology

The primary advantages of this technology are low cost and ease of use. Low analytical cost for the *in vitro* test removes the cost-constriction associated with *in vivo* testing which sometimes required that composite samples be taken (to reduce costs). With the proposed *in vitro* test, multiple sampling of small arms range soils allows definition of the perimeter of contamination by lead, which can be used in the design of clean-up strategies. When this technology is used with a portable X-Ray Fluorescence (XRF) device, the Innov-X, which measures lead in soil it can be very effective. The XRF can identify lead hotspots for sampling, while the *in vitro* method can determine the relative bioavailability of these hotspots, providing a rapid and flexible estimate of the risk from small arms ranges polluted with lead. This technology was demonstrated only at SARS, and is intended for use at those sites. In this sense, the technology is limited to ranges and is not intended for use at other sites, such as Superfund sites.

3. DEMONSTRATION DESIGN

3.1 Performance Objectives

The main performance objectives of this demonstration are detailed in Tables 3.1 and 3.2. Since this demonstration involves a new method for estimation of bioavailability that relies on laboratory generated data and statistical comparison, the performance criteria reflect the sampling, laboratory analysis, and statistical comparison of the data.

3.2 Selecting Test Sites

The test sites for this demonstration were ranges that used conventional bullets. Skeet ranges and ranges where shotguns have been used were debarred from the study. This was in the interest of uniform soil samples. The sites were in accordance with the criteria outlined in the Site Sampling Protocol. Since, this *in vitro* technology could be used to assess the risk from lead in ranges, the test sites could have been part of areas that currently need or are undergoing risk assessment. However, in practice it was difficult to get access to facilities to take samples from small arms ranges. During the initial stages of the demonstration soils collected at Aberdeen Proving Ground proved to have high bioavailability and it was decided from thereon to first submit collected samples to *in vitro* measurement so that samples with lower bioavailability could be pre-selected for the *in vivo* assessment. It was also decided to search for samples from sites that would vary in their characteristics (high organic matter, high CEC) so that the methods could be challenged as much as possible and areas with potentially low bioavailability would be included. This resulted in a total of over 30 samples being collected and tested using the *in vitro* method. Figure 4.9 shows the extractable lead as a function of total lead in the sample and demonstrate that all samples screen using the *in vitro* method had high bioavailability with a mean of 91%. This figure represents soil samples from small arms ranges in Maryland, Pennsylvania, New Jersey, New York, Tennessee, California, Oregon, Nebraska, Washington, Alaska, Louisiana, South Dakota, and Oregon.

Table 3.1 Performance Objectives for FY03/04.

Type of Performance Objective	Primary Performance Criteria	Expected Performance	Objective Met?
Sampling	1. Identification and selection of sites	4 small arms range sites	✓
	2. Site visits, screening, and sampling	SARS with lead levels >2000 ppm	✓
	3. Sample preparation and shipping	Tracking and chain-of-custody	✓
	4. <i>In vitro</i> analysis - 4 samples	Results	✓
	5. <i>In vitro</i> analysis - 4 samples	Results	✓
Analysis	1. Compare 4 SARS by both methods 2. Examine effect of pH 3. Examine effect of speciation	Preliminary statistics Is correlation > 0.8 Is pH a factor	✓

Table 3.2 Performance Objectives FY05/06.

Type of Performance Objective	Primary Performance Criteria	Expected Performance	Objective Met
Sampling	6. Identification and selection of sites	4 small arms range sites	✓
	7. Site visits, screening, and sampling	SARS with lead levels >2000 ppm	✓
	8. Sample preparation and shipping	Tracking and chain-of-custody	✓
	9. <i>In vitro</i> analysis - 4 samples	Results	✓
	10. <i>In vitro</i> analysis - 4 samples	Results	✓
Analysis	4. Compare 4 SARS by both methods 5. Examine effect of pH 6. Examine effect of speciation	Preliminary statistics Is correlation > 0.8 Is pH a factor	✓

Note: Linear correlation of 0.8 redundant because all samples had 100% bioavailability.

3.3 Test Site Description

The SARS sites were from a diverse group of ranges, but in the interest of convenience and ease of sampling was initially confined to the eastern half of the United States but was later extended to include all areas in which appropriate samples could be obtained. Physico-chemical characteristics were also measured. Soils were either taken by the PI or provided by the US Army Corps of Engineers, Vicksburg, MS, courtesy of Dr. Steve Larson. Soils that were taken by the PI were homogenized and sieved at US Army CHPPM soils laboratory, Edgewood. Soils that were provided had already been sieved to <250 µm in size. The soil characteristics are outlined in Table 3.3

The test site selection criteria were as follows;

1. Small arms range soil from military installations.
2. Sites should not have been disturbed or used as dump grounds.
3. Sites should be accessible for sampling
4. Sites could be part of a risk assessment or remediation (optional).

Table 3.3 Study Soil Characteristics

Site	pH	CEC	General Comments
MD1	6.3	0.954	taken
MD2	6.1	1.097	taken
LA	7.8	12.432	provided
AK	4.4	13.365	provided
NE	8.2	17.103	provided
WA	7.4	4.090	provided
SD	8.2	28.620	provided
OR	7.0	8.045	provided

3.4 Pre-Demonstration Testing and Analysis

Not applicable to this project.

3.5 Testing and Evaluation Plan

3.5.1 Demonstration Installation and Start-Up

Not applicable to this project.

3.5.2 Period of Operation

The original time frame of this project was FY02-FY04. However, difficulties encountered in obtaining both samples from small arms ranges and samples that had variable levels of bioavailability delayed the progress of the project until the final *in vivo* report was received from the contractor in June 2006.

3.5.3 Amount /Treatment Rate of Material to be Treated

Not applicable to this project.

3.5.4. Residuals Handling

Not applicable to this project.

3.5.5 Operating Parameters for the Technology

Not applicable to this project.

3.5.6 Experimental Design

The experimental design was a comparison of two methods using correlation. The sample size was small (n=8) because of the prohibitive costs of the *in vivo* analysis but the design was intended to show that the *in vitro* method could predict the *in vivo* value of RBA. Two pH values were used, pH 1.5 and pH 2.5, with the intention of examining the effect of pH on the RBA values and resulting correlation with the *in vivo* method. It was calculated that with this sample size, a linear correlation of 0.8 or greater would be sufficient to demonstrate that these methods agreed.

The experimental design was undermined by evidence, as the study proceeded, that bioavailability values were registering at essentially 100%, regardless of source of the soils. At this stage it was decided, in conjunction with ESTCP, to continue with the project but to find soils that were spread across a wide spectrum of possible conditions and locations. This would ascertain if the initial results from east coast soils would turn out to be the same for those from the heartland or west coast. It was decided to use the *in vitro* method to “screen” soils that might have low bioavailability and then to submit the samples for *in vivo* testing. However, no such range in bioavailability was revealed so samples from diverse states were used to demonstrate the project.

Sampling: Composite samples were taken by scooping the top few inches of soil across at least five sides on the impact sides of berms. At the CHPPM soils laboratory, samples were air-dried on trays at constant humidity to <2% residual moisture and then sieved to <250 µm size, using 2mm and then 250 µ sieves. For the *in vitro* method, samples were further dried to 60°C, and then analyzed according to the protocol found in the Appendix. For the *in vivo* method the soil sample was rolled 20 times to mix it evenly and then sub-sampled for swine dosing. The sieved samples were stored in Nalgene bottles. In some instances, soil samples that had been pre-sieved were forwarded to the P.I. These samples were sieved anyway and stored as above.

In Vitro Method:

Details of this method are found in the Appendix. Briefly, *in vitro* analysis was carried out at the laboratory of Dr. John Drexler at University of Colorado using a widely accepted *in vitro* method (Drexler and Brattin, In press). Triplicate sub-samples were taken from each well-mixed sieved soil. Then, 1.00 g was extracted in 100 mL of a solution of 0.4 M glycine buffer, tissue culture grade (Fisher Scientific Limited, PA) which had been adjusted to pH 1.5 using HCl, trace metal grade (Fisher Scientific Limited, PA). The closed 125 mL Nalgene bottles were placed in a heated extraction device and rotated end-over-end for 60 minutes at 37°C. After 1 hour, a 1 mL aliquot of the well-mixed solution was removed, filtered through a 0.45 µ cellulose acetate filter (BioExpress, UT) and analyzed for lead using a Varian ULTRAMASS ICP-MS (Varian, Inc., CA). *In vitro* bioavailability was expressed as ratio of extracted lead to the total lead in the sample, measure by nitric acid digestion and ICP-OES analysis. Lead speciation was carried out using a Joel 8600 electron microprobe) EMPA using a finely focused (1 F) producing characteristic X-rays for elemental analysis.

3.5.7 Sampling Plan

The sampling plan for this study required that 8 soil samples be collected from undisturbed SARS at different DoD facilities. Originally samples from only the east coast were to be collected, but (see Experimental Design) early results indicated that a wider range of sites should be used. The sample strategy is outlined in Appendix and is entitled Protocol for Soil Sampling of Firing Ranges. In several cases, samples were provided by other researchers, so sample treatment was adjusted depending on whether the samples had been sieved or not.

Sample Collection

Sampling sites were SAR which are active or retired, but had not been disturbed by heavy machinery. Sampling generally took place at the berm ends. Part of the sampling strategy was to use portable X-ray fluorescence technology to identify areas of highest lead concentration at the site of interest. The site was walked initially to identify the firing points and the berms. Areas where bullets or bullet holes are visible are, general, high in lead and these areas were field checked for soil lead concentration, using a portable XRF. Samples were composited only in the interests of increasing sample volume and obtaining a good representation of the soil material from which the high lead concentrations come. Maps were not necessary, since the technology and not the site was to be tested. Samples were transported to the CHPPM soils laboratory where they were dried, sieved, and stored in nalgene bottles.

Sample Analysis

The analytical methods used are listed in the protocols provided for *in vivo* and *in vitro* studies. Although no standard EPA method exists for these extractions, each has been published in peer review scientific literature and references are provided in the appendix.

Experimental Controls

The experimental controls are listed in the descriptions for each method.

Data Quality Parameters

These parameters are listed in the descriptions for each method.

Data Quality Indicators

A GLP review was completed on the *in vivo* laboratory during this study.

Calibration Procedures, Quality Control Checks, and Corrective Action.

These are described in each method.

3.5.8 Demobilization

Not applicable to this project.

3.6 Selection of Analytical/Testing Methods

The current swine model for determining the RBA was developed by U.S. EPA Region 8 in 1989 in cooperation with investigators at Michigan State University. The technology was refined and developed by Stan W. Casteel, D.V.M., Ph.D., at the University of Missouri (Casteel, Weis et al. 2006). Dr. Casteel has published several papers, in conjunction with EPA personnel, on *in vivo* bioavailability in soil from Superfund sites. The laboratory is focused on the question of bioavailability and remains the foremost facility for this type of investigation. For *in vitro* analysis, John Drexler, Ph.D., is considered one of the leading researchers in the field. Dr. Drexler was involved in the development of several of the methods currently in use for *in vitro* RBA and has published widely in this field, including papers with EPA personnel. Dr. Drexler carried out the *in vitro* model developed at his laboratory.

3.7 Selection of Analytical/Testing Laboratory

See 3.6.

4. PERFORMANCE ASSESSMENT

4.1 Performance Criteria

Table 4.1 General Performance Criteria Used to Evaluate the Technology.

Performance Criteria	Description	Primary or Secondary
Contaminant Reduction	The relative bioavailability of lead was assessed using this technology	Primary
Contaminant Mobility	No increase or decrease in Pb mobility resulted from this technology	Primary
Hazardous Materials	No hazardous materials were introduced, but the risk assessment of lead was refined by this technology	Primary
Process Waste	Since this is a technology for assessment of toxicity, no process waste was generated.	Primary
Factors that affect technology performance	<p>The following factors are important in the use of this technology</p> <ol style="list-style-type: none"> 1. Particle size should be < 250 μM 2. Sample size is limited to 1.0 g. 3. pH of the soil should not be a factor since, buffers are used in the extraction medium. 4. pH of the extraction solution may be a factor and was assessed by the study design. 	Primary
Reliability	The <i>in vitro</i> method has been published and has been used under widely varying conditions. It is considered a robust method.	Secondary

Ease of Use	The required equipment, a water bath with an orbital shaker is relatively easy to use.	Primary
Versatility	The technology is intended for use at all small arms ranges.	Primary

4.2 Performance Confirmation Methods

Soil collection and preparation was the responsibility of the PI at CHPPM. Soils were either taken by the PI or sent by other researchers to CHPPM, after which, if necessary, they were dried, and sieved to a particle size of <250 µm and stored in metal free Nalgene bottles. These were then shipped, in a round robin, to each investigator for *in vitro* and *in vivo* analysis. Sub-sampling by the PI prior to shipping was avoided to preclude bias due to settlement or sampling error. After arrival, each investigator was responsible for mixing the sample prior to sub sampling so that any settlement of sample in shipping would be avoided. Each co-investigator was responsible for their own QC, as outlined in the *in vitro* and *in vivo* method descriptions in the appendix.

The *in vivo* study contained a good deal of complexity and sample analysis, using 54 swine over a period of five days and requiring dosing schemes, blood sampling, and blood and tissue analysis for lead. Therefore, a site audit was conducted by the CHPPM GLP representative during the course of the project. The audit examined the protocols, methods, and facilities at the University of Missouri Veterinary Diagnostic Facility (Dr. Casteel’s laboratory) which were deemed adequate, though not compliant with GLP. It was not considered necessary to conduct a site visit to the *in vitro* laboratory of Dr. Drexler, as the process is simpler in application and could be judged by the quality of the submitted QC in reports. Triplicate analysis was carried out for the *in vitro* test and 5 animals were used for each dose for the *in vivo* study. Measures of precision and accuracy were determined by using either standard reference materials from NIST (environmental samples) or CDC (blood lead analysis). Since bioavailability is a relative measurement, there was no reference material that could be submitted to blindly test the accuracy of both methods. However, the consistently high values obtained using both methods were an indication of agreement. No linear correlative statistics could be carried out on these samples, since the bioavailability of all samples was high. The tests soils were screened for total metals using a portable XRF (Table 4.3).

Table 4.2 Performance Confirmation Methods.

Performance Criteria	Expected Performance Metric	Performance Confirmation
Contaminant Reduction	The relative bioavailability of lead was assessed using this technology	None needed
Contaminant Mobility	No increase or decrease in Pb mobility resulted from this technology	None needed
Hazardous Materials	No hazardous materials were introduced, but the risk assessment of lead was refined by this technology	None needed
Process Waste	Since this is a technology for assessment of toxicity, no process waste was generated.	None needed
Factors that affect technology performance	<p>The following factors are important in the use of this technology</p> <ol style="list-style-type: none"> 5. Particle size should be < 250 µM 6. Sample size is limited to 1.0 g. 7. pH of the soil should not be a factor since, buffers are used in the extraction medium. 8. pH of the extraction solution may be a factor and was assessed by the study design. 	<p>Comparison of <i>in vivo</i> and <i>in vitro</i> methods. Methods were not comparable over the expected range of concentrations because all soils tested had high bioavailability.</p>
Reliability	The <i>in vitro</i> method has been published and has been used under widely varying conditions. It is considered a robust method.	The <i>in vitro</i> method showed a significantly lower variance than the <i>in vivo</i> method.
Ease of Use	The required equipment, a water bath with an orbital shaker is relatively easy to use.	None needed

Versatility	The technology is intended for use at all small arms ranges.	Not applicable
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Table 4.3 Metals in Small Arms Range Soils from Eight Study Soils.

	MD1 SD	MD2 OR	LA	AK	NE	WA
Fe	21759 ±576 ±580	11960 ±198 29306 ±347	21211 ±272	42473 ±415	22200 ±280	45122
Pb	14654 ±151 ±303	21636 ±223 3170 ±39	12536 ±126	16113 ±154	10878 ±111	27156
Cu	1785 ±41 ±39	2388 ±50 183 ±13	941 ±28	1031 ±29	737 ±25	1893
Ti	6698 ±526 ±590	4520 ±482 2122 ±347	3487 ±416	2951 ±415	2744 ±415	4231
Zn	281 ±17 ±20	240 ±17 102 ±9	259 ±15	176 ±13	177 ±13	292
Sb	166 ±37 ±45	194 ±39 107 ±33	ND	ND	ND	453
Rb	33 ±4 62 ±6	ND 79 ±4	79 ±5	18 ±4	89 ±5	24 ±6
Zr	806 ±11 251 ±7	175 ±5 167 ±4	297 ±6	66 ±4	293 ±6	66 ±5
As	ND ±74	ND ND	ND	547 ±48	ND	550

Abbreviations: Fe=iron, Pb=lead, Cu=copper, Ti=titanium, Zn=zinc, Sb=antimony, Rb=rubidium, Zr=zirconium, As=arsenic. Values are mean of three subsamples taken from soil samples that had been sieved to <250 micron. Measurement was by portable XRF and is in ppm.

4.3 Data Analysis, Interpretation and Evaluation

This section comprises the following:

- A detailed description of the analysis behind two selected soils from NE and WA (pages 18-30). *In vivo* studies were carried out on two soils at a time and this avoids repetitive descriptions of the analysis of all soils. The detailed reports for each set of soils can be found in the Appendix.
- Overall summary figures and tables for all 8 soils (pages 31-34). This includes speciation analysis (Figure 4.8) for all soils, a summary of the *in vivo/in vitro* analysis (Table 4.6) and a figure showing the *in vitro* analysis of all sites sampled (Figure 4.9).

As outlined in preceding section, soils were analyzed independently by Dr. John Drexler (*in vitro*) and Dr. Stan Casteel (*in vivo*). Detailed reports for each analysis were presented by each and are included in the Appendix. To demonstrate the methodology, the report for NE and WA soils is described here, since each swine study encompassed two soils.

The NE and WA soils were obtained from Steve Larson at the Army Corps of Engineers and were already sieved to <250 μm on arrival. The samples were checked for particle size, and then stored in Nalgene bottles. After mixing thoroughly, three sub samples were used to determine the total metal analysis using the Innov-X soils XRF, and the results indicated levels of 10878 ± 111 , 27156 ± 303 ppm lead for NE and WA respectively. The other metals for these sites are also detailed in Table 4.3. Note that when the lead concentration doubled between samples, the copper concentration also doubled showing that lead and copper are being mobilized at relatively constant rates from copper jacketed bullets. The samples were then sent to Dr. Drexler for *in vitro* analysis and speciation. *In vitro* and *in vivo* results for each soil is presented in Table 4.6. Speciation analysis, Figures 4.1 and 4.2 showed that the samples were predominantly composed of oxidized compounds of lead such as lead carbonate (cerussite) or lead oxide, species that both result in high bioavailability. This was borne out by the *in vitro* measurements and the high bioavailability remained consistent for all eight samples in the study (Table 4.6). This high bioavailability is a consequence of the ease of extraction of carbonates or oxides of lead at pH 1.5. Other more refractory species such as lead sulfide would result in less extractable lead, reducing the numerator and thus the overall percentage of lead extracted.

The basic approach for measuring lead absorption *in vivo* was to administer an oral dose of lead to test animals and measure the increase in lead level in one or more body compartments (e.g., blood, soft tissue, bone). In order to calculate the RBA value of a test material, the increase in lead in a body compartment was measured both for that test material and an approximately equivalent dose of reference material (lead acetate). Because equal absorbed doses of lead (as Pb^{+2}) will produce equal responses (i.e., equal increases in concentration in tissues) regardless of the source or nature of the ingested lead, the RBA of a test material was calculated as the ratio of doses (test material and reference material) that produce equal increases in lead concentration in the body compartment. Thus, the basic data reduction task required to calculate an RBA for a test

material was 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.

In practice, the *in vivo* bioavailability consisted of twice daily dosing of juvenile swine for 14 days according to the dosing regime in Table 4.4. The change in blood lead values over the course of the 15 day period are shown in Figure 4.3. Four independent measurement endpoints were evaluated based on the concentration of lead observed in blood, liver, kidney, and bone (femur) (Figures 4.3 -4.7). For liver, kidney, and bone, the measurement endpoint was simply the concentration in the tissue at the time of sacrifice (day 15). The measurement endpoint used to quantify the blood lead response was the area under the curve (AUC) for blood lead vs. time (days 0-15). AUC was selected because it is the standard pharmacokinetic index of chemical uptake into the blood compartment, and is relatively insensitive to small variations in blood lead level by day. The AUC was calculated using the trapezoidal rule to estimate the AUC between each time point that a blood lead value was measured (days 0, 1, 2, 3, 5, 7, 9, 12, and 15). At the end of the study, the lead values in kidney, liver, and bone was measured in order to estimate the bioavailability in these tissues.

Model:

Basic Equations

It has been shown previously (USEPA, 2004) that nearly all blood lead AUC data sets can be well-fit using an exponential equation and most tissue (liver, kidney, and bone) lead data can be well-fit using a linear equation, as follow:

$$\text{Linear (liver, kidney, bone):} \quad \text{Response} = a + b \cdot \text{Dose}$$

$$\text{Exponential (blood lead AUC):} \quad \text{Response} = a + b \cdot [1 - \exp(-c \cdot \text{Dose})]$$

Simultaneous Regression

Because the data to be analyzed consist of three dose-response curves for each endpoint (the reference material and two test materials) and there is no difference between the curves when the dose is zero, all three curves for a given endpoint must have the same intercept. This requirement is achieved by combining the two dose response equations into one and solving for the parameters simultaneously, resulting in the following equations:

$$\text{Linear:} \quad y = a + b_r \cdot x_r + b_t \cdot x_t$$

$$\text{Exponential:} \quad y = a + b \cdot [(1 - \exp(-c_r \cdot x_r)) + (1 - \exp(-c_t \cdot x_t))]$$

where:

y = response

x = dose

a, b, c = empirical coefficients for the reference material (r) and test material (t).

All linear model fitting was performed in Microsoft® Office Excel using matrix functions. Exponential model fitting was performed using JMP® version 3.2.2, a commercial software package developed by SAS®.

Weighted Regression

Regression analysis based on ordinary least squares assumes that the variance of the responses is independent of the dose and/or the response (Draper and Smith, 1998). It has previously been shown that this assumption is generally not satisfied in swine-based RBA studies, where there is a tendency toward increasing variance in response as a function of increasing dose (heteroscedasticity) (EPA 2004). To deal with heteroscedasticity, the data are analyzed using weighted least squares regression. In this approach, each observation in a group of animals is assigned a weight that is inversely proportional to the variance of the response in that group:

$$w_i = (\sigma_i^2)^{-1}$$

where:

$$w_i = \text{weight assigned to all data points in dose group } i$$
$$\sigma_i^2 = \text{variance of responses of animals in dose group } i$$

(Draper and Smith, 1998).

As discussed in USEPA (EPA 2004), there are several alternative strategies for assigning weights. The preferred method identified by USEPA (EPA 2004) and the method used in this study estimates the value of σ_i^2 using an “external” variance model based on an analysis of the relationship between variance and mean response using data consolidated from ten different swine-based lead RBA studies. Log-variance increases as an approximately linear function of log-mean response for all four endpoints:

$$\ln(s_i^2) = k_1 + k_2 \cdot \ln(\bar{y}_i)$$

where:

$$s_i^2 = \text{observed variance of responses of animals in dose group } i$$
$$\bar{y}_i = \text{mean observed response of animals in dose group } i$$

Values of k_1 and k_2 were derived for each endpoint using ordinary least squares minimization, and the resulting values are shown below:

Endpoint	k1	k2
Blood AUC	-1.3226	1.5516
Liver	-2.6015	2.0999
Kidney	-1.8499	1.9557
Femur	-1.9713	1.6560

Goodness-of-Fit

The goodness-of-fit of each dose-response model was assessed using the F test statistic and the adjusted coefficient of multiple determination ($\text{Adj } R^2$) as described by Draper and Smith (1998). A fit is considered acceptable if the p-value is less than 0.05.

Assessment of Outliers

In biological assays, it is not uncommon to note the occurrence of individual measured responses that appear atypical compared to the responses from other animals in the same dose group. In this study, endpoint responses that yielded standardized weighted residuals greater than 3.5 or less than -3.5 were considered to be potential outliers (Canavos, 1984). When such data points were encountered in a data set, the RBA was calculated both with and without the potential outlier(s) excluded, and the result with the outlier(s) excluded was used as the preferred estimate.

Calculation of RBA Estimates

Endpoint-specific RBA Estimates

Lead RBA values were estimated using the basic statistical techniques recommended by Finney (1978). Each endpoint-specific RBA value was calculated as the ratio of a model coefficient for the reference material data set and for the test material data set:

$$\begin{aligned} \text{Linear endpoints:} & \quad \text{RBA}_t = b_t / b_r \\ \text{Exponential endpoint:} & \quad \text{RBA}_t = c_t / c_r \end{aligned}$$

The uncertainly range about the RBA ratio was calculated using Fieller's Theorem as described by Finney (1978).

RBA Point Estimate

Because there are four independent estimates of RBA (one from each measurement endpoint) for a given test material, the final RBA estimate for a test material involves combining the four endpoint-specific RBA values into a single value (point estimate) and estimating the uncertainty around that point estimate. As described in USEPA (EPA 2004), analysis of data from multiple studies suggests that the four endpoint-specific RBA values are all approximately equally reliable (as reflected in the average coefficient of variation in RBA values derived from each endpoint). Therefore, the RBA point estimate for the test material was calculated as the simple mean of all four endpoint-specific RBA values. The uncertainty bounds around this point estimate were estimated

using Monte Carlo simulation. Values for RBA were drawn from the uncertainty distributions for each endpoint with equal frequency. Each endpoint-specific uncertainty distribution was assumed to be normal, with the mean equal to the best estimate of RBA and the standard deviation estimated from Fieller's Theorem (Finney 1978). The uncertainty in the point estimate was characterized as the range from the 5th to the 95th percentile of the mean across endpoints.

The detailed *in vivo* studies for each pair of soils can be found in the Appendix. Table 4.5 shows a summary of the results of each *in vivo* study; details of each study can be found in the Appendix. A summary table of the final results for each of the eight soils in the study is presented in Table 4.9. The bioavailability of soils measured using the *in vitro* method was, as would be expected, more precise, since it simply involved extraction of lead from 1.00 g of soil at 37°C. With the *in vivo* method, biological variability introduced a wide estimate of RBA for each sample (WA; 0.67-1.55. NE; 0.59-1.35). However, the simple mean of all estimates give a point estimates of 93% and 107%, for NE and WA respectively. It is clear from the combined evidence in the speciation figures and the *in vitro* and *in vivo* measurements, that these soils have high bioavailability. Furthermore, the results for all eight soils in Table 4.6 show that regardless of the source of the small arms range soil, the outcome of bioavailability testing resulted in 100% bioavailability by either method. In addition, a number of soils that were sampled during the course of the study and subjected to *in vitro* measurements registered high bioavailability (Figure 4.9), confirming that a broad range of small arms soils have high bioavailability. This was independent of the source of the sample, whether high cation exchange capacity or high organic matter. Most berms are raised above the surrounding terrain and comprise sandy loams, making the conditions highly oxidizing. The table below summarizes the results of fitting the model to the data (see Figures 4.4-4.7).

Measurement Endpoint	TM1 (NE Soil)	TM2 (WA Soil)
Blood Lead AUC	0.89 (0.69 - 1.14)	1.11 (0.82 - 1.44)
Liver Lead	0.98 (0.45 - 2.49)	1.13 (0.54 - 2.91)
Kidney Lead	0.93 (0.72 - 1.22)	1.04 (0.81 - 1.36)
Femur Lead	0.92 (0.76 - 1.14)	0.98 (0.81 - 1.21)
Point Estimate	0.93 (0.59 - 1.35)	1.07 (0.67 - 1.55)

These results indicate that soils at small arms ranges, contain oxidized forms of lead, such as carbonates and oxides, and have high bioavailability. The speciation analysis for each site shows that the forms of lead found at small arms ranges are those known to have high bioavailability. The overall aim of the project, to verify the *in vitro* method as a good

predictor of the *in vivo* method, was not rejected and the results can be submitted as further verification of the *in vitro* method.

Figure 4.1 Speciation Analysis of Lead for NE Soil.

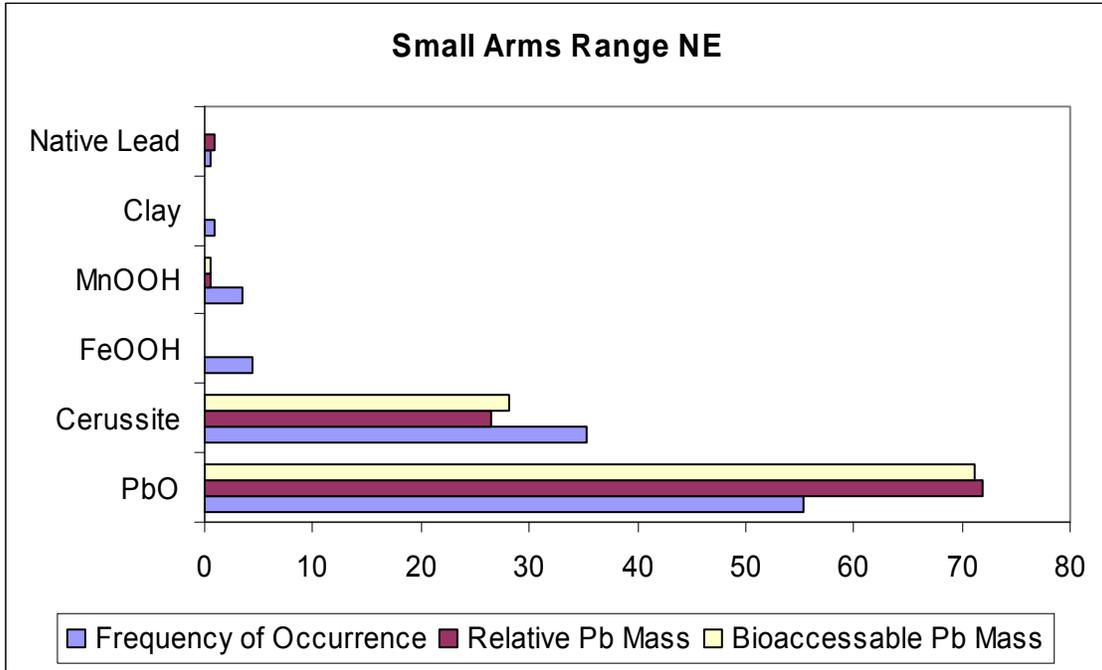


Figure 4.2 Speciation Analysis of Lead for WA Soil.

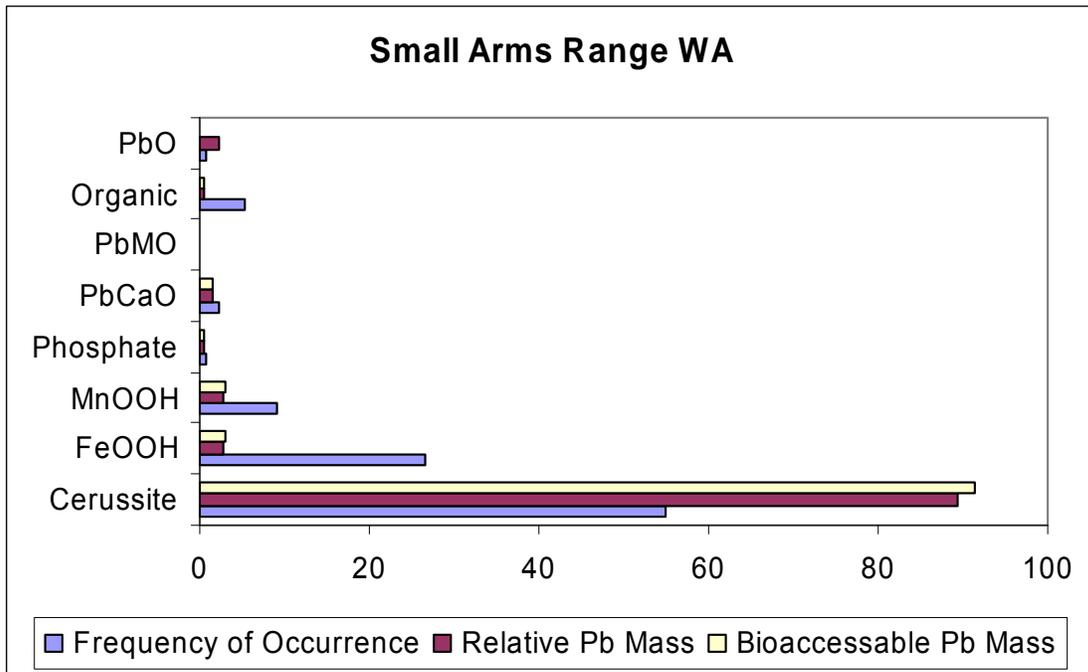


Table 4.4 Dosing Regimen for Test Soils NE and WA.

Group	Number of Animals	Dose Material Administered	Lead Dose ($\mu\text{g}/\text{kg}\cdot\text{day}$)	
			Target	Actual ^{a,b}
1	5	Lead Acetate	25	25.4
2	5	Lead Acetate	75	80.8
3	5	Lead Acetate	225	230.6
4	5	Small Arms Soil NE	75	81.2
5	5	Small Arms Soil NE	225	216.4
6	5	Small Arms Soil NE	675	738.8
7	5	Small Arms Soil WA	75	124.1
8	5	Small Arms Soil WA	225	398.5
9	5	Small Arms Soil WA	675	1170.7
10	3	Control	0	0.0

^a Calculated as the administered daily dose divided by the measured or extrapolated daily body weight, averaged over days 0-14 for each animal and each group.

^b Doses for Study 1 groups 7-9 (Test Material 2) are markedly higher than the target; doses were inadvertently calculated using the lead concentration of Test Material 1 instead of Test Material 2.

Doses were administered in two equal portions given at 9:00 AM and 3:00 PM each day. Doses were based on the mean weight of the animals in each group, and were adjusted every three days to account for weight gain.

Figure 4.3 Blood Lead Time Course after Dosing with NE and WA Soils.

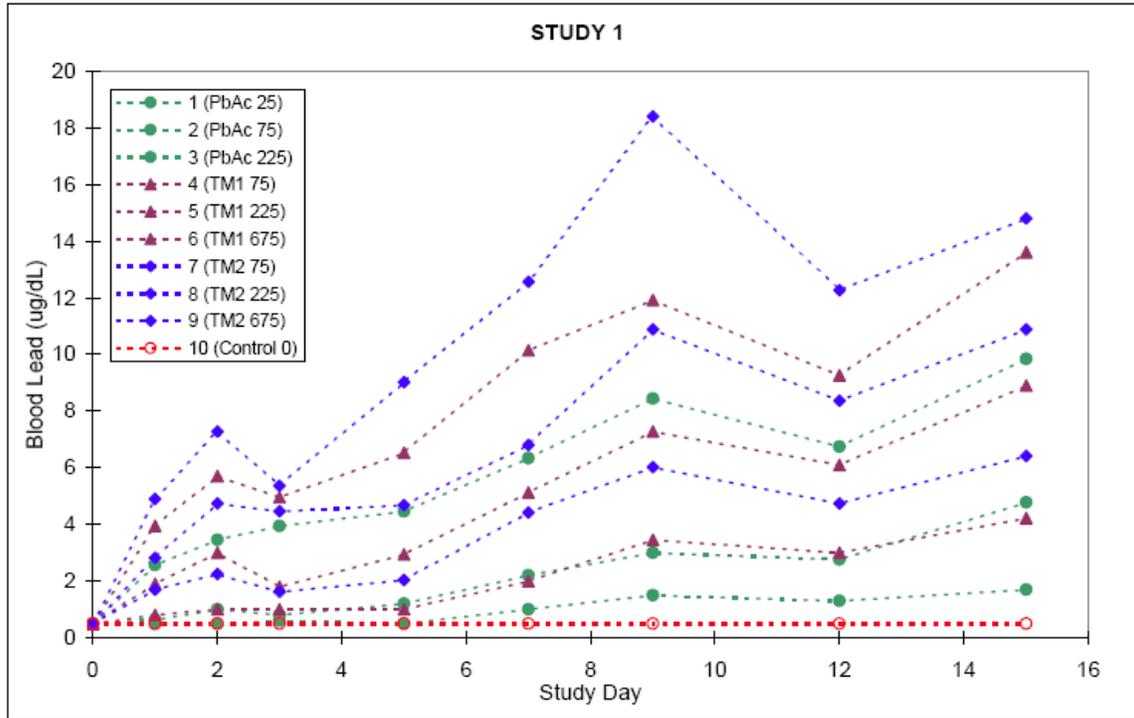
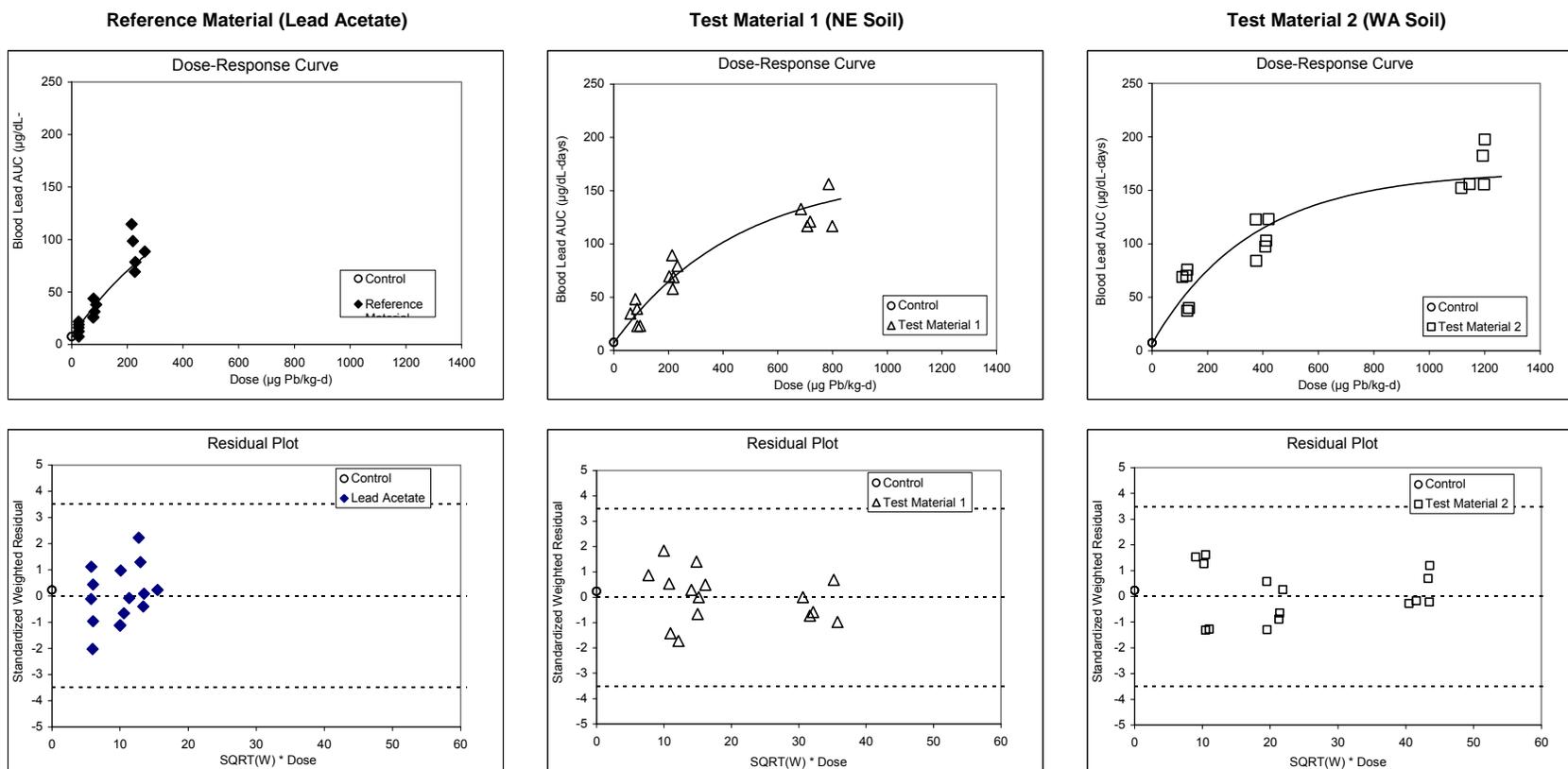


Figure 4.4 Blood Lead AUC Dose Response.



Summary of Fitting^a

Parameter	Estimate	Standard Error
a	6.89E+00	1.42E+00
b	1.61E+02	1.49E+01
c ₁	2.50E-03	4.06E-04
c ₁₁	2.22E-03	3.88E-04
c ₁₂	2.77E-03	5.37E-04
Covariance (c ₁ , c ₁₁)	0.6350	--
Covariance (c ₁ , c ₁₂)	0.6171	--
Degrees of Freedom	43	--

$$^a y = a + b(1 - \exp(-c_1 \cdot x_1)) + b(1 - \exp(-c_{11} \cdot x_{11})) + b(1 - \exp(-c_{12} \cdot x_{12}))$$

ANOVA

Source	SSE	DF	MSE
Fit	662.69	3	220.90
Error	54.97	44	1.25
Total	717.66	47	15.27

Statistic	Estimate
F	176.826
p	< 0.001
Adjusted R ²	0.9182

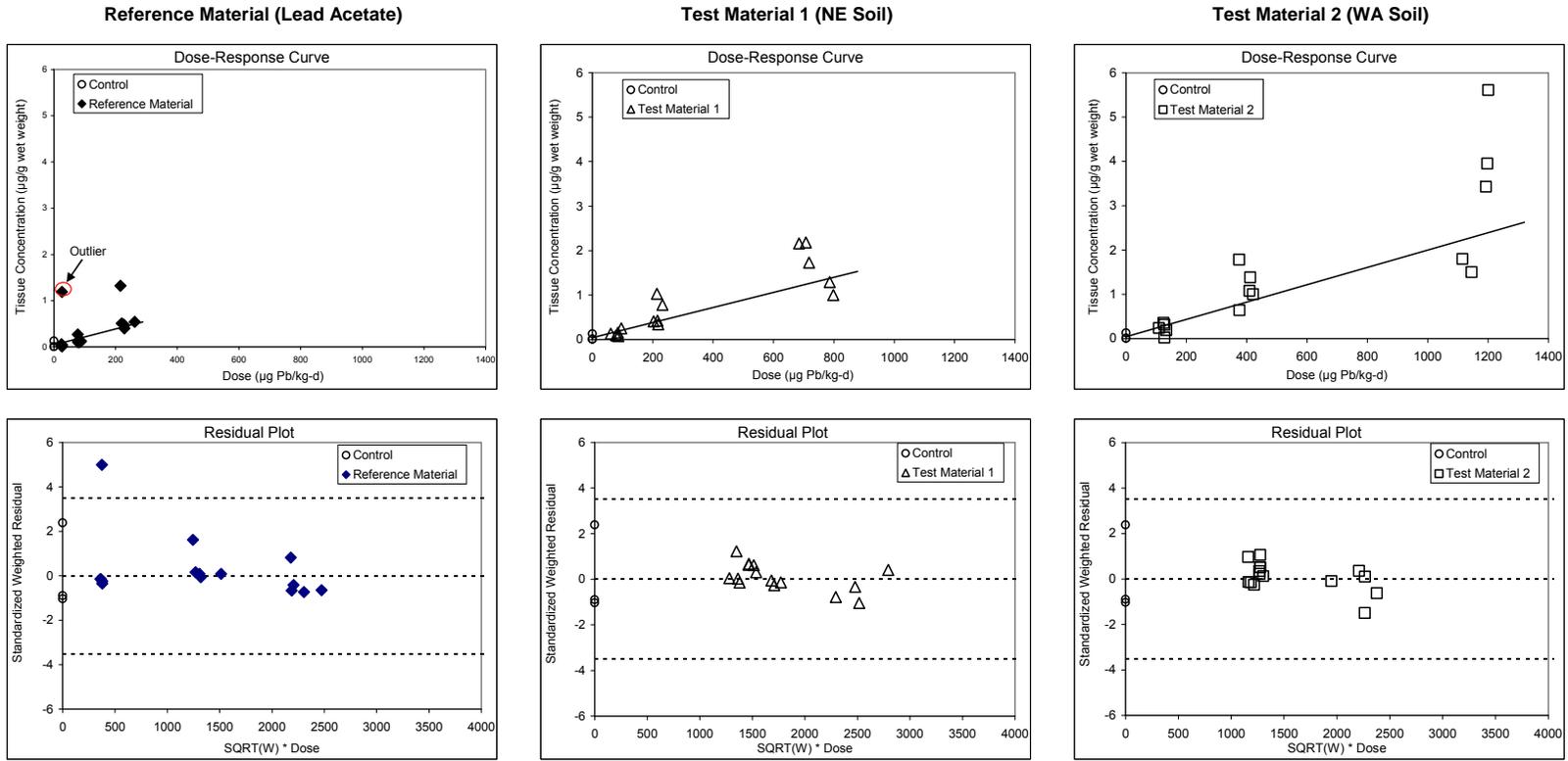
RBA and Uncertainty

	Test Material 1	Test Material 2
RBA	0.89	1.11
Lower bound ^b	0.69	0.82
Upper bound ^b	1.14	1.44
Standard Error ^b	0.128*	0.175*

^b Calculated using Fieller's theorem

* g ≥ 0.05, estimate is uncertain

Figure 4.5 Liver Lead Dose Response.



Summary of Fitting^a

Parameter	Estimate	Standard Error
a	4.27E-02	2.10E-02
b _i	1.72E-03	6.12E-04
b ₁₁	1.70E-03	5.04E-04
b ₁₂	1.96E-03	5.48E-04
Covariance (b _i , b ₁₁)	0.1239	--
Covariance (b _i , b ₁₂)	0.0793	--
Degrees of Freedom	45	--

^a $y = a + b_i \cdot x_i + b_{11} \cdot x_{i1} + b_{12} \cdot x_{i2}$

ANOVA

Source	SSE	DF	MSE
Fit	311.84	3	103.95
Error	503.55	44	11.44
Total	815.39	47	17.35

Statistic	Estimate
F	9.083
p	< 0.001
Adjusted R ²	0.3403

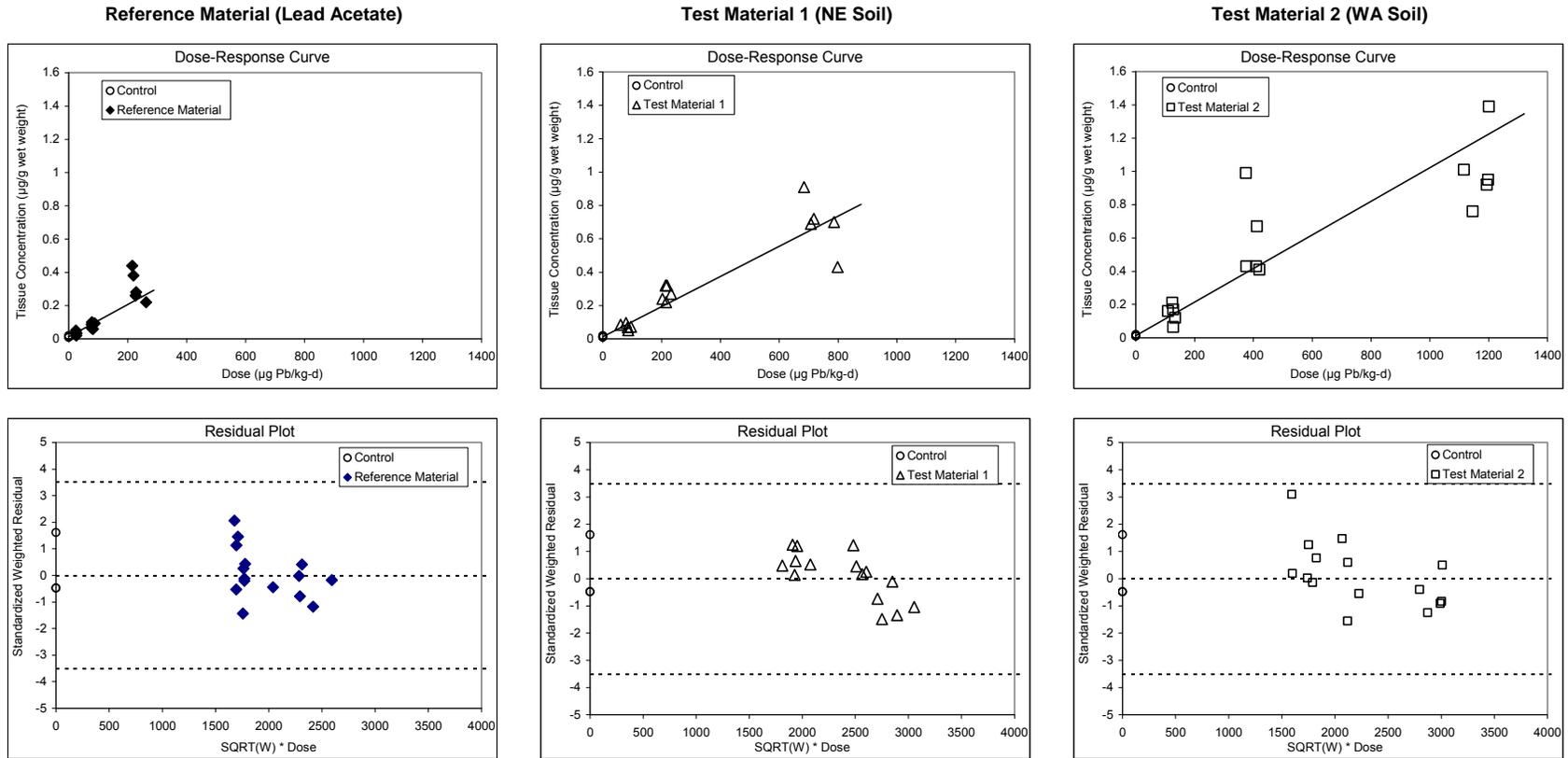
RBA and Uncertainty

	Test Material 1	Test Material 2
RBA	0.98	1.13
Lower bound ^b	0.45	0.54
Upper bound ^b	2.49	2.91
Standard Error ^b	0.427*	0.493*

^b Calculated using Fieller's theorem

* $g \geq 0.05$, estimate is uncertain

Figure 4.6 Kidney Lead Dose Response.



Summary of Fitting^a

Parameter	Estimate	Standard Error
a	1.23E-02	2.68E-03
b ₁	9.72E-04	1.20E-04
b ₁₁	9.05E-04	9.22E-05
b ₁₂	1.01E-03	9.67E-05
Covariance (b ₁ , b ₁₁)	0.0679	--
Covariance (b ₁ , b ₁₂)	0.0327	--
Degrees of Freedom	45	--

^a $y = a + b_1 \cdot x_1 + b_{11} \cdot x_{11} + b_{12} \cdot x_{12}$

ANOVA

Source	SSE	DF	MSE
Fit	185.71	3	61.90
Error	32.26	44	0.73
Total	217.97	47	4.64

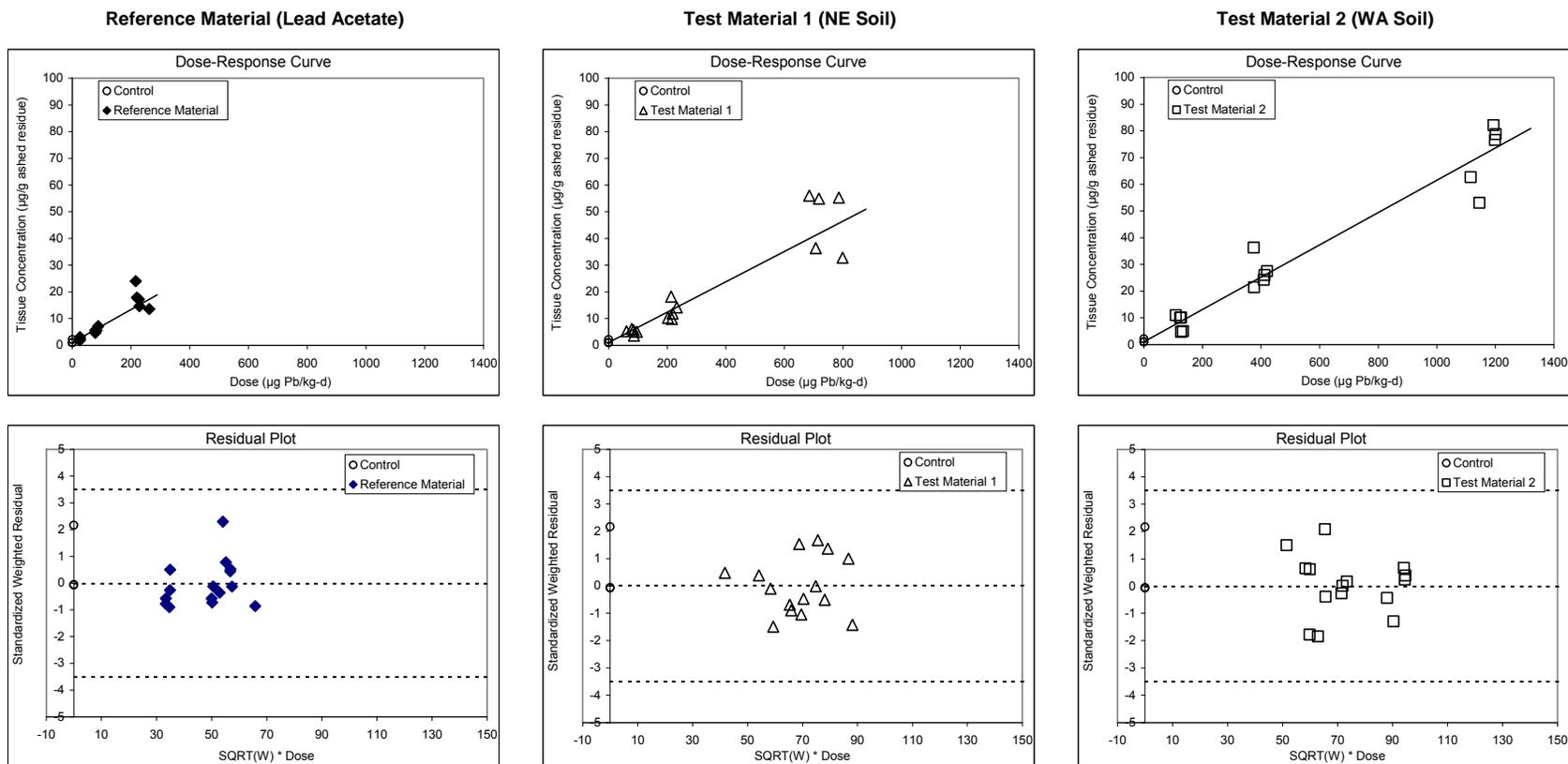
Statistic	Estimate
F	84.435
p	< 0.001
Adjusted R ²	0.8419

RBA and Uncertainty

	Test Material 1	Test Material 2
RBA	0.93	1.04
Lower bound ^b	0.72	0.81
Upper bound ^b	1.22	1.36
Standard Error ^b	0.144	0.160

^b Calculated using Fieller's theorem

Figure 4.7 Femur Lead Dose Response.



Summary of Fitting^a

Parameter	Estimate	Standard Error
a	1.04E+00	2.46E-01
b _r	6.17E-02	6.56E-03
b ₁₁	5.68E-02	4.19E-03
b ₁₂	6.05E-02	3.83E-03
Covariance (b _r , b ₁₁)	0.1354	--
Covariance (b _r , b ₁₂)	0.0872	--
Degrees of Freedom	45	--

^a $y = a + b_r \cdot x_r + b_{11} \cdot x_{11} + b_{12} \cdot x_{12}$

ANOVA

Source	SSE	DF	MSE
Fit	542.55	3	180.85
Error	52.48	44	1.19
Total	595.02	47	12.66

Statistic	Estimate
F	151.639
p	< 0.001
Adjusted R ²	0.9058

RBA and Uncertainty

	Test Material 1	Test Material 2
RBA	0.92	0.98
Lower bound ^b	0.76	0.81
Upper bound ^b	1.14	1.21
Standard Error ^b	0.111	0.117

^b Calculated using Fieller's theorem

Table 4.5 Table and Overall *In Vivo* Calculated Value for Eight Study Soils.

Measurement Endpoint	Estimated RBA (90% Confidence Interval)	
	Test Material 1 (NE Soil)	Test Material 2 (WA Soil)
Blood Lead AUC	0.89 (0.69 - 1.14)	1.11 (0.82 - 1.44)
Liver Lead	0.98 (0.45 - 2.49)	1.13 (0.54 - 2.91)
Kidney Lead	0.93 (0.72 - 1.22)	1.04 (0.81 - 1.36)
Femur Lead	0.92 (0.76 - 1.14)	0.98 (0.81 - 1.21)
Point Estimate	0.93 (0.59 - 1.35)	1.07 (0.67 - 1.55)

Measurement Endpoint	Estimated RBA (90% Confidence Interval)	
	Test Material 1 (MD1)	Test Material 2 (MD2)
Blood Lead AUC	127%	93%
Liver Lead	91%	71%
Kidney Lead	81%	66%
Femur Lead	68%	66%
Point Estimate	0.93 (0.59 - 1.35)	1.07 (0.67 - 1.55)

Measurement Endpoint	Estimated RBA (90% Confidence Interval)	
	TM3 (SD Soil)	TM4 (OR Soil)
Blood Lead AUC	0.70	1.03
Liver Lead	0.90 (0.63 - 1.30)	1.14 (0.80 - 1.64)
Kidney Lead	0.82 (0.62 - 1.08)	1.29 (0.99 - 1.70)
Femur Lead	0.67 (0.55 - 0.82)	1.01 (0.84 - 1.25)
Point Estimate	0.77 (0.55 - 1.08)	1.12 (0.81 - 1.51)

Measurement Endpoint	Estimated RBA (90% Confidence Interval)	
	TM3 (SD Soil)	TM4 (OR Soil)
Blood Lead AUC	0.70	1.03
Liver Lead	0.90 (0.63 - 1.30)	1.14 (0.80 - 1.64)
Kidney Lead	0.82 (0.62 - 1.08)	1.29 (0.99 - 1.70)
Femur Lead	0.67 (0.55 - 0.82)	1.01 (0.84 - 1.25)
Point Estimate	0.77 (0.55 - 1.08)	1.12 (0.81 - 1.51)

Figure 4.8. Speciation Results for All Soils.

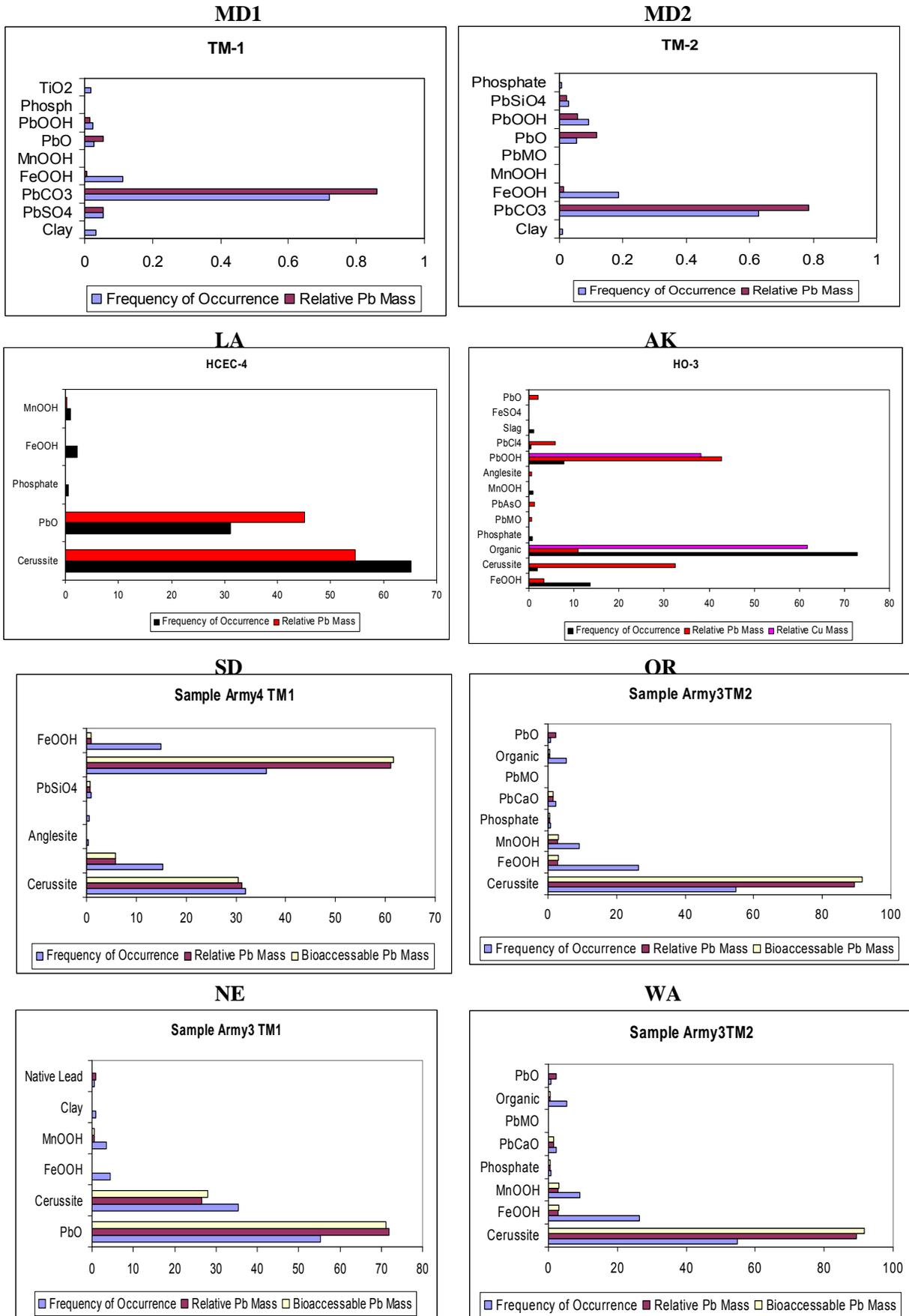


Table 4.6 Summary of Results for Eight Study Soils.

Site	Lead (ppm) mean \pm s.d.	<i>In vitro</i> (%) mean \pm s.d.	<i>In vivo</i> (%) mean (variance)
MD1	14645 \pm151	94 \pm2	115 (103-127)
MD2	21636 \pm223	98 \pm2	87 (80-93)
AK	16113 \pm154	93 \pm2	116 (86-160)
LA	12536 \pm126	90 \pm2	112 (75-155)
NE	10878 \pm111	100 \pm3	93 (59-135)
OR	3170 \pm39	100 \pm2	112 (81-151)
WA	27156 \pm303	83 \pm1	107 (67-155)
SD	21756 \pm247	99 \pm1	77 (55-108)

Table 4.6 shows the overall results for all 8 soils for this project. Small arms range soils sieved to $<250 \mu$ particle size were analyzed for total lead content. Sub-samples were then subjected to *in vitro* or *in vivo* analysis. *In vivo* analysis was carried out by Dr. Stan Casteel at University of Missouri using swine orally fed with a constant dose for 14 days. In a separate group, the same amount of lead was give as lead acetate. The response was measured as blood lead. The ratio of the dose-response (slope) of lead in soil and lead acetate at similar doses is the percent bioavailability. For the *in vitro* method, a 1.0 g aliquot of the sample was extracted at 37°C for 1 hour in a glycine-HCl buffer adjusted to pH 1.5. The assay was carried out in triplicate and at pH 1.5 and pH 2.5 (see Figure 4.10).

Figure 4.9 *In Vitro* Bioavailability of All Sites.

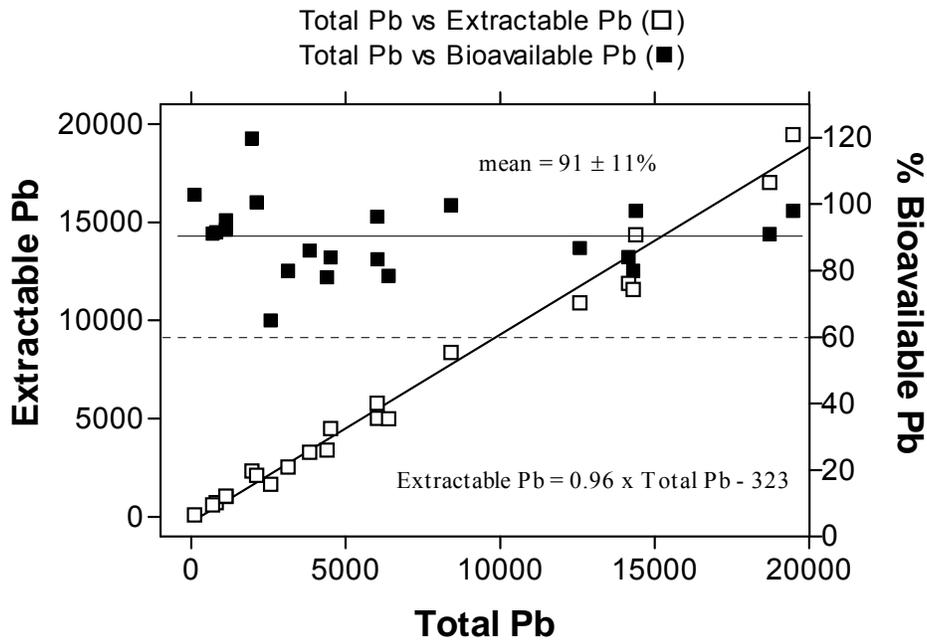


Figure 4.9 shows that results of *in vitro* bioavailability for all soils sampled for this study. The mean *in vitro* result was 91% \pm 11%. The extractable lead was expressed as a percentage of the total lead in the sample. The high extractability of lead using the *in vitro* method indicates that the metal can be easily solubilized at the pH 1.5. This is due to the fact that the metal species is predominantly oxidized forms of lead (oxides, carbonates) that are known to have high bioavailability.

Figure 4.10 Effect of pH on the *In Vitro* Method Results.

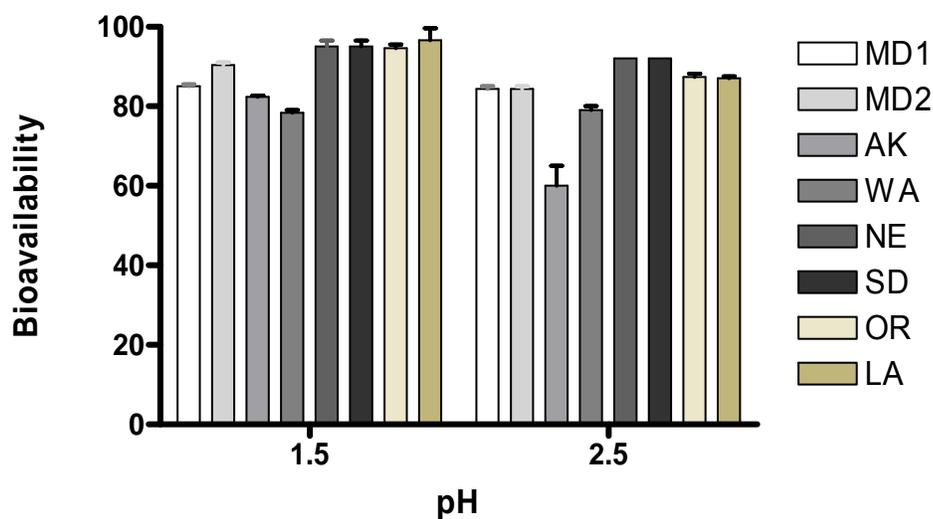


Figure 4.10. Analysis of soils at pH 1.5 and pH 2. The pH was adjusted to 2.5 to assess the effect of higher pH on the extraction method. These results show that except for one soil, there was no significant difference between soils as regards the pH of extraction. If anything, pH 1.5 seems to relate better (higher values) to the *in vivo* result than pH 2.5.

5. COST ASSESSMENT

The cost assessment of the *in vitro* and *in vivo* measurements is easily carried out by comparing the cost per sample. The initial contract for two soils samples for the *in vivo* test was \$73,149 for two soils samples, making it \$36, 574 per sample. The final contract for the last 4 soil samples was \$155, 337 for 4 soils making a cost of \$38, 834 dollars per soil sample. A cost breakout is shown below. The cost for the *in vitro* analysis was quoted as a unit cost per sample so there is no breakout. Samples were analyzed in triplicate so the total cost was \$600 dollars.

	<i>In vivo</i>	<i>In vitro</i>
Labor and Supplies	\$20,500	
Animals/Supplies	\$6,700	
Laboratory	\$8,350	
Consumables	\$750	
<hr style="border: 1px solid black;"/>		
Cost per soil sample	\$36,300	\$600 (3x200)

The *in vivo* costs can easily be contracted to a laboratory that has a heating bath with end-on-end rotation (Figure 1). Therefore there is no need for costly set-up. For the price of a single soil using *in vivo* analysis, 60 *in vitro* tests could be carried out. The question of whether the complexity captured by the *in vivo* test will be assessed under all conditions by the *in vitro* test has not been addressed by this study, since all of the samples contained lead species that have known high bioavailability.

Drexler has shown that there is a good correlation between both tests, regardless of soil sample type or origin. Since speciation costs about \$600 per sample, it would be feasible to check sites using only the *in vitro* test, and if it comes up low, to follow with a speciation study. Where there is high variability in soil types within a site, the *in vitro* test is ideal, allowing for numerous tests at relatively low cost.

6. IMPLEMENTATION ISSUES

Since this was not a demonstration of a technology in the field, there are no implementation issues. However, the study did assess the *in vitro* method, which has been widely used for assessment of solubility of lead. The method could be run by any inorganic analytical laboratory, since it requires minimal reagents, a water bath, and analytical spectroscopy for metals. The *in vitro* method is an ideal, low cost screening tool for large numbers of samples from ranges, and can detect low bioavailability if present. There are no significant issues in applying this technology; soil sampling can be carried out singly or in composite samples after which drying and sieving to a particle size of <250 μm is carried out. Analysis can be carried out by any metals laboratory that has a water bath and rotation device and spectroscopy.

7. REFERENCES

- Cao, X., L. Q. Ma, et al. (2003). "Weathering of Lead Bullets and Their Environmental Effects at Outdoor Shooting Ranges." Journal of Environmental Quality 32(2): 526-34.
- Casteel, S. W., R. P. Cowart, et al. (1997). "Bioavailability of Lead to Juvenile Swine Dosed with Soil from the Smuggler Mountain NPL Site of Aspen, Colorado." Fundamental and Applied Toxicology 36(2): 177-87.
- Casteel, S. W., C. P. Weis, et al. (2006). "Estimation of Relative Bioavailability of Lead in Soil and Soil-like Materials Using Young Swine." Environmental Health Perspectives 114(8): 1162-71.
- Drexler, J. and W. J. Brattin (In press). "An *In Vitro* Procedure for Estimation of Lead Relative Bioavailability with Validation." Human and Ecological Risk Assessment.
- Drexler, J. W. (1997). Validation of an *in vitro* method: A tandem approach to estimating the bioavailability of lead and arsenic in humans, IBC Conference on Bioavailability, Scottsdale, AZ.
- Drexler, J. W., C. Weis, and W. Brattin. (2003). "Relative Bioavailability of Lead: A validated *in vitro* procedure." Journal of Applied Toxicology.
- EPA (2004). Estimation of Relative Bioavailability of Lead in Soil and Soil like Materials Using *In Vivo* and *In Vitro* Methods. O. o. S. W. a. E. Response. OSWER 9285.7-77.
- EPA (2005). Best Management Practices for Lead at Outdoor Shooting Ranges. R. 2, US Environmental Protection Agency. EPA-902-B-01-001.
- Finney, D. J. (1978). Statistical Method in Biological Assay, Charles Griffin and Company.
- Jorgensen, S. S. (1987). "The fate of lead in soils: the transformation of lead pellets in shooting range soils." Ambio(16): 11-15.
- Smith, G. R. (1998). "Lead: Lead Statistics and Information, Mineral Commodity Summary." US Geological Survey Minerals Year Handbook 1998,
- US, E. P. A. (1994). Guidance Manual for the Integrated Exposure Uptake Biokinetic Model for Lead in Children. O. o. E. a. United States Environmental Protection Agency and R. Response.

9. POINTS OF CONTACT

POINT OF CONTACT	ORGANIZATION	PHONE/FAX	ROLE IN PROJECT
Desmond Bannon	US Army CHPPM	410-436-3387 ph 410-436-8258 fx	Principal Investigator
Mick Major	US Army CHPPM	410-436-7159 ph 410-436-8258 fx	Co-Investigator Advisor to the project
Stan Casteel	University of Missouri	573-882-6811	<i>In vivo</i> consultant
John Drexler	University of Colorado	303-492-5251	<i>In vitro</i> consultant

10. APPENDIX

The Appendix includes the following:

- Protocol for Sampling of Firing Ranges Soils.
- The Standard Operating Protocol for the in vitro method from Dr. John Drexler.
- Detailed reports from Dr. Stan Casteel on the analysis of each swine study, representing a pair of soils. Study 1 is for MD1 and MD2 soils. Study 2 is for soils from Louisiana and Alaska. Study 3 and 4 are for soils from NE, OR, WA, and SD.

Protocol for Soil Sampling of Firing Ranges.

Authors: Desmond Bannon and Mick Major, USArmy-CHPPM.

Date Verified: 07-30-2002

Revision number 2002-01

Background: This protocol is intended for composite sampling of small arms range soils to be used in the study entitled “Validation of a rapid and low-cost method for prediction of the oral bioavailability of lead from a soil matrix”. The samples collected will be homogenized, sieved to a particle size <250 μM and divided into duplicate samples for analysis by *in vivo* and *in vitro* methods. A small aliquot will be archived at USA-CHPPM.

Equipment: Lead is a ubiquitous contaminant and even though the proposed samples will have high lead concentration (1000’s ppm) it is better to avoid extraneous contamination. Use a clean stainless steel or plastic bucket (3-5 gallon size) and shovel to avoid external contamination of the sample.

Sampling: Examine the site to be sampled by walking the area around the berm. Identify areas of soil with the highest potential lead concentration, which may have visible bullet holes and/or bullets. A portable XRF instrument may be used to identify areas that have high Pb concentrations for sampling. Select enough sampling locations that will fill the sample container but do not take all the soil from one location. Instead, select at least four locations, taking approximately the same amount of soil from each location. Try to avoid taking extraneous material, such as grass, roots, and litter. Fill out the sample collection/traceability form attached to this protocol. After sampling the soils will be oven dried at 100° C. The samples will then be sieved through 2 mm and then 250 μ ASTM soil sieves. Sieved samples will then be stored in clean Nalgene containers for the duration of the study.

Post-sampling Disposition: The sample will be property of the Directorate of Toxicology, USA-CHPPM at Edgewood, Aberdeen, unless otherwise stated. After an initial lead analysis using portable XRF, the sample will be sieved to <250 μM at the CHPPM soils laboratory. The fraction <250 μM will be shipped to John Drexler or Stan Casteel, who will homogenize it for 30 minutes in a roller in order to prepare it for the *in vivo* assay.

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2200 Colorado Ave
Benson Earth Science
University of Colorado
Boulder, CO 80309

Phone: 303-492-5251

Stan W Casteel, DPhil, DVM, DABVT
Professor of Toxicology
University of Missouri
Dept of Veterinary Pathobiology
VMDL 1600 E Rollins
Columbia, MO 65205

Phone: 573-882-6811

Sample Collection Form – Oral Bioavailability Study

Date: ___ / ___ /2002

Location and POC for Site Entry: _____

Site Description: _____

Number of Locations Sampled to make Composite: _____

Comments: _____

Signature: _____

**University of Colorado
Dr. John Drexler.**

Relative Bioavailability Leaching Procedure (RBALP).

Revision 2.0 May, 2000

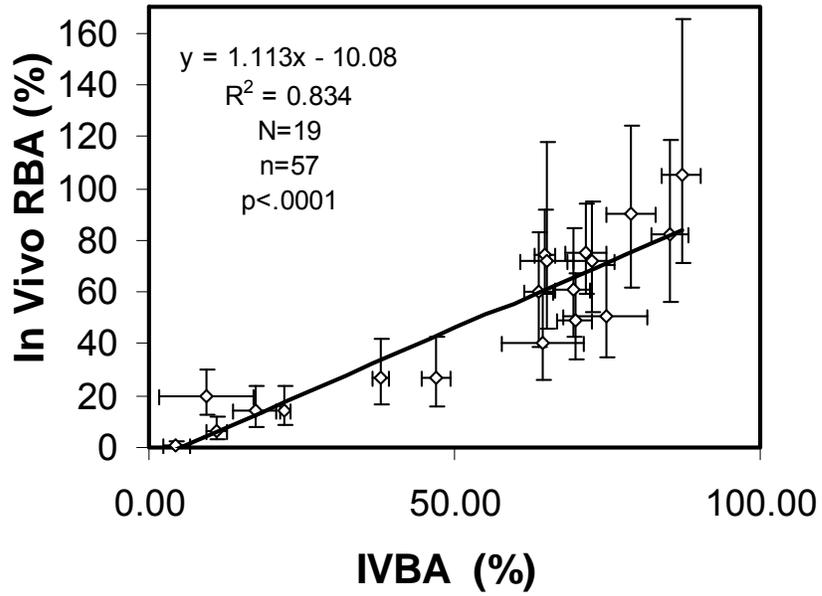
Standard Operating Procedure

1.0 Purpose

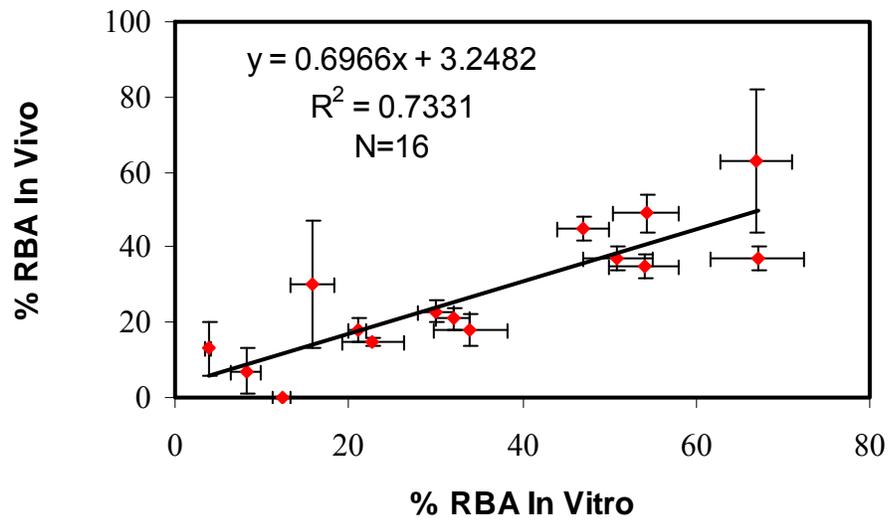
An increasingly important property of contaminated media found at environmental sites is the bioavailability of individual contaminants. Bioavailability is the fraction of a contaminant that is absorbed by an organism via a specific exposure route. Many animal studies have been conducted to experimentally determine oral bioavailability of individual metals, particularly lead and arsenic. During the period 1989-97, a juvenile swine model developed by USEPA Region VIII was used to predict the relative bioavailability of lead and arsenic in approximately 20 substrates (Weis and LaVelle 1991; Weis et al. 1994). The bioavailability determined was relative to that of a soluble salt (i.e. lead acetate trihydrate or sodium arsenate). The tested media had a wide range of mineralogy, and produced a range of lead and arsenic bioavailability values. In addition to the swine studies, other animal models (e.g. rats and monkeys) have been used for measuring the bioavailability of lead and arsenic from soils.

Several researchers have developed *in vitro* tests to measure the fraction of a chemical solubilized from a soil sample under simulated gastrointestinal conditions. The *in vitro* tests consist of an aqueous fluid, into which the contaminant is introduced. The solution then solubilizes the media under simulated gastric conditions. Once this procedure is complete, the solution is analyzed for lead and/or arsenic concentrations. The mass of the lead and/or arsenic found in the filtered extract is compared to the mass introduced into the test. The fraction liberated into the aqueous phase is defined as the bioavailable fraction of lead or arsenic in that media. To date, for lead-bearing materials tested in the USEPA swine studies, this *in vitro* assay has correlated well ($R^2 = 0.83$, $p = .0001$) with relative bioavailability. Arsenic has yet to be fully validated but shows a promising correlation with *in vivo* results.

LEAD



ARSENIC



2.0 Scope

This procedure has been developed to test contaminated media in animal studies, to determine the correlation between *in vitro* and *in vivo*. Only samples from which mineralogy has been fully characterized by EMPA techniques and for which bioavailability results from acceptable animal studies are available have been used for this study. A total of 20 substrates have been tested in validating the relative bioavailability leaching procedure (RBLP).

3.0 Relevant Literature

Background on the development and validation of *in vitro* test systems for estimating lead and arsenic bioaccessibility can be found in; Ruby et al. (1993, 1996); Medlin (1972); Medlin and Drexler, 1997; Drexler, 1998; and Drexler et al., 2005 and Casteel et al 2005..

Background information for the USEPA swine studies may be found in (Weis and LaVelle, 1991; Weis et al. 1994; and Casteel et al., 1997) and in the USEPA Region VIII Center in Denver, Colorado.

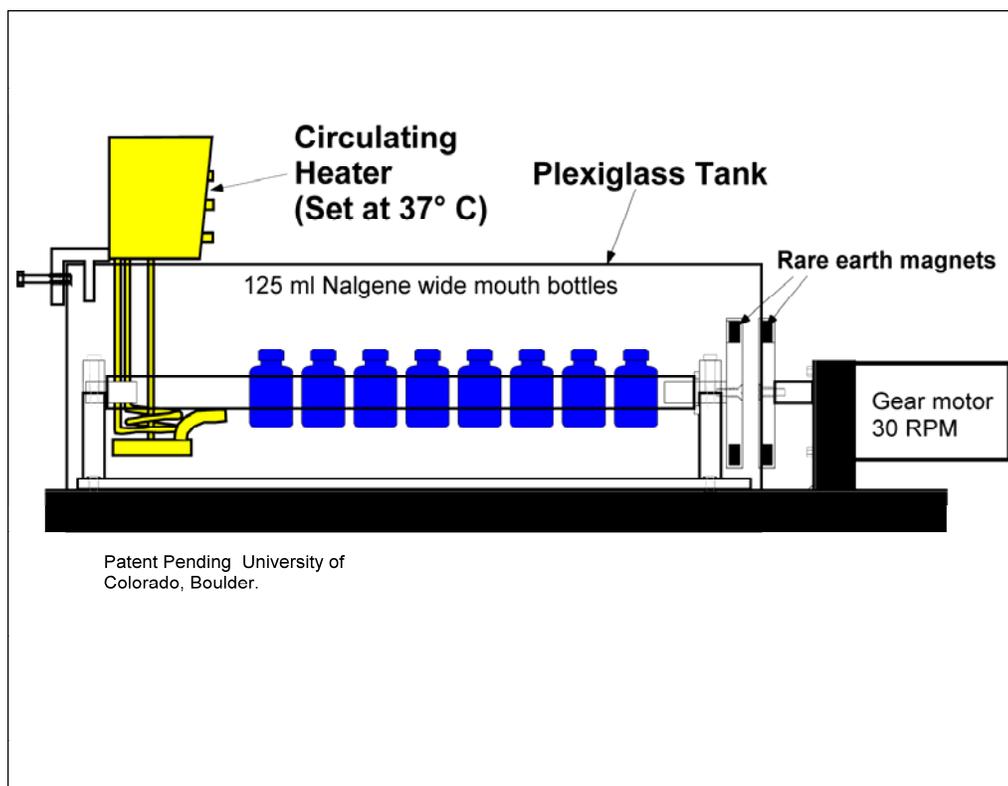
4.0 Sample Preparation

All media are prepared for the *in vitro* assay by first drying (<40 °C) all samples and then sieving to < 250 µm. The <250 micron size fraction was used because this is the particle size is representative of that which adheres to children's hands. Samples were thoroughly mixed prior to use to ensure homogenization. Samples are archived after the study completion and retained for further analysis for a period of six months unless otherwise requested. Prior to obtaining a subsample for testing in this procedure, each sample must be homogenized in its sample container by end-over-end mixing.

5.0 Apparatus and Materials

5.1 Equipment

The main piece of equipment required for this procedure is the extraction device illustrated in Figure 1. The device can be purchased from the Department of Geological Sciences, University of Colorado. For further information contact Dr. John W. Drexler, at (303) 492-5251 or drexlerj@colorado.edu. The device holds ten 125 ml, wide-mouth high-density polyethylene (HDPE) bottles. These are rotated within a Plexiglas tank by a TCLP extractor motor with a modified flywheel. The water bath must be filled such that the extraction bottles remained immersed. Temperature in the water bath is maintained at 37 +/- 2 °C using an immersion circulator heater (Fisher Scientific Model 730).



The 125-ml HDPE bottles must have an airtight screw-cap seal (Fisher Scientific #02-893-5C), and care must be taken to ensure that the bottles do not leak during the extraction procedure.

5.2 Standards and Reagents

The leaching procedure for this method uses an aqueous extraction fluid at a pH value of 1.5. The pH 1.5 fluid is prepared as follows:

Prepare 2 L of aqueous extraction fluid using ASTM Type II demonized (DI) water. The buffer is made up in the following manner. To 1.9 L of DI water, add 60.06 g glycine (free base, reagent grade), and bring the solution volume to 2 L (0.4M glycine). Place the mixture in the water bath at 37 °C until the extraction fluid reaches 37 °C. Standardize the pH meter (one should use both a 2.0 and a 4.0 pH buffer for standardization) using temperature compensation at 37 °C or buffers maintained at 37 °C in the water bath. Add trace metal grade, concentrated hydrochloric acid (12.1N) until the solution pH reaches a value of 1.50 +/- 0.05 (approximately 60 mL. All reagents must be free of lead and arsenic, and the final fluid must be tested to confirm that lead and arsenic concentrations are less than one-fourth the project required detection limits (PRDLs) of 100 and 20 µg/L, respectively (e.g., less than 25 µg/L lead and 5µg/L arsenic 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 must be properly cleaned, acid washed, and finally, triple-rinsed with demonized water prior to use.

6.0 Leaching Procedure

Measure 100 +/- 0.5 mL of the extraction fluid, using a graduated cylinder, and transfer to a 125 mL wide-mouth HPDE bottle. Add 1.00 +/- 0.5 g of test substrate (<250 m) to the bottle, ensuring that static electricity does not cause soil particles to adhere to the lip or outside threads of the bottle. If necessary, use an antistatic brush to eliminate static electricity prior to adding the media. Record the mass of substrate added to the bottle. Hand-tighten each bottle top and shake/invert to ensure that no leakage occurs, and that no media is caked on the bottom of the bottle.

Place the bottle into the modified TCLP extractor, making sure each bottle is secure and the lid(s) are tightly fastened. Fill the extractor with 125 mL bottles containing test materials or QA samples.

The temperature of the water bath must be 37 +/- 2 °C.

Turn on the extractor and rotate end-over-end at 30 +/- 2 rpm for 1 hour. Record the start time of rotation.

When extraction (rotation) is complete, immediately stop the extractor rotation and remove the bottles. Wipe them dry and place upright on the bench top.

Draw extract directly from the reaction vessel into a disposable 20 cc syringe with a Luer-Lok attachment. Attach a 0.45 µm cellulose acetate disk filter (25 mm diameter) to the syringe, and filter the extract into a clean 15 mL polypropylene centrifuge tube (labeled with sample ID) or other appropriate sample vial for analysis.

Record the time that the extract is filtered (i.e. extraction is stopped). If the total time elapsed is greater than 1 hour 30 minutes, the test must be repeated.

Measure the pH of the remaining fluid in the extraction bottle. If the fluid pH is not within +/- 0.5 pH units of the starting pH, the test must be discarded and the sample reanalyzed as follows:

If the pH has changed more than 0.5 units, the test will be re-run in an identical fashion. If the second test also results in a decrease in pH of greater than 0.5 s.u. this will be recorded, and the extract filtered for analysis. If the pH has increased by 0.5 s.u. or more, the test must be repeated, but the extractor must be stopped at specific intervals and the pH manually adjusted down to pH of 1.5 with dropwise addition of HCl (adjustments at 5, 10, 15, and 30 minutes into the extraction, and upon final removal from the water bath { 60 min}). Samples with rising pH values might better be run following the method of Medlin, 1997.

Store filtered samples in a refrigerator at 4 °C until they are analyzed. Analysis for lead and arsenic concentrations must occur within 1 week of extraction for each sample.

Extracts are to be analyzed for lead and arsenic, as specified in SOP #2, following EPA methods 6010B, 6020, or 7061A.

6.1 Quality Control/Quality Assurance

Quality Assurance for the extraction procedure will consist of the following quality control samples.

Reagent Blank-extraction fluid analyzed once per batch.

Bottle Blank-extraction fluid only run through the complete procedure at a frequency of 1 in 20 samples.

Duplicate sample-duplicate sample extractions to be performed on 1 in 10 samples.

Matrix Spike-a subsample of each material used will be spiked at concentrations of 10 mg/L lead and 1 mg/L arsenic and run through the extraction procedure (frequency of 1 in 10 samples).

National Institute of Standards and Testing (NIST) Standard Reference Material (SRM) 2711 will be used as a control soil. The SRM will be analyzed at a frequency of 1 in 25 samples.

Control limits are listed in Table 1. Limits for arsenic are only preliminary until final method is published

Table 1. RBALP Control Limits.

	Analysis Frequency	Control Limits
<u>Reagent Blank</u>	once per batch	< 25 □g/L lead < 5 □g/L arsenic
Bottle blank	5%	<50 □g/L lead <10 □g/L arsenic
Blank spike*	5%	85-115% recovery
Matrix spike*	10%	75-125% recovery
Duplicate sample	10%	+/- 20% RPD**
Control soil***	5%	+/- 10% RPD

* Spikes contained 10 mg/L lead 1 mg/l arsenic. ** RPD= relative percent difference. *** The National Institute of Standards and Technology (NIST) Standard Reference Material (SRM)

7.0 Chain-of-Custody Procedures

All media once received by the Laboratory must be maintained under standard chain-of-custody.

8.0 Data Handling and Verification

All sample and fluid preparation calculations and operations must be recorded on data sheets. Finally all key data will be entered into the attached EXCEL spreadsheet for final delivery and calculation of Bioavailability.

9.0 References

Casteel, S.W., R.P. Cowart, C.P. Weis, G.M. Henningsen, E.Hoffman and J.W. Drexler. 1997. Bioavailability of lead in soil from the Smuggler Mountain site of Aspen Colorado. Fund. Appl. Toxicol. 36: 177-187.

Casteel, S.W., C.P. Weis, G.M. Henningsen, and W.J. Brattin. 2005. Estimation of Relative Bioavailability of Lead in Soil and Soil-Like Materials Using Young Swine. Environmental Health Perspectives. (In Press).

Drexler, J.W., 1998. An *in vitro* method that works! A simple, rapid and accurate method for determination of lead bioavailability. EPA Workshop, Durham, NC..

Drexler, J.W., Brattin, W. and Weis, C.P., 2005. An *In Vitro* Procedure for Estimation of Lead Relative Bioavailability. Envir. Health Persp.. (In Press).

Medlin, E., and Drexler, J.W., 1995. Development of an *in vitro* technique for the determination of bioavailability from metal-bearing solids., International Conference on the Biogeochemistry of Trace Elements, Paris, France.

Medlin, E.A., 1997, An *In Vitro* method for estimating the relative bioavailability of lead in humans. Masters thesis. Department of Geological Sciences, University of Colorado, Boulder.

Ruby, M.W., A. Davis, T.E. Link, R. Schoof, R.L. Chaney, G.B. Freeman, and P. Bergstrom. 1993. Development of an *in vitro* screening test to evaluate the *in vivo* bioaccessability of ingested mine-waste lead. *Environ. Sci. Technol.* 27(13): 2870-2877.

Ruby, M.W., A. Davis, R. Schoof, S. Eberle. And C.M. Sellstone. 1996 Estimation of lead and arsenic bioavailbilty using a physiologically based extraction test. *Environ. Sci. Technol.* 30(2): 422-430.

Weis, C.P., and J.M. LaVelle. 1991. Characteristics to consider when choosing an animal model for the study of lead bioavailability. In: *Proceedings of the International Symposium on the Bioavailability and Dietary Uptake of Lead.* *Sci. Technol. Let.* 3:113-119.

Weis, C.P., R.H., Poppenga, B.J. Thacker, and G.M. Henningsen, 1994. Design of pharmacokinetic and bioavailability studies of lead in an immature swine model. In: *Lead in paint, soil, and dust: health risks, exposure studies, control measures, measurement methods, and quality assurance,* ASTM STP 1226, M.E. Beard and S.A. Iske (Eds.). American Society for Testing and Materials, Philadelphia, PA, 19103-1187.

REPORT 1

Bioavailability of Lead in US Army
Aberdeen Proving Ground, Maryland, Firing Ranges
US Army Experiment 1

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EXECUTIVE SUMMARY

A study using juvenile swine as test animals was performed to measure the gastrointestinal absorption of lead from two test materials, KD Range 1 and S Range 2 from the US Army Aberdeen Proving Ground, Maryland. The relative bioavailability of lead was assessed by comparing the absorption of lead from the test materials to that of a reference material (lead acetate). The lead concentrations of KD Range 1 and S Range 2 were 15,667 ug/g and 23,333 ug/g, respectively. Groups of five swine were given oral doses of lead acetate or a test material twice a day for 15 days. The amount of lead absorbed by each animal was evaluated by measuring the amount of lead in the blood (measured on days 0, 1, 2, 3, 5, 7, 9, 12, and 15) and the amount of lead in liver, kidney, and bone (measured on day 15 at study termination). The amount of lead present in blood or tissues of animals exposed to test materials was compared to that for animals exposed to lead acetate, and the results were expressed as relative bioavailability (RBA). For example, a relative bioavailability of 50% means that 50% of the lead in soil was absorbed equally as well as lead from lead acetate, and 50% behaved as if it were not available for absorption. Thus, if lead acetate were 40% absorbed, the test material would be 20% absorbed.

The RBA results for the two samples from this investigation are summarized below:

Measurement Endpoint	Test Soil	
	KD Range 1	S Range 2
Blood Lead AUC	127%	93%
Liver Lead	91%	71%
Kidney Lead	81%	66%
Bone Lead	68%	66%

However, because the estimates of RBA based on blood, liver, kidney, and bone do not agree in all cases, judgment must be used in interpreting the data. In general, we recommend greatest emphasis be placed on the RBA estimates derived from the blood lead data. This is because blood lead data are more robust and less susceptible to random errors than the tissue lead data, so there is greater confidence in RBA estimates based on blood lead. In addition, absorption into the central compartment is an early indicator of lead exposure, is the most relevant index of central nervous system exposure, and is the standard measurement endpoint in investigations of this sort. However, data from the tissue endpoints (liver, kidney, bone) also provide valuable information. We consider the plausible range to extend from the RBA based on blood AUC to the mean of the other three tissues (liver, kidney, bone). The preferred range is the interval from the RBA based on blood to the mean of the blood RBA and the tissue mean RBA. Our suggested point estimate is the mid-point of the preferred range. These values are presented below:

Relative Bioavailability of Lead	Test Soil	
	KD Range 1	SD Range 2
Plausible Range	127-80%	93-67%
Preferred Range	127-103%	93-80%
Suggested Point Estimate	115%	87%

Because of the inherent variability in the responses of different individual animals to lead exposure and the uncertainty in the relative accuracy and applicability of the different measurement endpoints, the values reported above should be interpreted with the understanding that the values may not be highly precise.

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1.0 INTRODUCTION

Absolute and Relative Bioavailability

Bioavailability is a concept that relates to the absorption of chemicals and how absorption depends upon the physical-chemical properties of the chemical and its medium (e.g., dust, soil, rock, food, water, etc.) and the physiology of the exposed receptor. Bioavailability is normally described as the fraction (or percentage) of a chemical which enters into the blood following an exposure of some specified amount, duration and route (usually oral). In some cases, bioavailability may be measured using chemical levels in peripheral tissues such as liver, kidney, and bone, rather than blood. The fraction or percentage absorbed may be expressed either in absolute terms (absolute bioavailability, ABA) or in relative terms (relative bioavailability, RBA). **Absolute bioavailability** is measured by comparing the amount of chemical entering the blood (or other tissue) following oral exposure to test material with the amount entering the blood (or other tissue) following intravenous exposure to an equal amount of some dissolved form of the chemical. Similarly, **relative bioavailability** is measured by comparing oral absorption of test material to oral absorption of some fully soluble form of the chemical (e.g., either the chemical dissolved in water, or a solid form that is expected to fully dissolve in the stomach). For example, if 100 ug of dissolved lead were administered in drinking water and a total of 50 ug entered the blood, the ABA would be 0.50 (50%). Likewise, if 100 ug of lead in soil were administered and 30 ug entered the blood, the ABA for soil would be 0.30 (30%). If the lead dissolved in water were used as the reference substance for describing the relative amount of lead absorbed from soil, the RBA would be $0.30/0.50 = 0.60$ (60%). These values (50% absolute bioavailability of dissolved lead and 30% absolute absorption of lead in soil) are the values currently employed as defaults in EPA's IEUBK model.

It is important to recognize that simple solubility of a test material in water or some other fluid (e.g., a weak acid intended to mimic the gastric contents of a child) may not be a reliable estimator of bioavailability due to the non-equilibrium nature of the dissolution and transport processes that occur in the gastrointestinal tract (Mushak 1991). For example, fluid volume and pH are likely to be changing as a function of time, and transport of lead across the gut will prevent an approach to equilibrium concentrations, especially for poorly soluble lead compounds. However, information on the solubility of lead in different materials is useful in interpreting the importance of solubility as a determinant of bioavailability. To avoid confusion, the term "bioaccessability" is used to refer to the relative amount of lead that dissolves under a specified set of test conditions.

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

Using Bioavailability Data to Improve Exposure Calculations for Lead

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

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

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}	=	RBA for soil measured in swine

Based on available information on lead absorption in humans and animals, the EPA estimates that the absolute bioavailability of lead from water and other fully soluble forms of lead is usually about 50% in children. Thus, when a reliable site-specific RBA value for soil is available, it may be used to estimate a site-specific absolute bioavailability as follows:

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

In the absence of site-specific data, the absolute absorption of lead from soil, dust and other similar media is estimated by EPA to be about 30%. Thus, the default RBA used by EPA for lead in soil and dust compared to lead in water is $30\%/50\% = 60\%$. 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 risks 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 risk from lead in these media may be higher than usually assumed.

2.0 STUDY DESIGN

A standardized study protocol for measuring absolute and relative bioavailability of lead was developed based upon previous study designs and investigations that characterized the young pig model (Weis et al. 1995). The study was performed as nearly as possible within the spirit and guidelines of Good Laboratory Practices (GLP: 40 CFR 792). A Quality Assurance Project Plan was prepared, approved and distributed to all study member prior to the study.

2.1 Test Materials

The two study test materials were taken from firing ranges at the US Army Aberdeen Proving Grounds, Maryland. Kd Range 1 was taken from the berm of an old long distance range which has been closed for about ten years. S Range 2 was from a sighting range about 300 yards from the known distance range. Ccomposite samples, of both test materials, were taken from 3-4 areas within 5 meters of each other, to a depth of 2-3 inches. The soils were dried at the Soils Lab of the Aberdeen Proving Ground and then sieved consecutively through a 2.0mm(#10) and a 250 micrometer (#60) sieves. The less than 250 micron friction was retained and used for the study. Samples were rolled for 30 minutes at a low speed, inverted 5 times, then sampled in triplicate for analysis for Pb content. The table below contains the results. The mean value was used for study dosing calculations.

Pb Concentration ppm	Test Soil	
	KD Range 1	S Range 2
Sample 1	15,000	26,000
Sample 2	16,000	20,000
Sample 3	16,000	24,000
Mean Value	15,667	23,333

2.2 Experimental Animals

Young swine were selected for use in these studies because they are considered to be a good physiological model for gastrointestinal absorption in children (Weis and LaVelle 1991). The animals were intact males of the Pig Improvement Corporation (PIC) genetically defined Line 26, and were purchased from Chinn Farms, Clarence, MO. The animals were held under quarantine to observe their health for one week before beginning exposure to test materials. To minimize weight variations between animals and groups, the number of animals purchased from the supplier was seven more than needed for the study, and the seven animals most different in body weight on day -4 (either heavier or lighter) and/or animals evidencing any signs of illness or injury, were excluded from further study. The remaining animals were assigned to dose groups at random. When exposure began (day zero), the animals were about 5-6 weeks old (juveniles, weaned at 3 weeks) and weighed an average

of about 13kg. Animals were weighed every three days during the course of the study. The group mean body weights over the course of the study are shown in Figure 2-1. On average, animals gained about 0.5 kg/day, and the rate of weight gain was comparable in all groups.

All animals were housed in individual lead-free stainless steel cages. Each animal was examined by a certified veterinary clinician (swine specialist) prior to being placed on study, and verified as PRRS (Porcine Reproductive and Respiratory Syndrome)-negative by testing. All animals were examined daily by an attending veterinarian while on study

2.3 Diet

Animals provided by the supplier were weaned onto standard pig chow purchased from MFA Inc., Columbia, MO. In order to minimize lead exposure from the diet, the animals were gradually transitioned from the MFA feed to a special low-lead feed (guaranteed less than 0.2 ppm lead, purchased from Zeigler Brothers, Inc., Gardners, PA) over the time interval from day - 7 to day -3, and this feed was then maintained for the duration of the study. The feed was nutritionally complete and met all requirements of the National Institutes of Health-National Research Council. The typical nutritional components and chemical analysis of the feed is presented in Table 2-2. Typically, the feed contained approximately 5.7% moisture, 1.7% fiber, and provided about 3.4 kcal of metabolizable energy per gram. Analysis of feed samples from other experiments indicate the mean lead level is typically below the quantitation limit of 0.09 ppm.

Each day every animal was given an amount of feed equal to 4% of the mean body weight of all animals on study. Feed was administered in two equal portions of 2% of the mean body weight at each feeding. Feed was provided at 11:00 AM and 5:00 PM daily. Drinking water was provided ad libitum via self-activated watering nozzles within each cage. Analysis of samples from randomly selected drinking water nozzles during previous studies indicate the mean lead concentration (treating non-detects at one-half the quantitation limit) is less than 1 ug/L.

Figure 2-1 Body Weights

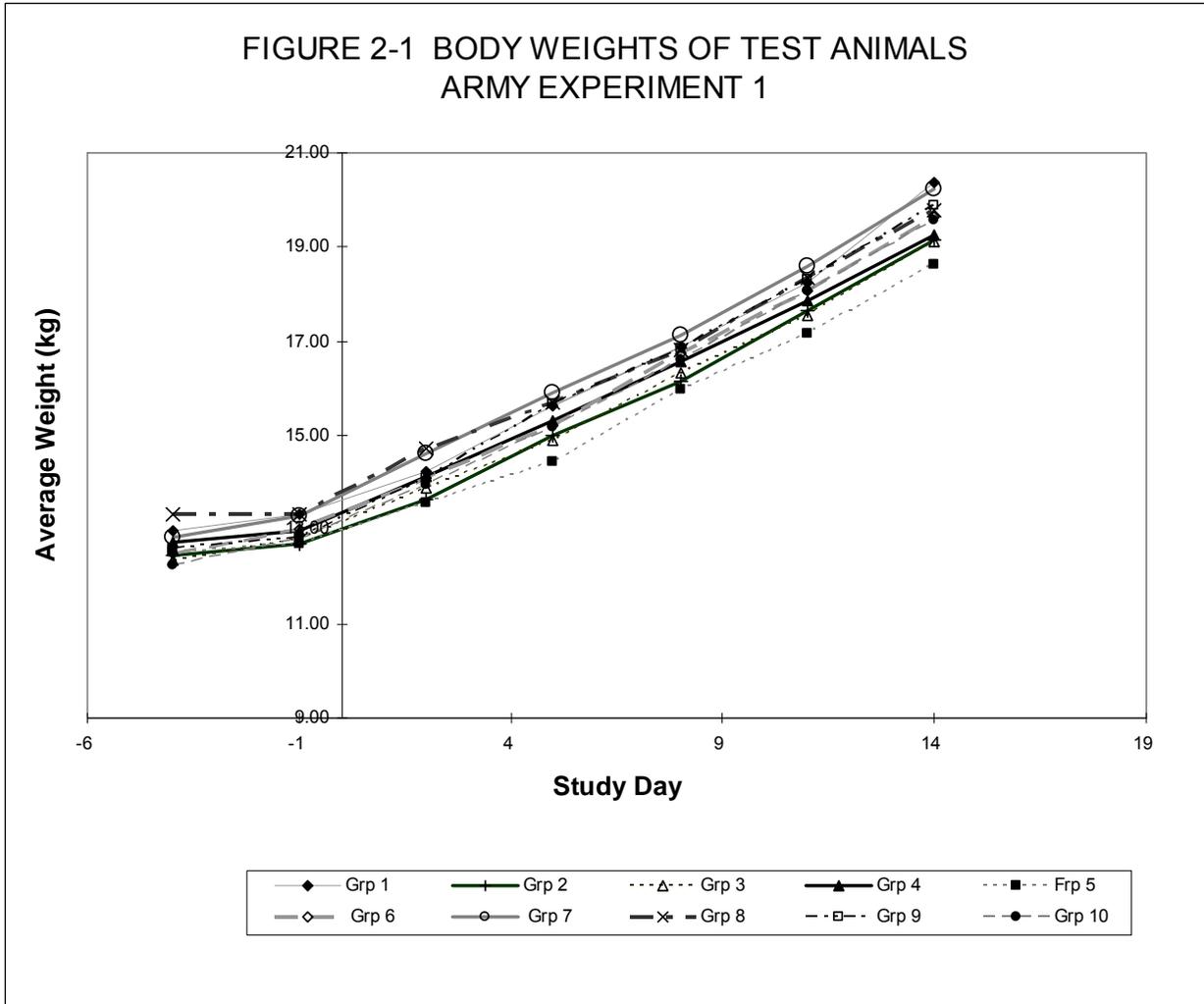


Table 2-1 Typical Feed Composition^a

Nutrient Name	Amount	Nutrient Name	Amount
Protein	20.1021%	Chlorine	0.1911%
Arginine	1.2070%	Magnesium	0.0533%
Lysine	1.4690%	Sulfur	0.0339%
Methionine	0.8370%	Manganese	20.4719 ppm
Met+Cys	0.5876%	Zinc	118.0608 ppm
Tryptophan	0.2770%	Iron	135.3710 ppm
Histidine	0.5580%	Copper	8.1062 ppm
Leucine	1.8160%	Cobalt	0.0110 ppm
Isoleucine	1.1310%	Iodine	0.2075 ppm
Phenylalanine	1.1050%	Selenium	0.3196 ppm
Phe+Tyr	2.0500%	Nitrogen Free Extract	60.2340%
Threonine	0.8200%	Vitamin A	5.1892 kIU/kg
Valine	1.1910%	Vitamin D3	0.6486 kIU/kg
Fat	4.4440%	Vitamin E	87.2080 IU/kg
Saturated Fat	0.5590%	Vitamin K	0.9089 ppm
Unsaturated Fat	3.7410%	Thiamine	9.1681 ppm
Linoleic 18:2:6	1.9350%	Riboflavin	10.2290 ppm
Linoleic 18:3:3	0.0430%	Niacin	30.1147 ppm
Crude Fiber	3.8035%	Pantothenic Acid	19.1250 ppm
Ash	4.3347%	Choline	1019.8600 ppm
Calcium	0.8675%	Pyridoxine	8.2302 ppm
Phos Total	0.7736%	Folacin	2.0476 ppm
Available Phosphorous	0.7005%	Biotin	0.2038 ppm
Sodium	0.2448%	Vitamin B12	23.4416 ppm
Potassium	0.3733%		

^a Nutritional values provided by Zeigler Bros., Inc.

2.4 Dosing

The protocol for exposing animals to lead is shown in Table 2-3. The dose levels for lead acetate were based on experience from previous swine investigations that showed that doses of 25-225 ug Pb/kg/day gave clear and measurable increases in lead levels in all endpoints measured (blood, liver, kidney, bone). The doses of test materials were set at the same level as lead acetate, with one higher dose (500 ug Pb/kg-day) included in case the test materials were found to yield very low responses. Actual mean doses, calculated from the administered doses and the measured body weights, are also shown in Table 2-3.

Animals were exposed to lead acetate or test material for 15 days, with the dose for each day being administered in two equal portions given at 9:00 AM and 3:00 PM (two hours before feeding). Doses were based on measured group mean body weights, and were adjusted every three days to account for animal growth. For animals exposed by the oral route, dose material was placed in the center of a small portion (about 5 grams) of moistened feed, and this was administered to the animals by hand. Most animals consumed their dose readily, but occasionally some animals delayed ingestion of the dose for up to two hours (the time the daily feed portion was provided). These delays are noted in the data provided in Appendix A, but are not considered to be a significant source of error. Occasionally, some animals did not consume some or all of the dose (usually because the dose dropped from their mouth while chewing). All missed or partial doses were recorded and the time-weighted average dose calculation for each animal was adjusted downward accordingly.

2.5 Collection of Biological Samples

Samples of blood were collected from each animal on the first day of exposure (day 0), and on days 1, 2, 3, 5, 7, 9, 12, and 15 following the start of exposure. All blood samples were collected by vena-puncture of the anterior vena cava, and samples were immediately placed in purple-top Vacutainer® tubes containing EDTA as anticoagulant. Although EDTA is a chelator of metals, its presence in the sampling tubes will not impact the analytical results for lead. This is because the nitric acid digest used in the analysis destroys the organic constituents in the blood, thereby freeing all lead for analysis. Blood samples were collected each sampling day beginning at 8:00 AM, approximately one hour before the first of the two daily exposures to lead on the sampling day and 17 hours after the last lead exposure the previous day. This blood collection time was selected because the rate of change in blood lead resulting from the preceding exposures is expected to be relatively small after this interval (LaVelle et al. 1991, Weis et al. 1993), so the exact timing of sample collection relative to last dosing is not likely to be critical.

Following collection of the final blood sample at 8:00 AM on day 15, all animals were humanely euthanized and samples of liver, kidney and bone (the right femur) were removed and stored in lead-free plastic bags at -40C until preparation for lead analysis. Samples of all biological samples collected were archived in order to allow for reanalysis and verification of lead levels, if needed, and possibly for future analysis for other metals (arsenic, cadmium, etc.). All animals were also subjected to detailed examination at necropsy by a certified veterinary pathologist in order to assess overall animal health.

2.6 Preparation of Biological Samples for Analysis

Blood

One mL of whole blood was removed from the purple-top Vacutainer and added to 9.0 mL of "matrix modifier", a solution recommended by the Centers for Disease Control and Prevention (CDCP) for analysis of blood samples for lead. The composition of matrix modifier is 0.2% (v/v) trace metal nitric acid, 0.5% (v/v) Triton X-100, and 0.2% (w/v) dibasic ammonium phosphate in deionized distilled water. Samples of the matrix modifier were routinely analyzed for lead to ensure the absence of lead contamination.

Liver and Kidney

One gram of soft tissue (liver or kidney) was placed in a lead-free screw-cap teflon container with 2 mL of concentrated (70%) trace metal nitric acid and heated in an oven to 90°C overnight (12-18 hours). After cooling, the digestate was transferred to a clean lead-free 10 mL volumetric flask and diluted to volume with deionized and distilled (DD) water.

Bone

The right femur of each animal was removed and defleshed, and dried at 100°C overnight (12-18 hours). The dried bones were then placed in a muffle furnace and dry-ashed at 450°C for 48 hours. Following dry ashing, the bone was ground to a fine powder using a lead-free mortar and pestle, and 200 mg was removed and dissolved in 10.0 mL of 1:1 (v:v) concentrated trace metal nitric acid/ DDwater. After the powdered bone was dissolved and mixed, 1.0 mL of the acid solution was removed and diluted to 10.0 mL by addition of deionized distilled water.

2.7 Lead Analysis

Samples of biological tissue (blood, liver, kidney, bone) and other materials (food, water, reagents and solutions, etc.) were arranged in a random sequence and provided to the analytical laboratory in a blind fashion (identified to the laboratory only by a chain of custody tag number). Each sample was analyzed for lead using a Perkin Elmer Model AAnalyst 800 graphite furnace atomic absorption spectrophotometer. Internal quality assurance samples were run every tenth sample, and the instrument was recalibrated every 15th sample. A blank, duplicate and spiked sample were run every 20th sample.

All results from the analytical laboratory were reported in units of ug Pb/L of prepared sample. The quantitation limit was defined as three-times the standard deviation of a set of seven replicates of a low-lead sample (typically about 2-5 ug/L). The standard deviation was usually about 0.3 ug/L, so the quantitation limit was usually about 0.9-1.0 ug/L (ppb). For prepared blood samples (diluted 1/10), this corresponds to a quantitation limit of 10 ug/L (1 ug/dL). For soft tissues (liver and kidney, diluted 1/10), this corresponds to a quantitation limit of 10 ug/kg (ppb) wet weight, and for bone (final dilution = 1/500) the corresponding quantitation limit is 0.5 ug/g (ppm) ashed weight.

Table 2-2 Dosing Protocol.

Group	Number of Animals	Dose Material Administered	Exposure Route	Lead Dose (ug Pb/kg-d)	
				Target	Actual ^a
1	5	Lead Acetate	Oral	25	24.7
2	5	Lead Acetate	Oral	75	38.0 ^b
3	5	Lead Acetate	Oral	225	160.3 ^b
4	5	KD Range 1	Oral	75	76.4
5	5	KD Range 1	Oral	225	200.2
6	5	KD Range 1	Oral	500	504.9
7	5	S Range 2	Oral	75	75.6
8	5	S Range 2	Oral	225	227.5
9	5	S Range 2	Oral	500	503.0
10	3	Control	Oral	0	0

Doses were administered in two equal portions given at 9:00 AM and 3:00 PM each day. Doses were based on the mean weight of the animals in each group, and were adjusted every three days to account for weight gain.

^a Calculated as the administered daily dose divided by the measured or extrapolated daily body weight, averaged over days 0-14 for each animal and each group.

^b Stock B of Lead Acetate for dose preparation was erroneously diluted to 100ml instead of 50ml. As this solution was used for all of the Group2 dose, it made the Target Dose for this group be 35.mg/kg-d. this solution was also used for the first 3 of the 5 dose preparations for Group 3. These were then dosed at 112.5mg/kg-d for Days 0- 9, and then using Stock C, dosed at 225mg/kg-d for days 10-15.

3.0 Data Analysis

3.1 Overview

Studies on the absorption of lead are often complicated because some biological responses to lead exposure may be non-linear functions of dose (i.e., tending to flatten out or plateau as dose increases). The cause of this non-linearity is uncertain but might be due either to non-linear **absorption kinetics** and/or to non-linear **biological response** per unit dose absorbed. When the dose-response curve for either the reference material (lead acetate) and/or the test material is non-linear, RBA is equal to the ratio of doses that produce equal responses (not the ratio of responses at equal doses). This is based on the simple but biologically plausible assumption that equal absorbed doses yield equal biological responses. Applying this assumption leads to the following general methods for calculating RBA from a set of non-linear experimental data:

1. Plot the biological responses of individual animals exposed to a series of oral doses of soluble lead (e.g., lead acetate). Fit an equation which gives a smooth line through the observed data points.
2. Plot the biological responses of individual animals exposed to a series of doses of test material. Fit an equation which gives a smooth line through the observed data.
3. Using the best fit equations for reference material and test material, calculate RBA as the ratios of doses of test material and reference material which yield equal biological responses. Depending on the relative shape of the best-fit lines through the lead acetate and test material dose response curves, RBA may either be constant (dose-independent) or variable (dose-dependent).

The principal advantage of this approach is that it is not necessary to understand the basis for a non-linear dose response curve (non-linear absorption and/or non-linear biological response) in order to derive valid RBA estimates. Also, it is important to realize that this method is very general, as it will yield correct results even if one or both of the dose-response curves are linear. In the case where both curves are linear, RBA is dose-independent and is simply equal to the ratio of the slopes of the best-fit linear equations.

3.2 Fitting the Curves

There are a number of different mathematical equations which can yield reasonable fits with the dose-response data sets obtained in this study. Conceptually, any equation which gives a smooth fit would be acceptable, since the main purpose is to allow for interpolation of responses between test doses. In selecting which equations to employ, the following principles were applied: 1) mathematically simple equations were preferred over mathematically complex equations, 2) the shape of the curves had to be smooth and biologically realistic, without inflection points, maxima or minima, and 3) the general form of the equations had to be able to fit data not only from this one study, but from all the studies that are part of this project. After testing a wide variety of different equations, it was found that all data sets could be well fitted using one of the following three forms:

<u>Linear (LIN):</u>	Response = a + b·Dose
<u>Exponential (EXP):</u>	Response = a + c·(1-exp(-d·Dose))
<u>Combination (LIN+EXP):</u>	Response = a + b·Dose + c·(1-exp(-d·Dose))

Although underlying mechanism was not considered in selecting these equations, the linear equation allows fitting data that do not show evidence of saturation in either uptake or response, while the exponential and mixed equations allow evaluation of data that appear to reflect some degree of saturation in uptake and/or response.

Each dose-response data set was fit to each of the equations above. If one equation yielded a fit that was clearly superior (as judged by the value of the adjusted correlation coefficient R^2) to the others, that equation was selected. If two or more models fit the data approximately equally well, then the simplest model (that with the fewest parameters) was selected. In the process of finding the best-fits of these equations to the data, the values of the parameters (a, b, c, and d) were subjected to some constraints, and some data points (those that were outside the 95% prediction limits of the fit) were excluded. These constraints and outlier exclusion steps are detailed in Appendix A (Section 3). In general, most blood lead AUC dose-response curves were best fit by the exponential equation, and most dose-response curves for liver, kidney and bone were best fit by linear equations. In evaluating spleen results, it was determined that data were best fit by the exponential equation.

3.3 Responses Below Quantitation Limit

In some cases, most or all of the responses in a group of animals were below the quantitation limit for the endpoint being measured. For example, this was normally the case for blood lead values in unexposed animals (both on day -4 and day 0), and in control animals. In these cases, samples were assigned a response equal to one-half the quantitation limit.

3.4 Quality Assurance

A number of steps were taken throughout this study and the other studies in this project to ensure the quality of the results. These steps are summarized below.

Duplicates

A randomly selected set of about 5% of all samples generated during the study were submitted to the laboratory in a blind fashion for duplicate analysis. The raw data are presented in Appendix A, and Figure 3-1 plots the results for blood (Panel A, upper) and for bone, liver and kidney (Panel B, lower). As seen, there was moderately good intra-laboratory reproducibility between duplicate samples for both blood and tissues, with linear regression lines having an R^2 value near 0.8.

Standards

The Centers for Disease Control and Prevention (CDCP) provides a variety of blood lead "check samples" for use in quality assurance programs for blood lead studies. Each time a group of

blood samples was prepared and sent to the laboratory for analysis, several CDCP check samples of different concentrations were included in random order and in a blind fashion.

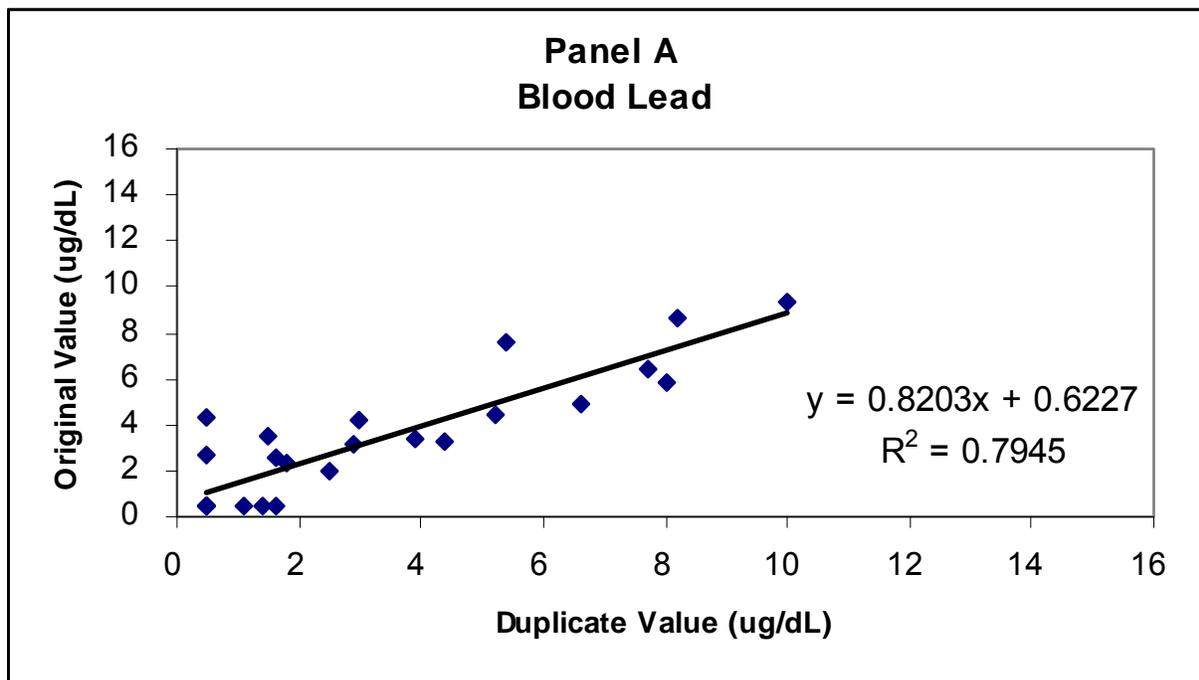
The results for the samples submitted during this study are presented in Appendix A, and the values are plotted in Figure 3-2. For the "low" standard (nominal = 1.7 ug/dL), the mean of the measured values was slightly above the detection limit (1 ug/dL) at 1.4ug/dL. For the "medium" and "high" standards, the means of the measured values were 3.9 ug/dL (nominal = 4.8 ug/dL) and 12.4 ug/dL (nominal = 13.9 ug/dL).

Data Audits and Spreadsheet Validation

All analytical data generated by the VMDL's research toxicology laboratory were validated prior to being released in the form of a database file. These electronic data files were "decoded" (linking the sample tag to the correct animal and day) using the sort function in Microsoft EXCEL®. To ensure that no errors occurred in this process, original electronic files were printed out and compared to printouts of the tag assignments and the decoded data.

All spreadsheets used to manipulate the data and to perform calculations (see Appendix A) were validated by hand-checking random cells for accuracy.

Figure 3-1 Comparison of Duplicate Analyses



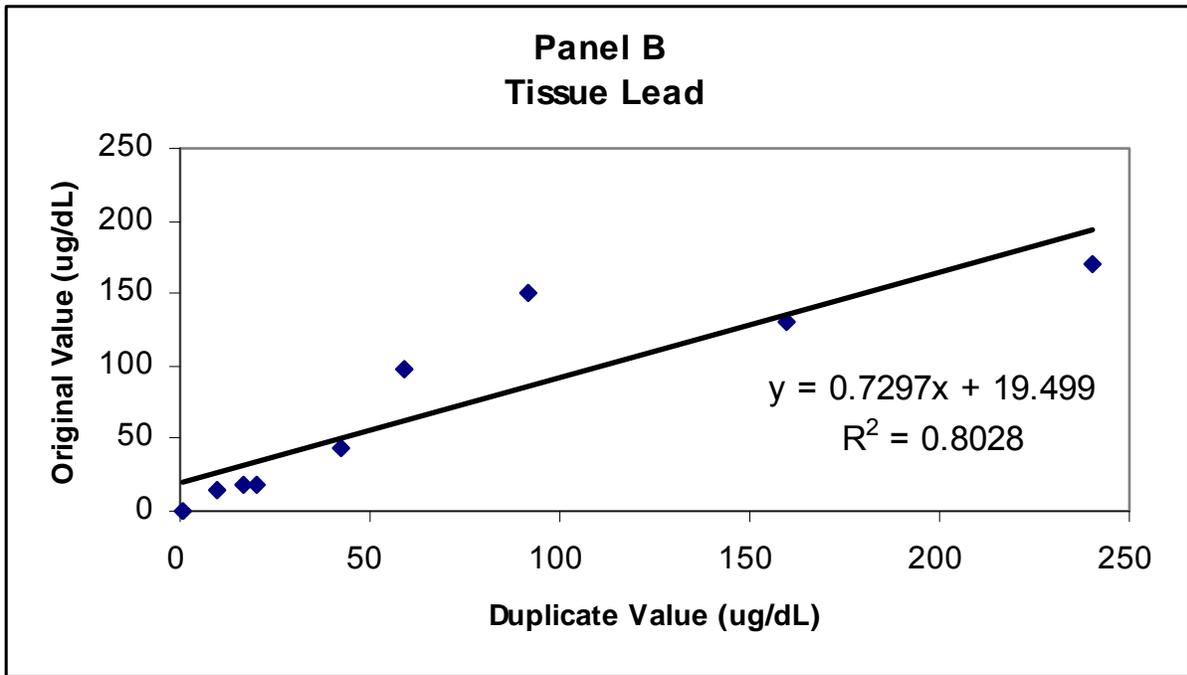
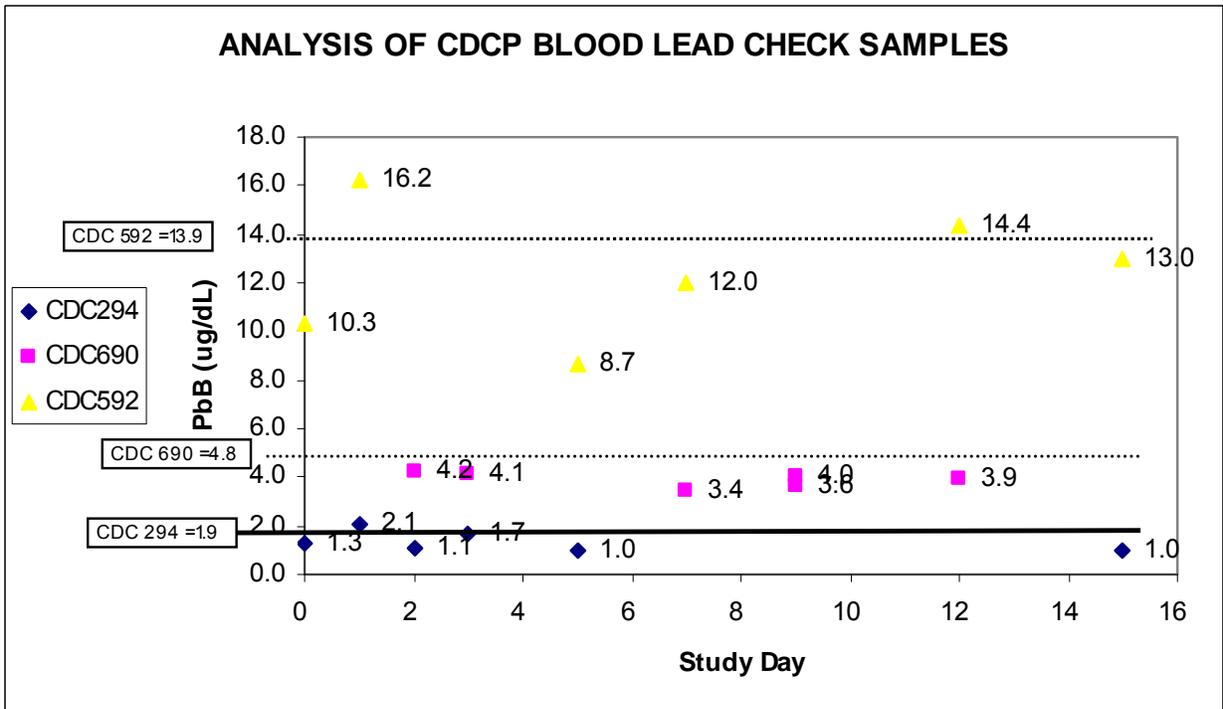


Figure 3-2 CDCP Check Samples

Army Experiment 1



4.0 Results

The following sections provide results based on the group means for each dose group investigated in this study. Appendix A provides detailed data for each individual animal. Results from this study will be compared and contrasted with the results from other studies in a subsequent report.

4.1 Blood Lead versus Time

Figure 4-1 shows the group mean blood lead values as a function of time during the study. As seen, blood lead values began at or below quantitation limits (about 1 ug/dL) in all groups, and remained at or below quantitation limits in control animals (Group 10). In animals given repeated oral doses of lead acetate (Groups 1-3), KD Range 1 soil (Groups 4-6), or S Range 2 soil (Groups 7-9), blood levels began to rise within 1-2 days, and tended to plateau by the end of the study (day 15). As seen, due to a dosing error in group 3 from day 0 through 8, there are deviations in the plateaus observed for the PbAc group.

4.2 Dose-Response Patterns

Blood Lead

The measurement endpoint used to quantify the blood lead response was the area under the curve (AUC) for blood lead vs time (days 0-15). AUC was selected because it is the standard pharmacokinetic index of chemical uptake into the blood compartment, and is relatively insensitive to small variations in blood lead level by day. The AUC was calculated using the trapezoidal rule to estimate the AUC between each time point that a blood lead value was measured (days 0, 1, 2, 3, 5, 7, 9, 12, and 15), and summing the areas across all time intervals in the study. The detailed data and calculations are presented in Appendix A, and the results are shown graphically in Figure 4-2. Each data point reflects the group mean exposure and group mean response, with the variability in dose and response shown by standard error bars. The figure also shows the best-fit equation through each data set.

As seen, the dose response pattern is non-linear for both the soluble reference material (lead acetate, abbreviated "PbAc"), and for each of the two test soils. Dose response curves for both the KD Range 1 and S Range 2 soil are similar to those seen for lead acetate.

Tissue Lead

The dose-response data for lead levels in bone, liver, and kidney (measured at sacrifice on day 15) are detailed in Appendix A, and are shown graphically in Figures 4-3 through 4-5, respectively

4-1

Group Mean PbB vs. Day, Army Experiment 1

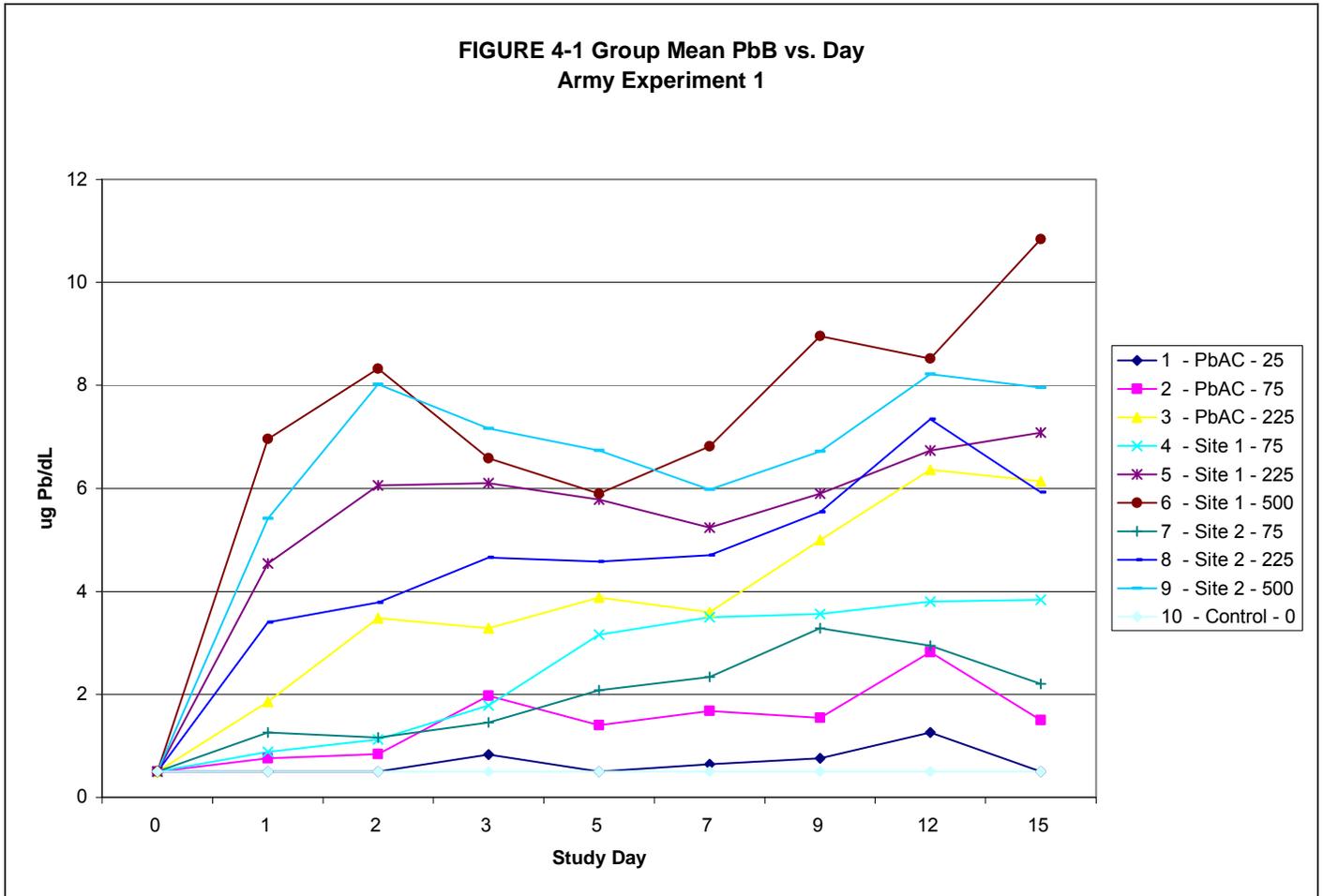


Figure 4-2 Blood Lead AUC Dose-response, Group Means +/-SEM for Army Experiment 1

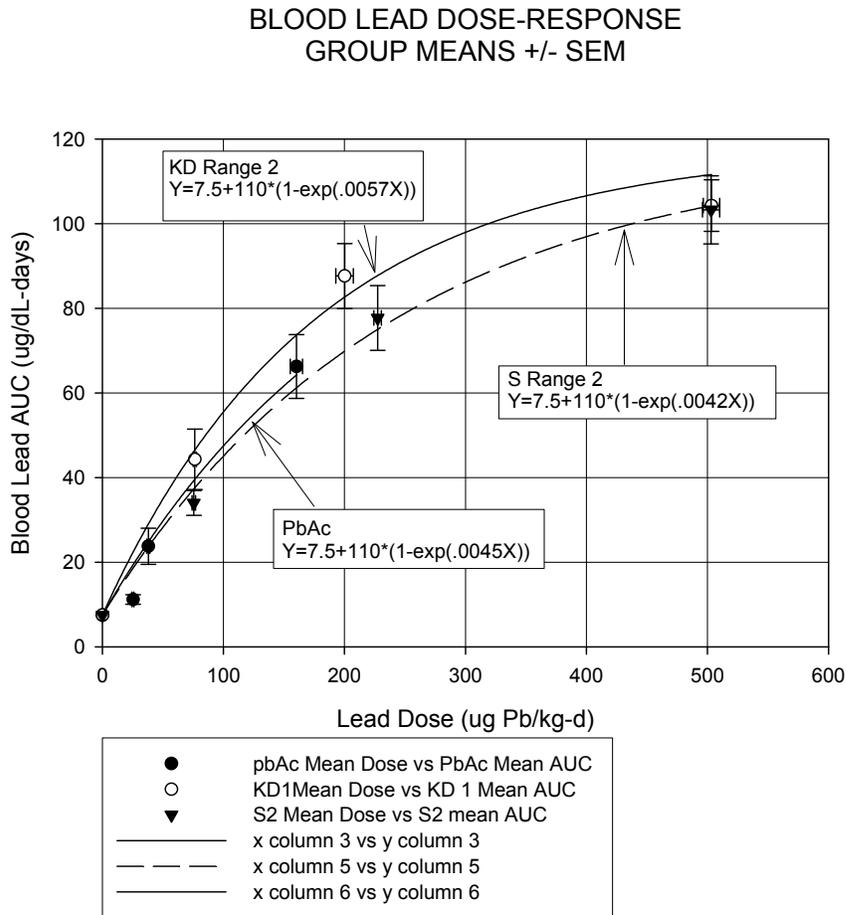


Figure 4-3 Bone Lead Dose Response, Group Means +/- SEM for Army Experiment 1

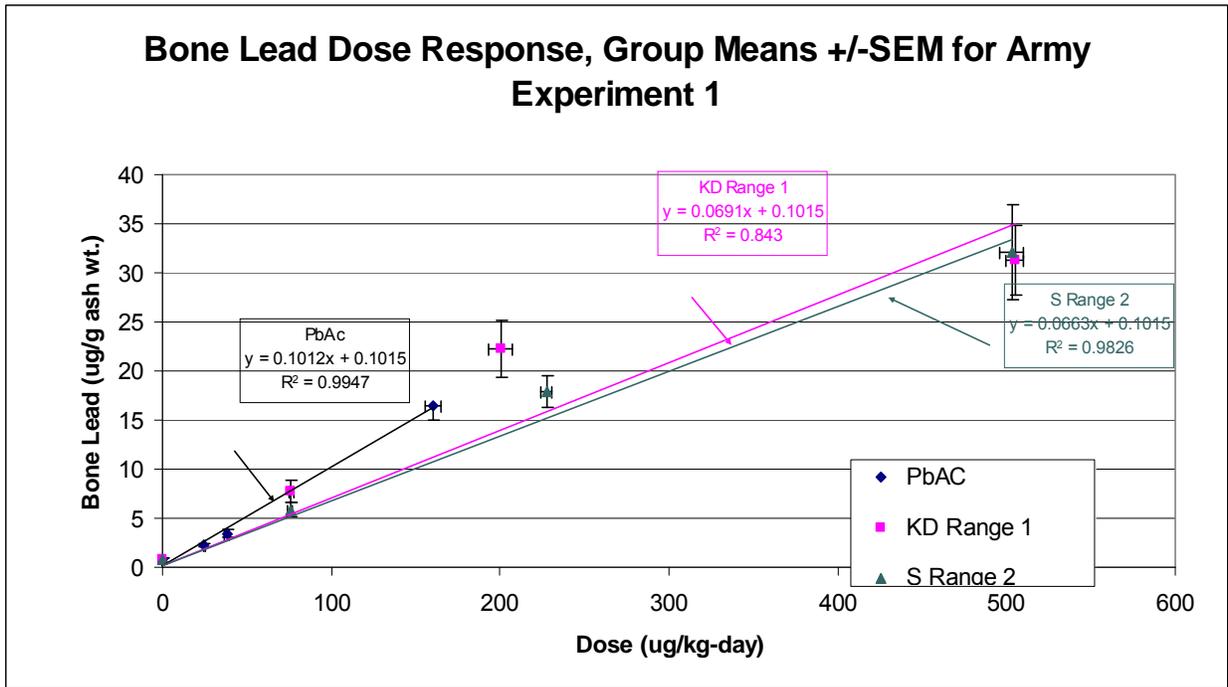


Figure 4-4 Liver Lead Dose-response, Group Means +/- SEM for Army Experiment 1

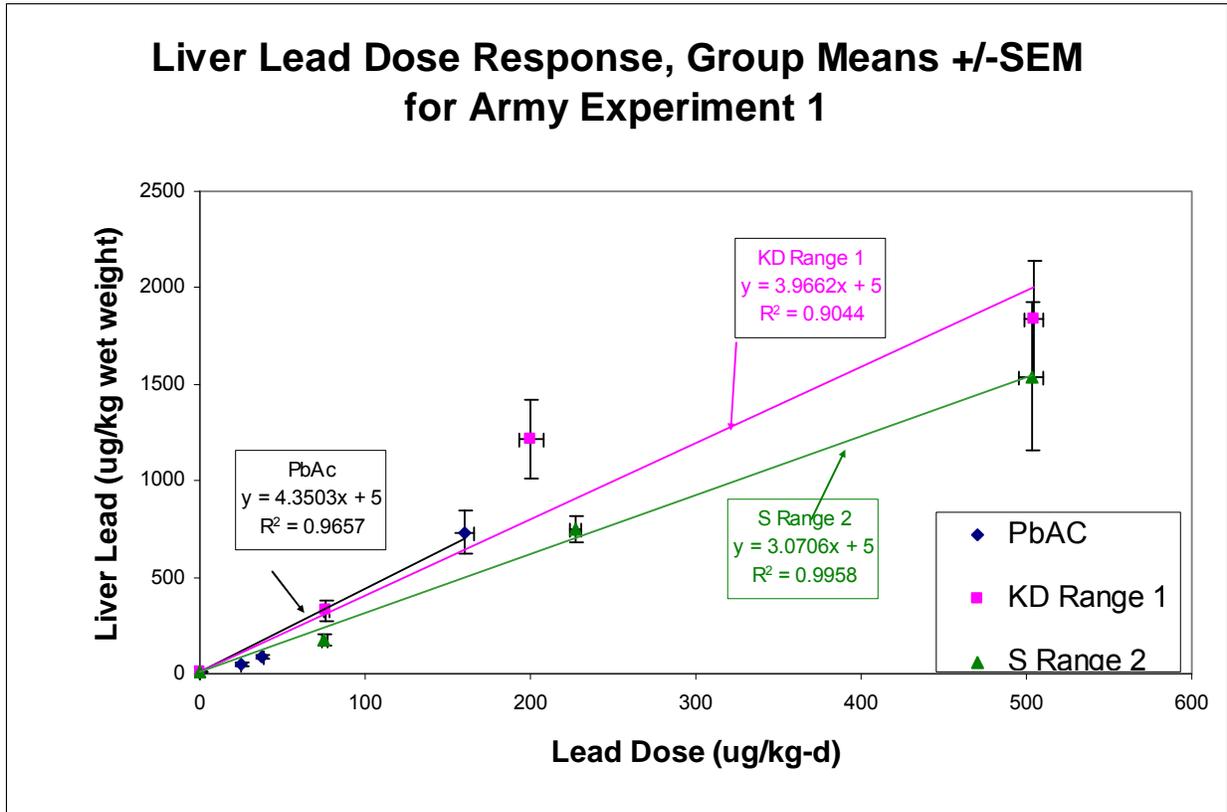
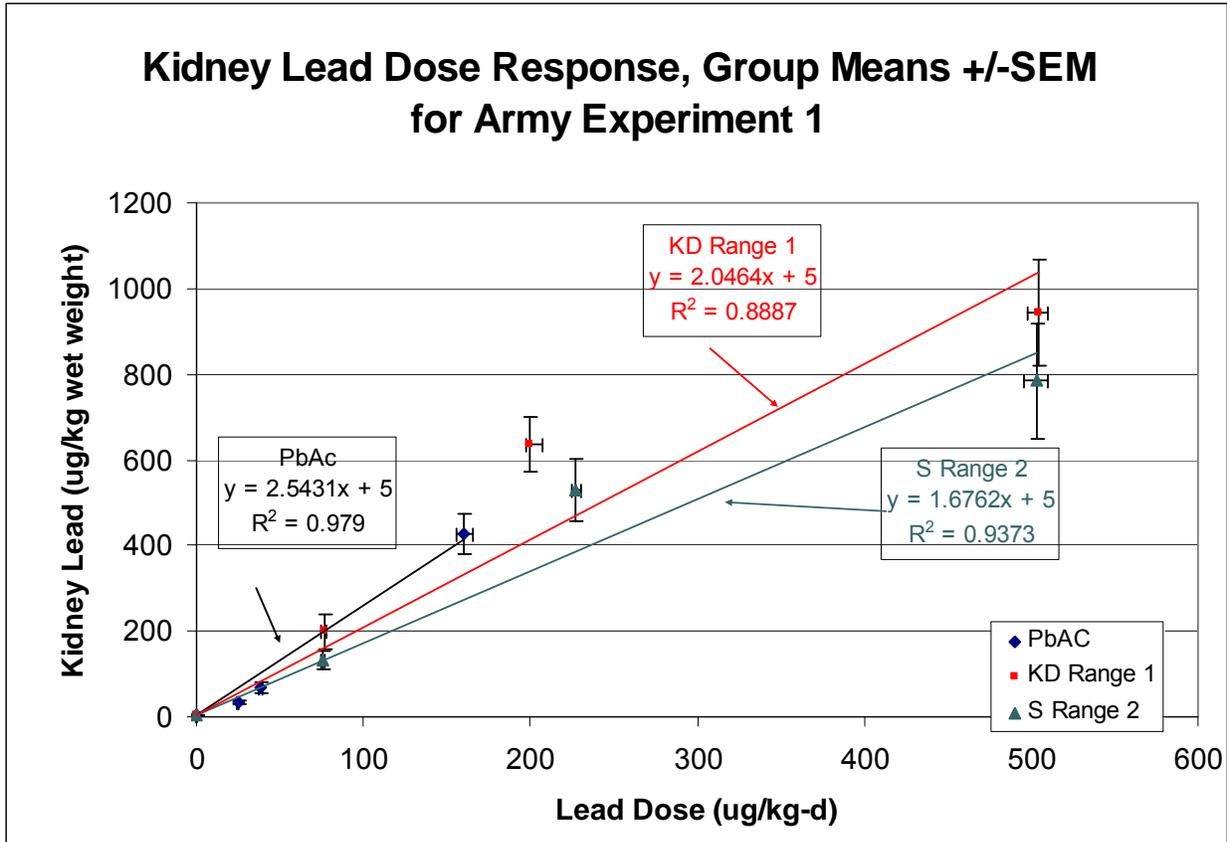


Figure 4-5 Kidney Lead Dose-response, Group Means +/- SEM for Army Experiment 1



As seen, all of these dose response curves for tissues are fit by linear equations, both for lead acetate and each of the two test soils.

4.3 Calculated RBA Values

Relative bioavailability values were calculated for each test material for each measurement endpoint (blood AUC, bone, liver, kidney) using the method described in Section 3.0. The results are shown below:

Measurement Endpoint	Estimated RBA	
	KD Range 1	S Range 2
Blood Lead AUC	1.27	.93
Liver Lead	.91	.71
Kidney Lead	.81	.66
Bone Lead	.68	.66

Recommended RBA Values

For each test soil, the estimates of RBA based on blood, liver, kidney, and bone are generally similar, but do not agree exactly in all cases. In general, we recommend greatest emphasis be placed on the RBA estimates derived from the blood lead data. There are several reasons for this recommendation, including the following:

- 1) Blood lead calculations are based on multiple measurements over time, and so are statistically more robust than the single measurements available for tissue concentrations. Further, blood is a homogeneous medium, and is easier to sample than complex tissues such as liver, kidney and bone. Consequently, the AUC endpoint is less susceptible to random measurement errors, and RBA values calculated from AUC data are less uncertain.
2. Blood is the central compartment and one of the first compartments to be affected by absorbed lead. In contrast, uptake of lead into peripheral compartments (liver, kidney, bone) depend on transfer from blood to the tissue, and may be subject to a variety of toxicokinetic factors that could make bioavailability determinations more complicated.
3. The dose-response curve for blood lead is non-linear, similar to the non-linear dose-response curve observed in children (e.g., see Sherlock and Quinn 1986). Thus, the response of this endpoint is known to behave similarly in swine as in children, and it is not known if the same is true for the tissue endpoints.

4. Blood lead is the classical measurement endpoint for evaluating exposure and health effects in humans, and the health effects of lead are believed to be proportional to blood lead levels.

However, data from the tissue endpoints (liver, kidney, bone) also provide valuable information. We consider the plausible range to extend from the RBA based on blood AUC to the mean of the other three tissues (liver, kidney, bone). The preferred range is the interval from the RBA based on blood to the mean of the blood RBA and the tissue mean RBA. Our suggested point estimate is the mid-point of the preferred range. These values are presented below:

Relative Bioavailability of Lead	Test Material	
	KD Range 1	S Range 2
Plausible Range	1.27-0.80	0.93-0.67
Preferred Range	1.27-1.03	0.93-0.80
Suggested Point Estimate	1.15	0.87

4.4 Uncertainty

The bioavailability estimates above are subject to uncertainty that arises from several different sources. First, differences in physiological and pharmacokinetic parameters between individual animals leads to variability in response, even when exposure is the same. Because of this inter-animal variability in the responses of different animals to lead exposure, there is mathematical uncertainty in the best fit dose-response curves for both lead acetate and test material. This in turn leads to uncertainty in the calculated values of RBA, because these are derived from the two best-fit equations. Second, there is uncertainty in how to weight the RBA values based on the different endpoints, and how to select a point estimate for RBA that is applicable to typical site-specific exposure levels. Third, there is uncertainty in the quantitative extrapolation of measured RBA values in swine to young children. Even though the immature swine is believed to be a useful and meaningful animal model for gastrointestinal absorption in children, it is possible that differences in stomach pH, stomach emptying time, and other physiological parameters may exist and that RBA values in swine may not be precisely equal to values in children.

5.0 REFERENCES

- Gibaldi, M. and D. Perrier. 1982. *Pharmacokinetics* (2nd edition) pp 294-297. Marcel Dekker, Inc, New York, New York.
- Goodman, A.G., T.W. Rall, A.S. Nies, and P. Taylor. 1990. *The Pharmacological Basis of Therapeutics* (8th ed.) pp. 5-21. Pergamon Press, Inc. Elmsford, New York.
- Klaassen, C.D., Amdur, M.O., and Doull, J. (eds). 1996. *Cassarett and Doull's Toxicology: The Basic Science of Poisons*. pp. 190. McGraw-Hill, Inc. New York, New York
- LaVelle, J.M., R.H. Poppenga, B.J. Thacker, J.P. Giesy, C. Weis, R. Othoudt and C. Vandervoot. 1991. Bioavailability of Lead in Mining Waste: An Oral Intubation Study in Young Swine. In: The Proceedings of the International Symposium on the Bioavailability and Dietary Uptake of Lead. Science and Technology Letters 3:105-111.
- Mushak, P. 1991. Gastro-intestinal Absorption of Lead in Children and Adults: Overview of Biological and Biophysico-chemical Aspects. In: The Proceedings of the International Symposium on the Bioavailability and Dietary Uptake of Lead. Science and Technology Letters 3:87-104.
- Sherlock, J.C., and M.J. Quinn. 1986. Relationship Between Blood Lead Concentration and Dietary Intake in Infants: the Glasgow Duplicate Diet Study 1979-1980. Food Additives and Contaminants 3:167-176.
- USEPA 1991. Technical Support Document on Lead. United States Environmental Protection Agency, Environmental Criteria and Assessment Office. ECAO-CIN-757.
- USEPA 1994. Guidance Manual for the Integrated Exposure Uptake Biokinetic Model for Lead in Children. United States Environmental Protection Agency, Office of Emergency and Remedial Response. Publication Number 9285.7-15-1. EPA/540/R-93/081.
- Weis, C.P. and J.M. LaVelle. 1991. Characteristics to consider when choosing an animal model for the study of lead bioavailability. In: The Proceedings of the International Symposium on the Bioavailability and Dietary Uptake of Lead. Science and Technology Letters 3:113-119.
- Weis, C.P., G.M. Henningsen, R.H. Poppenga, and B.J. Thacker. 1993. Pharmacokinetics of Lead in Blood of Immature Swine Following Acute Oral and Intravenous Exposure. The Toxicologist 13(1):175.
- Weis, C.P., R.H. Poppenga, B.J. Thacker, G.M. Henningsen, and A. Curtis. 1995. "Design of Pharmacokinetic and Bioavailability Studies of Lead in an Immature Swine Model." In: Lead in Paint, Soil, and Dust: Health Risks, Exposure Studies, Control Measures, Measurement Methods, and Quality Assurance. Astm Stp 1226, Michael E. Beard and S. D. Allen Iske, Eds., American Society for Testing and Materials, Philadelphia, 1995.

REPORT 2

***IN VIVO* BIOAVAILABILITY OF LEAD
IN U.S. ARMY SMALL ARMS RANGE SOILS:
EXPERIMENT 2**

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EXECUTIVE SUMMARY

A study using juvenile swine as test animals was performed to measure the gastrointestinal absorption of lead from two soil samples (Test Material 1 and Test Material 2) from U.S. Army small arms firing ranges from Louisiana and Alaska. The relative bioavailability of lead in the samples was assessed by comparing the absorption of lead from the test materials to that of a reference material (lead acetate). The lead concentrations of Test Material 1 and Test Material 2 were 13,992 $\mu\text{g/g}$ and 15,705 $\mu\text{g/g}$, respectively. Groups of five swine were given oral doses of lead acetate or a test material twice a day for 15 days. The amount of lead absorbed by each animal was evaluated by measuring the amount of lead in the blood (measured on days 0, 1, 2, 3, 5, 7, 9, 12, and 15) and the amount of lead in liver, kidney, and bone (measured on day 15 at study termination). The amount of lead present in blood or tissues of animals exposed to test materials was compared to that for animals exposed to lead acetate, and the results were expressed as relative bioavailability (RBA). The RBA results for the two samples in this study are summarized below:

Measurement Endpoint	Estimated RBA (90% Confidence Interval)	
	Test Material 1	Test Material 2
Blood Lead AUC	1.02 (0.83 - 1.21)	1.01 (0.81 - 1.19)
Liver Lead	1.32 (0.98 - 1.79)	1.27 (0.96 - 1.72)
Kidney Lead	1.36 (1.09 - 1.71)	1.33 (1.06 - 1.67)
Bone Lead	0.95 (0.83 - 1.09)	0.86 (0.76 - 0.99)
Point Estimate	1.16 (0.86 - 1.60)	1.12 (0.79 - 1.55)

These relative bioavailability estimates may be used to improve accuracy and decrease uncertainty in estimating human health risks from exposure to these test materials.

It is important to understand that the bioavailability estimates above are subject to uncertainty that arises from several different sources. One source of uncertainty stems from the inherent biological variability between different animals. This statistical variability is characterized by the confidence range around the endpoint-specific and the point estimate RBA values shown above. However, there is also uncertainty in the extrapolation of RBA values measured in juvenile swine to humans, and this uncertainty is not included in the statistical confidence bounds above. Even though juvenile swine are considered to be a good model for lead absorption in children, differences between swine and children could result in differences in RBA. In addition, RBA may depend on the amount and type of food in the stomach. In this regard, RBA values measured in this study are based on animals that have little or no food in their stomach at the time of lead exposure, and hence are likely to yield values of RBA that may be somewhat conservative for humans who ingest the soils along with food.

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***In Vivo* Bioavailability of Lead in U.S. Army Small Arms Range Soils: Experiment 2**

1.0 INTRODUCTION

1.1 Overview of Bioavailability

Reliable analysis of the potential hazard to humans from ingestion of lead depends upon accurate information on a number of key parameters, including lead concentration in environmental media (soil, dust, water, food, air, paint, etc.), intake rates of each medium, and the rate and extent of lead absorption by the body from an ingested medium (“bioavailability”). Knowledge of lead bioavailability is important because the amount of lead that 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 influence 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 is normally described as the fraction or percentage of a chemical that is absorbed by the body following an exposure of some specified amount, duration, and route (usually oral). 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:

$$\text{ABA} = (\text{Absorbed Dose}) / (\text{Ingested Dose})$$

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 the absolute bioavailability of lead in some appropriate reference material:

$$\text{RBA} = \text{ABA}(\text{test}) / \text{ABA}(\text{reference})$$

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 materials 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:

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

where:

- ABA_{soil} = Absolute bioavailability of lead in soil ingested by a human
- ABA_{soluble} = Absolute bioavailability in children of some dissolved or fully soluble form of lead
- RBA_{soil} = Relative bioavailability of lead in soil as measured in swine

Based on available information on lead absorption in humans and animals, the U.S. Environmental Protection Agency (USEPA) estimates that the absolute bioavailability of lead from water and other fully soluble forms of lead is usually about 50% in children (USEPA, 1991) and about 20% in adults (USEPA, 2003). 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:

$$ABA_{\text{soil}} (\text{Child}) = 50\% \cdot RBA_{\text{soil}}$$

$$ABA_{\text{soil}} (\text{Adult}) = 20\% \cdot RBA_{\text{soil}}$$

The default RBA used by USEPA for lead in soil and dust compared to lead in water is 60% for both children and adults. 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 the 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 Purpose of This Study

The objective of this study was to determine the oral bioavailability of lead in two soil samples collected from U.S. Army small arms firing ranges relative to the bioavailability of lead acetate, using juvenile swine as a test system. The relative bioavailability estimates may be used to improve accuracy and decrease uncertainty in estimating human health risks from exposure to these test materials.

2.0 Study Design

A standardized study protocol for measuring absolute and relative bioavailability of lead was developed based upon previous study designs and investigations that characterized the juvenile swine model (Weis et al., 1995). The study schedule is presented in Appendix A, Table A-1. The study was performed as nearly as possible within the spirit and guidelines of Good Laboratory Practices (GLP: 40 CFR 792).

2.1 Test Materials

2.1.1 Sample Description

The test materials used in this investigation were two soil samples from U.S. Army M16 rifle ranges in the Alabama/Mississippi region. Test Material 1 (HO-3) contains high levels of organic matter (OM) and Test Material 2 (HCEC-4) has a high cation exchange capacity (CEC).

2.1.2 Sample Preparation

The soil samples were tested as provided by the U. S. Army, without modification. Details on sample collection methods and processing prior to receipt by the testing facility were not provided.

2.1.3 Lead Concentration

Three subsamples (approximately 0.5 gram each) of each test material were digested in nitric acid and analyzed for lead by flame atomic absorption using a Perkin Elmer AAnalyst 800 high-performance atomic absorption spectrometer. The three results for each test material were then averaged together, resulting in final mean lead concentrations of 13,992 $\mu\text{g/g}$ for Test Material 1 and 15,705 $\mu\text{g/g}$ for Test Material 2.

2.2 Experimental Animals

Juvenile swine were selected for use in this study because they are considered to be a good physiological model for gastrointestinal absorption in children (Weis and LaVelle, 1991; Casteel et al., 1996). The animals were intact males of the Pig Improvement Corporation (PIC) genetically defined Line 26, and were purchased from Chinn Farms, Clarence, MO.

The number of animals purchased for the study was several more than required by the protocol. These animals were purchased at an age of about 5-6 weeks (weaning occurs at age 3 weeks) and housed in individual lead-free stainless steel cages. The animals were then held under quarantine for one week to observe their health before beginning exposure to test materials. Each animal was examined by a certified veterinary clinician (swine specialist) and any animals that appeared to be in poor health during this quarantine period were excluded from the study. To minimize weight variations among animals and groups, extra animals most different in body weight (either heavier or lighter) four days prior to exposure (day -4) were also excluded from the study. The remaining animals were assigned to dose groups at random (group assignments are presented in Appendix A, Table A-2). When exposure began (day zero), the animals were about 6-7 weeks old and weighed an average of about 13.5 kg. The animals were weighed every three days

during the course of the study. On average, animals gained about 0.46 kg/day and the rate of weight gain was comparable in all groups, ranging from 0.42 to 0.49 kg/day. These body weight data are summarized in Figure 2-1 and are also presented in Appendix A, Table A-3. All animals were examined daily by an attending veterinarian while on study.

2.3 Diet

Animals were weaned onto standard pig chow (purchased from MFA Inc., Columbia, MO) by the supplier. In order to minimize lead exposure from the diet, the animals were gradually transitioned from the MFA feed to a special low-lead feed (guaranteed less than 0.2 ppm lead, purchased from Zeigler Brothers, Inc., Gardners, PA), and this feed was maintained for the duration of the study. The feed was nutritionally complete and met all requirements of the National Institutes of Health–National Research Council. The typical nutritional components and chemical analysis of the feed are presented in Table 2-1. Each day every animal was given an amount of feed equal to 4% of the mean body weight of all animals on study. Feed amounts were adjusted every three days, when pigs were weighed. Feed was administered in two equal portions at 11:00 AM and 5:00 PM daily. Analysis of random feed samples indicated that the lead level did not exceed 0.15 µg/g.

Drinking water was provided *ad libitum* via self-activated watering nozzles within each cage. Analysis of samples from randomly selected drinking water nozzles indicated the lead concentration was less than 1.5 µg/L.

2.4 Dosing

The protocol for exposing animals to lead is shown in Table 2-2. The dose levels for lead acetate were based on experience from previous swine investigations that showed that lead doses of 25-225 µg/kg-day resulted in clear and measurable increases in lead levels in all endpoints measured (blood, liver, kidney, and bone). The actual administered doses were calculated based on the lead content of the material administered and the measured group mean body weights. Specifically, doses of lead for the three days following each weighing were based on the group mean body weight adjusted by the addition of 1 kg to account for the expected weight gain over the time interval. After completion of the study, body weights were estimated by interpolation for those days when measurements were not collected and the actual administered doses were calculated for each day and then averaged across all days. The actual mean doses for each dosing group are included in Table 2-2; the actual lead doses administered to each pig are presented in Appendix A, Table A-3.

Animals were exposed to lead acetate or a test material for 15 days, with the dose for each day being administered in two equal portions beginning at 9:00 AM and 3:00 PM (two hours before feeding), with two minute intervals allowed for individual pig dosing. Dose material was placed in the center of a small portion (about 5 grams) of moistened feed (this is referred to as a “doughball”), and this was administered to the animals by hand. If uneaten portions of doughballs were discovered, these were retrieved and offered again for consumption. Occasionally, some animals did not consume their entire dose. In these instances, the missed doses were estimated and recorded and the time-weighted average dose calculation for each animal was adjusted downward accordingly (see Appendix A, Table A-3).

2.5 Collection of Biological Samples

Samples of blood were collected from each animal on the first day of exposure (day 0) and on days 1, 2, 3, 5, 7, 9, 12, and 15 following the start of exposure. All blood samples were collected by vena-puncture of the anterior vena cava, and samples were immediately placed in purple-top Vacutainer® tubes containing EDTA (ethylenediaminetetra-acetic acid) as anticoagulant. Although EDTA is a chelator of metals, the nitric acid digest used in the analysis destroys the organic constituents in the blood, thereby freeing all lead for analysis. Thus, the presence of EDTA in the sampling tubes will not impact the analytical results for lead. Blood samples were collected each sampling day beginning at 8:00 AM, approximately one hour before the first of the two daily exposures to lead on the sampling day and 17 hours after the last lead exposure the previous day. This blood collection time was selected because the rate of change in blood lead resulting from the preceding exposures is expected to be relatively small after this interval (LaVelle et al., 1991; Weis et al., 1993), so the exact timing of sample collection relative to the last dosing is not likely to be critical.

Following collection of the final blood sample at 8:00 AM on day 15, all animals were humanely euthanized and samples of liver, kidney, and bone (the right femur, defleshed) were removed and stored at -80°C in lead-free plastic bags for lead analysis.

Samples of all biological samples collected were archived in order to allow for reanalysis and verification of lead levels, if needed. All animals were also subjected to detailed examination at necropsy by a certified veterinary pathologist in order to assess overall animal health.

2.6 Preparation of Biological Samples for Analysis

Blood

One mL of whole blood was removed from the purple-top Vacutainer® tube and added to 9.0 mL of “matrix modifier,” a solution recommended by the Centers for Disease Control and Prevention (CDC) for analysis of blood samples for lead. The composition of matrix modifier is 0.2% (v/v) ultrapure nitric acid, 0.5% (v/v) Triton X-100, and 0.2% (w/v) dibasic ammonium phosphate in deionized distilled water.

Liver and Kidney

One gram of soft tissue (liver or kidney) was placed in a lead-free screw-cap Teflon container with 2 mL of concentrated (70%) nitric acid and heated in an oven to 90°C overnight. After cooling, the digestate was transferred to a clean lead-free 10 mL volumetric flask and diluted to volume with deionized distilled water.

Bone

The right femur of each animal was broken and dried at 100°C overnight. The dried bones were then placed in a muffle furnace and dry-ashed at 450°C for 48 hours. Following dry ashing, the bone was ground to a fine powder using a lead-free mortar and pestle, and 200 mg was removed and dissolved in 10.0 mL of 1:1 (v:v) concentrated nitric acid/water. After the powdered bone

was dissolved and mixed, 1.0 mL of the acid solution was removed and diluted to 10.0 mL in deionized distilled water.

2.7 Lead Analysis

Samples of biological tissue (blood, liver, kidney, and bone) and other materials (e.g., food, water, reagents, solutions) were analyzed for lead by graphite furnace atomic absorption using a Perkin Elmer AAnalyst 800 high-performance atomic absorption spectrometer. Internal quality assurance samples (described below) were typically run every tenth sample, and the instrument was recalibrated at least every 20th sample.

All analytical results were reported in units of $\mu\text{g Pb/L}$ (ng/mL) of prepared sample. The quantitation limit was defined as three-times the standard deviation of a set of seven replicates of a low-lead sample (typically about 2-5 $\mu\text{g/L}$). The standard deviation was usually about 0.3 $\mu\text{g/L}$, so the quantitation limit was usually about 0.9-1.0 $\mu\text{g/L}$. For prepared blood samples (diluted 1/10), this corresponds to a quantitation limit of 10 $\mu\text{g/L}$ (1 $\mu\text{g/dL}$). For soft tissues (liver and kidney, diluted 1/10), this corresponds to a quantitation limit of 10 $\mu\text{g/kg}$ (ng/g) wet weight, and for bone (diluted 1/500) the corresponding quantitation limit is 0.5 $\mu\text{g/g}$ (ng/g) ashed weight. All responses below the quantitation limit were evaluated at one-half the quantitation limit.

Lead analytical results for study samples are presented in Appendix A, Table A-4; the results for quality assurance samples are presented in Appendix A, Table A-5, and are summarized below.

2.8 Quality Assurance

A number of quality assurance (QA) steps were taken during this project to evaluate the accuracy of the analytical procedures. These included:

Spike Recovery

Randomly selected samples were spiked with known amounts of lead (as lead acetate) and the recovery of the added lead was measured. Recovery for individual samples ranged from 80% to 114%, with an average across all analyses of $95 \pm 10\%$ ($N = 33$).

Duplicate Analysis of Sample Digestate

During sample analysis, approximately every tenth sample was analyzed in duplicate (i.e., the same prepared sample was analyzed twice). Duplicate results for blood samples were within 1 $\mu\text{g/L}$ ($N = 37$). Duplicate results for liver, kidney, and femur samples had a relative percent difference (RPD) of 0.3% to 13%, with an average of $5.6\% \pm 4.6\%$ ($N = 15$).

Sample Preparation Replicates

A random selection of about 7.5% of all tissue samples generated during the study were prepared for laboratory analysis in duplicate (i.e., two separate subsamples of blood/tissue were prepared for analysis). The results for these replicate preparations are summarized in Figure 2-2. As seen, the analytical results for replicate pairs of blood samples (Panel A of Figure 2-2) tend to follow

the line of equality, indicating that the replicate pairs are generally in good agreement, although there is some variability for individual samples. The absolute difference between replicate pairs of blood samples ranged from 0 to 3.4 µg/dL with an average of 0.9 µg/dL (N = 30). As seen, there was also good reproducibility between replicate samples for tissues (Panel B of Figure 2-2). The absolute difference between replicate pairs of tissue samples ranged from 0.01 to 3.2 µg/g with an average of 0.6 µg/g (N = 12).

Laboratory Control Standard

A laboratory control standard (a sample for which a certified concentration of lead has been established) was tested periodically during sample analysis. Results for this standard are summarized below:

Standard	Target Value	Acceptable Range	Mean	Range	Standard Deviation	Mean % Recovery	N
ERA 697, 1/5	17.5 µg/L	+/-10% (15.75 - 19.25 µg/L)	17.6	15.7 - 19.2	0.7	100.8%	104

As seen, recovery of lead from these standards was generally good and within the acceptable range.

Blood Lead Check Samples

The CDCP provides a variety of blood lead “check samples” for use in quality assurance programs for blood lead studies. Several CDCP check samples of different concentrations were analyzed periodically during sample analysis. The results are summarized in Figure 2-3. As seen, the results for the high and low standards (nominal values of 1.9 µg/dL and 13.9 µg/dL, respectively) tend to cluster around the line of equality as expected. The results of the middle standard (nominal value of 5.5 µg/dL) tend to be slightly lower than expected; the reason for this is not known.

Blanks

Samples of the sample preparation matrix for each endpoint (without added tissue) were routinely analyzed for lead to ensure the absence of lead contamination. These matrix blanks never yielded a measurable level of lead, with all values being reported as less than 1 µg/L.

Based on the results of all of the quality assurance samples and steps described above, it is concluded that the analytical results are of sufficient quality for derivation of reliable estimates of lead absorption from test materials.

3.0 Data Analysis

3.1 Overview

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 (e.g., 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). Because equal absorbed doses of lead (as Pb^{+2}) will produce equal responses (i.e., equal increases in concentration in tissues) regardless of the source or nature of the ingested lead, 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. Thus, 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.

Some biological responses to lead exposure may be non-linear functions of dose (i.e., tending to flatten out or plateau as dose increases). The cause of this non-linearity is uncertain but might be due either to non-linear absorption kinetics and/or to non-linear biological response per unit dose absorbed. However, the principal advantage of the approach described above is that it is not necessary to understand the basis for a non-linear dose response curve (non-linear absorption and/or non-linear biological response) in order to derive valid RBA estimates; in addition, this approach is general and yields reliable results for both non-linear and linear responses.

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, are presented in USEPA (2004) and are summarized below.

3.2 Measurement Endpoints

Four independent measurement endpoints were evaluated based on the concentration of lead observed in blood, liver, kidney, and bone (femur). For liver, kidney, and bone, the measurement endpoint was simply the concentration in the tissue at the time of sacrifice (day 15). The measurement endpoint used to quantify the blood lead response was the area under the curve (AUC) for blood lead vs. time (days 0-15). AUC was selected because it is the standard pharmacokinetic index of chemical uptake into the blood compartment, and is relatively insensitive to small variations in blood lead level by day. The AUC was calculated using the trapezoidal rule to estimate the AUC between each time point that a blood lead value was measured (days 0, 1, 2, 3, 5, 7, 9, 12, and 15):

$$AUC(d_i \text{ to } d_j) = 0.5 \cdot (r_i + r_j) \cdot (d_j - d_i)$$

where:

d = day number

r = response (blood lead value) on day i (r_i) or day j (r_j)

The areas were then summed across all time intervals in the study to yield the final AUC for each animal.

Occasionally blood lead values are obtained that are clearly different than expected. Blood lead values that were more than a factor of 1.5 above or below the group mean for any given day were flagged as potential outliers and are shaded gray in Appendix A, Table A-6. Each data point identified in this way was reviewed and professional judgment was used to decide if the value should be retained or excluded. In order to avoid inappropriate biases, blood lead outlier designations were restricted to values that were clearly aberrant from a time-course and/or dose-response perspective. In this study, a single value was judged to be a clear outlier; it is identified by a heavy black box outlining the value in Table A-6. This single blood lead value was excluded from the calculation of AUC; the missing value was replaced by a value interpolated from the preceding and following values from the same animal.

3.3 Dose-Response Models

Basic Equations

It has been shown previously (USEPA, 2004) that nearly all blood lead AUC data sets can be well-fit using an exponential equation and most tissue (liver, kidney, and bone) lead data can be well-fit using a linear equation, as follow:

$$\text{Linear (liver, kidney, bone):} \quad \text{Response} = a + b \cdot \text{Dose}$$

$$\text{Exponential (blood lead AUC):} \quad \text{Response} = a + b \cdot [1 - \exp(-c \cdot \text{Dose})]$$

Simultaneous Regression

Because the data to be analyzed consist of three dose-response curves for each endpoint (the reference material and two test materials) and there is no difference between the curves when the dose is zero, all three curves for a given endpoint must have the same intercept. This requirement is achieved by combining the two dose response equations into one and solving for the parameters simultaneously, resulting in the following equations:

$$\text{Linear:} \quad y = a + b_r \cdot x_r + b_{t1} \cdot x_{t1} + b_{t2} \cdot x_{t2}$$

$$\text{Exponential:} \quad y = a + b \cdot [(1 - \exp(-c_r \cdot x_r)) + (1 - \exp(-c_{t1} \cdot x_{t1})) + (1 - \exp(-c_{t2} \cdot x_{t2}))]$$

where:

y = response

x = dose

a, b, c = empirical coefficients for reference material (r), Test Material 1 (t1), and Test Material 2 (t2).

All model fitting was performed using JMP[®] version 3.2.2, a commercial software package developed by SAS[®].

Weighted Regression

Regression analysis based on ordinary least squares assumes that the variance of the responses is independent of the dose and/or the response (Draper and Smith, 1998). It has previously been shown that this assumption is generally not satisfied in swine-based RBA studies, where there is a tendency toward increasing variance in response as a function of increasing dose (heteroscedasticity) (USEPA, 2004). To deal with heteroscedasticity, the data are analyzed using weighted least squares regression. In this approach, each observation in a group of animals is assigned a weight that is inversely proportional to the variance of the response in that group:

$$w_i = (\sigma^2_i)^{-1}$$

where:

$$w_i = \text{weight assigned to all data points in dose group } i$$
$$\sigma^2_i = \text{variance of responses of animals in dose group } i$$

(Draper and Smith, 1998). As discussed in USEPA (2004), there are several alternative strategies for assigning weights. The preferred method identified by USEPA (2004), and the method used in this study, estimates the value of σ^2_i using an “external” variance model based on an analysis of the relationship between variance and mean response using data consolidated from ten different lead studies. Log-variance increases as an approximately linear function of log-mean response for all four endpoints:

$$\ln(s_i^2) = k1 + k2 \cdot \ln(\bar{y}_i)$$

where:

$$s_i^2 = \text{observed variance of responses of animals in dose group } i$$
$$\bar{y}_i = \text{mean observed response of animals in dose group } i$$

Values of k1 and k2 were derived for each endpoint using ordinary least squares minimization, and the resulting values are shown below:

Endpoint	k1	k2
Blood AUC	-1.3226	1.5516
Liver	-2.6015	2.0999
Kidney	-1.8499	1.9557
Femur	-1.9713	1.6560

Goodness-of-Fit

The goodness-of-fit of each dose-response model was assessed using the F test statistic and the adjusted coefficient of multiple determination (Adj R²) as described by Draper and Smith (1998). A fit is considered acceptable if the p-value is less than 0.05.

Assessment of Outliers

In biological assays, it is not uncommon to note the occurrence of individual measured responses that appear atypical compared to the responses from other animals in the same dose group. In this study, endpoint responses that yielded standardized weighted residuals greater than 3.5 or less than -3.5 were considered to be potential outliers (Canavos, 1984). When such data points were encountered in a data set, the RBA was calculated both with and without the potential outlier(s) excluded, and the result with the outlier(s) excluded was used as the preferred estimate.

3.4 Calculation of RBA Estimates

Endpoint-specific RBA Estimates

Lead RBA values were estimated using the basic statistical techniques recommended by Finney (1978). Each endpoint-specific RBA value was calculated as the ratio of a model coefficient for the reference material data set and for the test material data set:

$$\begin{aligned} \text{Linear endpoints:} \quad & \text{RBA}_{t1} = b_{t1} / b_r \\ & \text{RBA}_{t2} = b_{t2} / b_r \end{aligned}$$

$$\begin{aligned} \text{Exponential endpoint:} \quad & \text{RBA}_{t1} = c_{t1} / c_r \\ & \text{RBA}_{t2} = c_{t2} / c_r \end{aligned}$$

The uncertainty range about the RBA ratio was calculated using Fieller's Theorem as described by Finney (1978).

RBA Point Estimate

Because there are four independent estimates of RBA (one from each measurement endpoint) for each test material, the final RBA estimate for a test material involves combining the four endpoint-specific RBA values into a single value (point estimate) and estimating the uncertainty around that point estimate. As described in USEPA (2004), analysis of data from multiple studies suggests that the four endpoint-specific RBA values are all approximately equally reliable (as reflected in the average coefficient of variation in RBA values derived from each endpoint). Therefore, the RBA point estimate for each test material was calculated as the simple mean of all four endpoint-specific RBA values.

The uncertainty bounds around each point estimate were estimated using Monte Carlo simulation. For each test material, values for RBA were drawn from the uncertainty distributions for each endpoint with equal frequency. Each endpoint-specific uncertainty distribution was assumed to be normal, with the mean equal to the best estimate of RBA and the standard deviation estimated from Fieller's Theorem (Finney, 1978). The uncertainty in the point estimate was characterized as the range from the 5th to the 95th percentile of the mean across endpoints.

4.0 Results

4.1 Clinical Signs

The doses of lead administered in this study are below a level that is expected to cause toxicological responses in swine, and no clinical signs of lead-induced toxicity were noted in any of the animals used in the study.

4.2 Blood Lead vs. Time

Blood lead data for individual animals are presented in Appendix A, Figure A-1. Group mean blood lead values as a function of time are shown in Figure 4-1. As seen, blood lead values began at or below quantitation limits (about 1 µg/dL) in all groups, and remained at or below quantitation limits in control animals (Group 10). In animals given repeated oral doses of lead acetate (Groups 1-3), Test Material 1 (Groups 4-6), or Test Material 2 (Groups 7-9), blood levels began to rise within 1-2 days, and tended to plateau by the end of the study (day 15).

4.3 Dose-Response Patterns

Blood Lead AUC

As discussed in Section 3.2, the measurement endpoint used to quantify the blood lead response was the area under the curve (AUC) for blood lead vs. time (days 0-15). The AUC determinations are presented in Appendix A, Table A-7. The blood lead AUC dose-response data were modeled using an exponential equation (see Section 3.3). The results of this fitting are shown in Figure 4-2.

Tissue Lead

The dose-response data for lead in liver, kidney, and bone (measured at sacrifice on day 15) were modeled using a linear equation (see Section 3.3). The results of these fittings are shown in Figures 4-3 (liver), 4-4 (kidney), and 4-5 (femur). One outlier was identified in the reference material data for liver (as indicated in Figure 4-3a) and was excluded from the final calculation of RBA for liver (Figure 4-3b).

Data Variance

As discussed in Section 3.3, the data are analyzed using weighted least squares regression in order to deal with heteroscedasticity. The weights used in this analysis were derived using an external variance model based on an analysis of the relationship between variance and mean response using data consolidated from multiple lead studies, as presented in USEPA (2004). As shown in Figures 4-6 (AUC), 4-7 (liver), 4-8 (kidney), and 4-9 (femur), the variance of the data from this study is generally quite similar to that of the data used to generate the variance model.

4.4 Calculated RBA Values

Relative bioavailability values were calculated for each test material for each measurement endpoint (blood lead AUC, liver, kidney, and bone) using the method described in Section 3.4;

the suggested point estimate is calculated as the simple mean of the four endpoint-specific estimates. The results are shown below:

Measurement Endpoint	Estimated RBA (90% CI)	
	Test Material 1	Test Material 2
Blood Lead AUC	1.02 (0.83 - 1.21)	1.01 (0.81 - 1.19)
Liver Lead	1.32 (0.98 - 1.79)	1.27 (0.96 - 1.72)
Kidney Lead	1.36 (1.09 - 1.71)	1.33 (1.06 - 1.67)
Bone Lead	0.95 (0.83 - 1.09)	0.86 (0.76 - 0.99)
Point Estimate	1.16 (0.86 - 1.60)	1.12 (0.79 - 1.55)

CI = Confidence Interval

4.5 Uncertainty

The bioavailability estimates above are subject to uncertainty that arises from several different sources. One source of uncertainty is the inherent biological variability between different animals in a dose group, which in turn causes variability in the amount of lead in the tissues of the exposed animals. This between-animal variability in response results in statistical uncertainty in the best-fit dose-response curves and, hence, uncertainty in the calculated values of RBA. Such statistical uncertainty is accounted for by the statistical models used above and is characterized by the uncertainty range around the endpoint-specific and the point estimate values of RBA.

However, there is also uncertainty in the extrapolation of RBA values measured in juvenile swine to young children or adults, and this uncertainty is not included in the statistical confidence bounds above. Even though the immature swine is believed to be a useful and meaningful animal model for gastrointestinal absorption in children, it is possible that there are differences in physiological parameters that may influence RBA, and that RBA values in swine are not identical to values in children. In addition, RBA may depend on the amount and type of food in the stomach, since the presence of food can influence stomach pH, holding time, and possibly other factors that may influence lead solubilization. In this regard, it is important to recall that RBA values measured in this study are based on animals that have little or no food in their stomach at the time of lead exposure and, hence, are likely to yield high-end values of RBA. Thus, these RBA values may be somewhat conservative for humans who ingest the soils along with food. The magnitude of this bias is not known.

5.0 References

- Canavos, C. G. 1984. Applied Probability and Statistical Methods. Little, Brown and Co., Boston.
- Casteel, S. W., R. P. Cowart, C. P. Weis, G. M. Henningsen, E. Hoffman, W. J. Brattin, M. F. Starost, J. T. Payne, S. L. Stockham, S. V. Becker, and J. R. Turk. 1996. A swine model for determining the bioavailability of lead from contaminated media. In: Advances in Swine in Biomedical Research. Tumbleson and Schook, eds. Vol 2, Plenum Press, New York. Pp. 637-46.
- Draper, N. R., and H. Smith. 1998. Applied Regression Analysis (3rd Edition). John Wiley & Sons, New York.
- Finney, D. J. 1978. Statistical Method in Biological Assay (3rd Edition). Charles Griffin and Co., London.
- Gibaldi, M., and Perrier, D. 1982. Pharmacokinetics (2nd edition), pp 294-297. Marcel Dekker, Inc, New York, New York.
- Goodman, A.G., Rall, T.W., Nies, A.S., and Taylor, P. 1990. The Pharmacological Basis of Therapeutics (8th ed.), pp. 5-21. Pergamon Press, Inc. Elmsford, New York.
- Klaassen, C.D., Amdur, M.O., and Doull, J. (eds). 1996. Cassarett and Doull's Toxicology: The Basic Science of Poisons, pp. 190. McGraw-Hill, Inc. New York, New York.
- LaVelle, J.M., Poppenga, R.H., Thacker, B.J., Giesy, J.P., Weis, C., Othoudt R., and Vandervoot C. 1991. Bioavailability of Lead in Mining Waste: an oral intubation study in young swine. In: The proceedings of the international symposium on the bioavailability and dietary uptake of lead. Science and Technology Letters 3:105-111.
- Mushak, P. 1991. Gastro-intestinal absorption of lead in children and adults: overview of biological and biophysico-chemical aspects. In: The proceedings of the international symposium on the bioavailability and dietary uptake of lead. Science and Technology Letters 3:87-104.
- USEPA. 1991. Technical support document on lead. United States Environmental Protection Agency, Environmental Criteria and Assessment Office. ECAO-CIN-757.
- USEPA. 2003. Recommendations of the Technical Review Workgroup for Lead for an approach to assessing risks associated with adult exposures to lead in soil. United States Environmental Protection Agency Technical Review Workgroup for Lead. OSWER 9285.7-54, EPA-540-R-03-001. January.
- USEPA. 2004. Estimation of relative bioavailability of lead in soil and soil-like materials using *in vivo* and *in vitro* methods. United States Environmental Protection Agency, Office of Solid Waste and Emergency Response. OSWER 9285.7-77. June.

Weis, C.P., and J.M. LaVelle. 1991. Characteristics to consider when choosing an animal model for the study of lead bioavailability. In: The proceedings of the international symposium on the bioavailability and dietary uptake of lead. *Science and Technology Letters* 3:113-119.

Weis, C.P., G.M. Henningsen, R.H. Poppenga, and B.J. Thacker. 1993. Pharmacokinetics of lead in blood of immature swine following acute oral and intravenous exposure. *The Toxicologist* 13(1):175.

Weis, C.P., R.H. Poppenga, B.J. Thacker, G.M. Henningsen, and A. Curtis. 1995. Design of Pharmacokinetic and Bioavailability Studies of Lead in an Immature Swine Model. In: *Lead in Paint, Soil, and Dust: Health Risks, Exposure Studies, Control Measures, Measurement Methods, and Quality Assurance*. ASTM STP 1226, Michael E. Beard and S. D. Allen Iske (eds), American Society for Testing and Materials, Philadelphia, 1995

Figure 2-1 Body Weight Gain

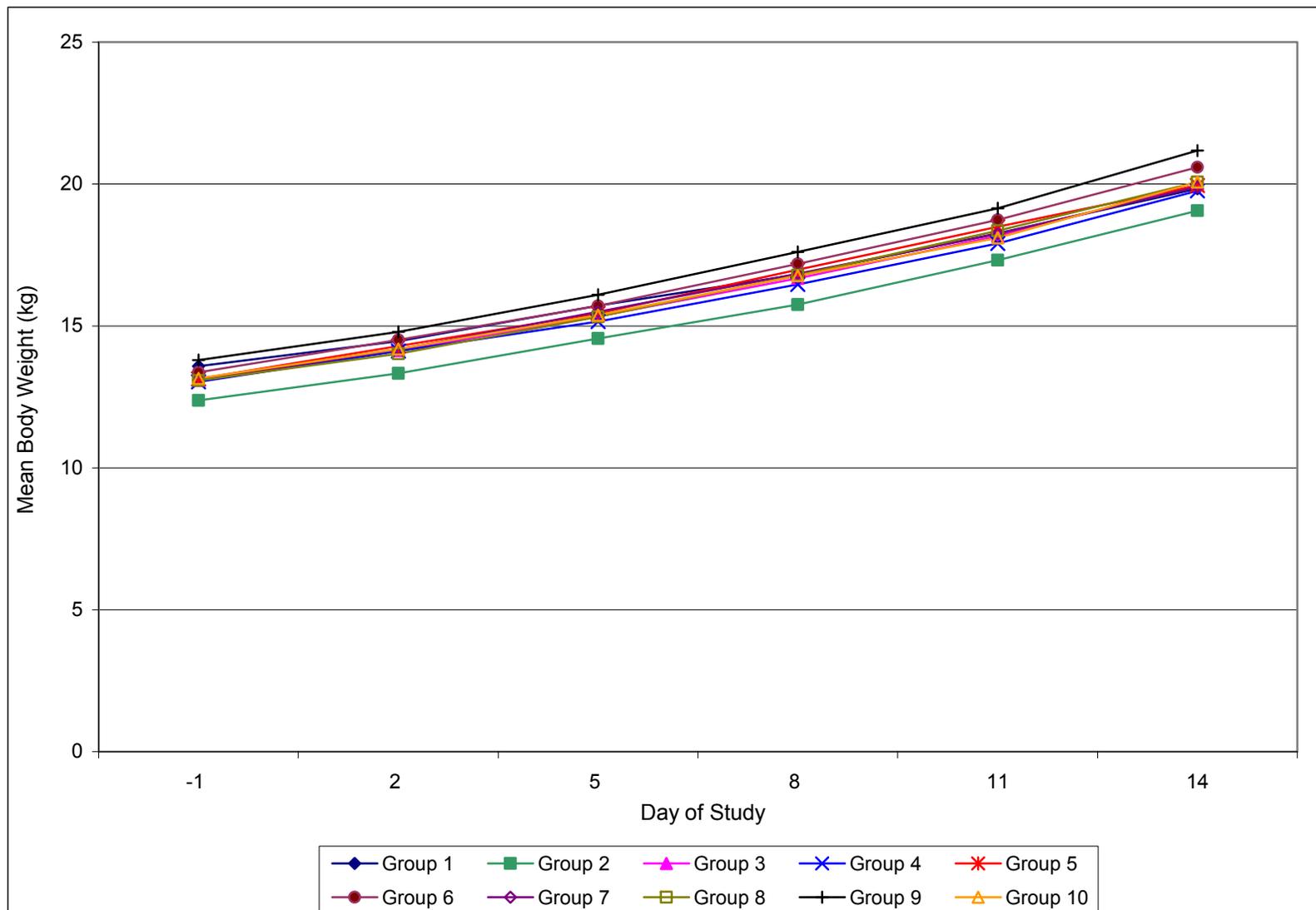


Figure 2-2 Sample Preparation Replicates

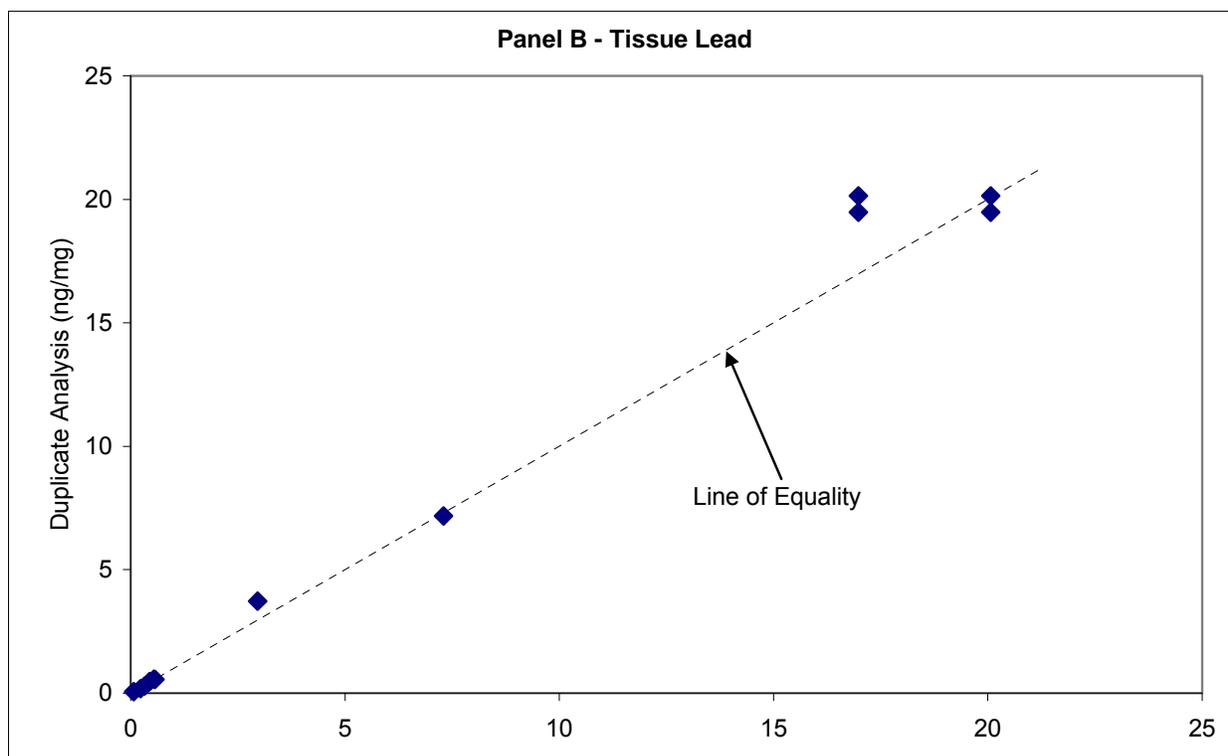
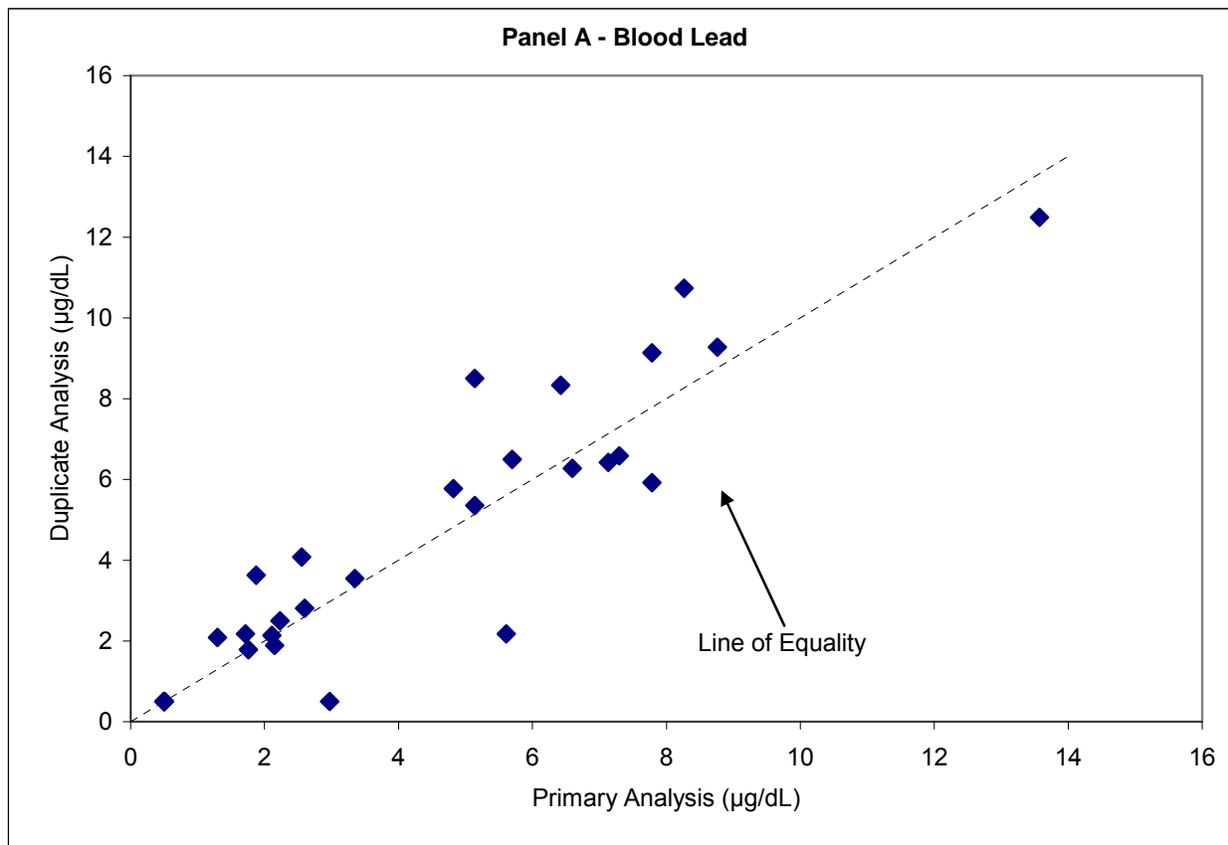


FIGURE 2-3 CDCP BLOOD LEAD CHECK SAMPLES

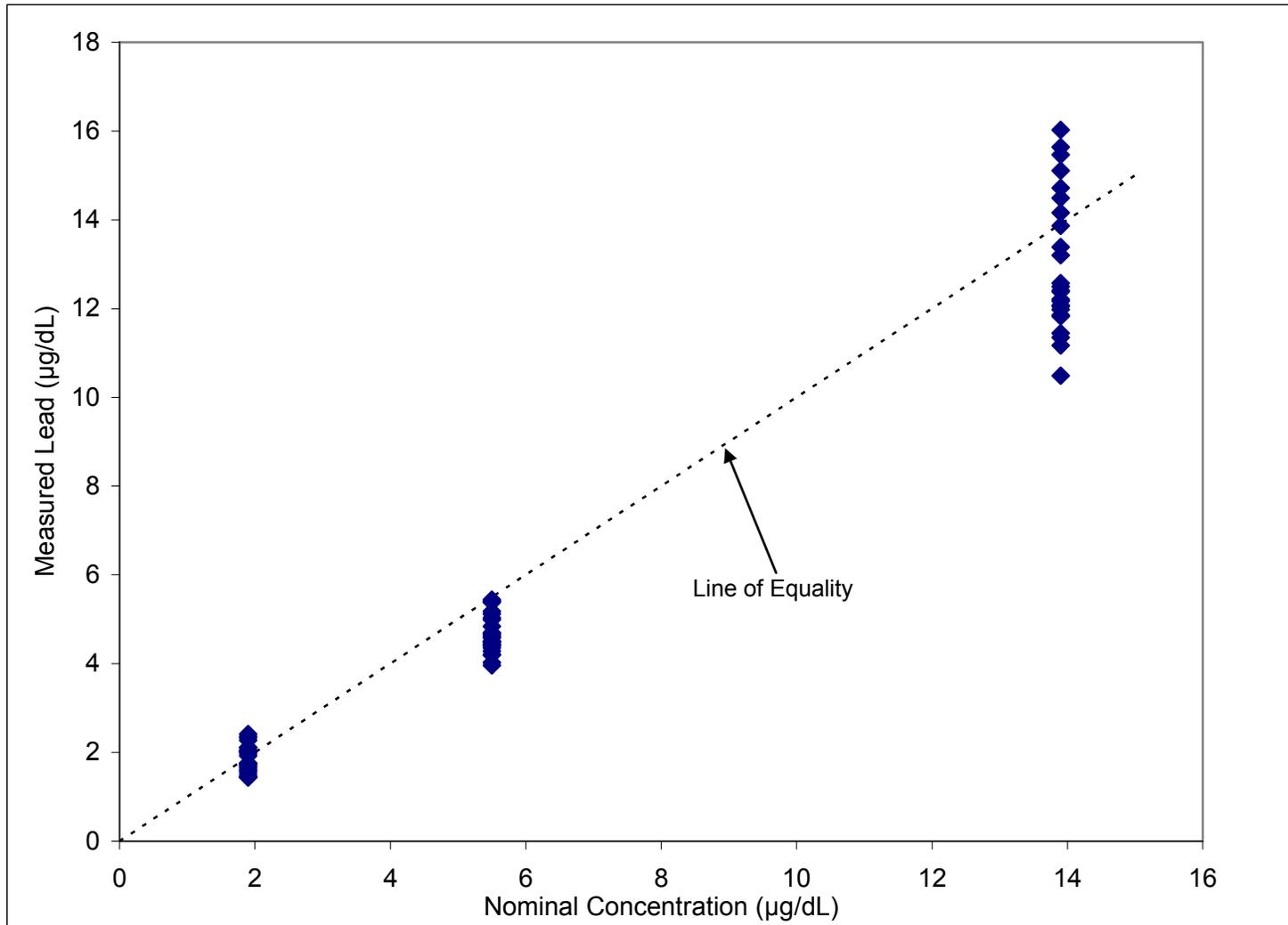
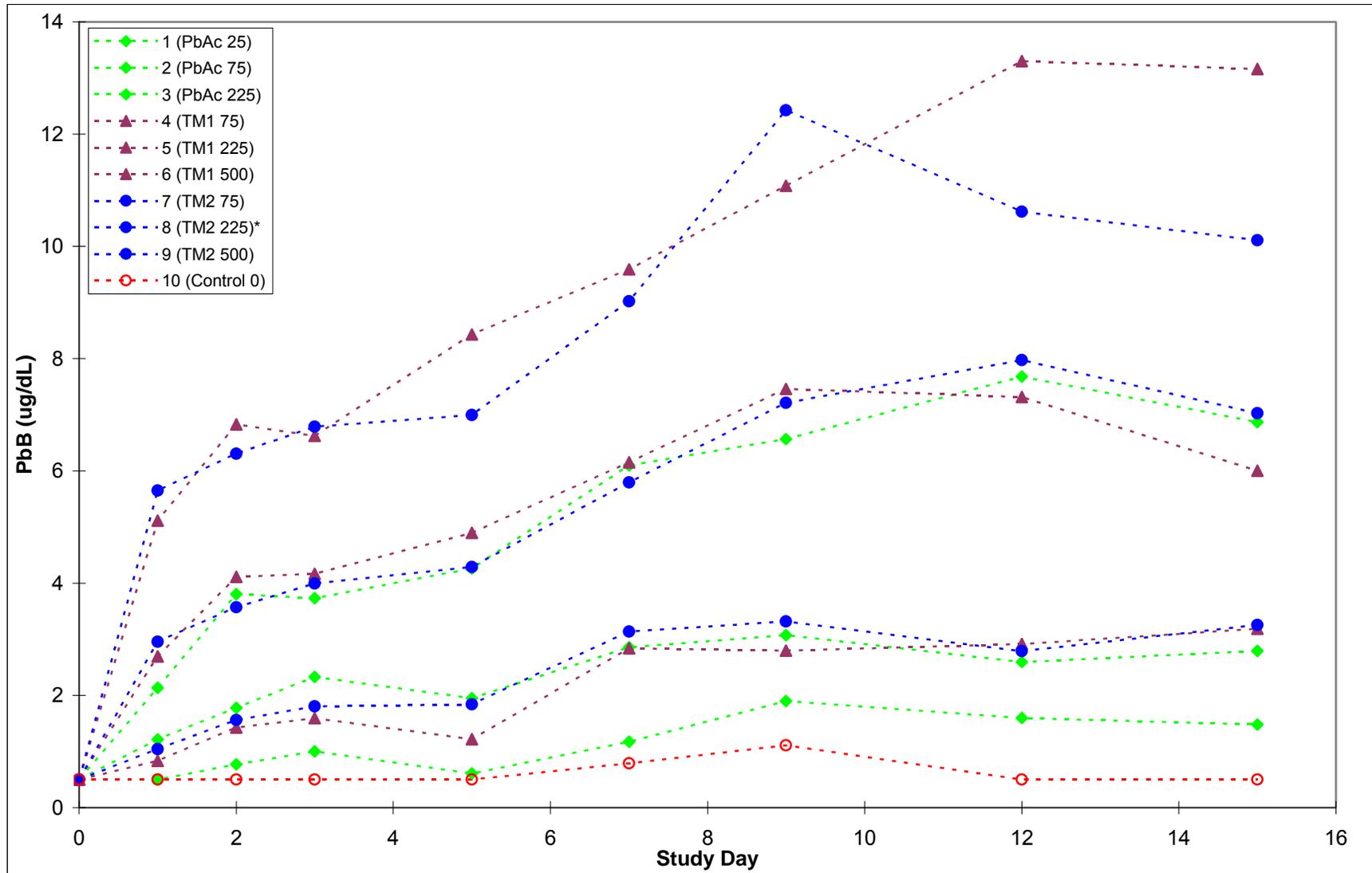
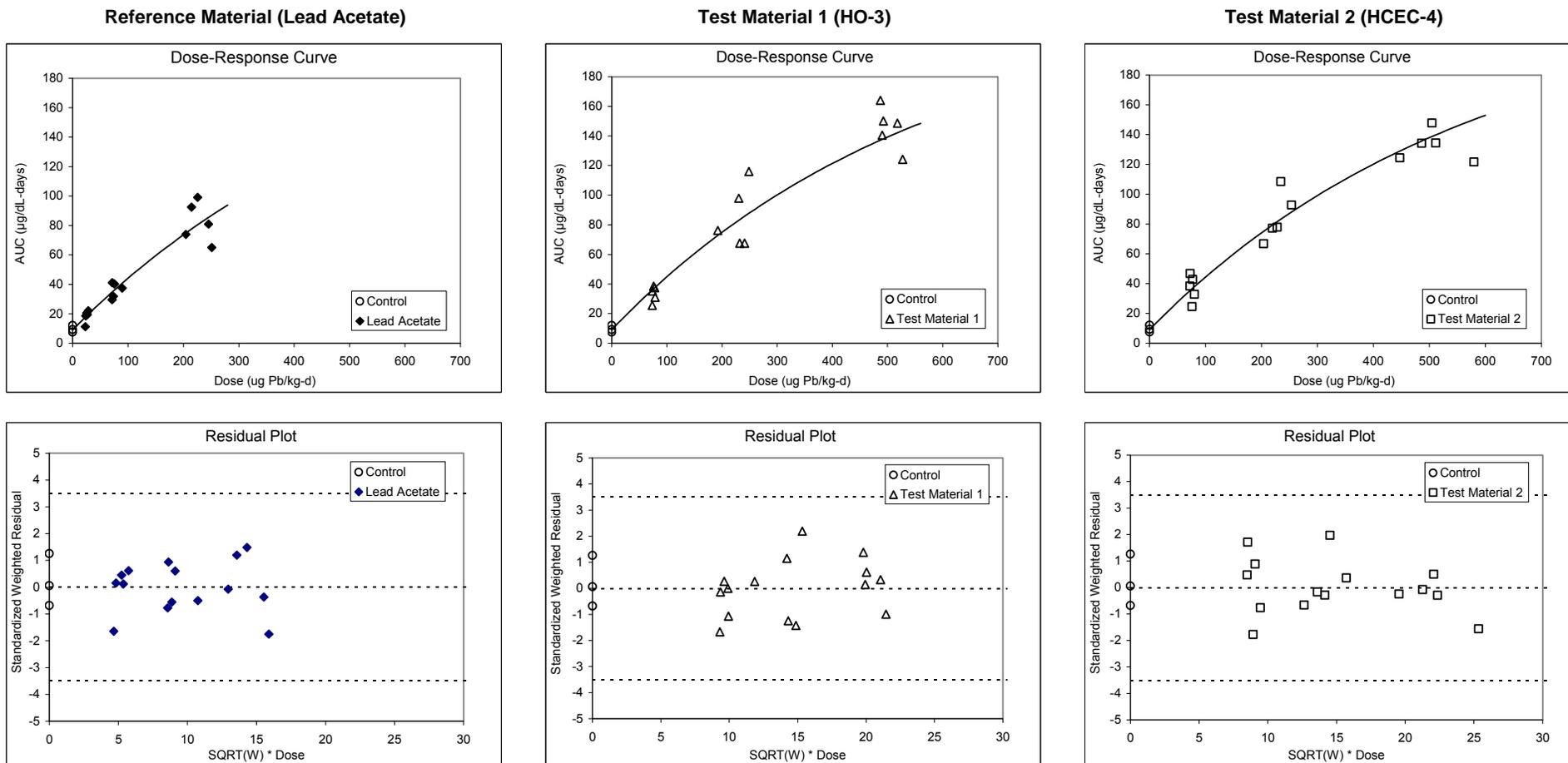


FIGURE 4-1 GROUP MEAN BLOOD LEAD BY DAY



*Group 8 (TM2 225): Outlier (pig #349, day 9) excluded

FIGURE 4-2 BLOOD LEAD AUC DOSE-RESPONSE



Summary of Fitting*

Parameter	Estimate	Standard Error
a	9.10E+00	1.24E+00
b	2.24E+02	4.64E+01
c _r	1.70E-03	4.50E-04
c _{t1}	1.74E-03	4.97E-04
c _{t2}	1.71E-03	4.96E-04
Covariance (c _r ,c _{t1})	0.9371	--
Covariance (c _r ,c _{t2})	0.9371	--
Degrees of Freedom	43	--

Goodness of Fit

Statistic	Estimate
F	197.942
p	< 0.001
Adjusted R ²	0.9437

RBA and Uncertainty

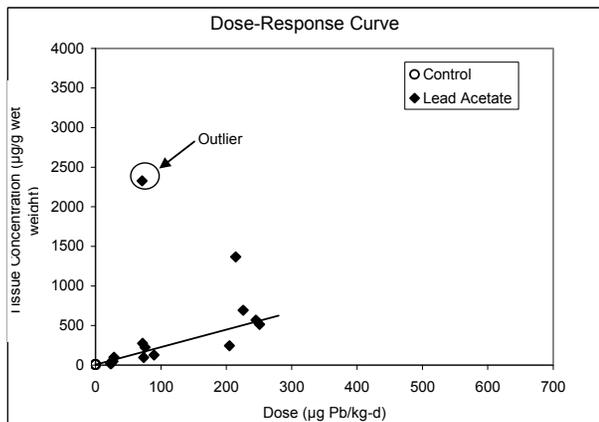
	Test Material 1	Test Material 2
RBA	1.02	1.01
Lower bound	0.83	0.81
Upper bound	1.21	1.19
Standard Error	0.102*	0.102*

*g ≥ 0.05, estimate is uncertain

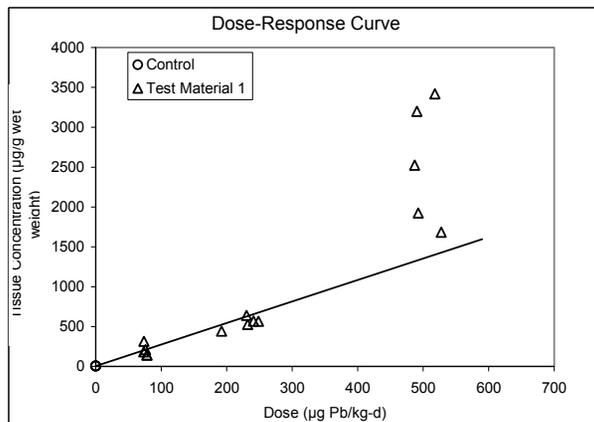
*Data were fit using the exponential model: $y = a + b \cdot (1 - \exp(-c_r \cdot x_r)) + b \cdot (1 - \exp(-c_{t1} \cdot x_{t1})) + b \cdot (1 - \exp(-c_{t2} \cdot x_{t2}))$

FIGURE 4-3a LIVER LEAD DOSE-RESPONSE: OUTLIER INCLUDED

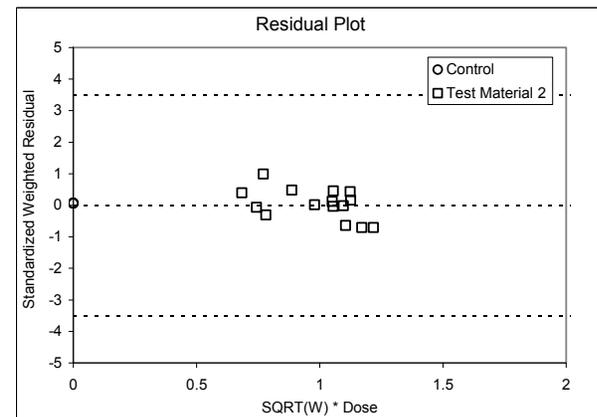
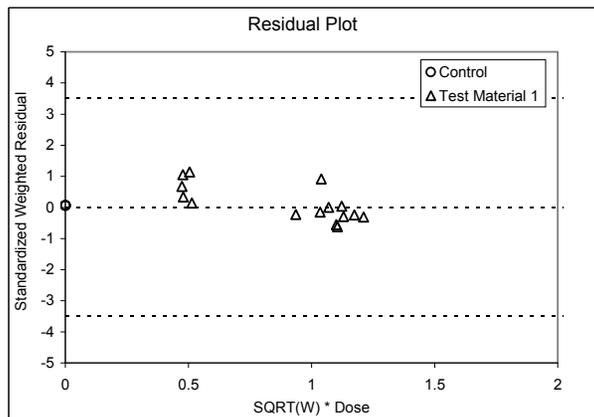
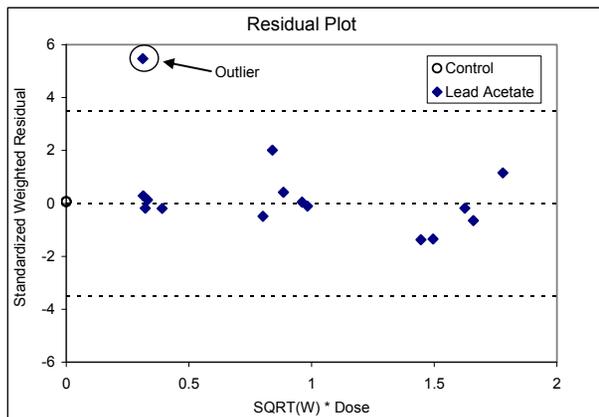
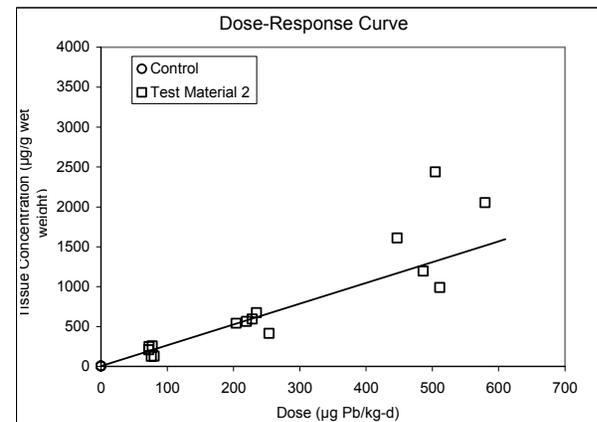
Reference Material (Lead Acetate)



Test Material 1 (HO-3)



Test Material 2 (HCEC-4)



Summary of Fitting*

Parameter	Estimate	Standard Error
a	4.83E+00	1.54E+00
b _r	2.22E+00	4.35E-01
b _{t1}	2.70E+00	4.98E-01
b _{t2}	2.61E+00	4.65E-01
Covariance (b _r ,b _{t1})	0.0026	--
Covariance (b _r ,b _{t2})	0.0026	--
Degrees of Freedom	44	--

Goodness of Fit

Statistic	Estimate
F	28.836
p	< 0.001
Adjusted R ²	0.6399

RBA and Uncertainty

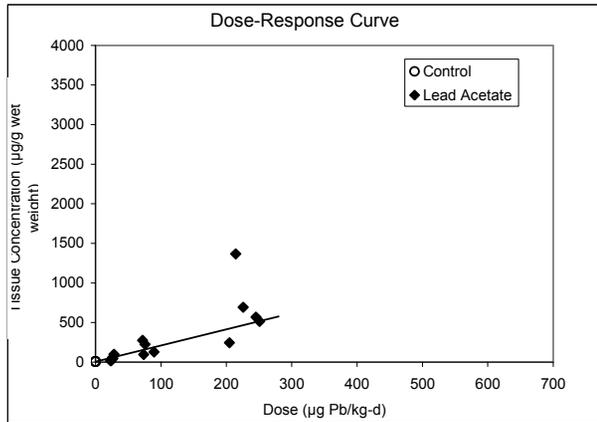
	Test Material 1	Test Material 2
RBA	1.22	1.18
Lower bound	0.76	0.75
Upper bound	1.97	1.89
Standard Error	0.327*	0.311*

* g ≥ 0.05, estimate is uncertain

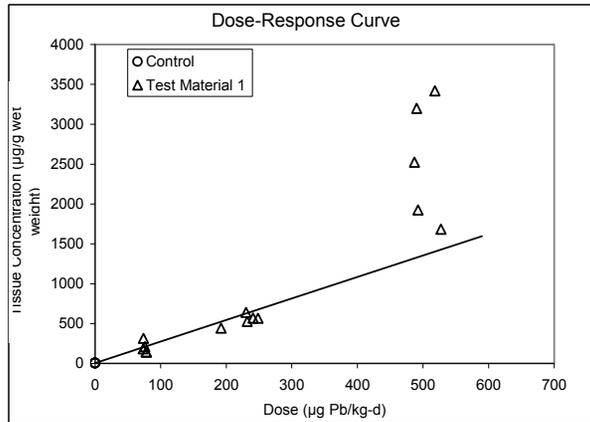
*Data were fit using the linear model: $y = a + b_r \cdot x_r + b_{t1} \cdot x_{t1} + b_{t2} \cdot x_{t2}$

FIGURE 4-3b LIVER LEAD DOSE-RESPONSE: OUTLIER EXCLUDED

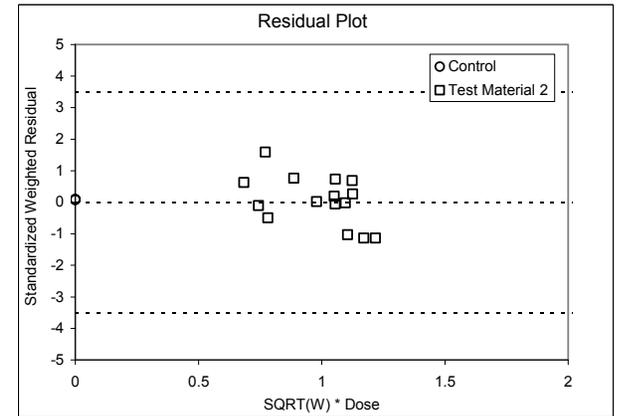
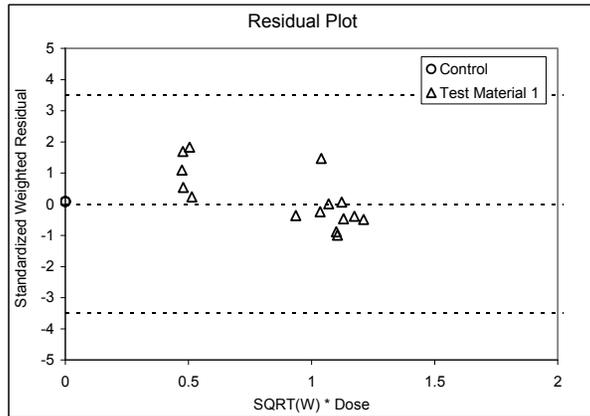
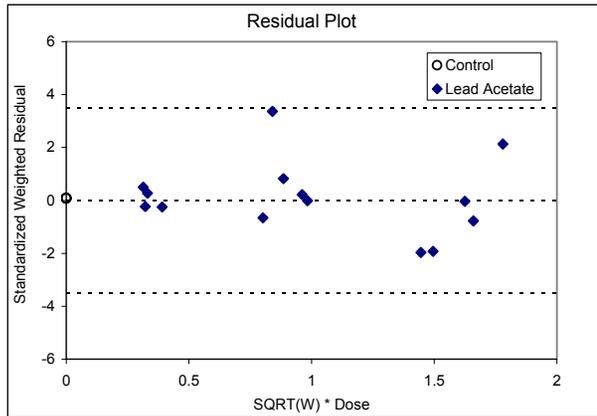
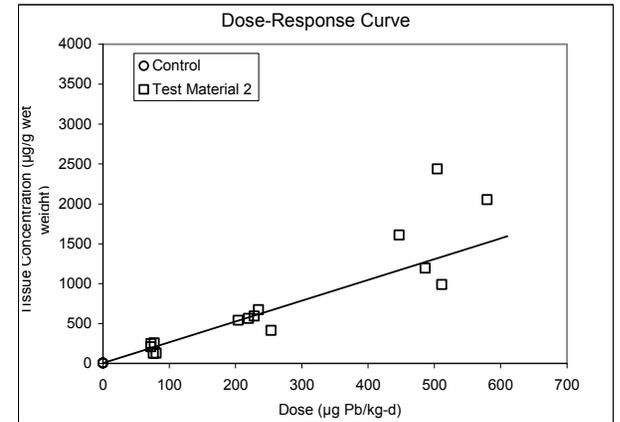
Reference Material (Lead Acetate)



Test Material 1 (HO-3)



Test Material 2 (HCEC-4)



Summary of Fitting*

Parameter	Estimate	Standard Error
a	4.87E+00	9.52E-01
b _r	2.05E+00	2.70E-01
b _{t1}	2.70E+00	3.08E-01
b _{t2}	2.61E+00	2.88E-01
Covariance (b _r ,b _{t1})	0.0026	--
Covariance (b _r ,b _{t2})	0.0026	--
Degrees of Freedom	43	--

Goodness of Fit

Statistic	Estimate
F	71.676
p	< 0.001
Adjusted R ²	0.8217

RBA and Uncertainty

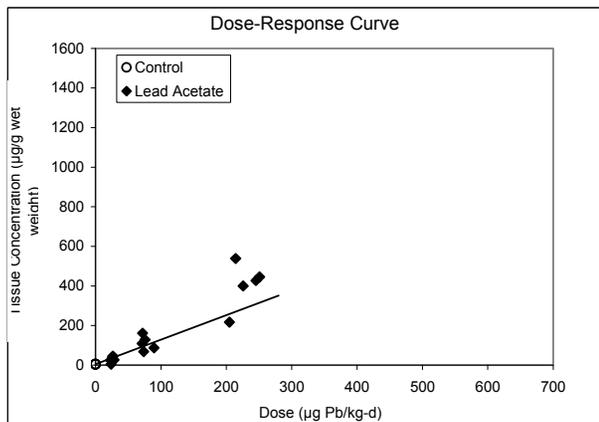
	Test Material 1	Test Material 2
RBA	1.32	1.27
Lower bound	0.76	0.75
Upper bound	1.97	1.89
Standard Error	0.327*	0.311*

*g ≥ 0.05, estimate is uncertain

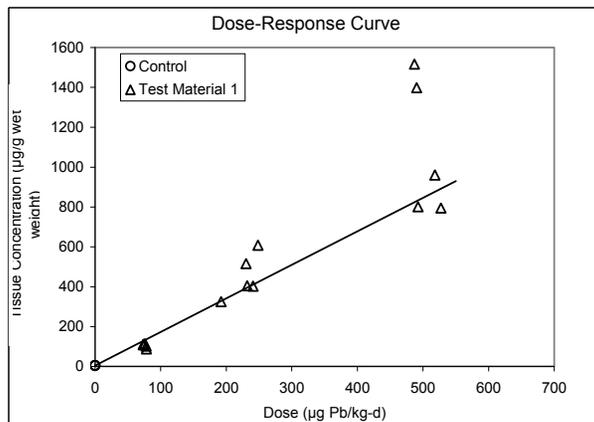
*Data were fit using the linear model: $y = a + b_r \cdot x_r + b_{t1} \cdot x_{t1} + b_{t2} \cdot x_{t2}$

FIGURE 4-4 KIDNEY LEAD DOSE-RESPONSE

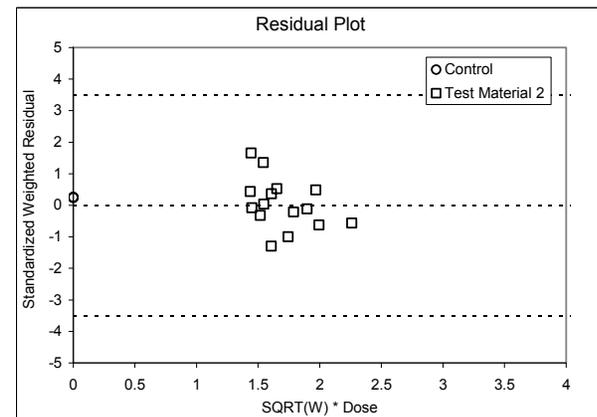
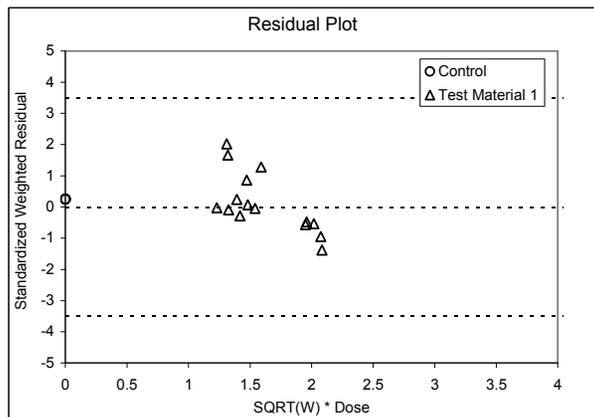
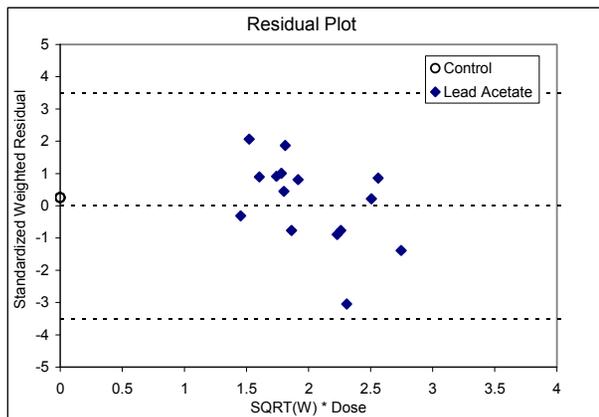
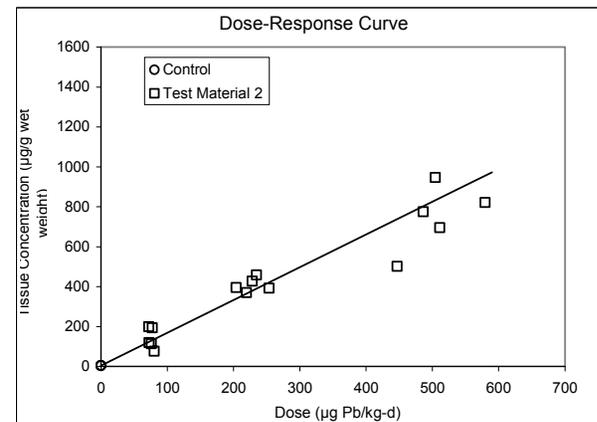
Reference Material (Lead Acetate)



Test Material 1 (HO-3)



Test Material 2 (HCEC-4)



Summary of Fitting*

Parameter	Estimate	Standard Error
a	4.56E+00	1.05E+00
b _r	1.24E+00	1.24E-01
b _{t1}	1.68E+00	1.51E-01
b _{t2}	1.64E+00	1.45E-01
Covariance (b _r ,b _{t1})	0.0116	--
Covariance (b _r ,b _{t2})	0.0082	--
Degrees of Freedom	44	--

Goodness of Fit

Statistic	Estimate
F	116.021
p	< 0.001
Adjusted R ²	0.8801

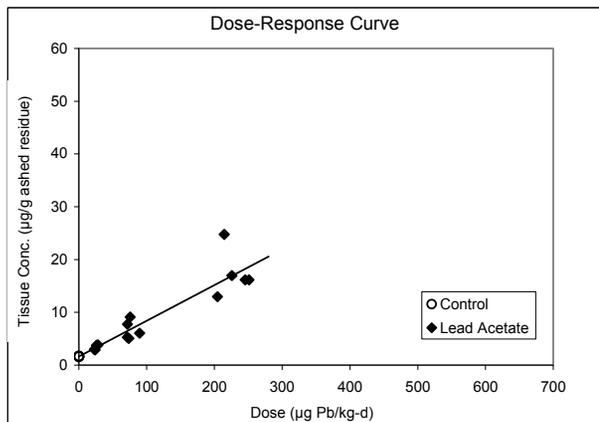
RBA and Uncertainty

	Test Material 1	Test Material 2
RBA	1.36	1.33
Lower bound	1.09	1.06
Upper bound	1.71	1.67
Standard Error	0.182	0.176

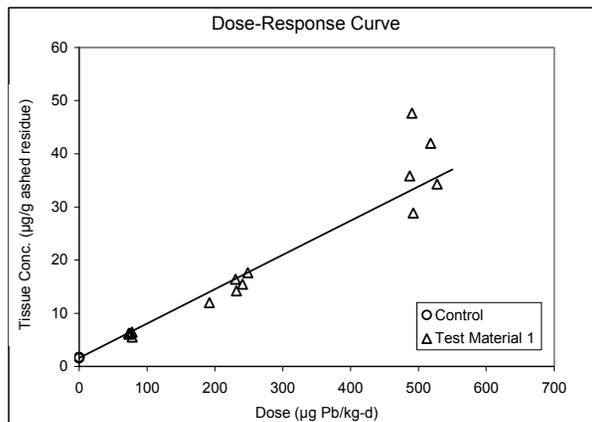
*Data were fit using the linear model: $y = a + b_r \cdot x_r + b_{t1} \cdot x_{t1} + b_{t2} \cdot x_{t2}$

FIGURE 4-5 FEMUR LEAD DOSE-RESPONSE

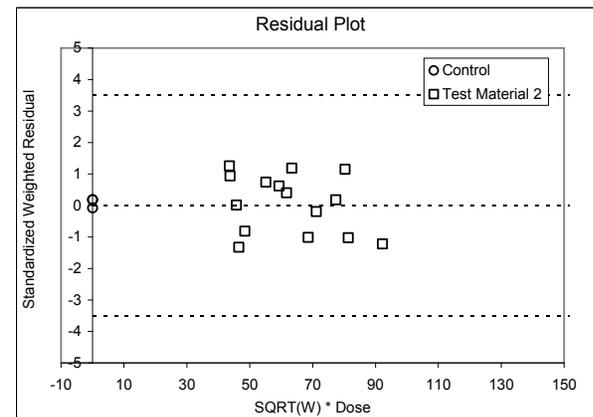
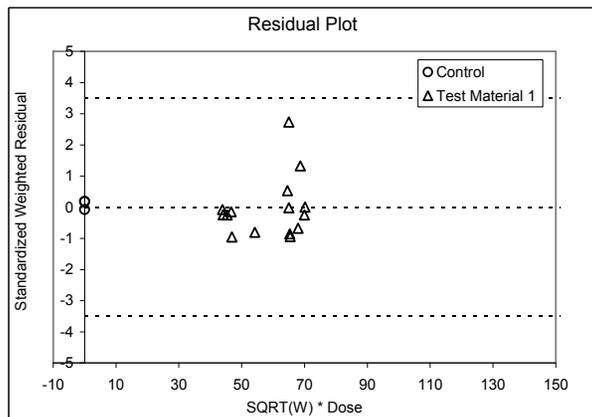
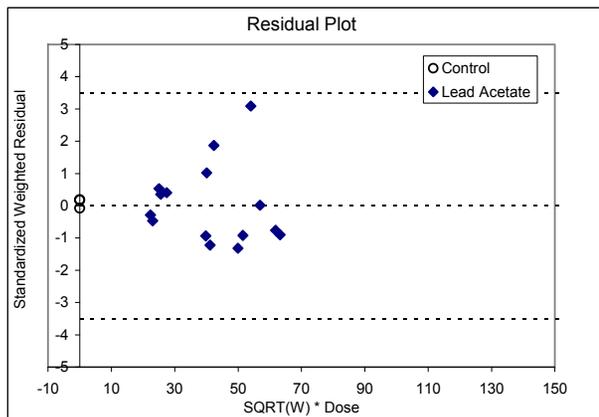
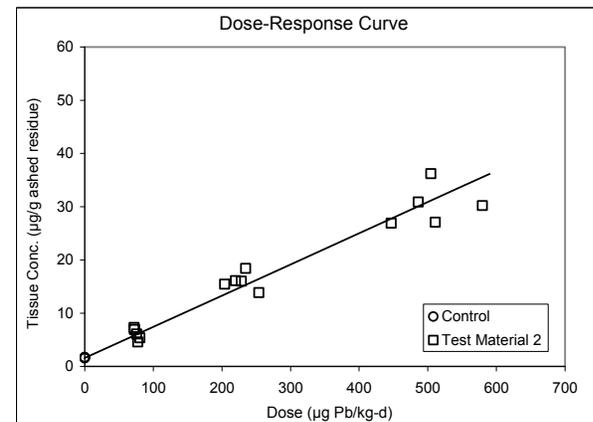
Reference Material (Lead Acetate)



Test Material 1 (HO-3)



Test Material 2 (HCEC-4)



Summary of Fitting*

Parameter	Estimate	Standard Error
a	1.61E+00	1.90E-01
b _r	6.78E-02	4.72E-03
b _{t1}	6.45E-02	3.27E-03
b _{t2}	5.86E-02	3.03E-03
Covariance (b _r ,b _{t1})	0.1271	--
Covariance (b _r ,b _{t2})	0.1242	--
Degrees of Freedom	44	--

Goodness of Fit

Statistic	Estimate
F	268.174
p	< 0.001
Adjusted R ²	0.9446

RBA and Uncertainty

	Test Material 1	Test Material 2
RBA	0.95	0.86
Lower bound	0.83	0.76
Upper bound	1.09	0.99
Standard Error	0.077	0.070

*Data were fit using the linear model: $y = a + b_r \cdot x_r + b_{t1} \cdot x_{t1} + b_{t2} \cdot x_{t2}$

FIGURE 4-7 VARIANCE MODEL FOR LIVER

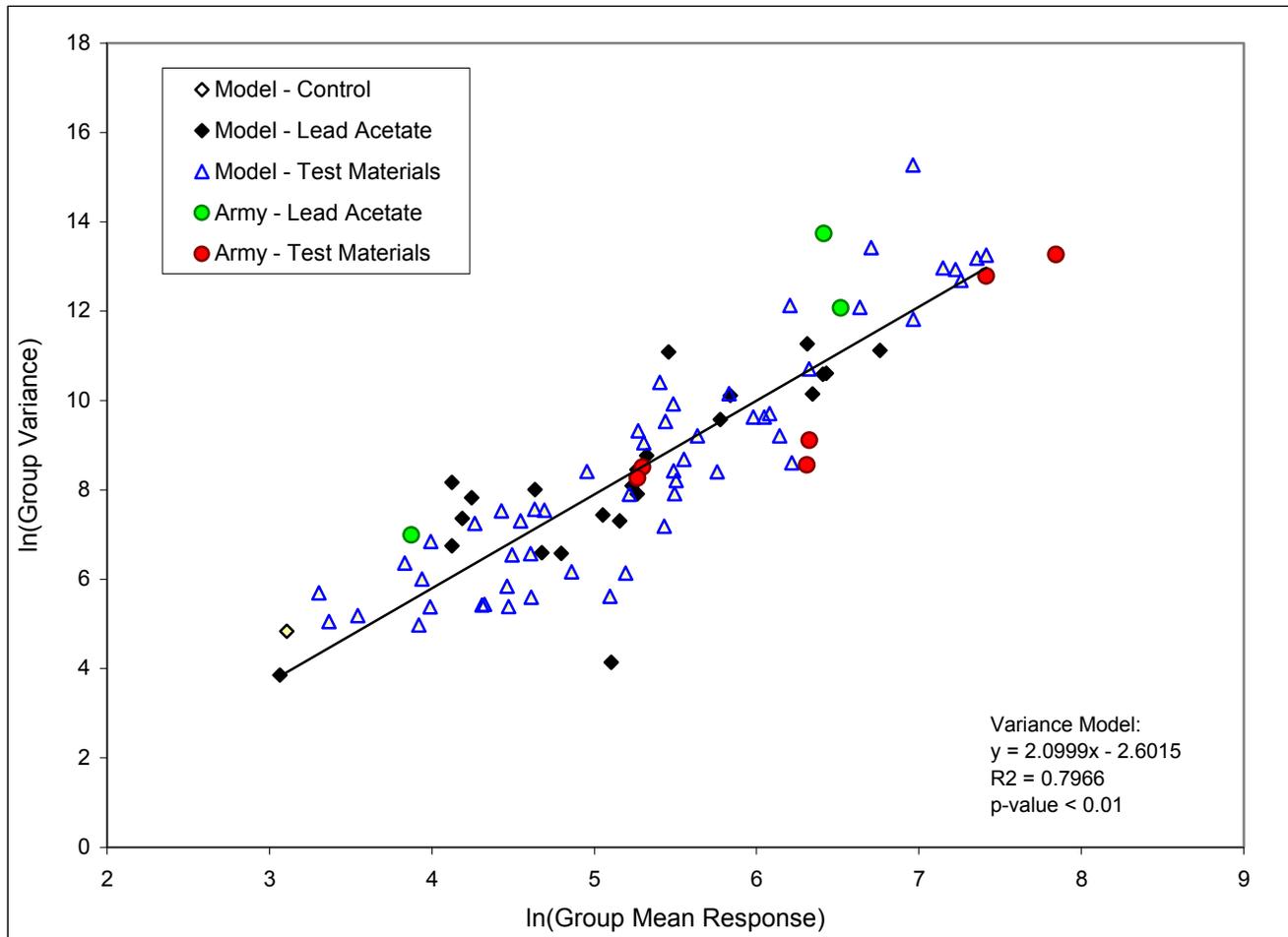


FIGURE 4-8 VARIANCE MODEL FOR KIDNEY

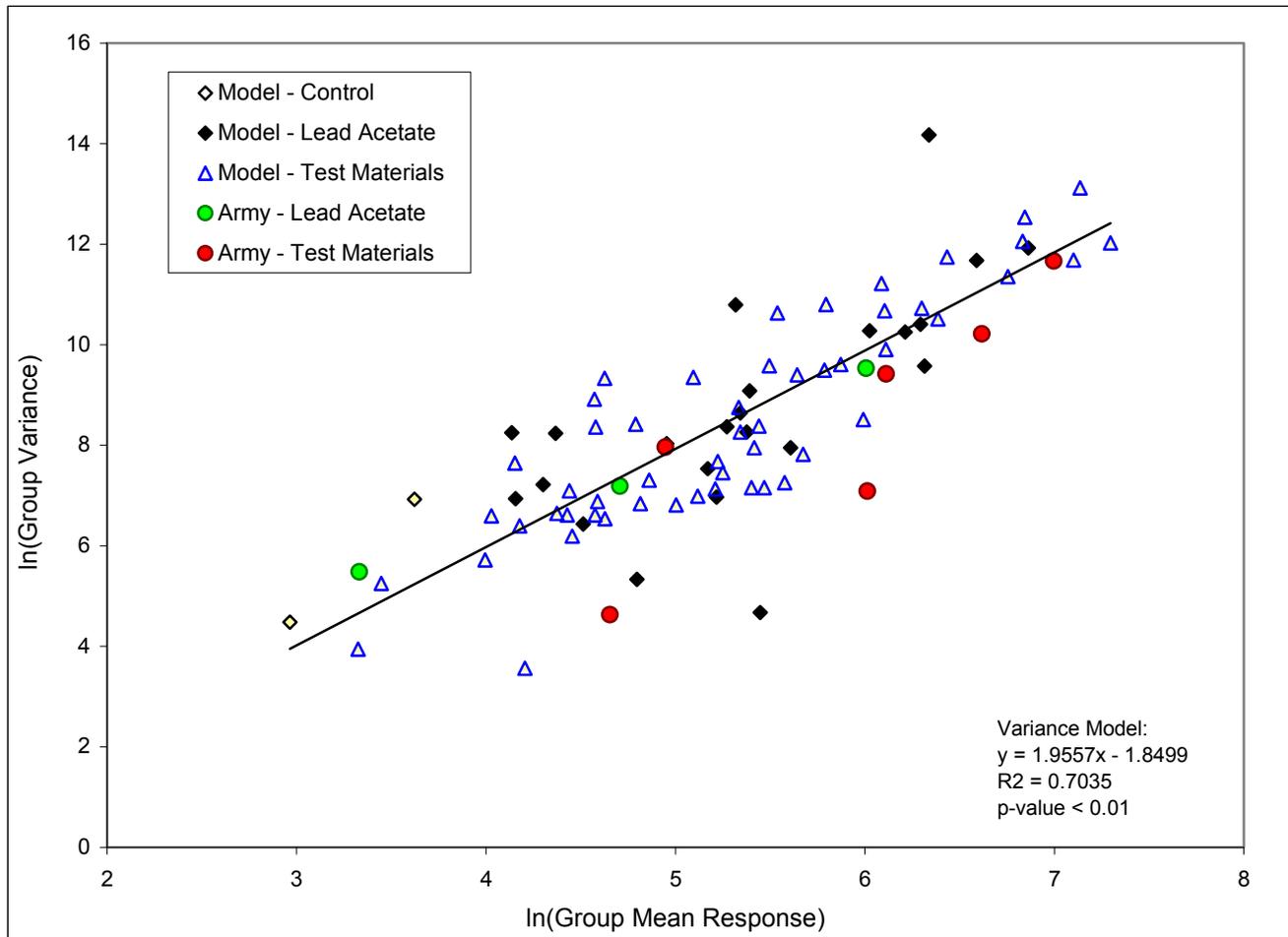
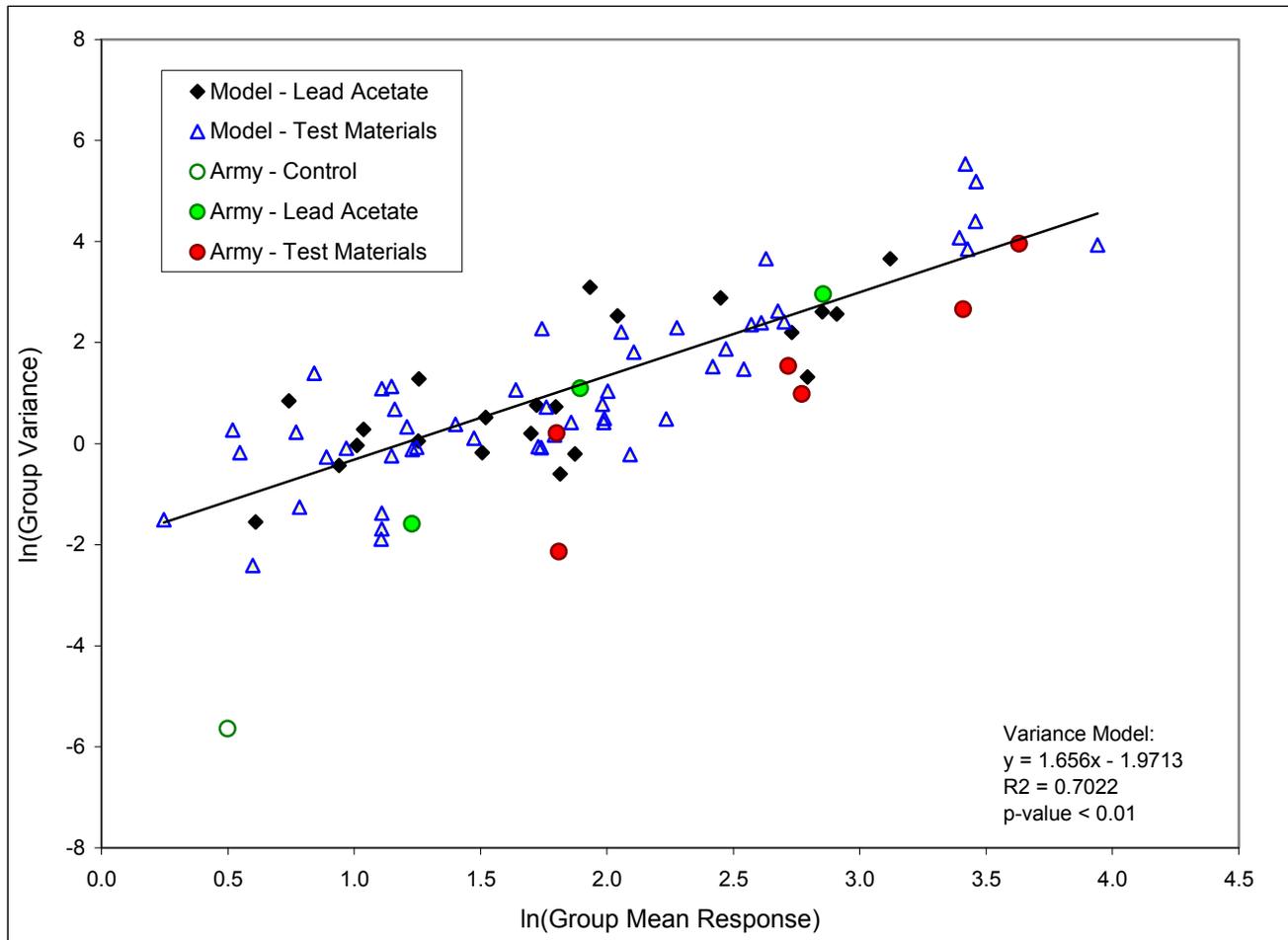
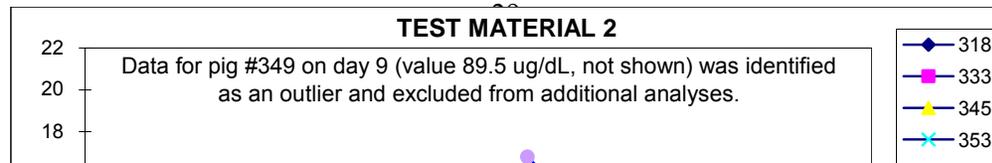
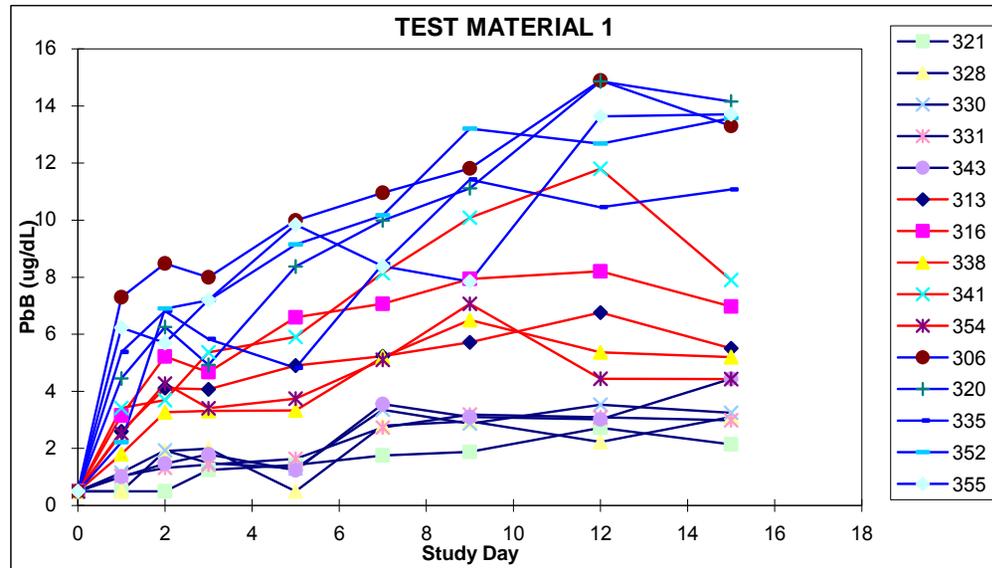
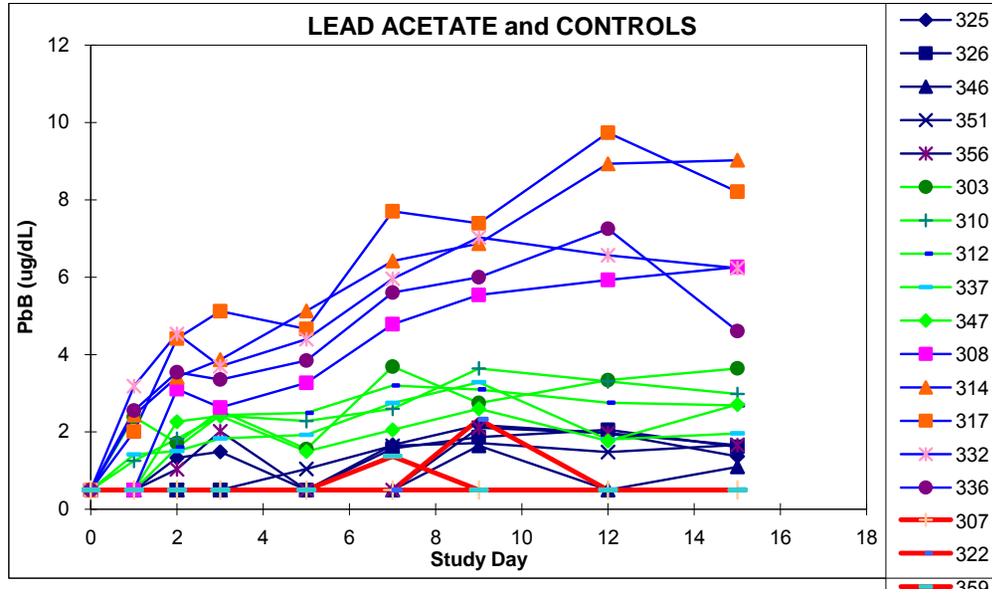


FIGURE 4-9 VARIANCE MODEL FOR FEMUR



DRAFT

FIGURE A-1 BLOOD LEAD DATA BY DAY



REPORTS 3 AND 4

**RELATIVE BIOAVAILABILITY OF LEAD IN SOILS
FROM SMALL ARMS FIRING RANGES IN NEBRASKA,
WASHINGTON, SOUTH DAKOTA, AND OREGON**

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EXECUTIVE SUMMARY

Two studies using juvenile swine as test animals were performed to measure the gastrointestinal absorption of lead from four test soils collected from small arms firing ranges in Nebraska, Washington, South Dakota, and Oregon. The lead concentrations in the four test soils are shown below:

Test Material (TM)	Lead Concentration (µg/g)
TM1 (NE Soil)	14,372
TM2 (WA Soil)	23,409
TM3 (SD Soil)	4,503
TM4 (OR Soil)	19,464

The relative bioavailability of lead in each test soil was assessed by comparing the absorption of lead from the test soil to that of a reference material (lead acetate).

Groups of five swine were given oral doses of lead acetate or the test soil twice a day for 15 days. The amount of lead absorbed by each animal was evaluated by measuring the amount of lead in the blood (measured on days 0, 1, 2, 3, 5, 7, 9, 12, and 15) and the amount of lead in liver, kidney, and bone (measured on day 15 at study termination). The amount of lead present in blood or tissues of animals exposed to test soil was compared to that for animals exposed to lead acetate, and the results were expressed as relative bioavailability (RBA). The RBA results for these four test soils are summarized below:

Measurement Endpoint	Estimated RBA (90% Confidence Interval)			
	TM1 (NE Soil)	TM2 (WA Soil)	TM3 (SD Soil)	TM4 (OR Soil)
Blood Lead AUC	0.89 (0.69 - 1.14)	1.11 (0.82 - 1.44)	0.70	1.03
Liver Lead	0.98 (0.45 - 2.49)	1.13 (0.54 - 2.91)	0.90 (0.63 - 1.30)	1.14 (0.80 - 1.64)
Kidney Lead	0.93 (0.72 - 1.22)	1.04 (0.81 - 1.36)	0.82 (0.62 - 1.08)	1.29 (0.99 - 1.70)
Femur Lead	0.92 (0.76 - 1.14)	0.98 (0.81 - 1.21)	0.67 (0.55 - 0.82)	1.01 (0.84 - 1.25)
Point Estimate	0.93 (0.59 - 1.35)	1.07 (0.67 - 1.55)	0.77 (0.55 - 1.08)	1.12 (0.81 - 1.51)

^aUpper and lower bounds could not be calculated.

As seen, using lead acetate as a relative frame of reference, the RBA estimates range from approximately 77% to 112% for the test soils. These relative bioavailability estimates may be

used to improve accuracy and decrease uncertainty in estimating human health risks from exposure to these test soils.

It is important to understand that these bioavailability estimates are subject to uncertainty that arises from several different sources. One source of uncertainty stems from the inherent biological variability between different animals, which is characterized by the confidence range around the endpoint-specific and the point estimate RBA values shown above. However, there is also uncertainty in the extrapolation of RBA values measured in juvenile swine to humans, and this uncertainty is not included in the statistical confidence bounds above. Even though juvenile swine are considered to be a good model for lead absorption in children, differences between swine and children could result in differences in RBA. In addition, RBA may depend on the amount and type of food in the stomach. In this regard, RBA values measured in these studies are based on animals that have little or no food in their stomach at the time of lead exposure, and hence are likely to yield values of RBA that may be somewhat conservative for humans who ingest the soils along with food.

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RELATIVE BIOAVAILABILITY OF LEAD IN SOILS FROM SMALL ARMS FIRING RANGES IN NEBRASKA, WASHINGTON, SOUTH DAKOTA, AND OREGON

6.0 INTRODUCTION

6.1 Overview of Bioavailability

Reliable analysis of the potential hazard to humans from ingestion of lead depends upon accurate information on a number of key parameters, including lead concentration in environmental media (e.g., soil, dust, water, food, air, paint), intake rates of each medium, and the rate and extent of lead absorption by the body from an ingested medium (“bioavailability”). Knowledge of lead bioavailability is important because the amount of lead that 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 influence 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 is normally described as the fraction or percentage of a chemical that is absorbed by the body following an exposure of some specified amount, duration, and route (usually oral). 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:

$$ABA = (\text{Absorbed Dose}) / (\text{Ingested Dose})$$

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 the absolute bioavailability of lead in some appropriate reference material:

$$RBA = ABA(\text{test}) / ABA(\text{reference})$$

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).

6.2 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 materials 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:

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

where:

- ABA_{soil} = Absolute bioavailability of lead in soil ingested by a human
- ABA_{soluble} = Absolute bioavailability in children of some dissolved or fully soluble form of lead
- RBA_{soil} = Relative bioavailability of lead in soil as measured in swine

Based on available information on lead absorption in humans and animals, the U.S. Environmental Protection Agency (USEPA) estimates that the absolute bioavailability of lead from water and other fully soluble forms of lead is usually about 50% in children (USEPA, 1991) and about 20% in adults (USEPA, 2003). 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:

$$ABA_{\text{soil}} (\text{child}) = 50\% \cdot RBA_{\text{soil}}$$

$$ABA_{\text{soil}} (\text{adult}) = 20\% \cdot RBA_{\text{soil}}$$

The default RBA used by USEPA for lead in soil and dust compared to lead in water is 60% for both children and adults. 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 the 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.

6.3 Purpose of this Study

The objective of these *in vivo* bioavailability studies was to determine the oral bioavailability of lead in four test soils collected from small arms firing ranges in Nebraska, Washington, South Dakota, and Oregon relative to the bioavailability of lead acetate using juvenile swine as a test system. The relative bioavailability estimates may be used to improve accuracy and decrease uncertainty in estimating human health risks from exposure to these test soils.

7.0 STUDY DESIGN

This investigation of lead absorption was performed in two sequential studies. The study design was patterned after the standardized study protocol for measuring relative bioavailability of lead (USEPA, 2004) using the juvenile swine model. The basic design for each of these two studies is presented in Table 2-1. As shown, each study investigated lead absorption from lead acetate (the reference material) and two site-specific soil samples (the test materials). Each material was administered to groups of five animals at three different dose levels for 15 days (detailed schedules for Study 1 and Study 2 are presented in Appendix Tables A-1 and B-1, respectively). Additionally, each study included a non-treated group of three animals to serve as a control for determining background lead levels. All doses were administered orally. Study details are provided below. The study was performed as nearly as possible within the spirit and guidelines of Good Laboratory Practices (GLP: 40 CFR 792).

7.1 Test Materials

7.1.1 Sample Description

The test materials for these studies consisted of four test soils collected from small arms firing ranges in Nebraska, Washington, South Dakota, and Oregon.

7.1.2 Sample Preparation

The soil samples were tested as provided by the U. S. Army, without modification. Details on sample collection methods and processing prior to receipt by the testing facility were not provided.

7.1.3 Lead Concentration

The concentration of lead in the soil test materials (TM) was measured in triplicate by flame atomic absorption. The resulting mean lead values were 14,372 $\mu\text{g/g}$ for TM1; 23,409 $\mu\text{g/g}$ for TM2; 4,503 $\mu\text{g/g}$ for TM3; and 19,464 $\mu\text{g/g}$ for TM4.

7.2 Experimental Animals

Juvenile swine were selected for use in this study because they are considered to be a good physiological model for gastrointestinal absorption in children (Weis and LaVelle, 1991; Casteel et al., 1996). The animals were intact males of the Pig Improvement Corporation (PIC) genetically defined Line 26, and were purchased from Chinn Farms, Clarence, MO.

The number of animals purchased for each study was several more than required by the protocol. These animals were purchased at an age of about 5-6 weeks (weaning occurs at age 3 weeks) and housed in individual lead-free stainless steel cages. The animals were then held under quarantine for one week to observe their health before beginning exposure to test materials. Each animal was examined by a certified veterinary clinician (swine specialist) and any animals that appeared to be in poor health during this quarantine period were excluded from the study. To minimize weight variations among animals and groups, extra animals most different in body weight (either heavier or lighter) four days prior to exposure (day -4) were also excluded from the study. The

remaining animals were assigned to dose groups at random (group assignments are presented in Appendix Tables A-2 and B-2).

When exposure began (day zero), the animals were about 6-7 weeks old and weighed an average of about 9.2 kg in Study 1 and 9.8 kg in Study 2. The animals were weighed every three days during the course of the study. On average, animals gained about 0.25 kg/day in both studies and the rate of weight gain was comparable in all dosing groups, ranging from 0.14 to 0.33 kg/day. These body weight data are summarized in Figure 2-1 and are also presented in Appendix Tables A-3 and B-3.

All animals were examined daily by an attending veterinarian while on study. Most animals (N = 89) exhibited no problems throughout the two studies. Several animals (3 on Study 1; 4 on Study 2) were treated for illness (e.g., fever, inappetance, diarrhea, vomiting) with Naxcel (see Appendix Tables A-4 and B-4).

7.3 Diet

Animals were weaned onto standard pig chow (purchased from MFA Inc., Columbia, MO) by the supplier. In order to minimize lead exposure from the diet, the animals were gradually transitioned from the MFA feed to a special low-lead feed (guaranteed less than 0.2 ppm lead, purchased from Zeigler Brothers, Inc., Gardners, PA), and this feed was maintained for the duration of the study. The feed was nutritionally complete and met all requirements of the National Institutes of Health–National Research Council. The typical nutritional components and chemical analysis of the feed are presented in Table 2-2. Each day every animal was given an amount of feed equal to 4% of the mean body weight of all animals on study. Feed amounts were adjusted every three days, when pigs were weighed. Feed was administered in two equal portions at 11:00 AM and 5:00 PM daily. Analysis of random low-lead feed samples indicated that the lead level did not exceed the detection limit of 0.01 µg/g.

Drinking water was provided *ad libitum* via self-activated watering nozzles within each cage. Analysis of samples from randomly selected drinking water nozzles indicated the lead concentration did not exceed the detection limit of 1 µg/L.

TABLE 2-2 TYPICAL FEED COMPOSITION

Nutrient Name	Amount	Nutrient Name	Amount
Protein	20.1021%	Chlorine	0.1911%
Arginine	1.2070%	Magnesium	0.0533%
Lysine	1.4690%	Sulfur	0.0339%
Methionine	0.8370%	Manganese	20.4719 ppm
Met+Cys	0.5876%	Zinc	118.0608 ppm
Tryptophan	0.2770%	Iron	135.3710 ppm
Histidine	0.5580%	Copper	8.1062 ppm
Leucine	1.8160%	Cobalt	0.0110 ppm
Isoleucine	1.1310%	Iodine	0.2075 ppm
Phenylalanine	1.1050%	Selenium	0.3196 ppm
Phe+Tyr	2.0500%	Nitrogen Free Extract	60.2340%
Threonine	0.8200%	Vitamin A	5.1892 kIU/kg
Valine	1.1910%	Vitamin D3	0.6486 kIU/kg
Fat	4.4440%	Vitamin E	87.2080 IU/kg
Saturated Fat	0.5590%	Vitamin K	0.9089 ppm
Unsaturated Fat	3.7410%	Thiamine	9.1681 ppm
Linoleic 18:2:6	1.9350%	Riboflavin	10.2290 ppm
Linoleic 18:3:3	0.0430%	Niacin	30.1147 ppm
Crude Fiber	3.8035%	Pantothenic Acid	19.1250 ppm
Ash	4.3347%	Choline	1019.8600 ppm
Calcium	0.8675%	Pyridoxine	8.2302 ppm
Phos Total	0.7736%	Folacin	2.0476 ppm
Available Phosphorous	0.7005%	Biotin	0.2038 ppm
Sodium	0.2448%	Vitamin B12	23.4416 ppm
Potassium	0.3733%		

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

7.4 Dosing

The protocol for exposing animals to lead is shown in Table 2-1. The dose levels for lead acetate were based on experience from previous swine investigations that showed that lead doses of 25-225 µg/kg-day resulted in clear and measurable increases in lead levels in all endpoints measured (blood, liver, kidney, and bone). The actual administered doses were calculated based on the lead

content of the materials administered and the measured group mean body weights¹. Specifically, doses of lead for the three days following each weighing were based on the group mean body weight adjusted by the addition of 1 kg to account for the expected weight gain over the time interval. After completion of the study, body weights were estimated by interpolation for those days when measurements were not collected and the actual administered doses were calculated for each day and then averaged across all days.

The actual mean doses for each dosing group are included in Table 2-1; the actual lead doses administered to each pig are presented in Appendix Tables A-3 and B-3.

Animals were exposed to lead acetate or a test material for 15 days, with the dose for each day being administered in two equal portions beginning at 9:00 AM and 3:00 PM (two hours before feeding), with two minute intervals allowed for individual pig dosing. Dose material was placed in the center of a small portion (about 5 grams) of moistened feed (this is referred to as a “doughball”), and this was administered to the animals by hand². If uneaten portions of doughballs were discovered, these were retrieved and offered again for consumption. Occasionally, some animals did not consume their entire dose. In these instances, the missed doses were estimated and recorded and the time-weighted average dose calculation for each animal was adjusted downward accordingly (see Appendix Tables A-3 and B-3).

¹ Doses for Groups 7-9 (Test Material 2) in Study 1 were inadvertently calculated using the lead concentration of Test Material 1 instead of Test Material 2 and, as a result, actual doses were markedly higher than the target doses.

² At the beginning of the Day 2 dose preparation of Study 1, about 25 g of Test Material 1 was inadvertently emptied onto the top of Test Material 2 in its bottle. However, because the color of the two soils was distinctly different, the mistake was immediately noticed and all of the Test Material 1 soil was removed as well as surrounding Test Material 2 soil; the removed soil was discarded. The Test Material 2 bottle was then inverted 5 times before being used to prepare any doughballs.

TABLE 2-1 DOSING PROTOCOL

Study 1

Group	Number of Animals	Dose Material Administered	Lead Dose (µg/kg-day)	
			Target	Actual ^{a,b}
1	5	Lead Acetate	25	25.4
2	5	Lead Acetate	75	80.8
3	5	Lead Acetate	225	230.6
4	5	Test Material 1	75	81.2
5	5	Test Material 1	225	216.4
6	5	Test Material 1	675	738.8
7	5	Test Material 2	75	124.1
8	5	Test Material 2	225	398.5
9	5	Test Material 2	675	1170.7
10	3	Control	0	0.0

Study 2

Group	Number of Animals	Dose Material Administered	Lead Dose (µg/kg-day)	
			Target	Actual ^a
1	5	Lead Acetate	25	26.1
2	5	Lead Acetate	75	79.4
3	5	Lead Acetate	225	236.3
4	5	Test Material 3	75	75.2
5	5	Test Material 3	225	235.7
6	5	Test Material 3	675	705.5
7	5	Test Material 4	75	78.5
8	5	Test Material 4	225	234.3
9	5	Test Material 4	675	679.7
10	3	Control	0	0.0

^a Calculated as the administered daily dose divided by the measured or extrapolated daily body weight, averaged over days 0-14 for each animal and each group.

^b Doses for Study 1 groups 7-9 (Test Material 2) are markedly higher than the target; doses were inadvertently calculated using the lead concentration of Test Material 1 instead of Test Material 2.

Doses were administered in two equal portions given at 9:00 AM and 3:00 PM each day. Doses were based on the mean weight of the animals in each group, and were adjusted every three days to account for weight gain.

7.5 Collection of Biological Samples

Samples of blood were collected from each animal on the first day of exposure (day 0) and on days 1, 2, 3, 5, 7, 9, 12, and 15 following the start of exposure. All blood samples were collected by vena-puncture of the anterior vena cava, and samples were immediately placed in purple-top Vacutainer® tubes containing EDTA (ethylenediaminetetra-acetic acid) as anticoagulant. Although EDTA is a chelator of metals, the nitric acid digest used in the analysis destroys the organic constituents in the blood, thereby freeing all lead for analysis. Thus, the presence of EDTA in the sampling tubes will not impact the analytical results for lead. Blood samples were collected each sampling day beginning at 8:00 AM, approximately one hour before the first of the two daily exposures to lead on the sampling day and 17 hours after the last lead exposure the previous day. This blood collection time was selected because the rate of change in blood lead resulting from the preceding exposures is expected to be relatively small after this interval (LaVelle et al., 1991; Weis et al., 1993), so the exact timing of sample collection relative to the last dosing is not likely to be critical.

Following collection of the final blood sample on day 15, all animals were humanely euthanized and samples of liver, kidney, and bone (the right femur, defleshed) were removed and stored at -80°C in lead-free plastic bags for lead analysis.

Samples of all biological samples collected were archived in order to allow for reanalysis and verification of lead levels, if needed. All animals were also subjected to detailed examination at necropsy by a certified veterinary pathologist in order to assess overall animal health.

7.6 Preparation of Biological Samples for Analysis

Blood

One mL of whole blood was removed from the purple-top Vacutainer® tube and added to 9.0 mL of “matrix modifier,” a solution recommended by the Centers for Disease Control and Prevention (CDC) for analysis of blood samples for lead. The composition of matrix modifier is 0.2% (v/v) ultrapure nitric acid, 0.5% (v/v) Triton X-100, and 0.2% (w/v) dibasic ammonium phosphate in deionized distilled water.

Liver and Kidney

One gram of soft tissue (liver or kidney) was placed in a lead-free screw-cap Teflon container with 2 mL of concentrated (70%) nitric acid and heated in an oven to 90°C overnight. After cooling, the digestate was transferred to a clean lead-free 10 mL volumetric flask and diluted to volume with deionized distilled water.

Bone

The right femur of each animal was defleshed, broken, and dried at 100°C overnight. The dried bones were then placed in a muffle furnace and dry-ashed at 450°C for 48 hours. Following dry ashing, the bone was ground to a fine powder using a lead-free mortar and pestle, and 200 mg was removed and dissolved in 10.0 mL of 1:1 (v:v) concentrated nitric acid/water. After the

powdered bone was dissolved and mixed, 1.0 mL of the acid solution was removed and diluted to 10.0 mL in deionized distilled water.

7.7 Lead Analysis

Samples of biological tissue (blood, liver, kidney, and bone) and other materials (e.g., food, water, reagents, solutions) were analyzed for lead by graphite furnace atomic absorption using a Perkin Elmer AAnalyst 800 high-performance atomic absorption spectrometer. Internal quality assurance samples are described in the following section (2.8).

The quantitation limit was defined as three-times the standard deviation of a set of seven replicates of a low-lead sample (typically about 2-5 $\mu\text{g/L}$). The standard deviation was usually about 0.3 $\mu\text{g/L}$, so the quantitation limit was usually about 0.9-1.0 $\mu\text{g/L}$. For prepared blood samples (diluted 1/10), this corresponds to a quantitation limit of 10 $\mu\text{g/L}$ (1 $\mu\text{g/dL}$). For soft tissues (liver and kidney, diluted 1/10), this corresponds to a quantitation limit of 10 $\mu\text{g/kg}$ (ng/g) wet weight, and for bone (diluted 1/500) the corresponding quantitation limit is 0.5 $\mu\text{g/g}$ (ng/g) ashed weight. All responses below the quantitation limit were evaluated at one-half the quantitation limit.

Lead analytical results for Study 1 samples are presented in Table A-5 of Appendix A; results for Study 2 are presented in Table B-5 of Appendix B. The results for quality assurance samples are presented in Appendix Tables A-6 and B-6; quality assurance results are summarized below.

7.8 Quality Assurance

A number of quality assurance (QA) steps were taken during this project to evaluate the accuracy of the analytical procedures. These included:

Spike Recovery

Randomly selected samples were spiked with known amounts of lead (as lead acetate) and the recovery of the added lead was measured. For Study 1, recovery for individual samples ranged from 70% to 147%, with an average of $93 \pm 13\%$ ($N = 72$). For Study 2, spike recoveries ranged from 75% to 103%, with an average of $90 \pm 6.2\%$ ($N = 54$).

Duplicate Analysis of Sample Digestate

Periodically during sample analysis, samples were randomly selected for duplicate analysis (i.e., the same prepared sample was analyzed twice). All duplicate results ($N = 123$) agreed within $\pm 15\%$ relative percent difference (RPD) (for analytical results greater than 10 $\mu\text{g/L}$) or $\pm 1 \mu\text{g/L}$ (for analytical results less than or equal to 10 $\mu\text{g/L}$), as required by the analytical protocol.

Sample Preparation Replicates

A random selection of about 7% of all biological samples generated during each study were prepared for laboratory analysis in duplicate (i.e., two separate subsamples of blood/tissue were prepared for analysis). The results for these replicate preparations are summarized in Figure 2-2. As seen, the analytical results for replicate pairs of blood samples (Panel A of Figure 2-2) tend to

follow the line of equality, indicating that the replicate pairs are generally in good agreement. The absolute difference between replicate pairs of blood samples ranged from 0 to 5.3 $\mu\text{g/dL}$ with an average of 0.6 $\mu\text{g/dL}$ (N = 60) across both studies. As seen, there was also good reproducibility between replicate samples for tissues (Panels B and C of Figure 2-2). The absolute difference between replicate pairs of liver and kidney samples ranged from 0 to 0.13 $\mu\text{g/g}$ with an average of 0.03 $\mu\text{g/g}$ (N = 14). The absolute difference between replicate pairs of femur samples ranged from 0.04 to 5.7 $\mu\text{g/g}$ with an average of 1.3 $\mu\text{g/g}$ (N = 6).

Laboratory Control Standards

Laboratory control standards (samples of reference materials for which a certified concentration of lead has been established) were tested periodically during sample analysis. Results for the standards are summarized below:

Standard	Target Value (Acceptable Range)	Mean	Range	SD	Mean % Recovery	N
DOLT-3 (dogfish liver)	0.319 (0.274 - 0.365)	0.297	0.25 – 0.38	0.072	93.0%	3
NIST SRM 1400 (bone ash)	9.07 (8.95 - 9.19)	10.03	9.76 – 10.3	00.38	110.6%	2

As seen, recovery of lead from these standards was generally good and within the acceptable range.

Blood Lead Check Samples

The CDCP provides a variety of blood lead “check samples” for use in quality assurance programs for blood lead studies. Several CDCP check samples of different concentrations were analyzed periodically during blood sample analysis. The results are summarized in Figure 2-3. In both studies, the results for all standards generally cluster around the line of equality, but tend to be slightly lower than expected; the reason for this is not known.

Blanks

Samples of the sample preparation matrix for each endpoint (without added tissue) were routinely analyzed for lead to ensure the absence of lead contamination. These matrix blanks never exceeded the detection limit of 1 $\mu\text{g/L}$ (N = 169).

Based on the results of all of the quality assurance samples and steps described above, it is concluded that the analytical results are of sufficient quality for derivation of reliable estimates of lead absorption from test materials.

8.0 DATA ANALYSIS

8.1 Overview

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 (e.g., 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). Because equal absorbed doses of lead (as Pb^{+2}) will produce equal responses (i.e., equal increases in concentration in tissues) regardless of the source or nature of the ingested lead, 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. Thus, 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.

Some biological responses to lead exposure may be non-linear functions of dose (i.e., tending to flatten out or plateau as dose increases). The cause of this non-linearity is uncertain but might be due either to non-linear absorption kinetics and/or to non-linear biological response per unit dose absorbed. However, the principal advantage of the approach described above is that it is not necessary to understand the basis for a non-linear dose response curve (non-linear absorption and/or non-linear biological response) in order to derive valid RBA estimates; in addition, this approach is general and yields reliable results for both non-linear and linear responses.

A detailed description of the curve-fitting methods and rationale, along with the methods used to quantify uncertainty in the RBA estimates for the test material, are presented in USEPA (2004) and are summarized below.

8.2 Measurement Endpoints

Four independent measurement endpoints were evaluated based on the concentration of lead observed in blood, liver, kidney, and bone (femur). For liver, kidney, and bone, the measurement endpoint was simply the concentration in the tissue at the time of sacrifice (day 15). The measurement endpoint used to quantify the blood lead response was the area under the curve (AUC) for blood lead vs. time (days 0-15). AUC was selected because it is the standard pharmacokinetic index of chemical uptake into the blood compartment, and is relatively insensitive to small variations in blood lead level by day. The AUC was calculated using the trapezoidal rule to estimate the AUC between each time point that a blood lead value was measured (days 0, 1, 2, 3, 5, 7, 9, 12, and 15):

$$AUC(d_i \text{ to } d_j) = 0.5 \cdot (r_i + r_j) \cdot (d_j - d_i)$$

where:

d = day number

r = response (blood lead value) on day i (r_i) or day j (r_j)

The areas were then summed across all time intervals in the study to yield the final AUC for each animal.

Blood Lead Outliers

Occasionally blood lead values are obtained that are clearly different than expected. Blood lead values that were more than a factor of 1.5 above or below the group mean for any given day were flagged as potential outliers and are shaded in Appendix Tables A-7 and B-7. Each data point identified in this way was reviewed and professional judgment was used to decide if the value should be retained or excluded. In order to avoid inappropriate biases, blood lead outlier designations are restricted to values that are clearly aberrant from a time-course and/or dose-response perspective. Two values in Study 1 and one value in Study 2 were judged to be clear outliers; these are identified by a heavy black box outlining the values in Appendix Tables A-7 (Study 1) and B-7 (Study 2). These blood lead values were excluded from the calculation of AUC; the missing values were replaced with values interpolated from the preceding and following values from the same animal.

8.3 Dose-Response Models

Basic Equations

It has been shown previously (USEPA, 2004) that nearly all blood lead AUC data sets can be well-fit using an exponential equation and most tissue (liver, kidney, and bone) lead data can be well-fit using a linear equation, as follow:

$$\text{Linear (liver, kidney, bone):} \quad \text{Response} = a + b \cdot \text{Dose}$$

$$\text{Exponential (blood lead AUC):} \quad \text{Response} = a + b \cdot [1 - \exp(-c \cdot \text{Dose})]$$

Simultaneous Regression

Because the data to be analyzed consist of three dose-response curves for each endpoint (the reference material and two test materials) and there is no difference between the curves when the dose is zero, all three curves for a given endpoint must have the same intercept. This requirement is achieved by combining the two dose response equations into one and solving for the parameters simultaneously, resulting in the following equations:

$$\text{Linear:} \quad y = a + b_r \cdot x_r + b_t \cdot x_t$$

$$\text{Exponential:} \quad y = a + b \cdot [(1 - \exp(-c_r \cdot x_r)) + (1 - \exp(-c_t \cdot x_t))]$$

where:

y = response

x = dose

a, b, c = empirical coefficients for the reference material (r) and test material (t).

All linear model fitting was performed in Microsoft® Office Excel using matrix functions. Exponential model fitting was performed using JMP® version 3.2.2, a commercial software package developed by SAS®.

Weighted Regression

Regression analysis based on ordinary least squares assumes that the variance of the responses is independent of the dose and/or the response (Draper and Smith, 1998). It has previously been shown that this assumption is generally not satisfied in swine-based RBA studies, where there is a tendency toward increasing variance in response as a function of increasing dose (heteroscedasticity) (USEPA, 2004). To deal with heteroscedasticity, the data are analyzed using weighted least squares regression. In this approach, each observation in a group of animals is assigned a weight that is inversely proportional to the variance of the response in that group:

$$w_i = (\sigma^2_i)^{-1}$$

where:

w_i = weight assigned to all data points in dose group i
 σ^2_i = variance of responses of animals in dose group i

(Draper and Smith, 1998).

As discussed in USEPA (2004), there are several alternative strategies for assigning weights. The preferred method identified by USEPA (2004) and the method used in this study estimates the value of σ^2_i using an “external” variance model based on an analysis of the relationship between variance and mean response using data consolidated from ten different swine-based lead RBA studies. Log-variance increases as an approximately linear function of log-mean response for all four endpoints:

$$\ln(s_i^2) = k1 + k2 \cdot \ln(\bar{y}_i)$$

where:

s_i^2 = observed variance of responses of animals in dose group i
 \bar{y}_i = mean observed response of animals in dose group i

Values of $k1$ and $k2$ were derived for each endpoint using ordinary least squares minimization, and the resulting values are shown below:

Endpoint	k1	k2
Blood AUC	-1.3226	1.5516
Liver	-2.6015	2.0999
Kidney	-1.8499	1.9557
Femur	-1.9713	1.6560

Goodness-of-Fit

The goodness-of-fit of each dose-response model was assessed using the F test statistic and the adjusted coefficient of multiple determination ($\text{Adj } R^2$) as described by Draper and Smith (1998). A fit is considered acceptable if the p-value is less than 0.05.

Assessment of Outliers

In biological assays, it is not uncommon to note the occurrence of individual measured responses that appear atypical compared to the responses from other animals in the same dose group. In this study, endpoint responses that yielded standardized weighted residuals greater than 3.5 or less than -3.5 were considered to be potential outliers (Canavos, 1984). When such data points were encountered in a data set, the RBA was calculated both with and without the potential outlier(s) excluded, and the result with the outlier(s) excluded was used as the preferred estimate.

8.4 Calculation of RBA Estimates

Endpoint-specific RBA Estimates

Lead RBA values were estimated using the basic statistical techniques recommended by Finney (1978). Each endpoint-specific RBA value was calculated as the ratio of a model coefficient for the reference material data set and for the test material data set:

$$\begin{array}{ll} \text{Linear endpoints:} & \text{RBA}_t = b_t / b_r \\ \text{Exponential endpoint:} & \text{RBA}_t = c_t / c_r \end{array}$$

The uncertainty range about the RBA ratio was calculated using Fieller's Theorem as described by Finney (1978).

RBA Point Estimate

Because there are four independent estimates of RBA (one from each measurement endpoint) for a given test material, the final RBA estimate for a test material involves combining the four endpoint-specific RBA values into a single value (point estimate) and estimating the uncertainty around that point estimate. As described in USEPA (2004), analysis of data from multiple studies suggests that the four endpoint-specific RBA values are all approximately equally reliable (as reflected in the average coefficient of variation in RBA values derived from each endpoint). Therefore, the RBA point estimate for the test material was calculated as the simple mean of all four endpoint-specific RBA values.

The uncertainty bounds around this point estimate were estimated using Monte Carlo simulation. Values for RBA were drawn from the uncertainty distributions for each endpoint with equal frequency. Each endpoint-specific uncertainty distribution was assumed to be normal, with the mean equal to the best estimate of RBA and the standard deviation estimated from Fieller's Theorem (Finney, 1978). The uncertainty in the point estimate was characterized as the range from the 5th to the 95th percentile of the mean across endpoints.

9.0 RESULTS

9.1 Clinical Signs

The doses of lead administered in these two studies are below a level that is expected to cause toxicological responses in swine, and no clinical signs of lead-induced toxicity were noted in any of the animals used in the studies.

9.2 Blood Lead vs. Time

Detailed results from Study 1 and Study 2 are presented in Appendix A and B, respectively; blood lead data for individual animals are presented in Figures A-1 and B-1. Group mean blood lead values as a function of time are shown in Figure 4-1. As seen, blood lead values began at or below quantitation limits (about 1 µg/dL) in all groups, and remained at or below quantitation limits in control animals (Group 10). In animals given repeated oral doses of lead acetate (Groups 1-3) or test soil (Groups 4-9), blood levels began to rise within 1-2 days, and tended to plateau by the end of the study (day 15).

9.3 Dose-Response Patterns

Variance

As discussed in Section 3.3, the dose-response data are analyzed using weighted least squares regression and the weights are assigned using an “external” variance model (USEPA, 2004). As shown in Figures 4-2 (Study 1) and 4-3 (Study 2), the variance of the data from these studies is generally quite similar to that of the data used to generate the variance model for all four measurement endpoints.

Blood Lead AUC

As discussed in Section 3.2, the measurement endpoint used to quantify the blood lead response was the area under the curve (AUC) for blood lead vs. time (days 0-15). The AUC determinations are presented in Appendix Tables A-8 and B-8.

The blood lead AUC dose-response data were modeled using an exponential equation (see Section 3.3). The results of this fitting are shown in Figure 4-4 (Study 1) and Figure 4-5 (Study 2).

Tissue Lead

The dose-response data for lead in liver, kidney, and bone (measured at sacrifice on day 15) were modeled using a linear equation (see Section 3.3). The results of these fittings are shown in Figures 4-6 (Study 1) and 4-7 (Study 2) for liver, 4-8 (Study 1) and 4-9 (Study 2) for kidney, and 4-10 (Study 1) and 4-11 (Study 2) for femur. In Study 2, one outlier was identified in the middle dose group of TM3 for femur (as indicated in Figure 4-11a) and was excluded from the final evaluation for lead (Figure 4-11b).

In Study 1, one outlier was identified in the low dose group of lead acetate for liver (as indicated in Figure 4-6a) and the data were re-fit with the outlier excluded (Figure 4-6b). Although excluding an outlier typically results in a better fit or little change, in this case it appears to yield a less reliable fit that fails to pass through the highest dose group³. In addition, the RBA estimate with the outlier included is much more consistent with those for the other three endpoints, while excluding the outlier results in RBA values around 1.5. While it is certainly possible for a material to have an RBA value greater than one, it is very unlikely for it to exceed one by 1.5 times. For these reasons, we recommend using the fit through all the data including the outlier for Study 1 liver.

9.4 Calculated RBA Values

Relative bioavailability values for the test soils were calculated for each measurement endpoint (blood lead AUC, liver, kidney, and bone) using the method described in Section 3.4; the suggested point estimate is calculated as the simple mean of the four endpoint-specific estimates. The results are shown below:

Measurement Endpoint	Estimated RBA (90% Confidence Interval)			
	TM1 (NE Soil)	TM2 (WA Soil)	TM3 (SD Soil)	TM4 (OR Soil)
Blood Lead AUC	0.89 (0.69 - 1.14)	1.11 (0.82 - 1.44)	0.70	1.03
Liver Lead	0.98 (0.45 - 2.49)	1.13 (0.54 - 2.91)	0.90 (0.63 - 1.30)	1.14 (0.80 - 1.64)
Kidney Lead	0.93 (0.72 - 1.22)	1.04 (0.81 - 1.36)	0.82 (0.62 - 1.08)	1.29 (0.99 - 1.70)
Femur Lead	0.92 (0.76 - 1.14)	0.98 (0.81 - 1.21)	0.67 (0.55 - 0.82)	1.01 (0.84 - 1.25)
Point Estimate	0.93 (0.59 - 1.35)	1.07 (0.67 - 1.55)	0.77 (0.55 - 1.08)	1.12 (0.81 - 1.51)

^aUpper and lower bounds could not be calculated, as Fieller's theorem failed; we are currently attempting to remedy this.

As seen, using lead acetate as a relative frame of reference, the RBA estimates range from approximately 77% to 112% for the test soils.

9.5 Uncertainty

The bioavailability estimates above are subject to uncertainty that arises from several different sources. One source of uncertainty is the inherent biological variability between different animals in a dose group, which in turn causes variability in the amount of lead in the tissues of the exposed animals. This between-animal variability in response results in statistical uncertainty in the best-fit dose-response curves and, hence, uncertainty in the calculated values

³ Upon exclusion of the outlier, the remaining four data points in this group have a very low sample variance, resulting in an extremely high weight being assigned to the group. The weight for this group is so high that the model fit is constrained to pass through it with very little deviation, and other dose groups exert very little influence. This outcome is judged to be inappropriate.

of RBA. Such statistical uncertainty is accounted for by the statistical models used above and is characterized by the uncertainty range around the endpoint-specific and the point estimate values of RBA.

However, there is also uncertainty in the extrapolation of RBA values measured in juvenile swine to young children or adults, and this uncertainty is not included in the statistical confidence bounds above. Even though the immature swine is believed to be a useful and meaningful animal model for gastrointestinal absorption in children, it is possible that there are differences in physiological parameters that may influence RBA and that RBA values in swine are not identical to values in children. In addition, RBA may depend on the amount and type of food in the stomach, since the presence of food can influence stomach pH, holding time, and possibly other factors that may influence lead solubilization. In this regard, it is important to recall that RBA values measured in this study are based on animals that have little or no food in their stomach at the time of lead exposure and, hence, are likely to yield high-end values of RBA. Thus, these RBA values may be somewhat conservative for humans who ingest the soils along with food. The magnitude of this bias is not known.

10.0 CONCLUSIONS AND RECOMMENDATIONS

When reliable site-specific data are lacking, the USEPA typically employs a default RBA value of 60% for lead in soil compared to soluble lead in water, for both children and adults. The RBA estimates for the soils tested in these two studies (77% to 112%) are higher than the default value of 60%, indicating that absorption of and hazards from lead in these soils may be higher than usually assumed. It is appropriate to take this into account when evaluating potential risks to humans from incidental ingestion of these soils.

These site-specific RBA estimates for lead are an improvement over the default value and should be considered for use in site-specific risk assessments. However, it is important to consider that the values are specific to the soils tested in these studies. Use of the RBA estimates may improve accuracy and decrease uncertainty in estimating human health risks from exposure to these test soils, as well as increase confidence in computations of site-specific risk-based cleanup levels.

11.0 REFERENCES

- Canavos, C. G. 1984. Applied Probability and Statistical Methods. Little, Brown and Co., Boston.
- Casteel, S. W., R. P. Cowart, C. P. Weis, G. M. Henningsen, E. Hoffman, W. J. Brattin, M. F. Starost, J. T. Payne, S. L. Stockham, S. V. Becker, and J. R. Turk. 1996. A swine model for determining the bioavailability of lead from contaminated media. In: Advances in Swine in Biomedical Research. Tumbleson and Schook, eds. Vol 2, Plenum Press, New York. Pp. 637-46.
- Draper, N. R., and H. Smith. 1998. Applied Regression Analysis (3rd Edition). John Wiley & Sons, New York.
- Finney, D. J. 1978. Statistical Method in Biological Assay (3rd Edition). Charles Griffin and Co., London.
- Gibaldi, M., and Perrier, D. 1982. Pharmacokinetics (2nd edition), pp 294-297. Marcel Dekker, Inc, New York, New York.
- Goodman, A.G., Rall, T.W., Nies, A.S., and Taylor, P. 1990. The Pharmacological Basis of Therapeutics (8th ed.), pp. 5-21. Pergamon Press, Inc. Elmsford, New York.
- Klaassen, C.D., Amdur, M.O., and Doull, J. (eds). 1996. Cassarett and Doull's Toxicology: The Basic Science of Poisons, pp. 190. McGraw-Hill, Inc. New York, New York.
- LaVelle, J.M., Poppenga, R.H., Thacker, B.J., Giesy, J.P., Weis, C., Othoudt R., and Vandervoot C. 1991. Bioavailability of Lead in Mining Waste: an oral intubation study in young swine. In: The proceedings of the international symposium on the bioavailability and dietary uptake of lead. Science and Technology Letters 3:105-111.
- Mushak, P. 1991. Gastro-intestinal absorption of lead in children and adults: overview of biological and biophysico-chemical aspects. In: The proceedings of the international symposium on the bioavailability and dietary uptake of lead. Science and Technology Letters 3:87-104.
- USEPA. 1991. Technical support document on lead. United States Environmental Protection Agency, Environmental Criteria and Assessment Office. ECAO-CIN-757.
- USEPA. 2003. Recommendations of the Technical Review Workgroup for Lead for an approach to assessing risks associated with adult exposures to lead in soil. United States Environmental Protection Agency Technical Review Workgroup for Lead. OSWER 9285.7-54, EPA-540-R-03-001. January.
- USEPA. 2004. Estimation of Relative Bioavailability of Lead in Soil and Soil-Like Materials Using *In Vivo* and *In Vitro* Methods – Draft Final. United States Environmental Protection Agency, Office of Solid Waste and Emergency Response, Washington, D.C. OSWER 9285.7-77, December 2004.

Weis, C.P., and LaVelle, J.M. 1991. Characteristics to consider when choosing an animal model for the study of lead bioavailability. In: The proceedings of the international symposium on the bioavailability and dietary uptake of lead. *Science and Technology Letters* 3:113-119.

Weis, C.P., Henningsen, G.M., Poppenga, R.H., and Thacker, B.J. 1993. Pharmacokinetics of lead in blood of immature swine following acute oral and intravenous exposure. *The Toxicologist* 13(1):175.

Weis, C.P., Poppenga, R.H., Thacker, B.J., Henningsen, G.M., and Curtis, A. 1995. Design of pharmacokinetic and bioavailability studies of lead in an immature swine model. In: *Lead in paint, soil, and dust: health risks, exposure studies, control measures, measurement methods, and quality assurance*. ASTM STP 1226, Michael E. Beard and S. D. Allen Iske (eds), American Society for Testing and Materials, Philadelphia, 1995.

FIGURE 2-1 BODY WEIGHT GAIN

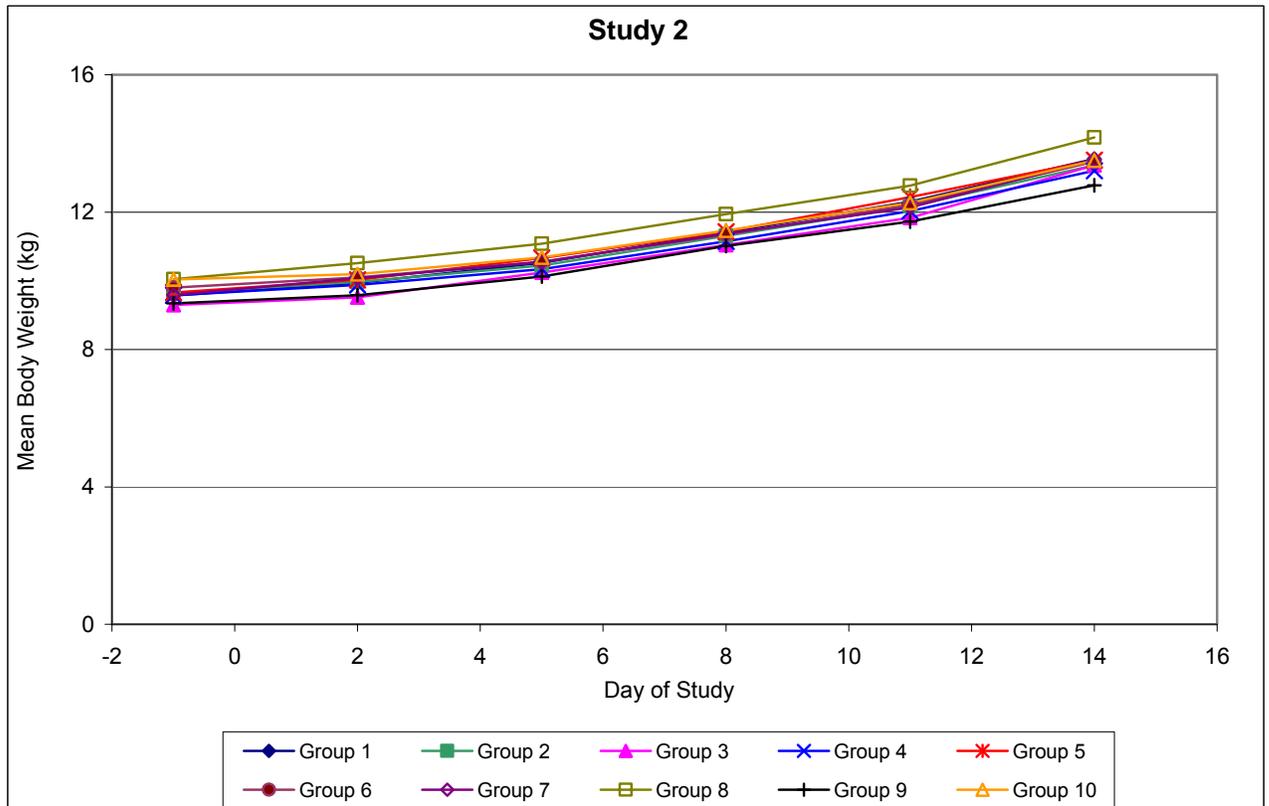
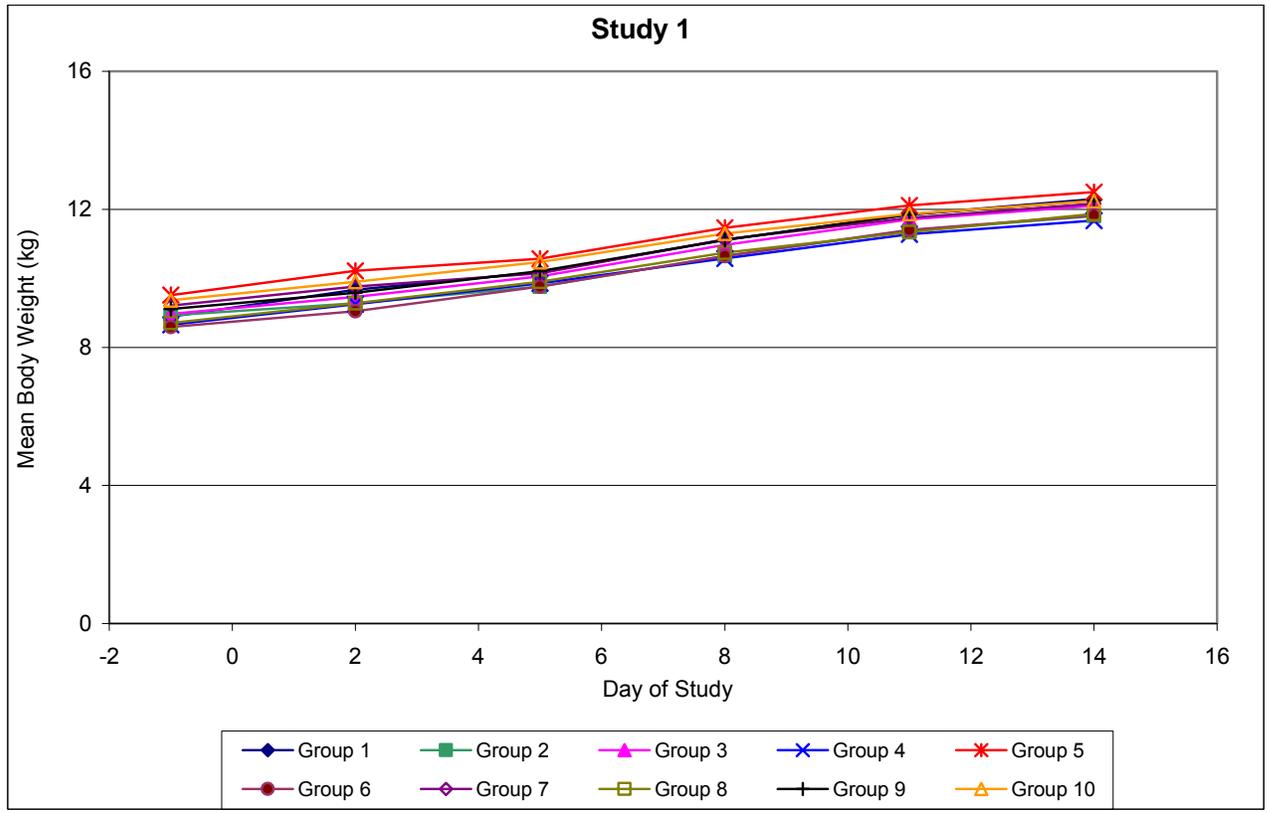


FIGURE 2-2 SAMPLE PREPARATION REPLICATES

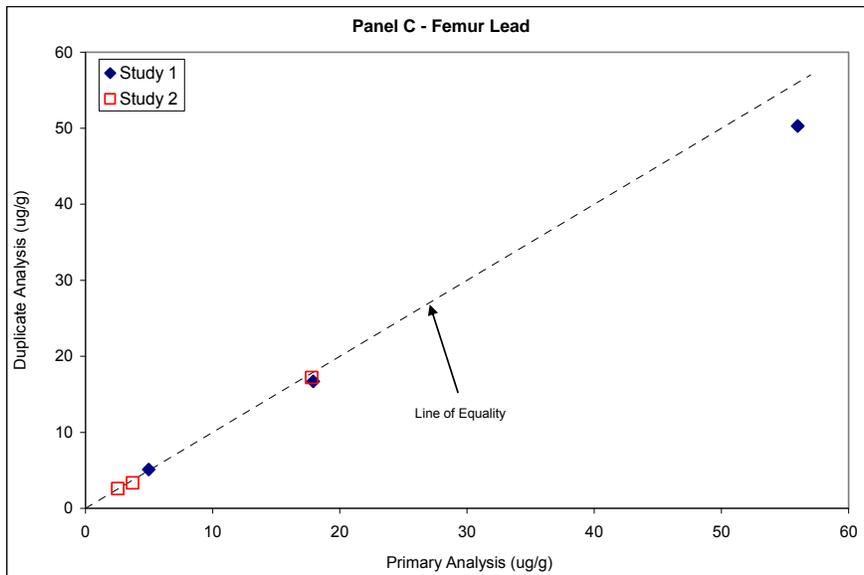
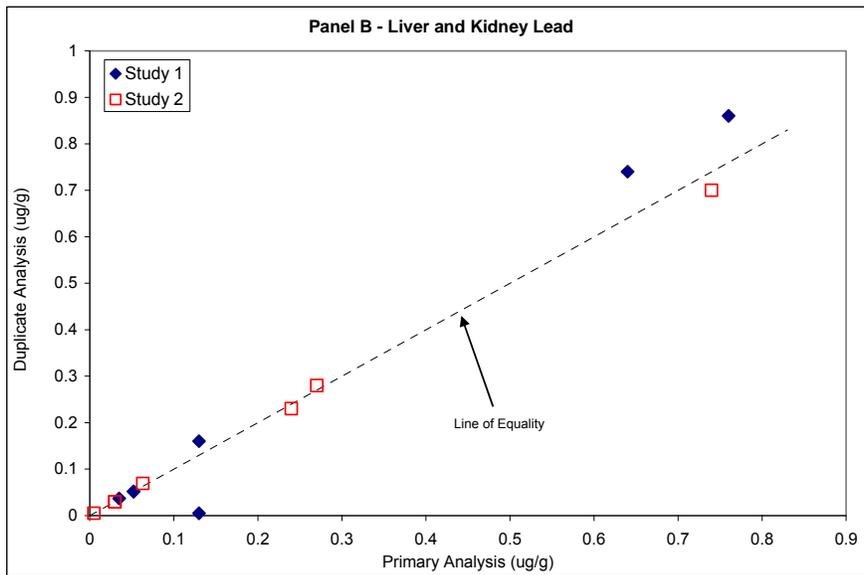
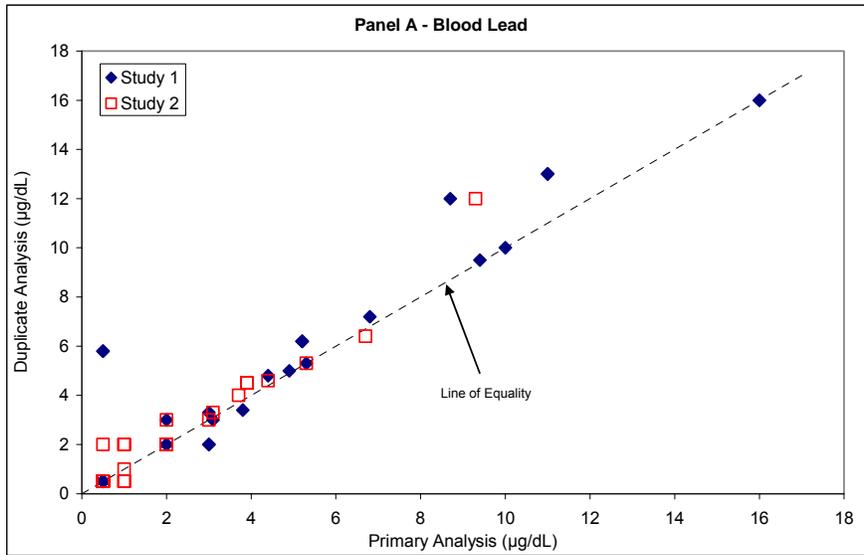


FIGURE 2-3 CDCP BLOOD LEAD CHECK SAMPLES

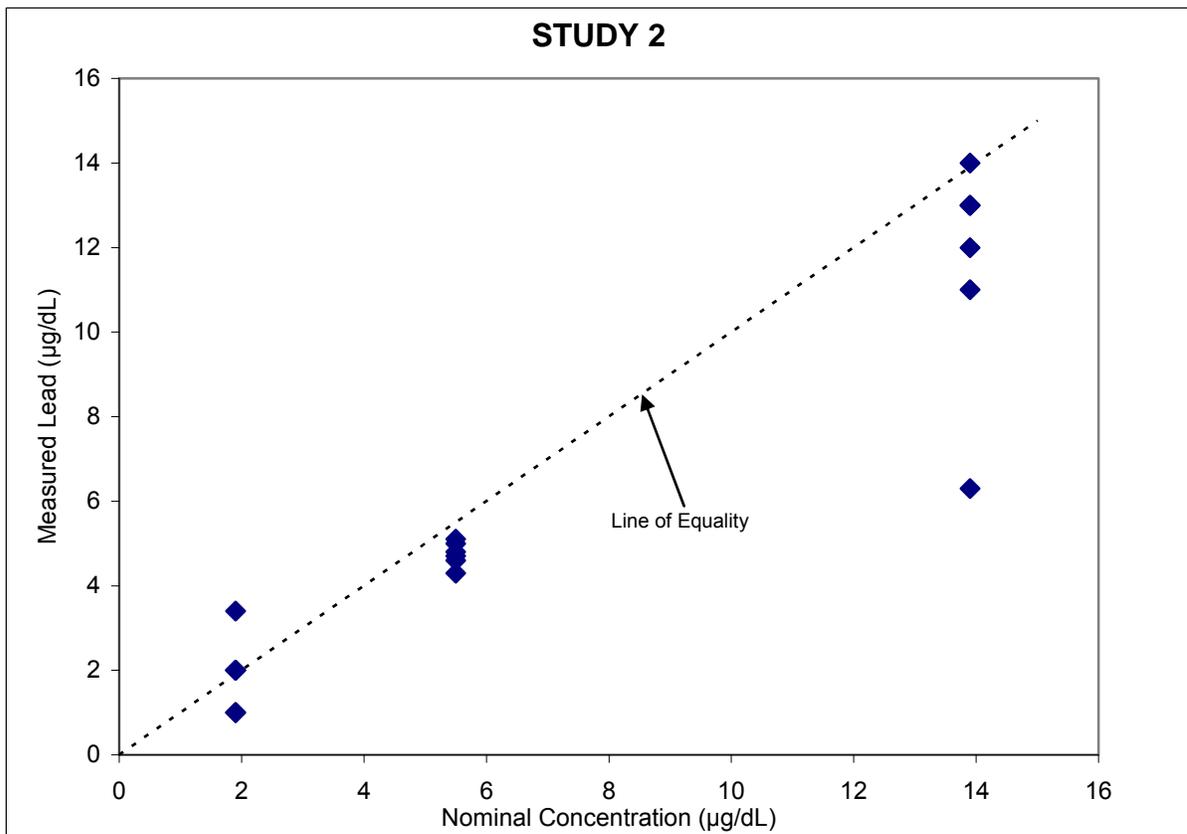
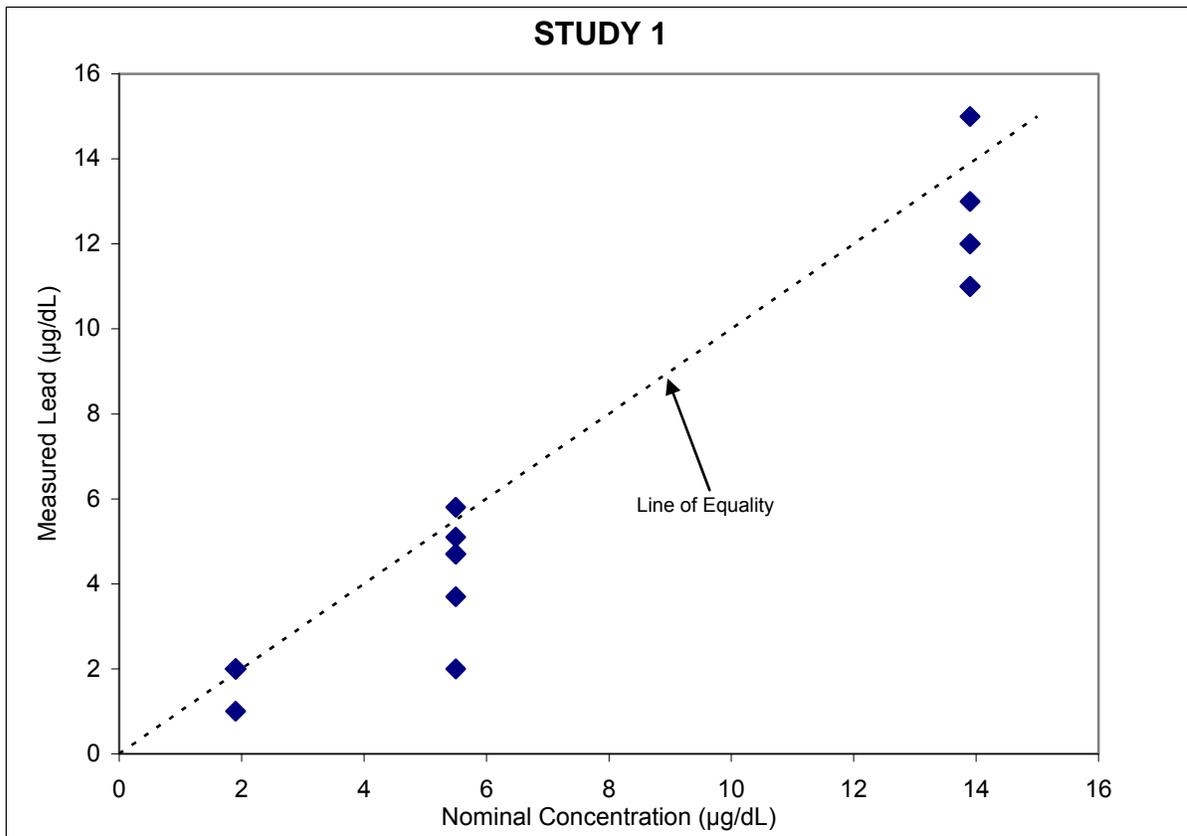
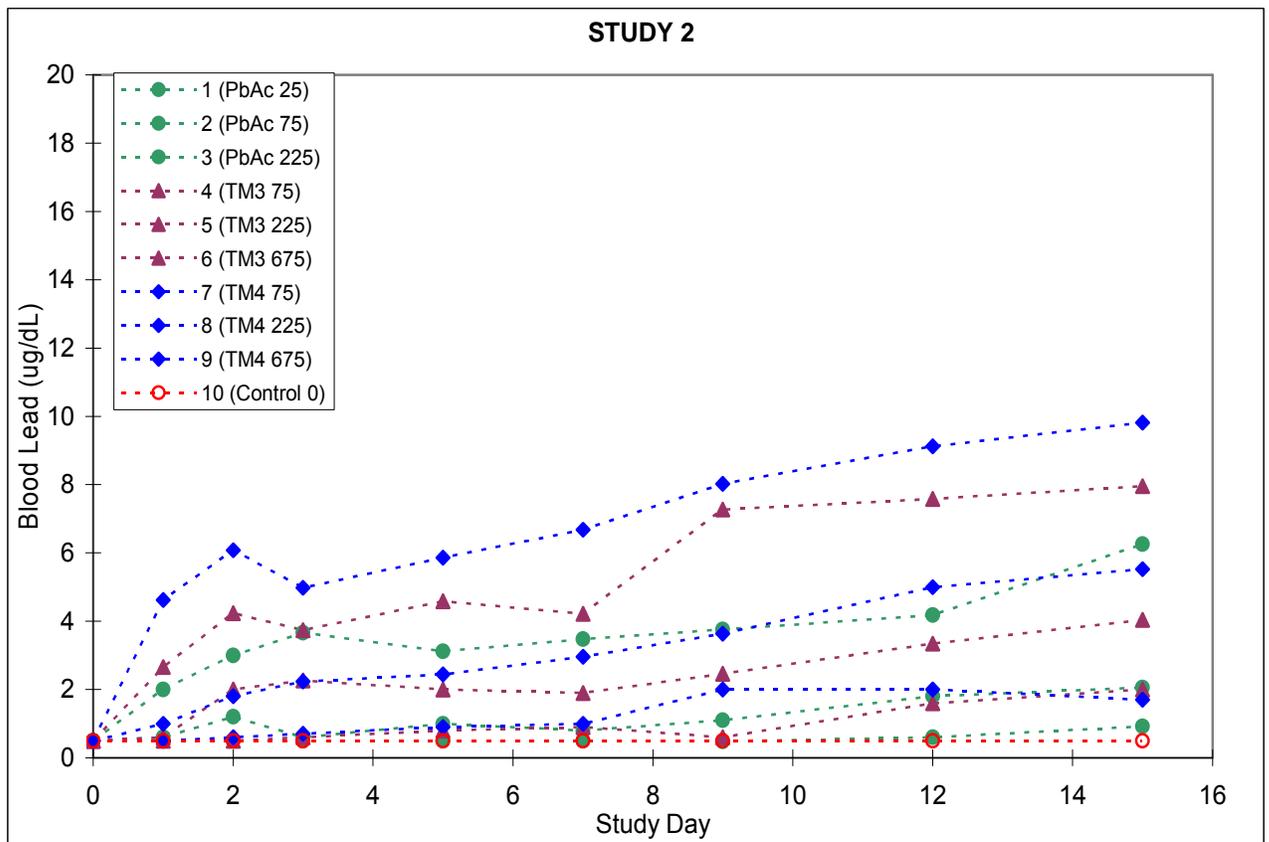
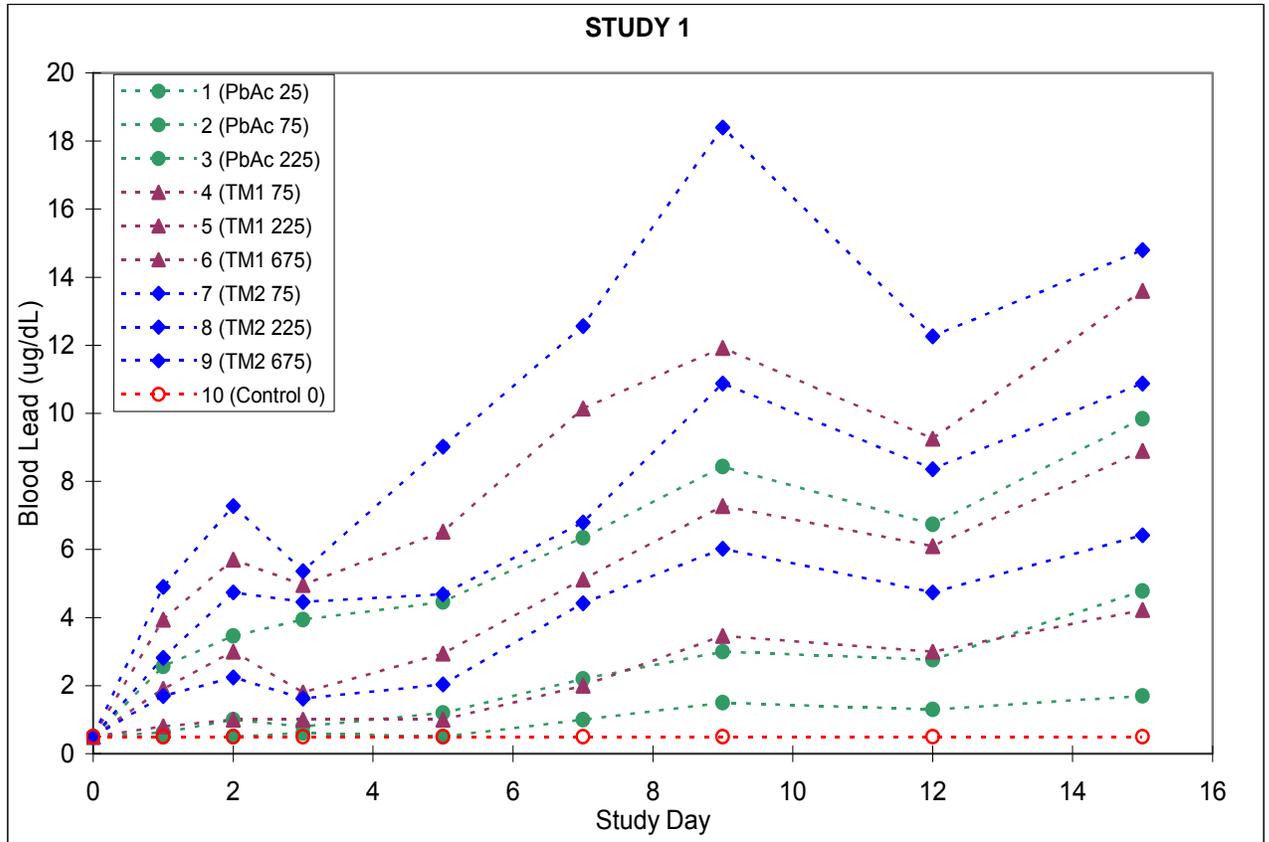


FIGURE 4-1 GROUP MEAN BLOOD LEAD BY DAY



Individual blood lead outliers excluded.

FIGURE 4-2 VARIANCE MODELS (STUDY 1)

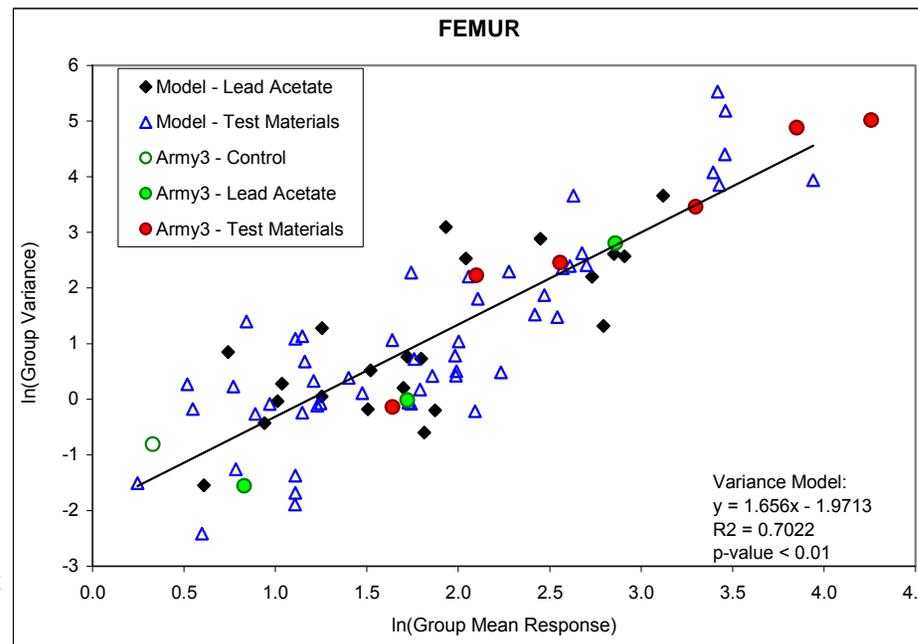
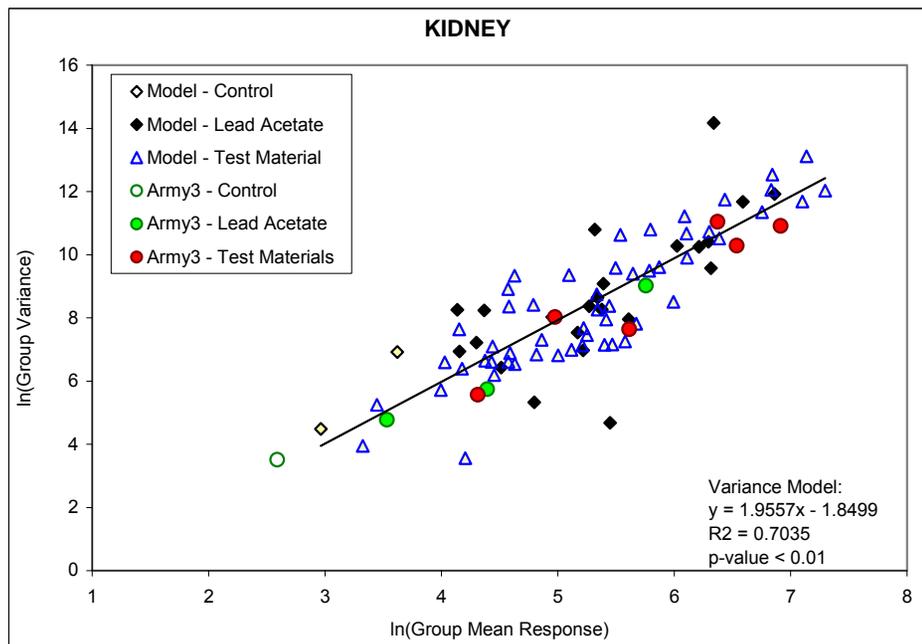
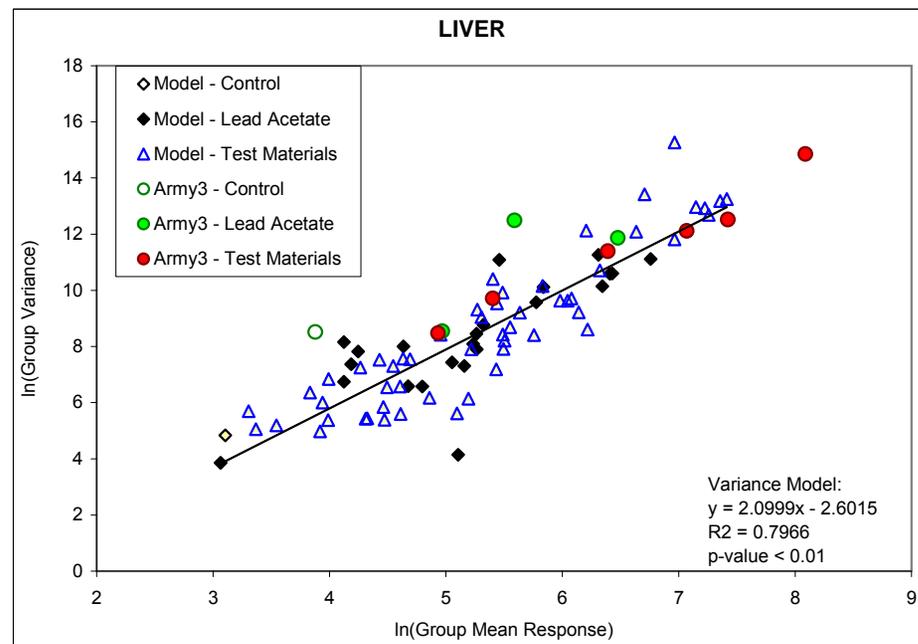
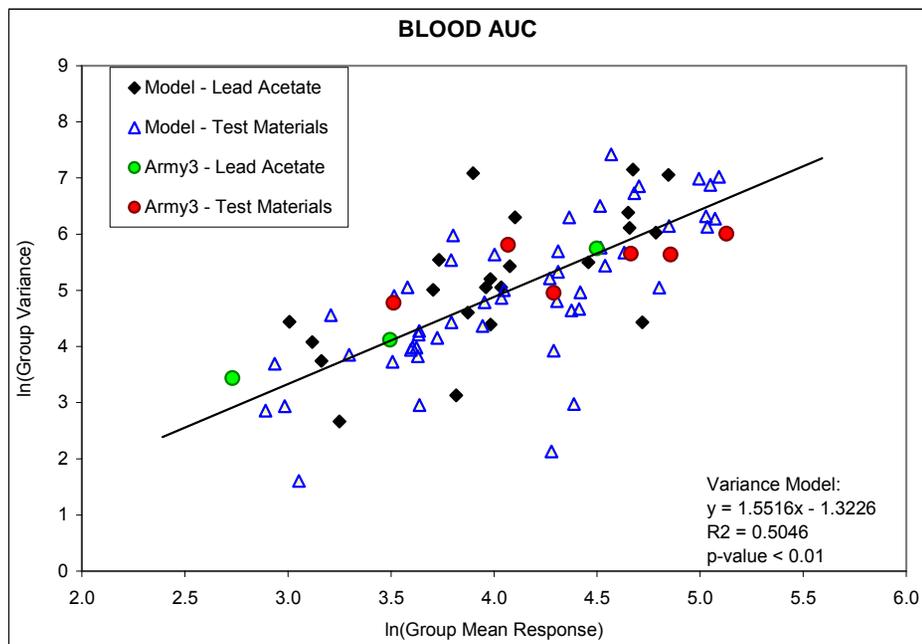


FIGURE 4-3 VARIANCE MODELS (STUDY 2)

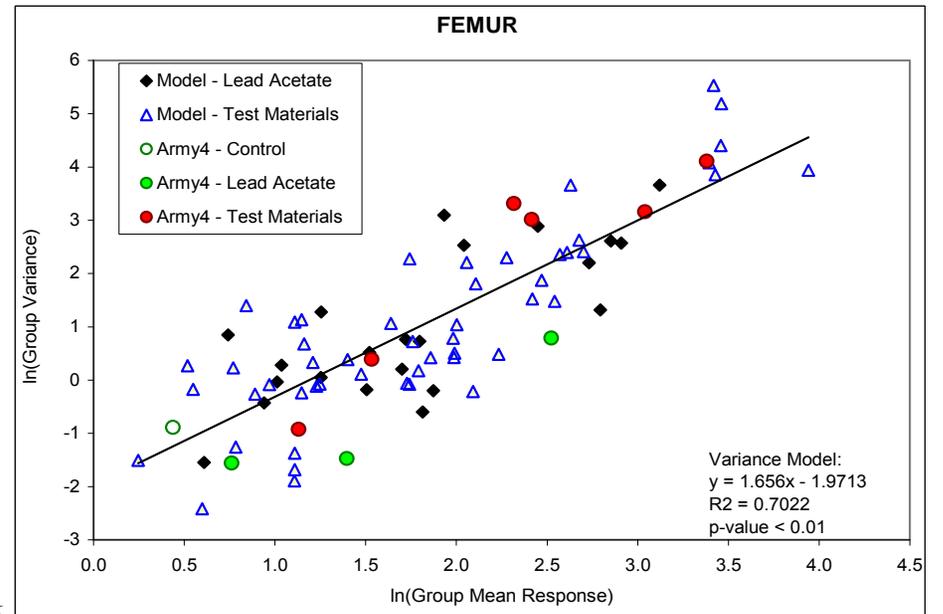
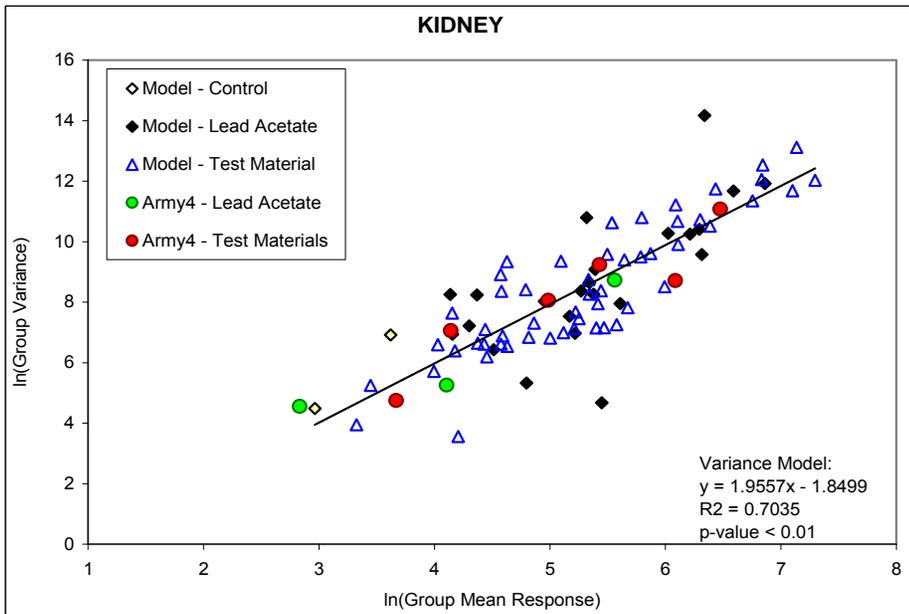
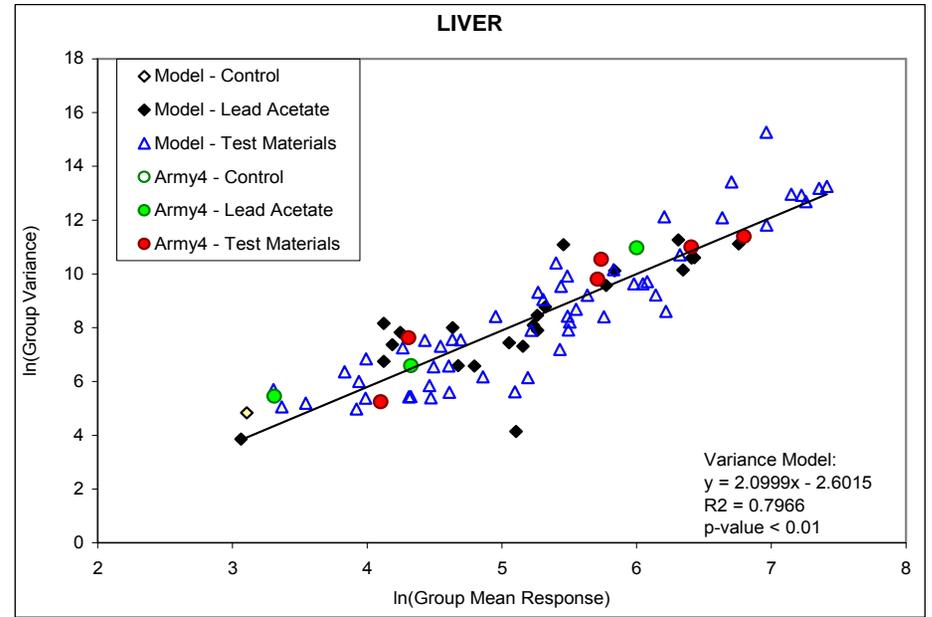
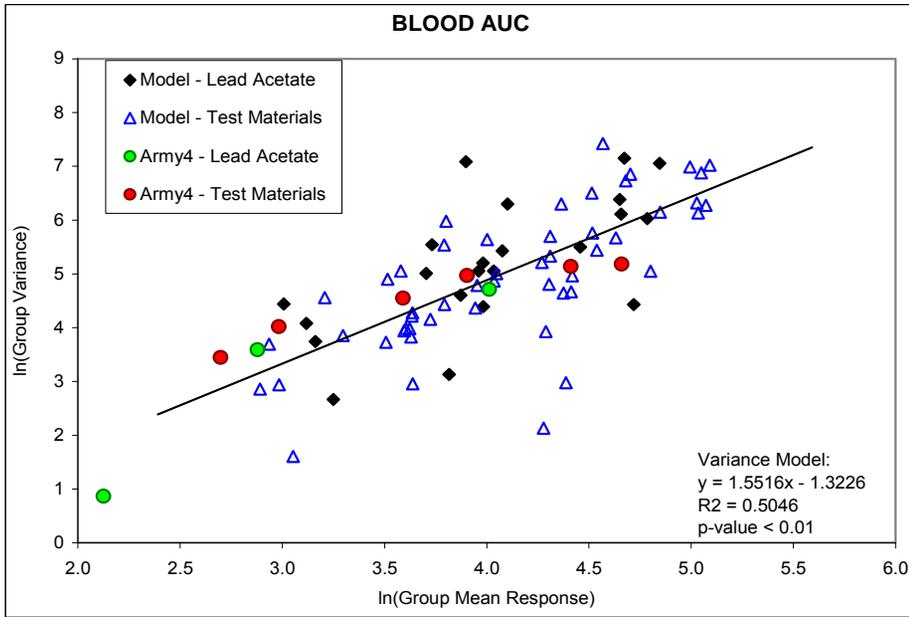
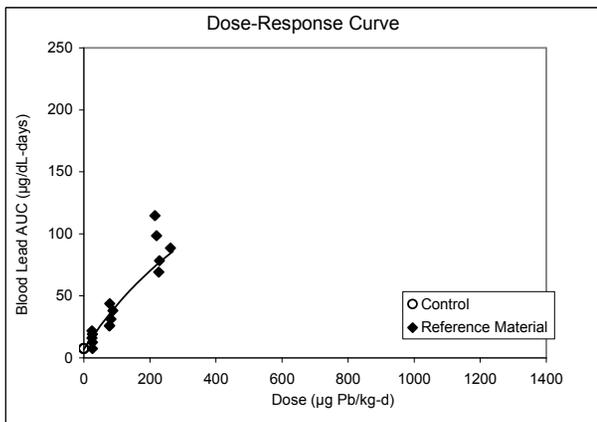
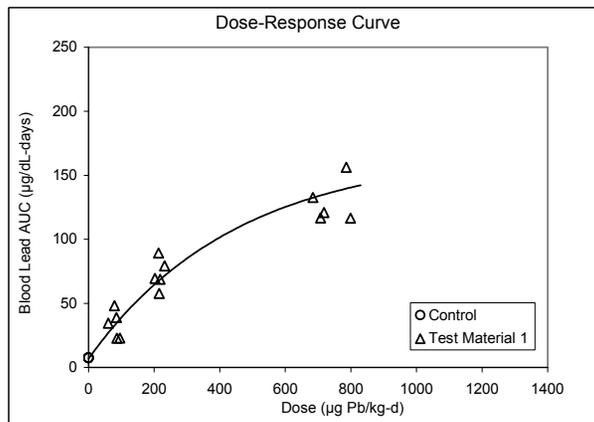


FIGURE 4-4 BLOOD LEAD AUC DOSE-RESPONSE: STUDY 1 (All Data)

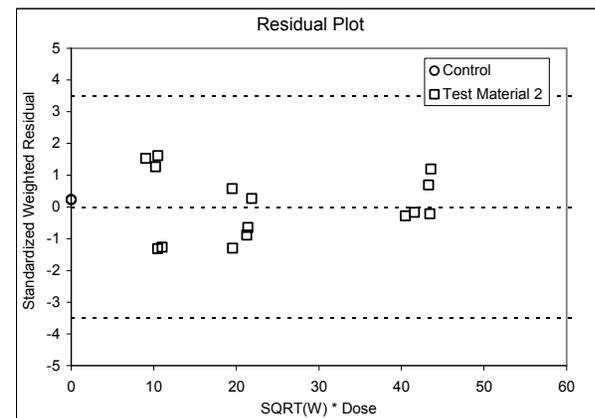
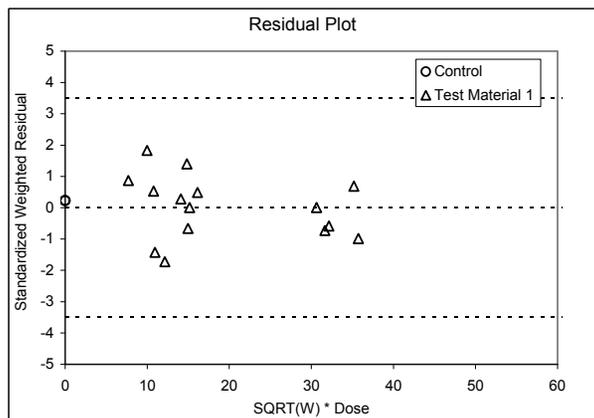
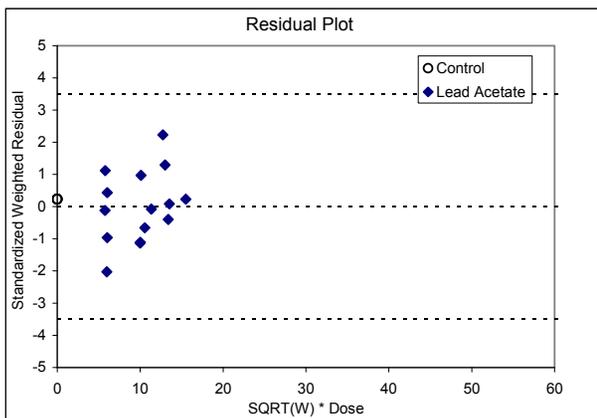
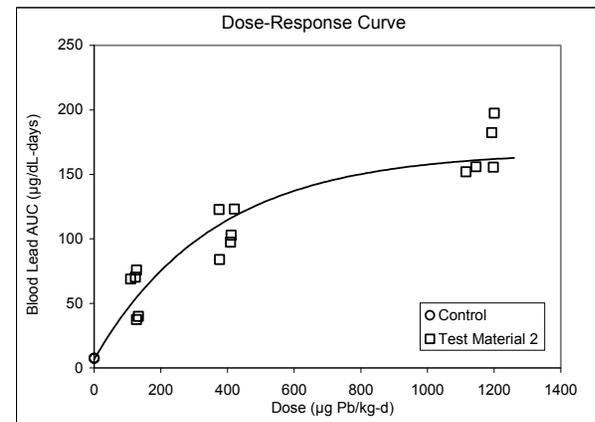
Reference Material (Lead Acetate)



Test Material 1 (NE Soil)



Test Material 2 (WA Soil)



Summary of Fitting^a

Parameter	Estimate	Standard Error
a	6.89E+00	1.42E+00
b	1.61E+02	1.49E+01
c _r	2.50E-03	4.06E-04
c _{t1}	2.22E-03	3.88E-04
c _{t2}	2.77E-03	5.37E-04
Covariance (c _r , c _{t1})	0.6350	--
Covariance (c _r , c _{t2})	0.6171	--
Degrees of Freedom	43	--

ANOVA

Source	SSE	DF	MSE
Fit	662.69	3	220.90
Error	54.97	44	1.25
Total	717.66	47	15.27

Statistic	Estimate
F	176.826
p	< 0.001
Adjusted R ²	0.9182

RBA and Uncertainty

	Test Material 1	Test Material 2
RBA	0.89	1.11
Lower bound ^b	0.69	0.82
Upper bound ^b	1.14	1.44
Standard Error ^b	0.128*	0.175*

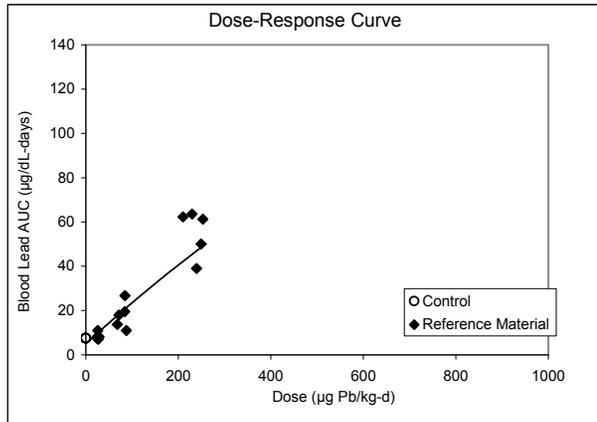
^b Calculated using Fieller's theorem

* g ≥ 0.05, estimate is uncertain

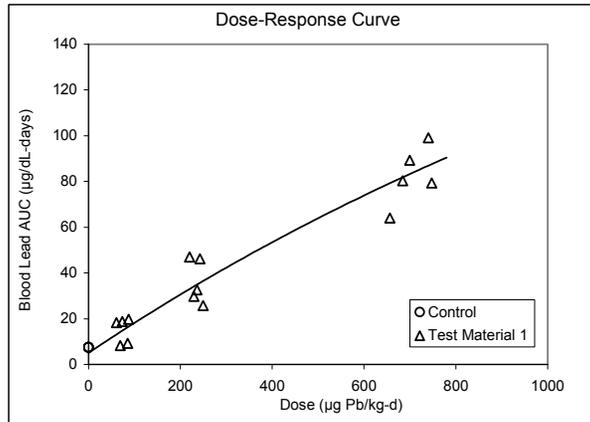
^a $y = a + b \cdot (1 - \exp(-c_r \cdot x_r)) + b \cdot (1 - \exp(-c_{t1} \cdot x_{t1})) + b \cdot (1 - \exp(-c_{t2} \cdot x_{t2}))$

FIGURE 4-5 BLOOD LEAD AUC DOSE-RESPONSE: STUDY 2 (All Data)

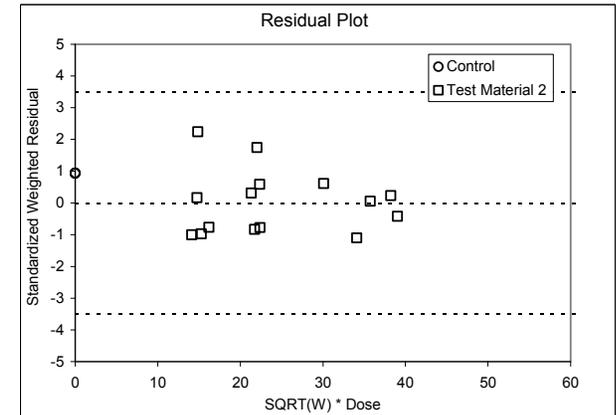
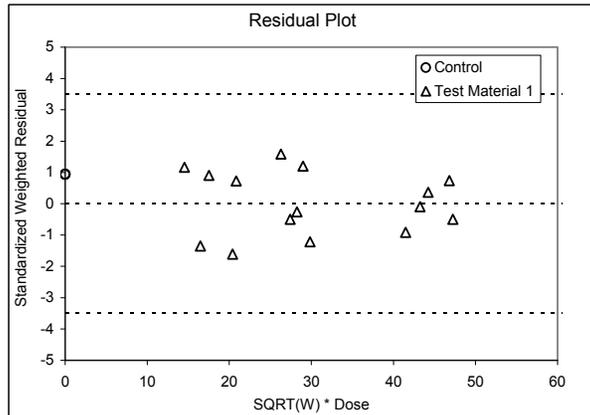
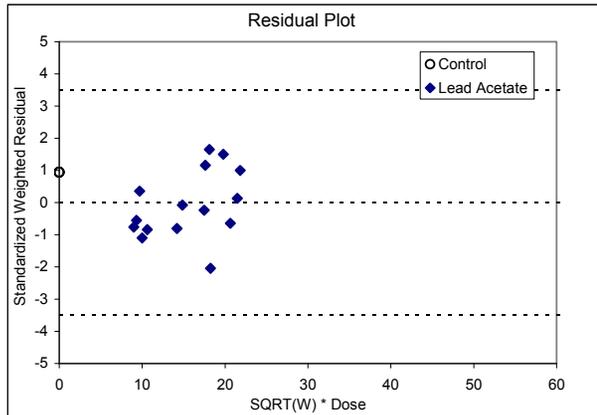
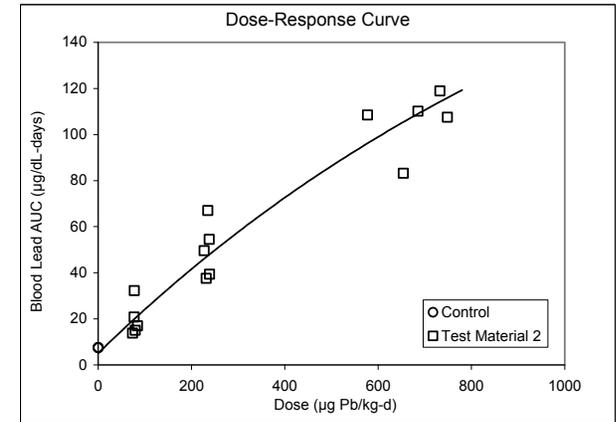
Reference Material (Lead Acetate)



Test Material 3 (SD Soil)



Test Material 4 (OR Soil)



Summary of Fitting^a

Parameter	Estimate	Standard Error
a	5.06E+00	1.14E+00
b	2.43E+02	1.34E+02
c _r	7.89E-04	4.99E-04
c _{t1}	5.55E-04	3.62E-04
c _{t2}	8.14E-04	5.52E-04
Covariance (c _r , c _{t1})	0.9766	--
Covariance (c _r , c _{t2})	0.9771	--
Degrees of Freedom	43	--

ANOVA

Source	SSE	DF	MSE
Fit	400.91	3	133.64
Error	51.95	44	1.18
Total	452.85	47	9.64

Statistic	Estimate
F	113.196
p	< 0.001
Adjusted R ²	0.8775

RBA and Uncertainty

	Test Material 3	Test Material 4
RBA	0.70	1.03
Lower bound ^b	--	--
Upper bound ^b	--	--
Standard Error ^c	0.099*	0.152*

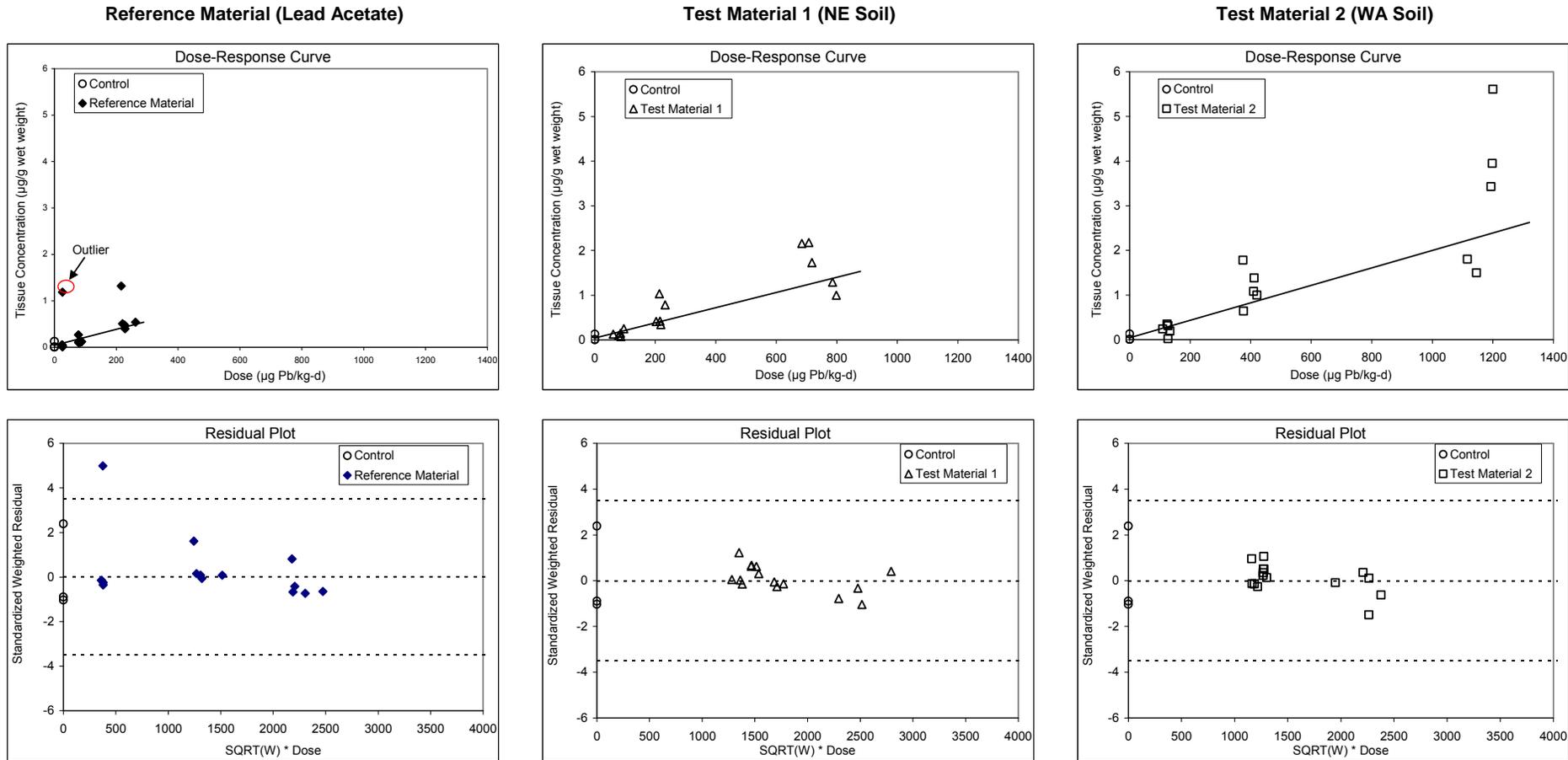
^b Upper and lower bounds could not be calculated, as Fieller's theorem failed

^c Calculated using Fieller's theorem

* g ≥ 0.05, estimate is uncertain

^a $y = a + b \cdot (1 - \exp(-c_r \cdot x_r)) + b \cdot (1 - \exp(-c_{t1} \cdot x_{t1})) + b \cdot (1 - \exp(-c_{t2} \cdot x_{t2}))$

FIGURE 4-6a LIVER LEAD DOSE-RESPONSE: STUDY 1 (All Data)



Summary of Fitting^a

Parameter	Estimate	Standard Error
a	4.27E-02	2.10E-02
b _r	1.72E-03	6.12E-04
b _{t1}	1.70E-03	5.04E-04
b _{t2}	1.96E-03	5.48E-04
Covariance (b _r , b _{t1})	0.1239	--
Covariance (b _r , b _{t2})	0.0793	--
Degrees of Freedom	45	--

^a $y = a + b_r \cdot X_r + b_{t1} \cdot X_{t1} + b_{t2} \cdot X_{t2}$

ANOVA

Source	SSE	DF	MSE
Fit	311.84	3	103.95
Error	503.55	44	11.44
Total	815.39	47	17.35

Statistic	Estimate
F	9.083
p	< 0.001
Adjusted R ²	0.3403

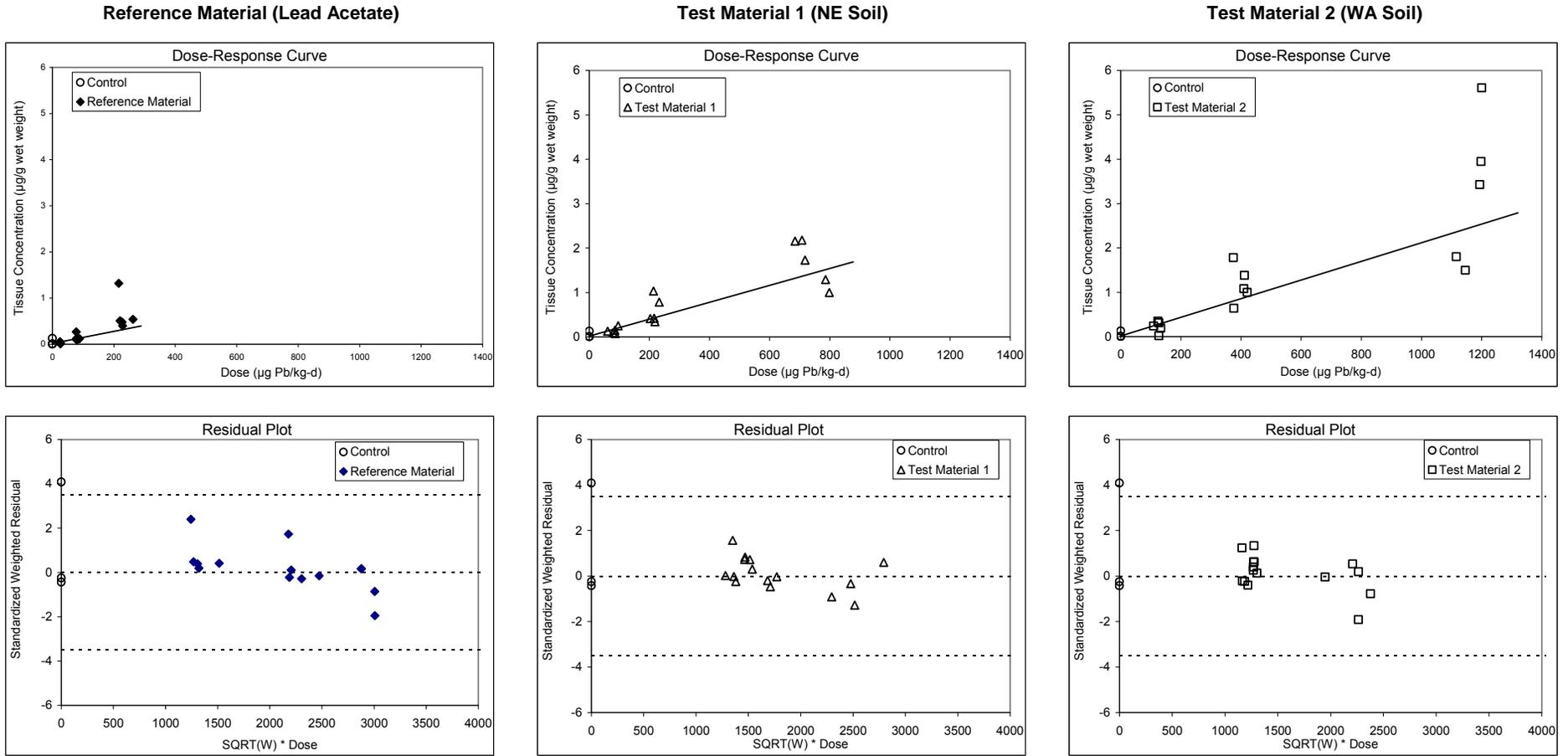
RBA and Uncertainty

	Test Material 1	Test Material 2
RBA	0.98	1.13
Lower bound ^b	0.45	0.54
Upper bound ^b	2.49	2.91
Standard Error ^b	0.427*	0.493*

^b Calculated using Fieller's theorem

* $g \geq 0.05$, estimate is uncertain

FIGURE 4-6b LIVER LEAD DOSE-RESPONSE: STUDY 1 (Outlier Excluded)



Summary of Fitting^a

Parameter	Estimate	Standard Error
a	1.69E-02	1.29E-02
b _r	1.32E-03	4.45E-04
b _{t1}	1.90E-03	3.77E-04
b _{t2}	2.10E-03	4.14E-04
Covariance (b _r , b _{t1})	0.1965	--
Covariance (b _r , b _{t2})	0.1243	--
Degrees of Freedom	44	--

^a $y = a + b_r \cdot X_r + b_{t1} \cdot X_{t1} + b_{t2} \cdot X_{t2}$

ANOVA

Source	SSE	DF	MSE
Fit	339.32	3	113.11
Error	286.26	43	6.66
Total	625.58	46	13.60

Statistic	Estimate
F	16.990
p	< 0.001
Adjusted R ²	0.5105

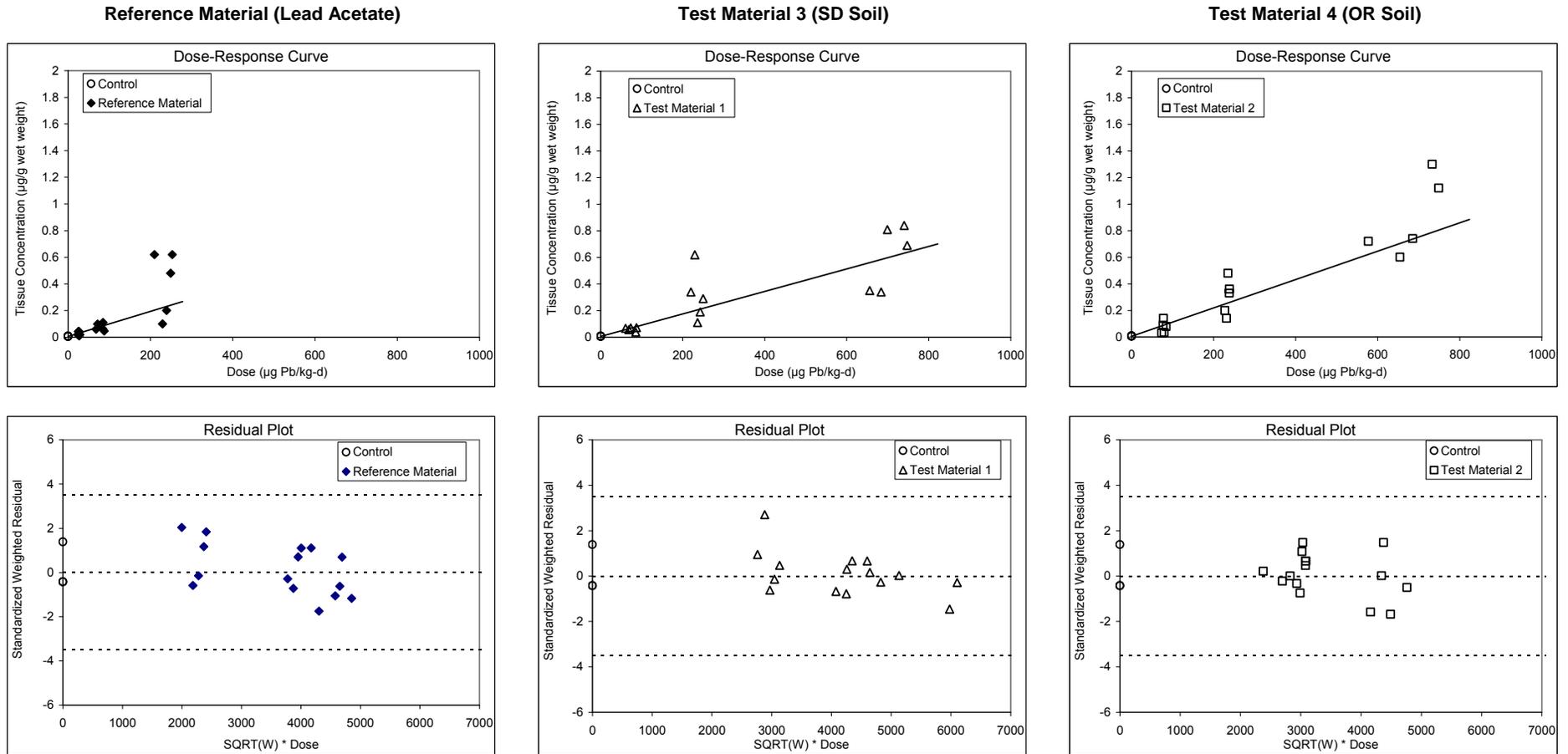
RBA and Uncertainty

	Test Material 1	Test Material 2
RBA	1.44	1.59
Lower bound ^b	0.84	0.90
Upper bound ^b	3.26	3.69
Standard Error ^b	0.515*	0.589*

^b Calculated using Fieller's theorem

* $g \geq 0.05$, estimate is uncertain

FIGURE 4-7 LIVER LEAD DOSE-RESPONSE: STUDY 2 (All Data)



Summary of Fitting^a

Parameter	Estimate	Standard Error
a	6.17E-03	1.63E-03
b _r	9.35E-04	1.45E-04
b _{t1}	8.46E-04	1.22E-04
b _{t2}	1.07E-03	1.50E-04
Covariance (b _r , b _{t1})	0.0257	--
Covariance (b _r , b _{t2})	0.0221	--
Degrees of Freedom	45	--

^a $y = a + b_r \cdot X_r + b_{t1} \cdot X_{t1} + b_{t2} \cdot X_{t2}$

ANOVA

Source	SSE	DF	MSE
Fit	557.42	3	185.81
Error	181.23	44	4.12
Total	738.65	47	15.72

Statistic	Estimate
F	45.111
p	< 0.001
Adjusted R ²	0.7379

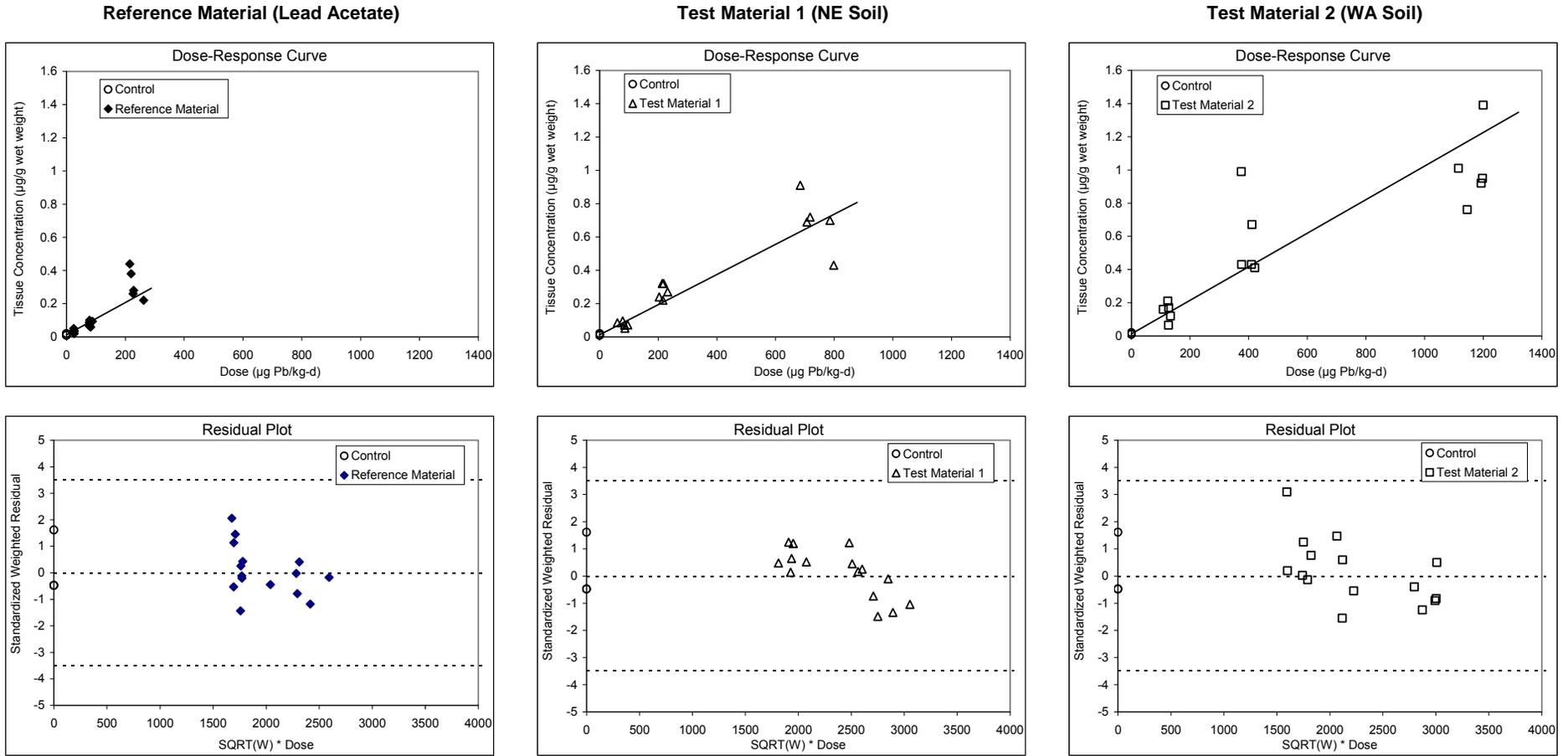
RBA and Uncertainty

	Test Material 3	Test Material 4
RBA	0.90	1.14
Lower bound ^b	0.63	0.80
Upper bound ^b	1.30	1.64
Standard Error ^b	0.189*	0.236*

^b Calculated using Fieller's theorem

* $g \geq 0.05$, estimate is uncertain

FIGURE 4-8 KIDNEY LEAD DOSE-RESPONSE: STUDY 1 (All Data)



Summary of Fitting^a

Parameter	Estimate	Standard Error
a	1.23E-02	2.68E-03
b _r	9.72E-04	1.20E-04
b _{t1}	9.05E-04	9.22E-05
b _{t2}	1.01E-03	9.67E-05
Covariance (b _r , b _{t1})	0.0679	--
Covariance (b _r , b _{t2})	0.0327	--
Degrees of Freedom	45	--

^a $y = a + b_r \cdot X_r + b_{t1} \cdot X_{t1} + b_{t2} \cdot X_{t2}$

ANOVA

Source	SSE	DF	MSE
Fit	185.71	3	61.90
Error	32.26	44	0.73
Total	217.97	47	4.64

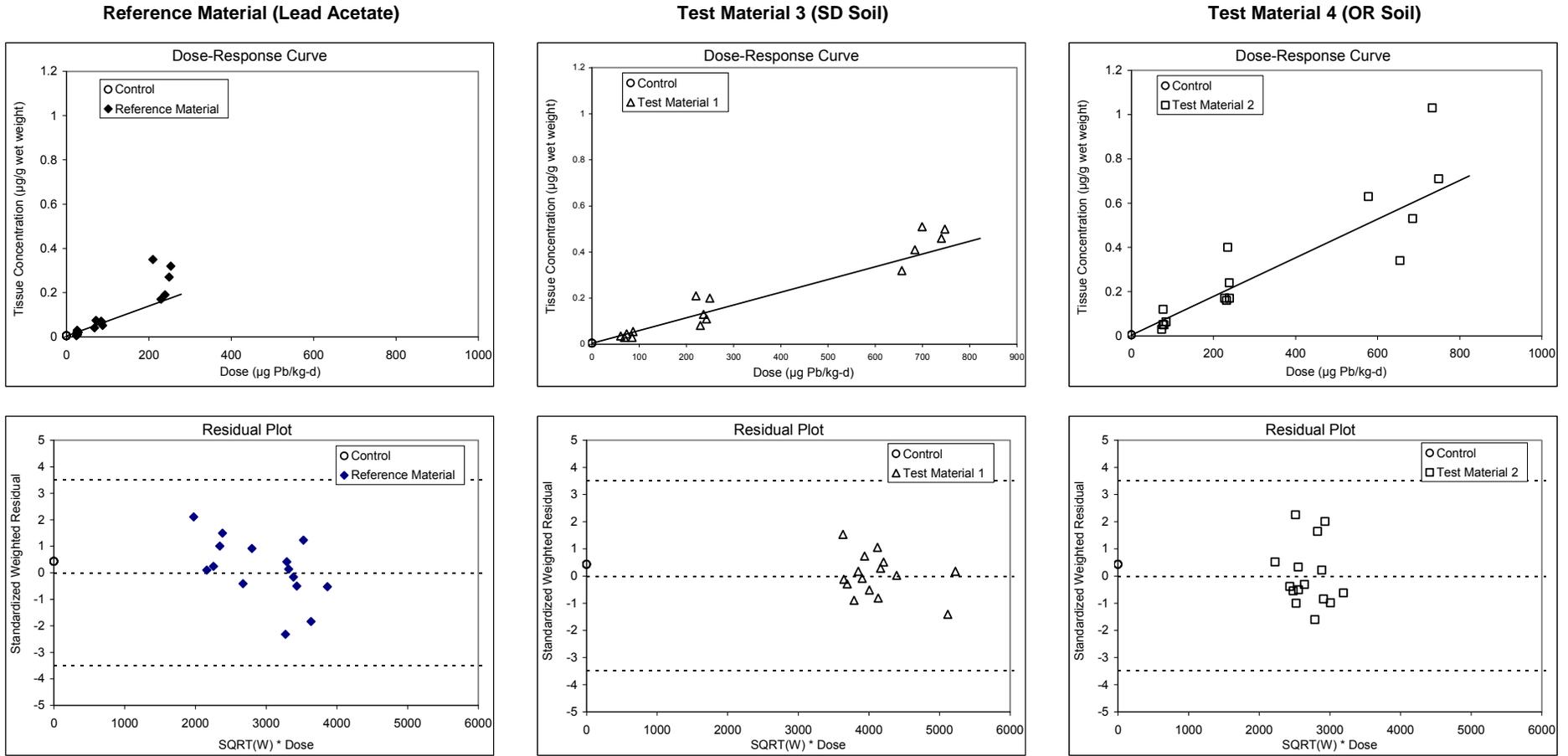
Statistic	Estimate
F	84.435
p	< 0.001
Adjusted R ²	0.8419

RBA and Uncertainty

	Test Material 1	Test Material 2
RBA	0.93	1.04
Lower bound ^b	0.72	0.81
Upper bound ^b	1.22	1.36
Standard Error ^b	0.144	0.160

^b Calculated using Fieller's theorem

FIGURE 4-9 KIDNEY LEAD DOSE-RESPONSE: STUDY 2 (All Data)



Summary of Fitting^a

Parameter	Estimate	Standard Error
a	4.12E-03	1.17E-03
b _r	6.78E-04	8.48E-05
b ₁₁	5.53E-04	5.91E-05
b ₁₂	8.73E-04	8.99E-05
Covariance (b _r , b ₁₁)	0.0432	--
Covariance (b _r , b ₁₂)	0.0278	--
Degrees of Freedom	45	--

^a $y = a + b_r \cdot X_r + b_{11} \cdot X_{11} + b_{12} \cdot X_{12}$

ANOVA

Source	SSE	DF	MSE
Fit	206.25	3	68.75
Error	38.87	44	0.88
Total	245.12	47	5.22

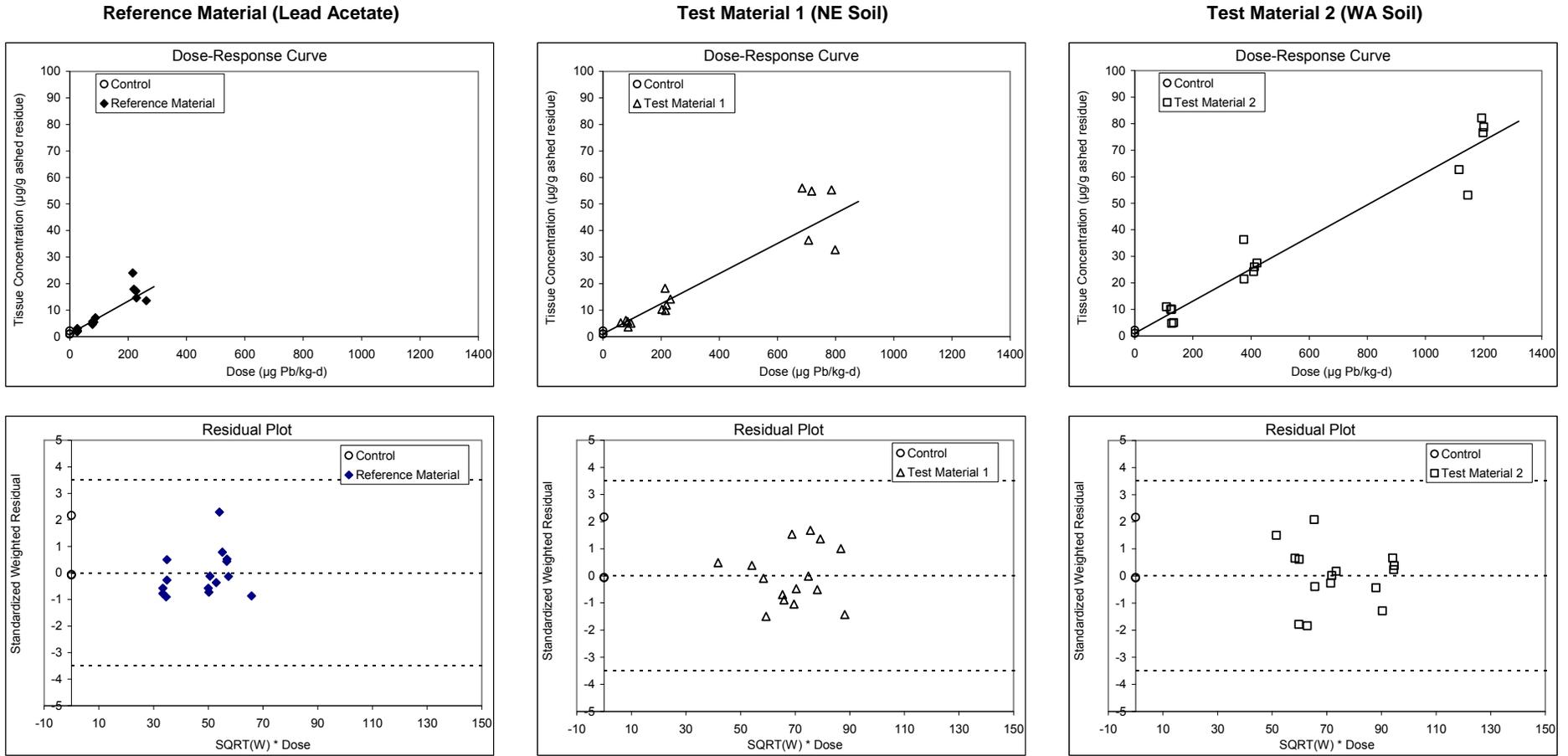
Statistic	Estimate
F	77.833
p	< 0.001
Adjusted R ²	0.8306

RBA and Uncertainty

	Test Material 3	Test Material 4
RBA	0.82	1.29
Lower bound ^b	0.62	0.99
Upper bound ^b	1.08	1.70
Standard Error ^b	0.131	0.206

^b Calculated using Fieller's theorem

FIGURE 4-10 FEMUR LEAD DOSE-RESPONSE: STUDY 1 (All Data)



Summary of Fitting^a

Parameter	Estimate	Standard Error
a	1.04E+00	2.46E-01
b _r	6.17E-02	6.56E-03
b ₁₁	5.68E-02	4.19E-03
b ₁₂	6.05E-02	3.83E-03
Covariance (b _r , b ₁₁)	0.1354	--
Covariance (b _r , b ₁₂)	0.0872	--
Degrees of Freedom	45	--

^a $y = a + b_r \cdot X_r + b_{11} \cdot X_{11} + b_{12} \cdot X_{12}$

ANOVA

Source	SSE	DF	MSE
Fit	542.55	3	180.85
Error	52.48	44	1.19
Total	595.02	47	12.66

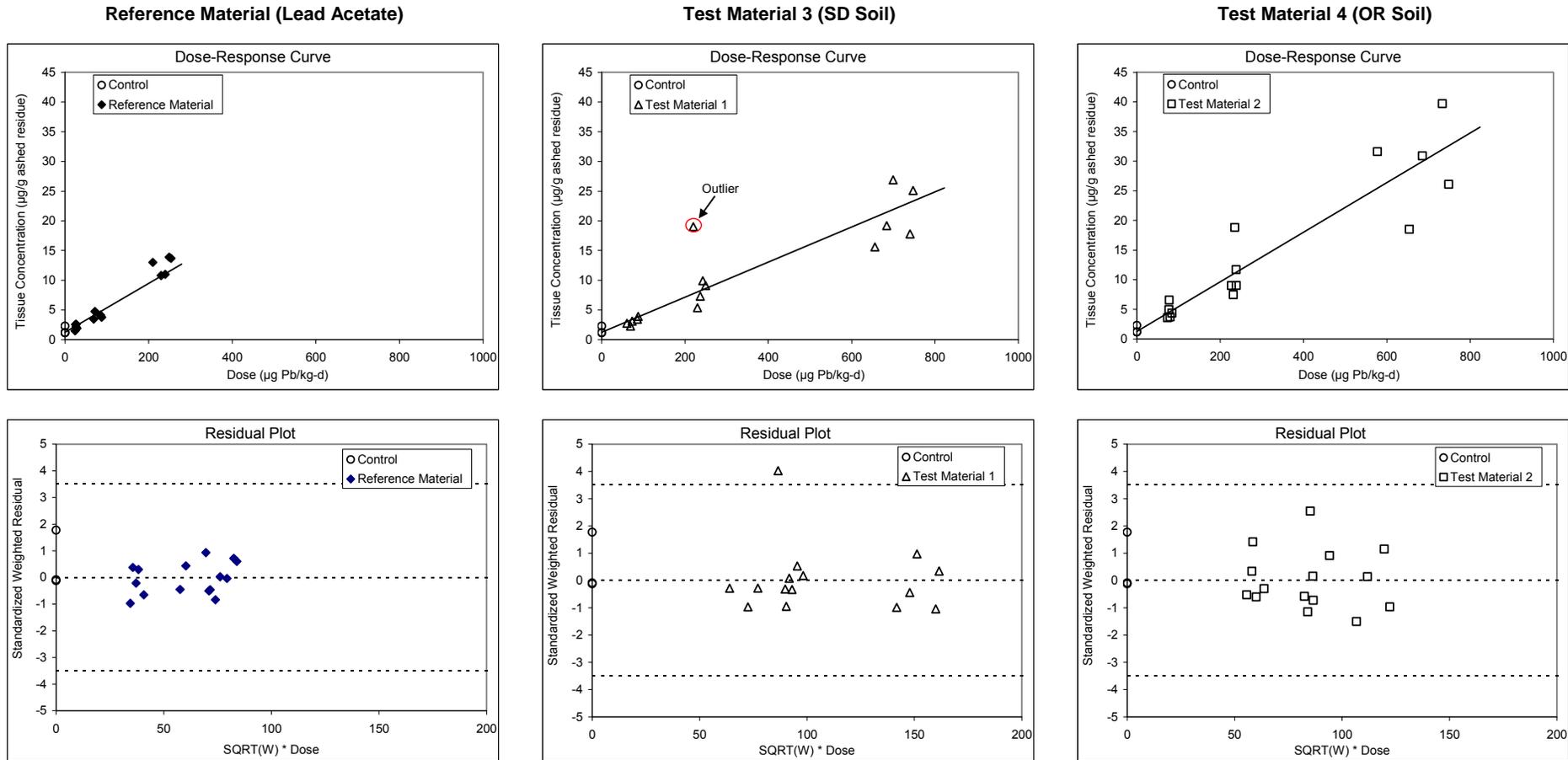
Statistic	Estimate
F	151.639
p	< 0.001
Adjusted R ²	0.9058

RBA and Uncertainty

	Test Material 1	Test Material 2
RBA	0.92	0.98
Lower bound ^b	0.76	0.81
Upper bound ^b	1.14	1.21
Standard Error ^b	0.111	0.117

^b Calculated using Fieller's theorem

FIGURE 4-11a FEMUR LEAD DOSE-RESPONSE: STUDY 2 (All Data)



Summary of Fitting^a

Parameter	Estimate	Standard Error
a	1.24E+00	2.41E-01
b _r	4.13E-02	5.36E-03
b _{t1}	2.96E-02	2.77E-03
b _{t2}	4.19E-02	3.49E-03
Covariance (b _r , b _{t1})	0.1744	--
Covariance (b _r , b _{t2})	0.1407	--
Degrees of Freedom	45	--

^a $y = a + b_r \cdot X_r + b_{t1} \cdot X_{t1} + b_{t2} \cdot X_{t2}$

ANOVA

Source	SSE	DF	MSE
Fit	332.20	3	110.73
Error	57.00	44	1.30
Total	389.20	47	8.28

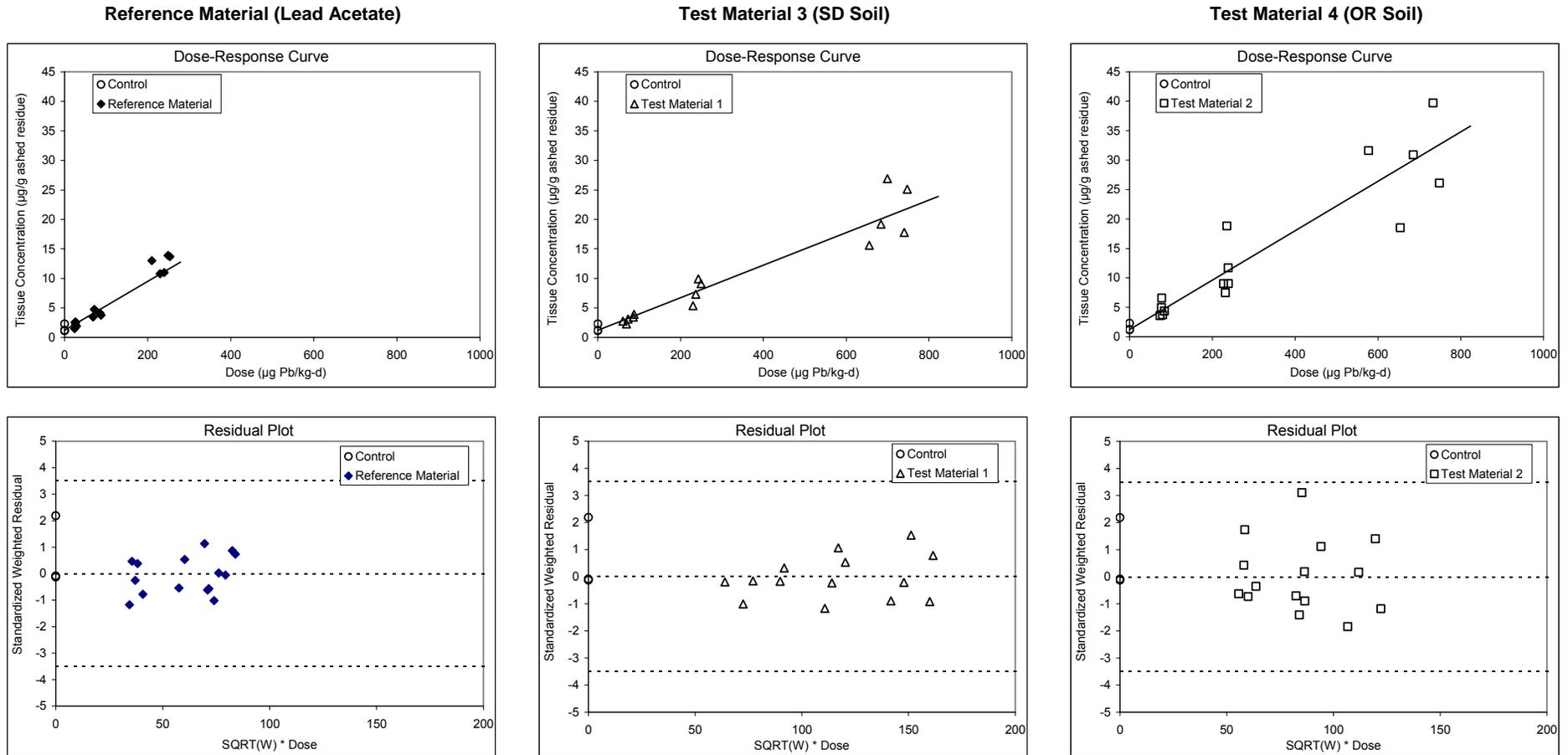
Statistic	Estimate
F	85.480
p	< 0.001
Adjusted R ²	0.8436

RBA and Uncertainty

	Test Material 3	Test Material 4
RBA	0.72	1.02
Lower bound ^b	0.56	0.81
Upper bound ^b	0.93	1.32
Standard Error ^b	0.105	0.146

^b Calculated using Fieller's theorem

FIGURE 4-11b FEMUR LEAD DOSE-RESPONSE: STUDY 2 (Outlier Excluded)



Summary of Fitting^a

Parameter	Estimate	Standard Error
a	1.23E+00	1.97E-01
b _r	4.14E-02	4.39E-03
b _{t1}	2.76E-02	2.22E-03
b _{t2}	4.19E-02	2.85E-03
Covariance (b _r ,b _{t1})	0.1784	--
Covariance (b _r ,b _{t2})	0.1407	--
Degrees of Freedom	44	--

^a $y = a + b_r \cdot X_r + b_{t1} \cdot X_{t1} + b_{t2} \cdot X_{t2}$

ANOVA

Source	SSE	DF	MSE
Fit	321.19	3	107.06
Error	37.36	43	0.87
Total	358.55	46	7.79

Statistic	Estimate
F	123.212
p	< 0.001
Adjusted R ²	0.8885

RBA and Uncertainty

	Test Material 3	Test Material 4
RBA	0.67	1.01
Lower bound ^b	0.55	0.84
Upper bound ^b	0.82	1.25
Standard Error ^b	0.081	0.119

^b Calculated using Fieller's theorem

FIGURE A-1 BLOOD LEAD DATA BY DAY

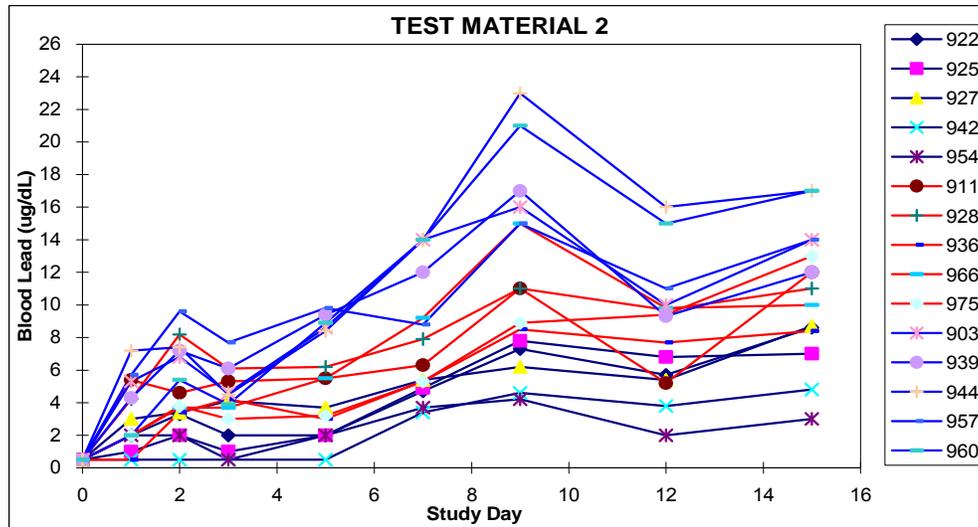
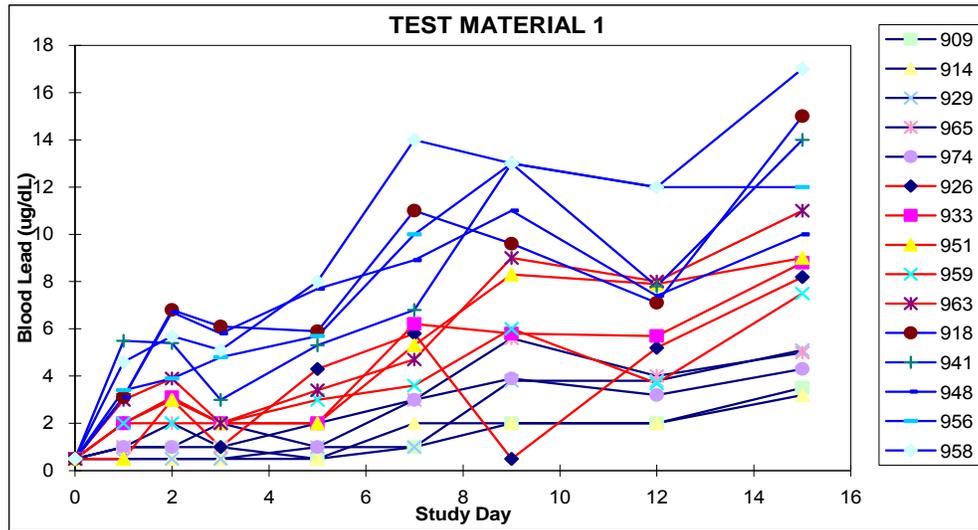
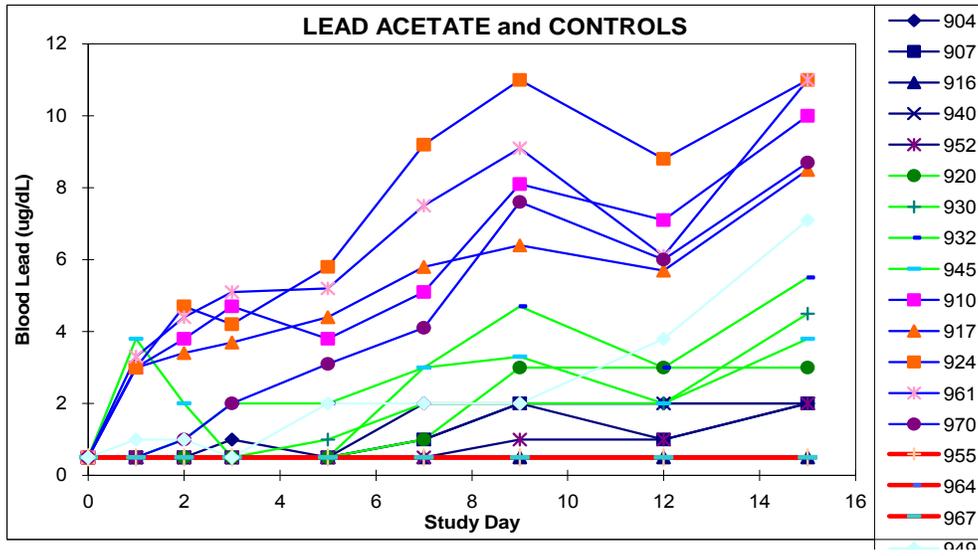


FIGURE B-1 BLOOD LEAD DATA BY DAY

