Relative Bioavailability of Lead from Mining Waste Soil in Rats


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The purposes of this study were to determine the extent of absorption of lead (Pb) in mining waste soil from Butte, Montana, and to investigate the effect of mining waste soil dose (g soil/day) on tissue lead concentrations. Young, 7- to 8-week-old male and female Sprague-Dawley rats (5/sex/group) were given mixing waste soil that contained 810 or 3908 ppm lead mixed in a purified diet (AIN-76) at four different dose levels (0.2, 0.5, 2, and 5% dietary soil) for 30 consecutive days. Standard groups included untreated controls and dosed feed soluble lead acetate groups (1, 10, 25, 100, and 250 μg Pb/g feed). The soil test levels bracketed a pica child's soil exposure level and the lead acetate concentrations bracketed the test soil dose levels of lead. Liver, blood, and femur were analyzed for total lead concentration using graphite furnace atomic absorption spectroscopy. Clinical signs, body weight, food consumption, and liver weights for test soil and standard groups were similar to control. Tissue lead concentrations from test soil animals were significantly lower than the tissue concentrations for the lead acetate group. Relative percentage bioavailability values, based on lead acetate as the standard, were independent of the two different test soils, dose levels, and sex and were only slightly dependent on the tissue (blood > bone, liver). Mean relative percentage bioavailability values of lead in the Butte mining waste soil were 20% based on the blood data, 9% based on the bone data, and 8% based on the liver data. The results of this study will provide the information needed to determine the significance of lead exposure from Butte soils in human health as part of the Superfund Remedial Investigation/Feasibility Study process. © 1992 Society of Toxicology.

This study was initiated to address Superfund health risk assessment issues related to the bioavailability of lead from Butte, Montana, mining waste soils. Sources of lead representing potential risk to human health are the waste rock piles from previous underground mining activities that are scattered throughout the Butte area. Material from these piles has been used over the years for residential fill material and has, therefore, become indistinguishably mixed with residential soils. An important source of lead exposure for children is the inadvertent ingestion of soil containing lead as a result of normal hand-to-mouth activity and the mouthing of objects that have come in contact with soil containing lead (ATSDR, 1988). Previous research on the bioavailability of lead in ingested soil and dust was summarized by Chaney et al. (1989). Chaney (1991) has extended this review and presented the hypothesis that soil in the diet could adsorb lead during passage through the intestinal system. Thus, in stead of the generally expected linear effect of dose on concentration of lead in tissue for soluble lead salts added to a purified diet, it is reasonable that the response (tissue lead) should approach a plateau with increasing soil dose. Soil contains hydrous iron oxides, organic matter, and other adsorbing surfaces which may bind lead, thereby reducing absorption in the small intestine. Thus, the inherent chemical properties of soil-lead adsorption sites may reduce the bioavailability of soil-lead compared to soluble lead-salts and lead compounds ingested without soil. This type of pattern was found for plant root uptake of metals from soils with added sewage sludge because the sludge added specific metal adsorption capacity to the soil-sludge mixture (Corey et al., 1987).

The specific objective of this study was to determine the extent of adsorption of lead into the blood, bone, and liver of young male and female Sprague-Dawley rats that were fed various concentrations of lead-contaminated mining waste soil mixed with AIN-76 purified diet for 30 consecutive days. In addition to the mining waste soil treatment groups, a control group (purified diet only) and a standard group (dosed feed lead acetate) were included in the experimental design. At termination, blood, liver, and femur specimens were collected from each animal. Because lead is not homogeneously distributed in the body, but rather is dispersed among several distinct compartments (blood, soft tissue, and bone) (Rabinowitz et al., 1976; Marcus, 1985 a,b,c), measuring lead in multiple compartments more accurately reflects total body distribution of lead. Lead concentrations have been used over the years for residential fill material and has, therefore, become indistinguishably mixed with residential soils. An important source of lead exposure for children is the inadvertent ingestion of soil containing lead as a result of normal hand-to-mouth activity and the mouthing of objects that have come in contact with soil containing lead (ATSDR, 1988). Previous research on the bioavailability of lead in ingested soil and dust was summarized by Chaney et al. (1989). Chaney (1991) has extended this review and presented the hypothesis that soil in the diet could adsorb lead during passage through the intestinal system. Thus, in stead of the generally expected linear effect of dose on concentration of lead in tissue for soluble lead salts added to a purified diet, it is reasonable that the response (tissue lead) should approach a plateau with increasing soil dose. Soil contains hydrous iron oxides, organic matter, and other adsorbing surfaces which may bind lead, thereby reducing absorption in the small intestine. Thus, the inherent chemical properties of soil-lead adsorption sites may reduce the bioavailability of soil-lead compared to soluble lead-salts and lead compounds ingested without soil. This type of pattern was found for plant root uptake of metals from soils with added sewage sludge because the sludge added specific metal adsorption capacity to the soil-sludge mixture (Corey et al., 1987).
were analyzed by graphite furnace atomic absorption spectroscopy and/or inductively coupled plasma atomic emission spectroscopy. Relative percentage bioavailability values were estimated by comparing tissue lead concentrations of the test soil to the standard treatment groups. These data provide the information needed to determine the significance of lead exposure from Butte soils in assessing human health risks as part of the Superfund Remedial Investigation/Feasibility Study process.

METHODS

Materials

The original test substances were two mining waste soils (Test Soil I, 810 ppm lead; Test Soil II, 8858 ppm lead) that were composites of soils collected from residential areas in Butte, Montana, during November, 1989. Test Soil II had a higher lead concentration than was targeted for this study and, therefore, was blended with Test Soil I to produce Test Soil III which contained 3908 ppm lead. Test Soils I and III were used for the dosed feed preparations. Lead (II) acetate hydrate (CH₃CO₂ Pb·3H₂O) was obtained from Aldrich Chemical Co. (Milwaukee, WI) and was used to prepare the dosed feed formulations for the standard groups.

Test Substance Composition

Moisture. Moisture content was determined by weighing and driving a 5-g sample (±0.1 mg) at 108°C for 2 hr, followed by reweighing. Percentage moisture was determined by calculating the difference between the pretrained and dried samples.

Organic matter. Percentage organic matter in the test soils was estimated from loss-on-ignition at 430°C until constant weight or after heating for 24 hr (Dawes, 1974). The results after heating for 20 hr are reported because no change in the weight of the sample occurred between 6 and 20 hr.

pH. The pH of the test soils was determined using EPA SW 846 Method 9045. The pH was measured with an appropriately calibrated pH meter (Orion, Model 501).

Total element content. Total element and lead in the test soils were determined using EPA Test Method SW 846 (Method 3050, Acid Digestion of Sediments, Sludges, and Soils) for sample preparation; and EPA Test Method SW 846 (Method 6010, Inductively Coupled Plasma Atomic Emission Spectroscopy) for metal determination. Method 7000, Atomic Absorption Method, was also used for trace metal analysis by electrothermal furnace technique (EAA).

Mineralogic evaluation. Lead mineralogy in Test Soils I and III was determined using a JEOL 8600 electron microprobe (electron beam diameter of 1 μm) in the wavelength dispersive mode. Samples were prepared by impregnating the soil with epoxy and polishing the surface with kerosene rather than water to avoid partial dissolution of soluble phases.

Particle size. Particle size analysis of the test soils was determined using the electrozone method (Particle Data Laboratories, Ltd., Elmhurst, IL).

Test System and Animal Maintenance

This was a nonclinical laboratory study performed in compliance with the EPA Good Laboratory Practice Regulations, 40 CFR Part 792 (U.S. EPA, 1988a). Seventy male and 70 female Sprague-Dawley rats (5/sex/dose group; 7-8 weeks of age at initiation of dosing) were supplied by Charles River Laboratories (Kingston, NY). The animals were housed in an environmentally controlled room where the temperature and relative humidity specifications were 19 to 25°C and 40 to 70%, respectively. Lighting conditions were set to provide 12 hr of fluorescent light in the AM and 12 hr of darkness in the PM. Animals were individually housed in standard polycarbonate cages, the dimensions of which were 26 x 20 x 20 cm. Cages contained hardwood bedding (San-Chips. P J Murphy Forest Products, Rochelle Park, NJ). All animals were provided deionized water ad libitum (≤20 μg Pb/liter) by glass bottle reservoirs filled with stainless steel sipper tubes. All rats were fed ad libitum in metal feeders. The untreated control group animals were fed a purified diet (AIN-76 complete meal (Zeigier Brothers, Garners, PA)). The dosed feed treatment group animals were fed AIN-76 sucrose-free meal into which the appropriate amount of sucrose and specific test substance, i.e., test soil or lead acetate, were added. The soil replaced part of the sucrose rather than replacing part of, or diluting, the complete mixed diet. The AIN-76 complete meal and AIN-76 sucrose-free meal contained <0.20 μg Pb/g.

Dosing Regimen and Administration

Dosed feed formulations were prepared for the animals fed lead acetate and the test soils mixed in the diet. The dose levels selected for use on this study were based on reported soil exposure levels for pica children. For a 15-kg child with pica-for-soil, approximately 10 g of soil and 250 g dry diet are estimated to be consumed daily (U.S. EPA, 1989b). Based on these values, soil constitutes approximately 4% of the pica child's diet. Thus, at levels of 2 and 5% soil in the diet were selected in this study to bracket pica child exposure levels. The lower dose levels of 0.2 and 0.5% soil reflected logarithmic decreases from the higher dose levels above the lowest exposure level believed to produce tissue concentrations that are above the analytical detection level. Typical children (exclusive of those with pica-for-soil) ingest less than 100 mg of soil per day, on average, which is equivalent to approximately 0.04% soil in their diet (Calabrese et al., 1989). However, feeding rats a comparable dose would result in tissue lead concentrations indistinguishable from background concentrations. For the Test Soil I group, the 0.2, 0.5, 2, and 5% soil dosed corresponded to 1.62, 4.05, 16.2, and 40.5 ppm lead. For the Test Soil III group, the concentration of lead in the four soil dose levels was 7.82, 19.5, 78.2, and 195 ppm. The exposure levels of the lead acetate groups (1, 10, 25, 100, and 250 ppm) were chosen to bracket the estimated exposure levels for the two dosed feed test soil groups. Dosed feed concentrations, stability, and homogeneity were verified during the study. All doses were homogeneous with relative standard deviations of less than 5%, except the 1-ppm lead acetate preparation. Actual concentrations ranged from 93 to 116% of the target concentrations. Appropriate dosed feed mixtures were presented to the rats at approximately the same time each day.

In-Live Parameters

Animals were observed twice daily for any signs of morbidity or mortality and once daily for signs of toxicity. Individual body weight determinations were made once weekly and individual food consumption determinations were made daily.

Tissue Collection and Preparation

Animals were euthanized with a single overdose injection of sodium pentobarbital. Blood was collected into a syringe by a cardiac puncture. Animals were necropsied and liver and bone (right femur) were collected, weighed, and frozen at approximately −20°C. Cardiac blood specimens were transferred to heparinized containers and refrigerated until the time of preparation for analysis. Each blood sample was well mixed in a rotating tumbler for at least 30 min prior to removing an aliquot of known weight for lead analyses. Liver specimens were homogenized with a tissue homogenizer (Brinkman Polytron, Westbury, NY) and frozen (approximately −20°C) until analyses. The whole bone was placed in 2 N NaOH to digest any soft residual tissue, dried, and weighed. The defleshed bone was then placed in 6 N HCl until it
Determination of Lead in Biological Fluids, Tissues, Soils, and Dosed Feed Mixtures

Samples were analyzed using a Perkin-Elmer Model 5000 Zeeman atomic absorption spectrometer equipped with a Model 500 graphite furnace (GFAS) or an inductively coupled plasma atomic emission spectrometer (ICP-AES) (Thermo Jarrell-Ash Model 61-975) for low or high concentrations of lead, respectively. Blood specimens were digested with 0.05% Triton X-100 in 0.2 M HNO₃. Aliquots (20 µl) of all the test solutions were injected onto the platform of the graphite furnace with 5 µl of matrix modifier solution (0.4% monosodium ammonium phosphate and 0.14% magnesium nitrate hexahydrate in 1% (v/v) HNO₃). Solubilized bone specimens were digested in 15 M HNO₃ in a boiling water bath, diluted with water, and injected into the graphite furnace with a matrix modifier solution (same as blood except also containing 1% calcium). Liver samples were digested with three parts 15 M HNO₃ to one part 70% HClO₄ and lead was determined by graphite furnace as described previously for blood. Actual soils and dosed feed mixtures were digested with 15 M HNO₃; 30% H₂O₂, and 12 M HCl on a hot plate. The digested samples were redissolved in 15 M HNO₃ and diluted to a proper concentration within the linear range of the instrument.

Lead in all samples was calculated from linear regression equations using the method of standard additions (Klein and Hach, 1977). The method detection limits (MDLs) for the blood, bone, and liver lead concentrations were 1.1 µg/liter, 0.56 µg/g, and 0.04 µg/g, respectively. The method detection limit is defined as the lead concentration that yields an absorbance equal to three times the standard deviation of a sample with a concentration of lead that is distinctly detectable above, but close to, the blank absorbance measurement. All lead concentrations below the MDL were set at the detection limits for statistical analysis.

Statistics

The principal objectives of the statistical analysis were to characterize the nature of the trends in tissue lead concentrations with increasing delivered concentrations of lead in the diet and to characterize the variability of the responses about these trends in tissue uptake. Regression models were fitted to the data to quantify the dose-response trends and to provide smoothed estimates of the tissue uptake concentrations and their variability.

Nonlinear regression models were fitted to the results from the lead acetate and test soil groups to describe the trends between the delivered dose (mg Pb/kg BW) and tissue lead concentrations (µg/liter or µg/g). Each model simulates the dose-response relationship, plateauing as the input dose increases. Separate fits were carried out for males and for females. This resulted in six fits per tissue type.

The regression models related the group mean tissue lead concentrations to the external-delivered lead dose. The data suggested that the tissue concentrations increased to a limiting value (i.e., an asymptote) as the external lead dose increased and maintained a nonzero level even in the absence of lead in the diet (i.e., a background level).

For blood lead concentrations, the model fitted the data ($p > 0.05$) in five of the six cases; there was borderline lack of fit ($p = 0.04$) for the lead acetate males. For bone lead concentrations, the model fitted each treatment and sex combination. For liver lead concentrations, the model fitted the lead acetate groups for both males and females. There was insufficient lead uptake in the test soil groups to warrant fitting regression models. Tissue concentrations were estimated based on the unsmoothed average concentrations among the five animals per sex at each dose.

Each animal had background levels of lead in its blood, bone, and liver apart from any lead that was fed to it during the study. These background levels were adjusted for when the amounts of dosed lead taken up from the study diet were estimated. For the liver lead concentrations, the low dose concentrations for each treatment and sex were, for the most part, at or below the detection limit, 0.04 µg/g. The background level for liver was, therefore, set at the detection limit, 0.04 µg/g, for both males and females.

The relative bioavailability for a particular dose of a test soil was defined as the ratio of the lead uptake from that dose of the test soil to the lead uptake of the same dose of the lead acetate treatment. The lead uptake estimates from the test soil and from the lead acetate standard were adjusted for background levels before being compared. The regression curves for the lead acetate groups were interpolated to obtain predictions at doses corresponding to the test soil group doses.

RESULTS

Test Soil Characterization

Test soil characterization results (lead concentration, percentage moisture content, percentage organic matter, pH, total element content, mineralogic evaluation, and particle size) for Test Soils I and III are summarized in Table 1. Total lead concentrations in the two test soils mixed in the feed and fed to the animals were 810 ± 21 ppm for Test Soil I and 3908 ± 31 ppm for Test Soil III which are typical of many mining waste soils. The percentage organic matter content for Test Soils I and III was 3.0 and 4.1, respectively. The pH of the soils was strongly to extremely acidic (according to USDA-SCS Soil pH Categories), with pH values of approximately 4.5 and 3.7 for Test Soils I and III, respectively, which are typical of noncalcareous soils derived from mining waste. Several elements were present at high concentrations that may have affected lead absorption. These included, aluminum, arsenic, cadmium, calcium, copper, iron, manganese, and zinc.

The lead phase mineralogy provides a useful indicator of the important mineral and noncrystalline phases controlling lead solubility of ingested soils. In Test Soil I, 45% of the lead mass was present as anglesite (PbSO₄) and galena (PbS). Coronadite (MnPbMnO₄) and lead oxide phosphate (Pb₃(PO₄)₂) accounted for 50% while iron elyrite ([Cu-Fe]Pb₂(SO₄)₂(OH)₄) accounted for the remaining 5%. Galena is a common ore mineral while anglesite is an oxidation product that forms a rind around galena in acid environments produced by dissolution of pyrite in mining waste rock (Nordstrom, 1982) (Fig. 1). The low lead concentration in Test Soil I resulted in a limited number of lead-bearing crystals in the sample. Test Soil III, however, contained a large number of lead minerals, resulting in better counting statistics and a more representative distribution. The lead mineralogy observed in Test Soil III consisted primarily of anglesite and galena (77%) with a variety of minor phases. Both samples displayed alteration products and rindings (coating or armoring of the original mineral grain by a reaction product) of the primary lead phases, e.g., galena (PbS) altering to anglesite (PbSO₄) (Fig. 1), cerussite (PbCO₃) altering to anglesite, lead oxide (PbO) altering to anglesite, and further alteration of anglesite to plumbojarosite
Mineralogic analysis:
- Anglesite (PbSO₄) - 28% 53%
- Galena (PbS) - 17% 24%
- Coronadite (MnPbMn₂(OH)₄) - 28% —
- Lead oxide phosphate (Pb₃(OH)(PO₄)₂) - 22% —
- Iron elyte (Cu-Fe-Pb(SO₄)OH₄) - 5% —
- Barite (Pb-BaSO₄) - — 5%
- Lead phosphate (Pb₃(PO₄)₂) - — 4%
- Thorium-lead phosphate (Th-Pb(PO₄)) - — 4%
- Plumboferrite (PbFe₂O₄) - — 4%
- Plumbojarosite (PbFe₄(SO₄)OH₁₂) - — 3%
- Lead oxide - — 3%

* The concentration of lead in the soils was determined by ICP-AES using EPA SW 846 Method 6010. Triplicate aliquots of the soils were digested according to EPA Method 3050 and duplicate aliquots of each digestate were analyzed. Tabled values are reported as the mean ± standard deviation.

The pH of the soil was determined using EPA SW 846 Method 9045. Tabled values are reported as the mean ± standard deviation of duplicate analyses.

The overall group mean food consumption values for the untreated control group rats during the 30-day in-life period was 23 g feed/day/animal. Group mean food consumption values for all treatment groups were not significantly different (p < 0.05) from the control group values for both sexes. Although there were a few treatment groups with subgroups that had elevated group mean food consumption values, the increases were generally slight in magnitude and did not reflect dose-dependent changes. In addition, the data suggested that no palatability problems occurred with the lead acetate or soil lead mixed in the diet. After 4 weeks, group mean body weight gain values ranged from 27 to 40 g/week for the males and 6 to 18 g/week for the females. Thus, exposure occurred during a rapid growth phase and the lead acetate and soil lead did not compromise growth patterns.

**Signs of Toxicity**

Treatment group animals were similar in appearance and behavior to the control group animals and no overt signs of toxicity were observed.

**Food Consumption Determinations**

The overall group mean food consumption values for the untreated control group rats during the 30-day in-life period was 23 g feed/day/animal. Group mean food consumption values for all treatment groups were not significantly different (p < 0.05) from the control group values for both sexes. Although there were a few treatment groups with subgroups that had elevated group mean food consumption values, the increases were generally slight in magnitude and did not reflect dose-dependent changes. In addition, the data suggested that no palatability problems occurred with the lead acetate or soil lead mixed in the diet. After 4 weeks, group mean body weight gain values ranged from 27 to 40 g/week for the males and 6 to 18 g/week for the females. Thus, exposure occurred during a rapid growth phase and the lead acetate and soil lead did not compromise growth patterns.

**Daily Exposure Index**

The group mean daily exposure index values (milligrams lead consumed per kilogram body weight per day) by study...
week for male and female rats are summarized in Table 2. The group mean daily exposure index data indicated approximately proportional increases in the level of exposure (absolute amount and per body weight) at the different dose levels for each treatment group and both sexes. The exposure index differed slightly between the sexes for many of the dose levels of each treatment group, with the exposure level being higher for females than for males. The sex difference was attributed to similar group mean daily food consumption values between the sexes but substantially lower body weight values for the female animals when compared to the male animals.

**Tissue Lead Levels**

**Blood.** Overall, the group mean whole blood lead concentration values for the Test Soil I and III groups were significantly lower than the blood lead concentration values for comparable exposure levels of the lead acetate group (Fig. 2). The group mean whole blood lead concentration values increased with increasing dose levels for all treatment groups but were not proportional, reaching a plateau at the higher dose levels in the soil treatment groups. A plateau was less apparent for the male and female lead acetate groups. For the most part, similar group mean blood lead concentration values were observed for male and female rats within the same treatment group.

**Bone.** The group mean bone lead concentration values increased with increasing dose levels for all treatment groups (Fig. 3). Bone lead levels were very low following dietary ingestion of Test Soils I and III compared to bone lead levels after ingestion of lead acetate. Lead absorption and distri-
Lead consumption values were calculated by multiplying the concentration of lead in the dosed feed by the daily food consumption. The equation for daily exposure index (mg Pb/kg BW) is:

\[
\text{Daily exposure index} = \frac{\text{group mean daily lead consumption (mg) \times week}}{\text{group mean body weight (kg), week}}.
\]

**Test Soil I and III**

Similar group mean bone lead concentrations were observed for male and female rats within the same treatment group, except for the lead acetate treatment group, whereby the male group mean liver lead concentrations were approximately twofold higher than the females.

**Relative Percentage Bioavailability**

**Blood.** Relative percentage bioavailability at the low dose levels (0.2 and 0.5%) could not be accurately determined due to the very low blood lead concentrations attained at these exposure levels. Only at the higher dose levels, i.e., 2 and 5%, were blood lead levels following lead acetate and test soil ingestion elevated sufficiently above background to permit precise estimation of bioavailability values (Table 3). Table 3 presents the relative percentage bioavailability values for Test Soil I and III, and 5% dietary soil dose levels bracket the pica child's daily soil ingestion. For Test Soils I and III, relative percentage bioavailability values ranged from 12 to 26% and 20 to 27%, respectively, for the male and female 2 and 5% dose levels groups based on the lead acetate standard. There were no statistically significant differences between the dose levels or sexes. Based on blood data, the overall mean relative percentage bioavailability value for Test Soils I and III at 2 and 5% soil in the diet was approximately 20%.

**Bone.** Similar to the blood, relative percentage bioavailability values for the bone data at the low dose levels (0.2 and 0.5% dietary soil) were highly variable. Data for the 2 and 5% dose levels provided more reliable relative percentage bioavailability results (Table 3). For Test Soils I and III, relative percentage bioavailability values ranged from approximately 5 to 11% and 8 to 13%, respectively, for the male groups.
BIOAVAILABILITY OF LEAD FROM MINING SOIL

FIG. 2. Lead concentration in blood (µg Pb/liter) versus dose (mg Pb/kg BW) in (A) male and (B) female rats. Data are expressed as the mean ± standard deviation, n = 5 (duplicate analyses per animal). Method detection limit is 1.1 µg Pb/liter.

FIG. 3. Lead concentration in bone (mg Pb/kg FW) versus dose (mg Pb/kg BW) in (A) male and (B) female rats. Data are expressed as the mean ± standard deviation, n = 5 (duplicate analyses per animal). Method detection limit is 0.56 mg Pb/kg.

This study was conducted to determine the extent of absorption (relative percentage bioavailability) of lead from two different mining soils using young Sprague-Dawley rats fed soil mixed with a purified diet. It is the first study to fully investigate the bioavailability of lead in soils containing mine waste using a soil dose-response approach. Male and female Sprague-Dawley rats (5 animals/sex/group) were fed mining waste soil (810 and 3908 ppm lead) from Butte, Montana, mixed in an AIN-76 purified diet at four dose levels for 30 consecutive days. The soil was mixed with a purified diet to lower the background levels of lead found in control animals and to allow the detection of the soil lead in the animal’s tissues even at low lead levels. This diet also maximizes lead absorption because it is relatively low in calcium. Low dietary calcium increases lead absorption because calcium and lead are absorbed through similar mechanisms (Six and Goyer.

Liver. For Test Soil I, liver lead levels were slightly above or at method detection limits at dose levels of 0.2, 0.5, and 2% dietary soil for both sexes. Thus, the extent of absorption and eventual accumulation of lead in the liver could not be accurately determined. This resulted in relative percentage bioavailability estimates for these dose levels that were highly variable and imprecise. At the 5% dose level, the relative percentage bioavailability values were approximately 9% (males) and 8% (females) based on the lead acetate standard (Table 3). For Test Soil III, liver lead levels were near method detection limits at dose levels of 0.2 and 0.5% dietary soil for both sexes. Thus, relative percentage bioavailability estimates for the 0.2 and 0.5% dose levels were variable due to relatively low absorption and accumulation of lead to the liver following ingestion of Test Soil III. For the male 2 and 5% Test Soil III dose levels, group mean relative percentage bioavailability values were approximately 7 and 8%, respectively, and, for the females, 14 and 10%, respectively, based on the lead acetate standard (Table 3). The overall mean relative percentage bioavailability value for Test Soils I and III at 2 and 5% soil in the diet was approximately 8%.

DISCUSSION

and female 2 and 5% dose level groups based on the lead acetate standard. There were no statistically significant differences between dose levels or sexes. Using bone data, the overall mean relative percentage bioavailability value for Test Soils I and III at 2 and 5% soil in the diet was approximately 9%.
The study was 30 days in length because of the long biological half life of lead. Thirty days appeared to be an adequate compromise between the need for sufficient time for accumulation of lead in the blood and tissue, while still balancing the need for an exposure period which ensured that animals remained in a rapid growth phase. The target tissues measured were whole blood, liver, and bone.

**TABLE 3**

Relative Percentage Bioavailability Values of Lead for Male and Female Rats Administered Test Soils I and III Mixed with Feed

<table>
<thead>
<tr>
<th>Group*</th>
<th>Test soil I</th>
<th>Test soil III</th>
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<tbody>
<tr>
<td></td>
<td>Blood</td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2%</td>
<td>18.1 (6.0)</td>
<td>19.6 (3.3)</td>
</tr>
<tr>
<td>5%</td>
<td>12.1 (3.6)</td>
<td>21.3 (3.8)</td>
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<tr>
<td>Females</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2%</td>
<td>25.7 (7.8)</td>
<td>26.8 (4.8)</td>
</tr>
<tr>
<td>5%</td>
<td>13.8 (4.7)</td>
<td>22.1 (4.4)</td>
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<tr>
<td></td>
<td>Bone</td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2%</td>
<td>8.0 (3.5)</td>
<td>7.5 (1.4)</td>
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<tr>
<td>5%</td>
<td>4.8 (1.3)</td>
<td>7.5 (1.4)</td>
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<tr>
<td>Females</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2%</td>
<td>10.6 (3.3)</td>
<td>13.3 (2.2)</td>
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<tr>
<td>5%</td>
<td>6.1 (1.9)</td>
<td>13.0 (2.9)</td>
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<td></td>
<td>Liver</td>
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<tr>
<td>Males</td>
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<tr>
<td>2%</td>
<td>4.3 (2.4)</td>
<td>7.1 (1.5)</td>
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<tr>
<td>5%</td>
<td>8.7 (2.0)</td>
<td>7.5 (1.5)</td>
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<tr>
<td>Females</td>
<td></td>
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</tr>
<tr>
<td>2%</td>
<td>0.6 (3.1)</td>
<td>13.6 (3.1)</td>
</tr>
<tr>
<td>5%</td>
<td>8.2 (2.8)</td>
<td>9.8 (2.1)</td>
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</tbody>
</table>

* Relative percentage bioavailability values were determined using the lead acetate group as the standard. Tabulated data are reported as the mean with the standard error of the mean in parentheses.

* 2 and 5% refer to the percentage soil in the diet.
the important compartments for lead distribution. In addition, the chronic feeding design allowed us to keep dietary lead levels similar to those of pica children, and to keep the ratio of lead to other elements close to those of these children. Other study designs have used 10 times or higher dietary lead for shorter periods, potentially confusing interactions between lead and other elements such as calcium and phosphorus.

The clinical appearance, body weight, food consumption, and liver weight values in the soil-treated animals were similar to those in the control group animals, indicating no overt toxicity that may have affected the relative percentage bioavailability values estimated for this study. Significantly higher lead concentrations were measured in the blood, bone, and liver of animals fed lead acetate when compared to animals fed the test soils mixed in diet. Thus, under comparable dosing conditions, the bioavailability of lead in the Butte soils was considerably less than that of lead acetate administered in the diet. Over the dose range tested (0.2 to 5% soil in diet), the blood, bone, and liver lead concentration versus dose profiles generally plateaued for animals of both sexes when fed the test soils mixed in diet. The plateau response to soil lead is extremely apparent if one compares the pattern of response of tissue lead for the soil animals to the high linear slope at the start of the lead acetate curves. These data are consistent with results from epidemiological studies that show there is a weak relationship between soil lead concentration and human blood lead levels at other mining sites with a high proportion of lead sulfide in the mineral assemblage (Bornschein et al., 1990).

Relative percentage bioavailability values, based on lead acetate as the standard, were independent of the two different test soils, dose levels, and sex, and only slightly dependent on the tissue (blood > bone, liver). It is appropriate to compare lead acetate with the forms of lead in mine waste soils because lead acetate is highly soluble (thus maximizing bioavailability) and can serve as a surrogate for soluble lead compounds ingested in the human diet. Overall relative percentage bioavailability values (assuming no biologically significant differences between sexes, dose levels, or soils) were 20% based on the blood data; 9% based on the bone data; and 8% based on the liver data (2 and 5% soil dose levels only). The low bioavailability of lead in the Butte soil-treated animals agreed favorably with low blood lead levels (average of 3.5 µg/dl) found in children from Butte, Montana (Butte-Silver Bow County Environmental Health Study, 1991). Negligible lead absorption (i.e., only slightly above background concentrations) occurred at test soil dose levels of 0.2 and 0.5%. These lower dose levels, although more closely approximating soil lead exposure to that of normal children, were still above the average lead level intake of a child. Thus, uptake of lead from soil at dose levels similar to those of a normal child’s exposure would not be detectable under this study protocol.

This study demonstrates that lead in the Butte mining waste soil is less bioavailable than lead from an automotive or paint lead source. For example, rats fed soil lead from a roadside (auto exhaust lead) and from along the edge of a lead-paint house demonstrated lead concentrations in blood, bone, liver, kidney, and brain after 30 days that were similar to rats fed lead acetate: while at 90 days, tissue concentrations in bone and kidney were about 70 to 80% those in lead acetate rats (Dacre and Ter Haar, 1977). Chaney et al. (1984) reported relative percentage lead bioavailability values (based on bone) in Baltimore garden soils ranging from 15 to 70% (average of 33.4%) of the dietary lead acetate. Based on bone lead, the bioavailability of lead in Butte soils was about one-quarter that in the Baltimore garden soils. These comparisons underscore the importance of evaluating lead source and species when predicting bioavailability values for lead in soils. Soil mineralogy is a critical parameter in assessing lead dissolution because the different lead-bearing solids characteristic of different sources have varying solubilities under the different pH regimes of the stomach prior to and after ingestion of food. The low bioavailability is due in part to the pervasive encapsulation and alteration of the common lead-bearing solids (galena and anglesite) that preclude ready dissolution of these minerals over their residence time in the GI tract. Mineralogy of the Butte soils demonstrates that galena has oxidized to anglesite, with peripheral precipitation of K-jarosite coating the lead grains. This arming process serves to inhibit dissolution of lead-bearing solids both physically through a reduction in the surface area of anglesite exposed to GI tract fluids and chemically due to the less soluble nature of K-jarosite in an acidic medium, i.e., pH < 4 (Vlek et al., 1974; Davis et al., 1992).

The bioavailability of lead is influenced by the species of lead incorporated into the soil (which varies depending on the source of lead), the size of the lead-containing soil particles, the matrix incorporating the lead species, and nutrients or other compounds ingested with the lead (Steele et al., 1990; Chaney et al., 1989). The toxicokinetics of lead can be affected by interactions with essential elements. Increased levels of iron decrease lead uptake (Barton et al., 1978). Low dietary levels of zinc (Cerklewski and Forbes, 1976; El-Gazzar et al., 1978) and copper (Klauder et al., 1973; Klauder and Petering, 1975) increase lead absorption. High levels, therefore, may act to impede the absorption of lead (Edshall and Wyman, 1958; Underwood, 1977; Brewer et al., 1985). Thus, high levels of aluminum, arsenic, cadmium, calcium, copper, iron, manganese, and zinc may have affected lead absorption, indicating the importance of determining the elemental content and mineralogy of the soils.

The lead in the original Butte soils may actually be even less bioavailable than the results of the present experiment.
show. The test soils were passed through a 250-μm sieve and blended to obtain a more homogeneous lead concentration. However, the sieved portion of the soil (<250 μm fraction) represented only about 10% of the original soil sample, with the remaining 90% of larger particle size. In addition, by mechanically mixing the soil into the feed, the soil particle sizes were reduced further. For example, Test Soil I had a geometric mean volume-based diameter of 48 μm ranging from 1 to 194 μm while Test Soil III had a geometric mean volume-based diameter of 42 μm ranging from 1 to 182 μm. Thus, the actual mean particle size of the test soils was well below the 250-μm size. In general, the lower the particle size, the greater the absorption of lead because smaller particles (higher surface area to mass) will dissolve more rapidly in the GI tract, thus producing more solubilized lead. Decreasing the particle size of the test soil, therefore, facilitates the absorption of lead into the systemic circulation. Thus, the bioavailability of lead in the indigenous Butte soils is likely to be less than that measured in the present study due to larger particle sizes found in the original Butte soil samples. In this study, the mean particle size of both test soils was below the upper limit for the size of soil particles reported to adhere to children’s hands (<100 μm) that constitutes the major route of ingestion (Duggan et al., 1985; Chaney et al., 1989). Therefore, the bioavailability of lead in the indigenous Butte soils may be less than that determined for the present study for a pica child because the particle sizes of the test soils in this study were of a size which mimicked reported soil particle size values for normal human exposure but also optimized the dissolution rate and extent of absorption. Thus, for a child with pica-for-soil, this study represents a worst case scenario because pica children ingest large particles during bulk soil ingestion and not exclusively the <100 μm size fraction.

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