

Bioavailability of Lead in Mining Wastes: An Oral Intubation Study in Young Swine

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Introduction

The incidental ingestion of soil as a result of hand-to-mouth and object-to-mouth behaviors is recognized as a significant source of exposure of young children to toxicants present in soil and household dust (Calabrese *et al.*, 1987). In particular, it is generally accepted that these exposure pathways may be the most important contributors to non-dietary lead exposures (Bartrop, 1973; USEPA, 1989). Thus, where there is significant contamination of residential soils, a crucial determining factor for systematic exposure is the availability of soil/dust lead for absorption from the gastrointestinal (GI) tract. Recently, the bioavailability of ingested lead has become a significant issue in establishing clean-up levels for soils contaminated with wastes from mining and/or milling operations. Two hypotheses have been put forward which suggest that lead from mining/milling activities may be less bioavailable than that from other lead sources such as smelter emissions and lead paint dust (see Steele *et al.*, 1989). Basically, these arguments are (1) lead at many mining sites may occur as lead sulfide (PbS) which is relatively insoluble in water at neutral pH compared to other common lead containing compounds, and, thus, may pass through the GI tract without appreciable dissolution, and (2) lead in soils at mining sites may exist as particles which are relatively large, and are thus both less likely to be ingested and to dissolve in GI fluids. However, the basis for these assumptions lacks an empirical foundation. For example, there is little published data on the forms of lead that occur in soils near smelter, milling or mining sites. Similarly, little data exist on the distribution of lead in different particle size fractions at either smelter sites or mining/milling sites.

In order to further elucidate the bioavailability of lead from mining/milling wastes, EPA Region VIII initiated a study in collaboration with Michigan State University of GI uptake of lead in mill tailings taken from Midvale, UT. The lead present in deposits adjacent to the community of Midvale is derived primarily from galena ore and thus should consist mostly of lead

as lead sulfide crystals. Further, because past milling processes often produced wastes with relatively large grain size (Benedict, 1955), most of the lead was predicted to be associated with relatively large (greater than about 100 μm) soil particles. Particles larger than 100 μm probably will not adhere efficiently to skin and thus will not be available for transfer to the mouth during typical hand-to-mouth behaviors (Que Hee *et al.*, 1985). Thus, based on the above arguments, lead in the Midvale tailings should be relatively unavailable for absorption from the GI tract, compared with absorption of a water soluble lead salt such as lead nitrate. In fact, one would predict that availability would be similar to that of reagent grade lead sulfide, providing that tailings lead was not 'buried' in a silicate or pyrite matrix which could further reduce bioavailability. The EPA/MSU study was designed to test the following null hypothesis: (1) that lead in the mine tailings is unavailable for absorption from the gut of pigs and (2) that absorption of lead in tailings is not different than that of lead sulfide and lead nitrate.

Materials and Methods

Animal model

Because lead intoxication is a matter of concern primarily for young children (age 6 months to 6 years, EPA 1989), an animal model chosen for research on bioavailability should reflect as much as possible the behavior, and GI physiology and biochemistry of young children. As discussed in detail in another report in this symposium (Weis and LaVelle 1991), rodent and lagomorph models seemed not to meet the above criteria and so were rejected as candidate models for the study. Several other potential models were investigated, and the one which seemed to most clearly meet study criteria was the recently weaned pig (Weis and LaVelle, 1991). Young pigs have been used extensively as a model for children's GI function (Dodds, 1982; Miller and Ullrey, 1987; Weis and LaVelle, 1991). Moreover, physiology and biochemistry of

Table 1 Doses of lead for mixtures of control soil and lead from different sources.

	Dose group				
	Low tailings	Medium tailings	High tailings	PbS	PbNO ₃
Nominal lead dose	0.44	0.88	1.76	1.76	1.76
Measured lead dose	0.44	0.88	1.84	1.52	1.67

All values are in mg/kg

calcium and, by experimental metabolism, appear to be similar in young pigs and humans (Weiss and LaVelle, 1991).

Weaned, male, cross-bred swine (*Sus scrofa*) used for the study were selected according to age (45 to 47 days) from the nursery area of the Michigan State University swine facility. Animals were allowed to acclimate in experimental cages for 5 to 10 days. Animals were fed 4 % of body weight per day, an amount which met or exceeded all nutrient requirements for this age of swine set by the Committee on Animal Nutrition of the National Research Council. Animals were fed twice daily and water was provided by mixing equal volumes with the food to make a slurry. This minimized food spillage by the pigs. Additional water was provided at midday.

Lead source

Midvale, UT, a community of about 30,000 located 12 miles south of Salt Lake City, is the site of past milling and smelting activities. The milling operation was active from 1910 to 1970. It produced about 14,000,000 cubic yards of mine tailings from the processing of lead, copper and zinc ores. The tailings pile now covers over 260 acres and, in places, is over 50 feet deep.

Lead from tailings was obtained from an archived grab sample taken from the surface of the tailings deposit. The surface sample was deemed appropriate since it is surface material which is transported via wind and which children may contact when playing on contaminated soil. The tailings sample was screened through a 100 mesh sieve to obtain a fraction of particles smaller than about 150 μm , close to the size fraction expected to adhere to human skin. Analysis of the material before and after sieving indicated that lead concentration in the whole tailings sample (16,900 ppm, USEPA, 1990) was similar to that in the sieved fraction (17,200 ppm, Billing, 1990).

Reagent grade lead nitrate and lead sulfide were used as positive controls and were obtained from Fluka Chemicka-Biochemica, 980 S 2nd Street, Nonkonkoma, NY 11779 and Sigma Chemical Co, PO Box 14508, St Louis, MO 63178, respectively.

Control soil was obtained from a surface grab sample taken in an area of Midvale, UT where lead contamination was expected to be minimal. This soil was also passed through a 100 mesh sieve. Analysis of the sieved fraction of the control soil indicated a lead concentration of 150 ppm.

To obtain different doses of lead, control soil was mixed

with different proportions of the sieved tailings or the reagent grade lead salts. Doses were mixed such that the amount of lead to be given to each animal on a mg per kg basis was contained in 292 mg of soil mixture. Knowing the concentrations of lead in the control soil and mine tailings and the desired concentration of lead in the final mixture, a Pearson Square was used to calculate the ratio of control soil to tailings required. A similar approach was used with the positive controls although, in that case, the lead concentration was calculated from the formula weight. Appropriate aliquants of mixtures were weighed on a top-loading gram scale and mixed thoroughly by manual shaking for at least 5 minutes in acid-washed polyethylene containers.

Actual lead concentrations in the tailings sample, the control soil and the mixtures were determined by three different methods, X-ray fluorescence, and atomic absorption following nitric or hydrofluoric digestions.

Dosing protocol

Animals were assigned randomly to one of five treatment groups, three animals per group, for each of two identical experimental blocks completed sequentially. Animals in one group were administered a single dose of one of the above soils mixes, 292 mg soil mix per kg body weight, as a slurry in 40 mL of distilled water, by intubation. The mixing vessel and the stomach tube were then rinsed with an additional 20 mL of distilled water to ensure complete delivery of the dose. Animals were watched closely for emesis for the first hour after dosing. After 120 hours, the same dose was readministered to each pig using the same procedures. Thus, each animal was given the same dose twice, as a check on intra-animal variability.

Three groups of animals received mixtures of tailings and control soil. In addition, one group of animals received lead sulfide mixed with control soil and the final group received lead nitrate mixed with control soil. Nominal and measured lead doses for each group are provided in Table 1. Pilot studies indicated that background lead concentrations in the blood of pigs were very low (below the quantification limit of 40 $\mu\text{g L}^{-1}$), and apparently stable over time, and that administration of control soil alone did not cause a measurable increase in background blood lead concentrations after a single dose. Thus, in this study, each animal served as its own control, the first blood lead measurements serving to establish the basal blood lead level.

Table 2 Methods for atomic absorption spectrophotometry

Wavelength	283.4 nm
Slit width	1.4 nm
Conditions	Dry - 60 s, 35135 (C ramp) Ashing - 30 s, 600 (C) Atomization - 7 s, 2,000 (C)
Matrix Modifier	Triton X-100, 0.5 % wt/v Nitric Acid, 0.2 % v/v Ammonium phosphate dibasic, 0.2 % wt/v

Blood sampling and analysis (see Tables 2 and 3)

Immediately before administering the soil mixes, and at 1, 2, 4, 6, 8, 12, 24, 48, 72, and 96 hours post dosing, blood samples were taken from the jugular vein with a closed Vacutainer system (Becton-Dickinson Vacutainer Systems, Rutherford, NJ 07070). Samples were taken initially in tubes containing EDTA as an anticoagulant and stored at 4° C. As soon as possible after collection, samples were split into three 1 mL aliquots. One tube was stored at 4° C for future use, a second was analyzed by AA (see below) and the third was sent to CDC for confirmatory lead analysis.

Whole blood lead concentrations were determined by atomic absorption spectroscopy with an Hitachi model 180-80, Zeeman effect, atomic absorption spectrophotometer equipped with a graphite furnace. Whole blood (100 µL) was mixed with a matrix modifier of triton X-100 and dibasic ammonium phosphate in type II distilled water acidified with ultra pure 16 M nitric acid (Table 2). Blanks, 50, 100, 200 and 300 ppb standards were included in each analytical run.

Results*Interlaboratory blood lead analysis comparisons*

The studies were conducted under strict quality assurance procedures. Ninety three percent of the duplicate analyses were within 10 % of one another, with a mean difference of 4.0 %. Ninety six percent of the proficiency standards were within 10 % of the certified values in an interlaboratory comparison (University of Wisconsin).

The results of analyses of blood lead by MSU were plotted against the results obtained on splits of the same samples from CDC (Figure 1). With the exception of one low value obtained by CDC, there was excellent agreement between the two laboratories. The slope of the line is, as predicted, near 1 and the y intercept near 0. The close agreement suggests that the blood lead values obtained in the study, even those near the limits of quantification, are accurate.

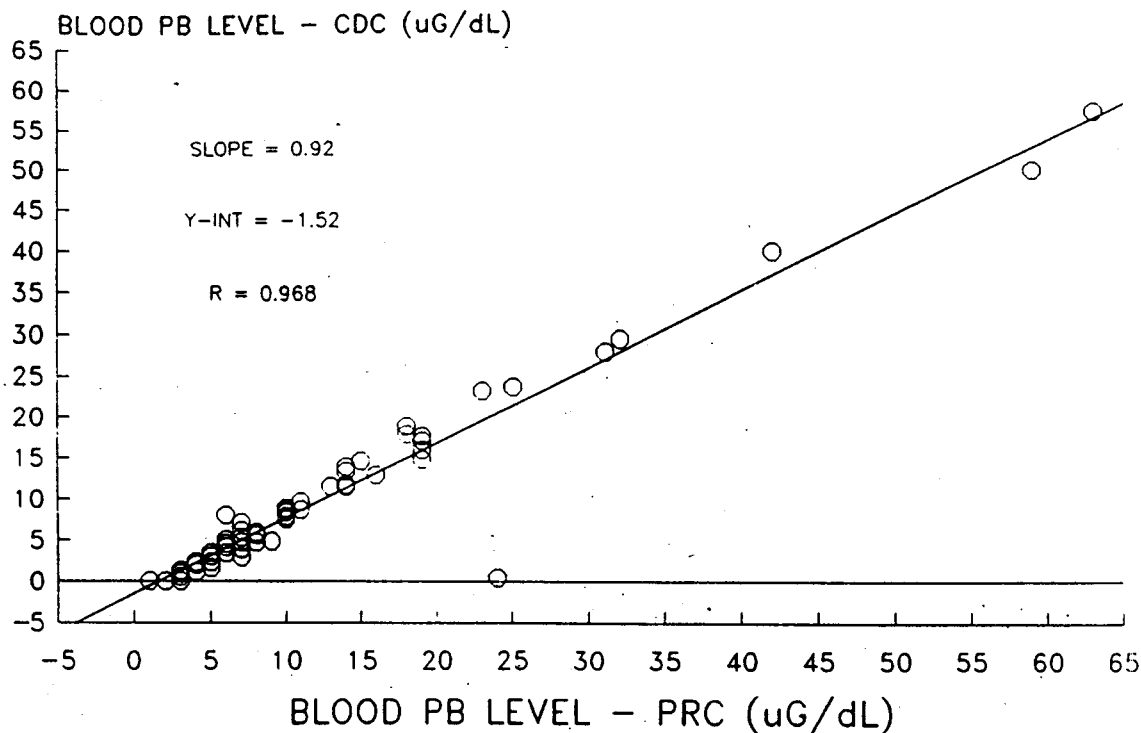


Figure 1 Comparison of blood Pb analyses run by the Pesticide Research Center (PRC) at Michigan State University with analyses run on split samples by the Centers for disease Control (CDC) in Atlanta. Line is a linear regression from all data points.

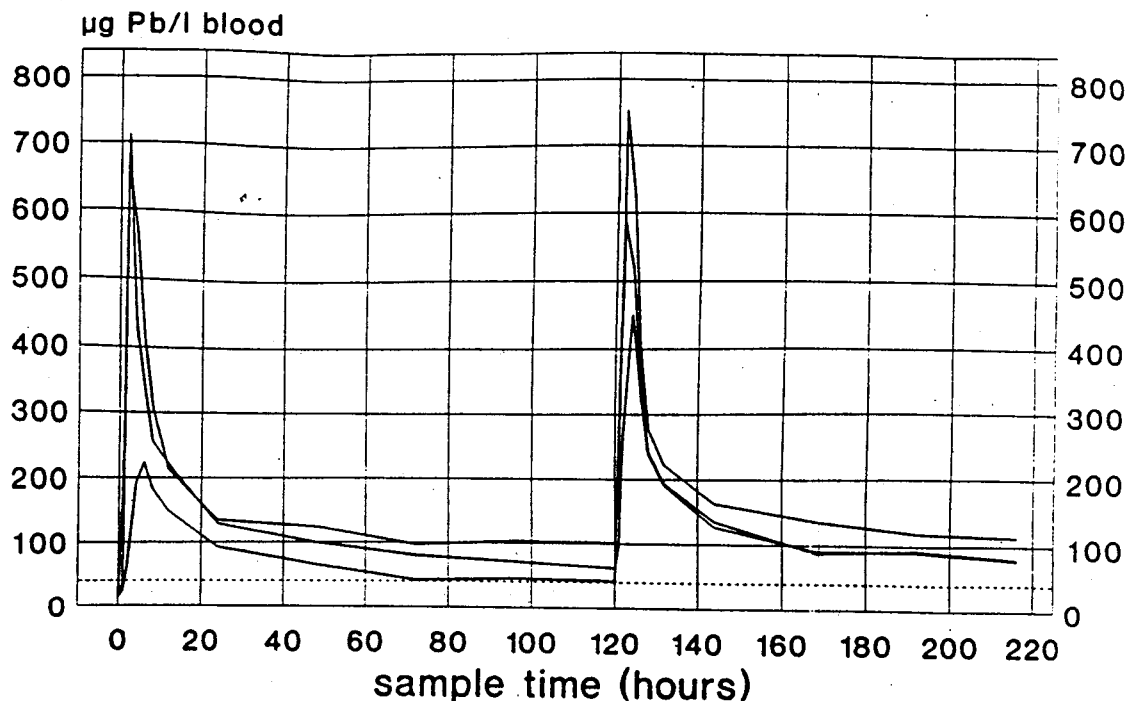


Figure 2 Representative blood lead concentration curves for three pigs given control soil mixed with the greatest amounts of Midvale tailings. Dose given to all animals was 1.54 mg Pb/kg body weight. They were dosed by oral introduction at 0 and 120h. The dashed line is the quantification limit.

Blood lead vs time curves

Uptake kinetics of lead were similar among treatments, but variation among individuals was observed (Figure 2). The maximum concentration of lead in blood occurred between 4 and 6 h after dosing, and blood lead levels returned to near background within 96 h. Since relative bioavailability was estimated using area-under-the-curve (AUC) calculations (see below), it is important to note that extrapolation of blood lead curves past 96 hours suggested that contribution to total AUC from 96 to 168 hours (three more days) might have averaged about 8 to 10 %. Thus, estimates of bioavailability should not be greatly influenced by the small amounts of lead which might still be in the blood after 96 hours.

Area under the blood lead/time curves

An approximately linear relationship was observed between dose of lead in tailings and the amount of lead absorbed, as measured by AUC (Figure 3). (AUCs were estimated using Sigma Scan software (Jandel Scientific, Corte Madera, CA 94925) and a digital plotter.) Estimates of AUC for individual blood lead/time curves were combined for all animals (Figures 3 and 4). Lead in tailings at the greatest administered dose was absorbed to a greater extent than was lead nitrate mixed with control soil, although the statistical analysis did not take into account the lower actual dose received by the animals receiving lead nitrate (Figure 4). However, reagent grade lead sulfide mixed with soil was absorbed to a significantly lesser extent (p

< 0.05). Thus, contrary to the predictions of Steele *et al.* 1989 the lead in tailings from the Midvale site more closely resembled that of a soluble lead salt (PbNO_3) than that of the less soluble lead sulfide. It is interesting to note, however, that even lead sulfide was absorbed to a measurable extent. By way of comparison, absorption of lead in animals administered lead in tailings at 0.56 mg kg^{-1} was slightly less than that absorbed by animals given reagent grade lead sulfide at a dose of 1.5 mg kg^{-1} . Thus, lead in the tailings may be about 2 to 3 times more available than reagent grade lead sulfide in this study.

The relative absorption of lead from tailings and from lead nitrate is interesting, but may be due, in part, to saturation of transport mechanisms in the gut. If the doses of lead for the high-dose tailings group and the lead nitrate group were at or near those necessary to saturate active transport of lead, estimations of relative bioavailability could be compromised to some degree. Thus, it is possible that some difference in relative bioavailability between lead nitrate and lead in tailings could have been encountered if both had been administered at lesser doses.

Discussion

The results reported at this symposium are still being analyzed and this paper presents only a partial and preliminary interpretation of the data. Even so, it seems that several interesting conclusions can be reached concerning the relative bioavailabilities of lead in smelting vs mining/milling wastes.

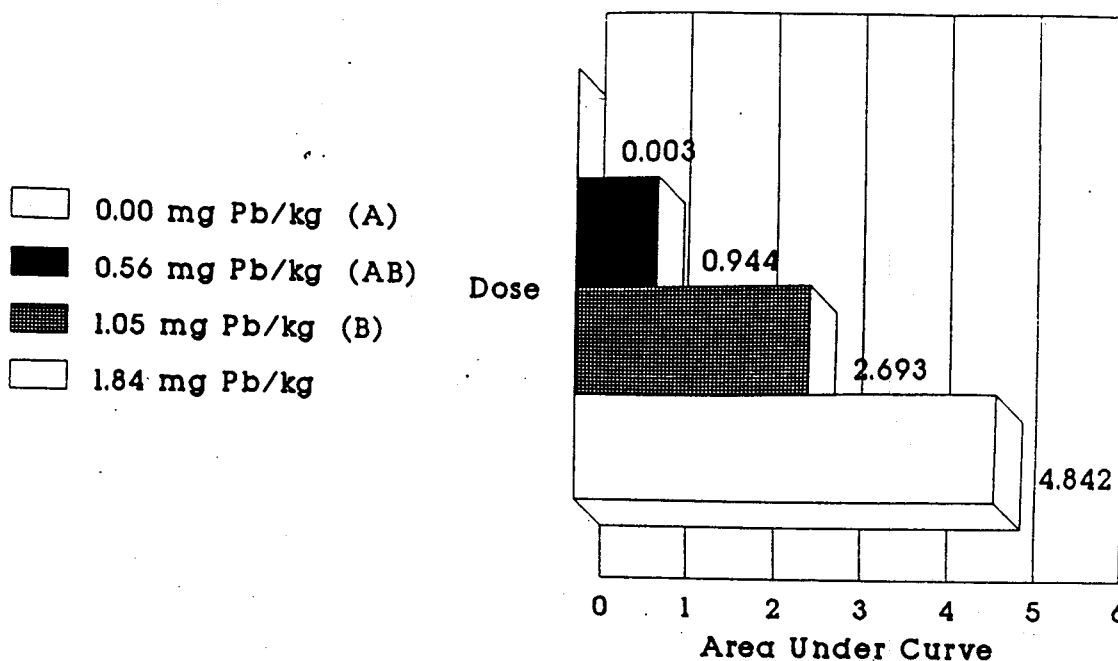


Figure 3 Average blood lead concentrations for three groups of pigs given control soil mixed with different proportions of tailings from Midvale, UT. The group associated with a dose of 0.00 mg Pb/kg represents the AUC for a hypothetical control group. Since animals which received no dose of lead always had levels of lead in the blood less than the quantitation limit of $40 \mu\text{g L}^{-1}$, this AUC was calculated assuming a blood lead concentration of $40 \mu\text{g L}^{-1}$. Means of doses with same letter (A/B) are not significantly different ($p < 0.05$).

The apparently greater absorption of lead in tailings compared with the lead nitrate control is unexpected. The difference between the two lead forms is partly due to the lesser dose given to the animals receiving lead as lead nitrate. However, other factors may play a role. If the dose of lead in both tailings and lead nitrate were sufficiently high to saturate active lead transport, reversible binding of lead to constituents of stomach and intestinal contents could play a significant role in determining lead absorption. When the amount of lead is sufficiently low that active transport is not saturated, absorption of lead will, in theory, be independent of free lead concentrations, and reversibly bound lead will be fully available for absorption. Active transport will serve to continuously pull the binding equilibrium toward dissociation. Provided that gut transit times are not too short, all bound lead would be available for uptake. At greater lead doses, near or in excess of those that might saturate active processes, bound lead might not be fully available for absorption. Where active transport is saturated, further absorption will depend on the free lead concentration. Bound lead under these conditions may not be completely available. In preparation of material for dosing, control soil was substantially diluted with tailings. The tailings have a low organic content and would be expected to have fewer binding sites for lead than control soil which was taken from the surface of a vegetated area. With fewer binding sites, high doses of lead may lead to a greater free lead concentration in the intestine and, hence, greater lead absorption. When reagent grade lead

nitrate was mixed with soil there was little dilution. In this case, increased binding could have led to somewhat decreased absorption.

It is interesting to note that in rats greater than 8 weeks of age, where active transport of calcium (and presumably lead) is absent (Weis and LaVelle, 1991), binding of lead to soil components has been shown to reduce absorption, at least in cases where the amount of soil administered to the pigs in this study (over 2 grams for a 10 kg animal) suggest that similar effects could also have occurred here. Comparison of absorption at lower doses might help determine if binding effects were important in this study.

Regardless of potential differences between availability of lead in tailings and lead nitrate in this study, the results using pigs as an experimental model for lead bioavailability demonstrate that soil in tailings is absorbed by young pigs to a significant extent. Absorption of lead from tailings was significantly greater than that from reagent grade lead sulfide mixed with control soil and appeared to be similar to that of lead nitrate in the control soil mixture. This is consistent with chemical and physical measurements made on the tailings sample. Small particles appeared to contain at least as great a concentration of lead as did the total sample. This suggests that lead is not confined primarily to larger particles at this tailings site. Furthermore, independent electron microprobe analysis of sieved tailings from the Midvale site showed the presence of large numbers of galena crystals with estimated diameters less

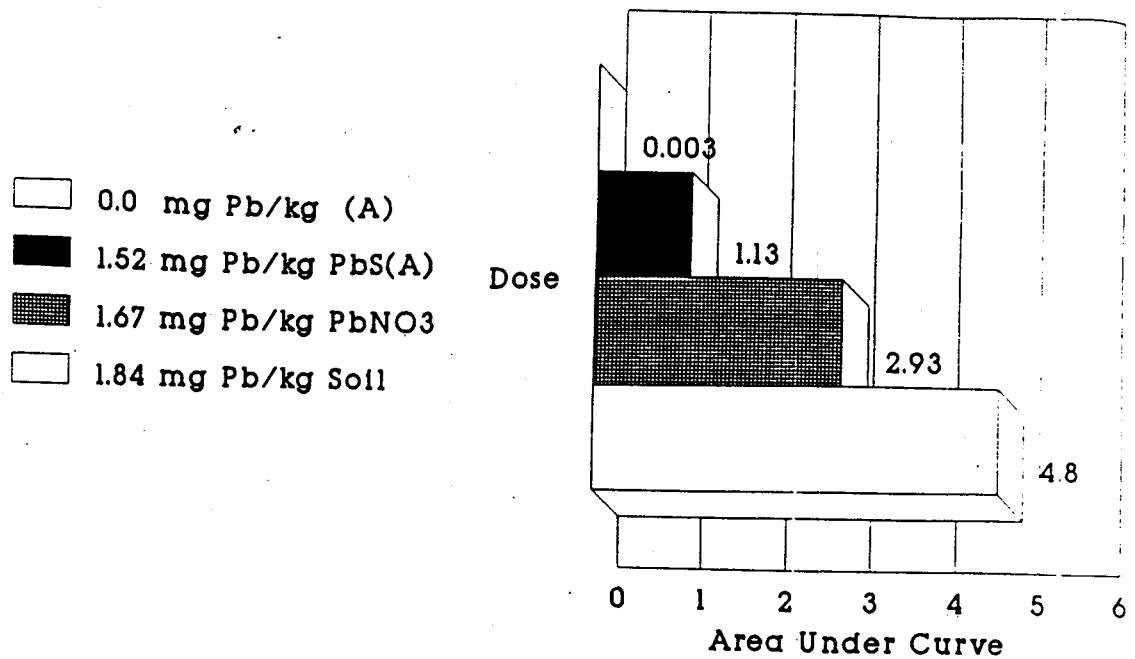


Figure 4 A. Concentrations for three groups of pigs given control soil mixed with tailings from Midvale, UT, reagent grade lead sulfide. The group associated with a dose of 0.00 mg Pb/kg is identical to that in Figure 3. The statistics were run on the combined data without normalization for differences in dose. Means of doses with same letter (A/B) are not significantly different ($p < 0.05$).

than 10 μm (Drexler, personal communication). Particles of this size might be completely dissolved within 50 to 100 minutes in gastric fluids (Healy et al., 1982). In the stomachs of young children, where there would be continued secretion of gastric acids, dissolution could be even more efficient. Finally, the results of the electron microprobe analyses indicate that considerable oxidation of lead in tailings has and is taking place, such that less soluble forms of lead such as PbS are replaced by sulfates and oxides that are considerably more soluble (Drexler 1990, personal communication). Thus, for this milling site, the basic arguments described by Steele et al., 1989 may not hold true. First, much of the lead is present in more soluble forms, which appear to be highly available at, e.g., smelting sites, and second, large amounts of lead are present even in the smallest particles.

The above results are similar to those being collected at other mining/milling sites. For instance, in material from Butte, MT, there seems to be a consistent enrichment of lead and other metals in smaller (100 mesh) particles, a finding opposite of that predicted by the Steele et al. analysis for such a mining site. Further, current studies of smelter sites, like East Helena, MT, seem to indicate that considerable contamination may be due to dusts blown from concentrate piles (most of which comes from galena ore deposits). Thus, even at 'pure' smelting sites, such as the smelter facility in East Helena, MT, considerable contamination may come from sources other than stack emissions. The emerging picture seems to indicate that the

division between smelting sites on one hand and mining/milling sites on the other may be somewhat artificial. It seems possible that exposures at all sites associated with mining and/or processing of lead-containing ores will be mixed, coming from both small particle, lead oxides typical of smelters, and from sulfide containing particles and their oxidation products typical of mining and milling activity. This implies that it may not be possible to separate smelters from other mining activities on the basis of bioavailability.

If the above findings in young pigs can be extended to young children, lead in mining/milling wastes may present a similar hazard as does lead from other sources. Anatomical, physiological and biochemical similarities between young children and the pigs used in this study are presented in detail elsewhere in this volume (Weis and LaVelle, 1991). These similarities support the extrapolation of the results to children. In addition, the overall pattern of lead uptake from the GI tract seems similar in pigs and humans. Based on excretion patterns of Pb-203 in adult volunteers, maximum concentrations of lead in blood probably occur before about 6 hours after dosing, since peak excretion seems to occur at this time or slightly later (Kehoe, 1961). This is very similar to the timing of peak blood lead concentrations in young pigs in this study. Assuming no major differences between adults and young children vis-a-vis the shape of the blood lead/time curve, young pigs appear to model children satisfactorily.

In summary, swine seem to be an appropriate model for

young children. Initial results using this model suggest that tailings material from Midvale, UT is more available to young pigs than is reagent grade PbS when presented as a single large dose by intubation. Chemical and physical characterization of the lead in the tailings material suggest that lead is present in small particles, consistent with this relatively high availability. Further, weathering (oxidation) appears to produce lead forms which are more soluble and, thus, potentially more bioavailable. This initial study does not lend support to current arguments that lead from mining/milling activity is significantly less bioavailable than that from other sources and activities.

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