Uses and Limits of Empirical Data in Measuring and Modeling Human Lead Exposure

Paul Mushak
PB Associates, Durham, North Carolina

This paper examines the uses and limits of empirical data in evaluating measurement and modeling approaches to human lead exposure. Empirical data from experiment or observation or both have been used in studies of lead exposure. For example, experimental studies have elucidated and quantified physiologic or biokinetic parameters of lead exposure under controlled conditions. Observation, i.e., epidemiology, has been widely applied to study population exposures to lead. There is growing interest in the use of lead exposure prediction models and their evaluation before use in risk assessment. Empirical studies of lead exposure must be fully understood, especially their limits, before they are applied as "standards" or reference information for evaluation of exposure models, especially the U.S. Environmental Protection Agency's lead biokinetic model that is a focus of this article. Empirical and modeled datasets for lead exposure may not agree due to a) problems with the observational data or b) problems with the model; caution should be exercised before either a model or observational data are rejected. There are at least three sources of discordance in cases where there is lack of agreement: a) empirical data are accurate but the model is flawed; b) the model is valid but reference empirical data are inaccurate; or c) neither empirical data nor model is accurate, and each is inaccurate in different ways. This paper evaluates some of the critical empirical inputs to biokinetic models, especially lead bioavailability.

— Environ Health Perspect 106(Suppl 6):1467-1484 (1998).  http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-6/1467-1484mushakabstract.html

Key words: lead exposure modeling, lead models, blood lead

This paper describes uses and limits of both empirical data and predictive exposure models in assessing lead exposure; it was prepared as part of the proceedings for a U.S. Environmental Protection Agency (U.S. EPA) meeting on lead biokinetic models. The earlier portion is more concise and summarizing, owing to the huge amount of data. Some later sections are of necessity more detailed because of the evolving nature of the topics and their importance, their current stage of development, and need for a current assessment. Critical current assessment is particularly required for the topic of lead bioavailability, a significant element in reliable lead exposure measurement and modeling. Lead bioavailability in its many multidisciplinary complexities and nuances is still poorly understood and misunderstood by many, is interpretively misused by others, and continues to feed a growing, increasingly jumbled literature. These differing tasks involve different styles of writing on the respective topics, differences the reader hopefully will not find too abrupt in flow of thought and information.

Lead exposure in this paper refers to both the amount of lead entering various receiving compartments of the body through inhalation and ingestion, and the subsequent absorption of some fraction of the lead intake. Intake is sometimes defined by others as exposure, whereas the amount absorbed is defined as dose. There are several ways one can attempt to quantitate lead exposure in humans. One can measure the amount of lead in biologic media from these subjects or lead in environmental media from their surroundings. One can also estimate, i.e., predict, the extent of lead exposure using a mathematical model and available environmental lead or exposure information, e.g., biologic data, for input.

As noted by Mushak (1), models are abstract constructions and depictions of complex systems that permit easier comprehension for study and application. Biokinetic models of substance uptake, disposition, and removal are the subject of regulatory and health policy interest and increasingly figure in risk assessment practices, per the guidelines of the National Research Council (NRC) (2). The guidelines are categorized as hazard identification, dose-response relationships, exposure assessment, and risk characterization. The first two are typically general or generic; the remaining two are case- or site-specific. In the case of lead, the first two components are relatively well studied, but it is the site-specific lead exposures that often drive the utility of the overall paradigm. Modeling of lead exposure in human populations is especially useful in those situations where there are limits on field measurements of lead exposure.

Models of human lead exposure are of various categorical and computational types and they differ in their relative complexity and range of application. The earliest are purely ad hoc models based on specific datasets in the form of equations derived from regression analyses. These involve predicting a dependent measure, such as blood lead concentration, when regressed against an environmental measure such as soil or dust lead concentrations. These statistically defined models integrate measured data into an inferential statement about the relationship of lead in some medium to some measure of body lead burden, e.g., lead in blood (PbB). We infer some overall relationship but cannot delineate the mechanisms by which that relationship operates physiologically, anatomically, or biochemically. Descriptively, these models are cross-sectional in nature and assume that the depiction of lead's behavior represents steady-state conditions. An example of an ad hoc statistical model based on regression data is that of Angle et al. (3) linking child PbB to urban environmental lead data.
A relatively more sophisticated form of (multiple) regression analysis, structural equation modeling, represents a pathway approach in which specific pathways via which environmental lead sources provide lead to human intake and uptake can be ascertained to some extent. Here, we can fill in some of the intermediate, external steps between a lead source such as airborne lead or lead paint and eventual lead uptake.

The predictive value of statistical, regression-based models is often limited to the particular set of conditions prevailing in the study producing the model. The more complex and widely applicable model approach is the physiologic mechanistic model, where one constructs as accurately as feasible a quantitative, mathematical simulation of what happens when there is lead intake and uptake. This simulation assumes biokinetic mechanisms of lead behavior in vivo. Historically, these models have conceptually and computationally consisted of the earlier classical compartment models, the more recent physiologically based pharmacokinetic (PBPK) model, and hybrids of the two. The latter include the U.S. EPA's integrated exposure uptake bio-kinetic (IEUBK) model for childhood lead exposure (4–7), the variable-age Leggett model (8) and the O'Flaherty PBPK model (9,10). These biokinetic mathematical models have focused on blood lead as the predictive internal exposure marker. The IEUBK model is a particular focus of this paper.

**Uses and Limits of Empirical Data in Lead Exposure Measurement**

Empirical data refer to measurement information gathered by experiment or observation. Both forms of empirical information have commonly been gathered in studies of human lead exposure. Experimental approaches typically entail *in vitro* or animal model studies of specific elements of exposure or exposure parameters not possible or appropriate in human studies. Direct human experimental data in adult volunteers and under institutional oversight have been reported. Observational data are numerous in the lead exposure literature and have typically consisted of different types of epidemiologic assessments of environmental lead exposures and exposures in occupational settings. Observations, i.e., epidemiologic information, include both measurements of lead in environmental media and measurement of biologic markers in exposed populations. Subsumed within biologic monitoring are the various components that are quantitatively significant in contributing to an integrated expression of exposure as determined by one example of biologic monitoring, PbB.

Use of empirical data in lead exposure assessment can take several forms: measurement in a research study, preliminary data collection to establish more systematic measurements, and lead exposure monitoring frameworks through serial, systematic measurements. These especially apply for PbB data collection.

With reference to the utility and limits of empirical data in exposure model assessment, the accuracy and validity of empirical data used for calibrating or validating the model is itself important. A model for estimating PbB values first requires we validate or calibrate its output of PbB estimates by determining the relative reliability or accuracy of PbB sampling and measurement methods employed for gathering PbB data for direct comparison.

With regard to actual field measurements of human lead exposures, such data gathering can be of two types: environmental measurement and biologic measurement. Environmental data can be obtained by limited measurements at one or few time points or by monitoring levels of lead in environmental media within some framework and serially over time.

**Environmental Lead Measurements**

Environmental measurements of lead represent potential exposures of populations rather than actual exposures of some specific population. When such data are combined with measurements of systemic exposure, or dose, such as lead in some physiologic medium like blood, one is able to carry out inferential statistical analysis of the relationships between the source and the specific pathways of lead movement into the body and eventually the bloodstream and target organs. Although elevated lead levels in environmental media encountered by humans do not prove systemic exposure and associated toxicity, the reverse situation is equally problematic. Available biologic data such as PbB screening results in the absence of environmental measurements severely hinder quantitative conclusions about where the lead is coming from and what lead source or pathway one must remediate to reduce or remove the exposure risk.

Environmental assessment necessarily figures in the development and use of lead exposure models. Statistical, ad hoc models for sites such as those depicted in multiple regression analyses require environmental lead concentrations as the independent variable in subsequent regression analyses. Mechanistic physiologic, i.e., biokinetic, models require environmental data for input of all the various lead intakes prior to computational integration of the inputs. This is just as true of the reported classical compartment kinetic models as it is of the PBPK models. Although certain biokinetic models can make use of default, best estimate or generic concentrations for uniform or centralized media sources such as diet or drinking water, certain critical inputs such as soil and dust lead are preferably derived from site-specific measurements if such values have been reliably determined. Reliable and accurate site-specific data, preferable for any predictive model that is still being evaluated, should produce better concordance between measured and predicted results, compared to some default value.

Environmental measurement produces results that are affected by a variety of factors, e.g., the context or framework of the data gathering, the sampling design for media lead measurements, and the laboratory measurement methods. Environmental lead assessments have been used in programmatic or systematic analyses over time and space, i.e., site-specific monitoring over some time period, and in the form of site-specific measurements at some single point in time. An example of the former is the U.S. EPA's air monitoring network that gathers multiple-site data for a variety of purposes. A second example is groundwater monitoring around or on a hazardous waste site by monitoring wells. The latter monitoring is typically part of the resolution of regulatory or litigatory action for Superfund sites in which monitoring groundwater for determining off-site migration is to be done for many years.

Although air or groundwater lead measurements may be done in some systematic monitoring framework, a number of other lead-containing media of interest at some particular site are more commonly examined on a single or limited repeat sampling basis. This would typically be the case for soil, dust, and paint samples.

The environmental media sampling design for subsequent lead measurements can often be the largest source of uncertainty and variability in arriving at most exposure-representative statistical depictions of environmental lead contamination. These include the "what" and the "how" of
media lead sampling. This is best illustrated in the first instance by the question, What does one collect for lead measurement in the way of environmental sampling? This will be a difficult question to answer when there is lack of understanding or information about what the sources and pathways of lead are to the subjects under study. Lead readily undergoes environmental cycling into and out of environmental compartments that may also serve as exposure media for humans.

The complexity and interrelationships among these compartments for lead are presented in Figure 1. This environmental lead flow scheme permits one to determine some of the environmental media that may need to be analyzed for lead given some information about the history of the locale vis-à-vis sources of lead contamination. To illustrate, soil lead contamination from a nearby stationary source such as a lead smelter means that not only soil lead but also interior and exterior dust, garden crops used as food, and so forth should be measured. There may be multiple lead source inputs to pathway media such as soil and tapwater, e.g., air lead fallout and leaded paint for soil, and lead-soldered household plumbing joints producing contamination of tapwater drunk directly and contamination of any foods cooked in the tapwater.

An equally vexing question in sampling design has to do with how to sample a particular potential exposure locale. With dusts and soils, does one use only grab samples or gridded composites? What sampling protocol best captures any potential high heterogeneity of lead distribution in these site media, e.g., soil contamination by lead from multiple sources? With soils, there are critical questions of where to sample, what should be composited, and what form of compositing is most likely to reflect child exposures. What depth of soil should be examined? Soil plots used for food crop gardening should be sampled differently than areas where children mainly contact the uppermost centimeter or two of soil during extended play. Various reports have appeared addressing these issues in a regulatory context and they include but are not limited to the U.S. EPA’s 1989 Risk Assessment Guidance for Superfund (11). This source includes guidance on how to deal with various sampling design issues for environmental contaminants in human health risk assessment.

Sampling protocols should also reflect the physical and physicochemical characteristics of the media that are most relevant to intake and subsequent internal exposure by such risk groups as infants and toddlers. For example, soil samples of diverse particle sizes should be tested for lead only after those particle sizes within soil samples that reflect likely human intakes of soil lead are first isolated and analyzed. The smaller size soil particles, i.e., those ≤ 150 μm, are those most likely to be ingested, most likely to be transformed in terms of lead release, most likely to have the highest relative lead content, and the fraction(s) most likely to be associated with biomarkers of exposure and of adverse effect (12-14).

Sampling protocols ideally should include measurements that may say something about the source of lead in that medium. For example, proximity of soils to a curb versus soils at a house roof drip line may reveal lead concentration patterns that reflect input sources. High lead levels at medians between sidewalk and roadway but lower lead levels farther from the road suggest auto exhaust lead from past gasoline lead use. High lead concentrations in a soil band corresponding to the drip line suggest paint lead or washing off of deposited particles from air lead fallout. A declining soil lead gradient with increasing distance from exterior building surfaces suggests lead paint. Similarly, tapwater lead that varies markedly with flushing would implicate unit plumbing, e.g., lead-soldered copper plumbing fittings, rather than an elevated water lead concentration entering the unit from outside (15).

In the case of dust sampling, there are the questions of where to sample and how much to sample, particularly in contact areas for children. These questions should be linked to what one knows about the interactions of exposed individuals with soil and dust-contaminated areas. If large quantities of household dust are to be collected, sampling in residences where general housekeeping practices prevent accumulating dust on surfaces in children’s play areas may force collections in...
historical dust accumulation areas such as under furniture, and in unused rooms, attics, basements. These areas might show less association with ongoing child lead exposures than areas of child activity.

Although laboratory methods that pass quality assurance and quality control protocols are generally available for lead in a number of media, laboratory methodological issues remain for dusts and soils (16). Laboratory methods for lead analysis are now relatively standardized and prescriptive in the contract laboratory program of the U.S. EPA for those sites that figure or may figure in regulatory or litigatory actions. A further issue is how to quantitate the lead contamination in samples collected inside residences, especially dust samples. Traditionally, one measured the concentration of lead in soils and dusts per unit mass, typically as parts per million. Milar and Mushak (17) first used lead measurements based on concentrations of lead per unit surface area as well as the conventional expression in mass concentration. Lead content per unit area for quantitation helps to better define the parameter of dust loading rates and the highly variable parameter of household cleaning practices (17). The quantification by unit area approach in subsequent investigations has been shown to be a better reflection of likely human lead exposure than the concentration-per-unit mass method (18).

**Measurable Biomarkers of Human Lead Exposure**

The NRC (19,20) defines a biologic marker of exposure as an exogenous substance or its metabolite (in some testable biological medium) or the product of an interaction between some xenobiotic agent and some target molecule or cell. There are also biomarkers of lead effect and of lead susceptibility, but these are outside the interests of this paper.

Figure 2 depicts two types of lead exposure biomarkers: physiologic fluids such as whole blood (PbB) or plasma lead (PbP), and mineral tissue markers (lead in bone [PbBone] and teeth [PbT]). PbB has been and remains the most popular biomarker. It is also the one that is both readily understood as to methodology and for which generally accepted dose–response relationships exist in terms of PbB thresholds associated with adverse effects. This dose–response relationship is depicted in Figure 3 as an analogy to a thermometer, as depicted by Mushak (21) and as adapted from the Agency for Toxic Substances and Disease Registry (ATSDR) (22). The amount of body lead burden determines the reading in the form of the number of effects whose thresholds have been crossed. Analogous depictions have been reported (23). Figure 3 shows thresholds in PbB for the full spectrum of adverse health effects in infants and toddlers. At a PbB of 10 μg/dl, a battery of effects discernible on a group basis and involving the developing nervous system begins to be apparent. At the other end of the PbB range, 100 μg/dl and higher, convulsions, coma, and death become increasingly likely. PbB represents mainly recent lead intake and uptake in infants and toddlers having modest lead exposures, with an associated biologic half-life range on the order of 3 to 6 weeks (1,24–28). With increasing age and/or body lead burden, the fraction of body lead accumulated in bones begins to increasingly contribute to the total quantity of PbB through resorption.

Two human exposure categories that represent the extremes of current versus cumulative lead inputs to blood can be identified. The infant and toddler with low exposure would have PbB reflecting almost all recent lead intake, whereas retired adult lead workers with a long working history of heavy lead exposure would show practically

![Figure 2. Characteristics of lead exposure biomarkers.](image-url)

![Figure 3. Full spectrum lead dose response. Modified from ATSDR (22).](image-url)
EMPIRICAL DATA IN LEAD EXPOSURE MODELING

all PbB arising from PbBone lead resorption after leaving the workplace exposure. Adults with mainly moderate environmental lead exposures will have PbB concentrations arising from variable inputs from both current intakes and historical PbBone releases, the latter being further increased during pregnancy, lactation, and post-menopausal periods (1,24). The labile nature of PbB in infants and toddlers in absolute terms makes it difficult to reconstruct earlier exposures without the availability of, e.g., serial PbB data collected over a reasonable time.

Infants and toddlers with relatively high lead exposures, as might occur in deteriorated housing in inner-city neighborhoods in America's largest cities, have been shown in some studies to have a higher fraction of total PbB from some slower kinetic component than that which reflects recent absorption and that probably includes PbBone, even though the PbBone turnover rate is assumed to be quite high in this age group. Succop et al. (29) employed statistical analysis of serial blood data to calculate a half-life of 10 months in PbB of inner-city Cincinnati children. Angle et al. (30) noted that analysis of stable lead isotope ratios in inner-city Omaha toddlers indicated that a measurable, significant part of PbB arose from endogenous sources rather than recent intake sources. These reports have obvious implications for lead remediation strategies where PbB reduction is the biologic index of reduced exposure. That is, the total PbB decline that would be associated with remediation will require a period of time to be realized if the starting body lead burden lodged in bone before remediation is relatively high.

A second toxicokinetic issue for human exposures is the nature of the PbB relationship to lead in environmental media. It is generally observed (25) that over a large range of environmental lead levels, the corresponding group PbB is curvilinear downward at the higher end of the lead intake range. Experimental data suggest one factor that may be operating is the dependence of lead uptake on the lead concentration in ingested or inhaled media (31,32). Chamberlain (33), however, also invokes an exposure-dependent increase in urinary excretion, based on his studies of workers with occupational lead exposures. Flanagan and co-workers (34) reported that human adult volunteers acutely ingesting lead as a single dose over a range of 4 to 400 μg/day had the same uptake rate, but it is unclear whether chronic intakes over this range would actually show dose dependency of uptake after achieving a stable PbB value.

Although overall PbB may show a declining slope relationship at high intakes, the overall direction of PbB is still upward. The fractional distribution of total PbB to plasma increases with a PbB increase above 50 to 60 μg/dl (35,36). Because this is the point along the PbB versus lead intake curve where change in slope also occurs, the nature of the curve at high intakes may be reflecting more efficient removal of circulating lead through plasma. This would indicate that tissues continue to receive a relatively linear lead load through plasma. This, in fact, appears to occur. Relative linearity in tissue lead increase with intake is seen when one compares dosing lead levels with selected tissue lead levels for rodents (37) or dogs (38). Increased urinary lead output may occur as well with increasing intake, but its magnitude is not enough to void the linearity of the tissue lead–lead intake relationship, or the upward curvilinearity of the PbP versus increasing lead intake relationship.

The relative accuracy of PbB measurements on individuals or population group basis is determined by a host of factors. It is imperative to comprehend the impact of these factors on PbB data before one can use such information to draw conclusions about the extent of lead exposure in a community or to evaluate (validate and calibrate) lead exposure prediction models. These factors include a) the nature of the PbB gathering process with respect to existence or absence of any programmatic infrastructure, b) the biokinetic nature of the PbB measurement itself and how it's affected by the type of PbB survey; and c) the quality of the epidemiologic and biostatistical design employed for carrying out the PbB survey program.

A critical factor in blood lead measurements is the nature of the PbB data gathering. PbB screening within a programmatic public health framework and including serial testing, i.e., PbB monitoring, has a number of advantages over a "single-shot" testing effort specifically looking at a particular community's lead exposure sources. With the latter, there can be considerable uncertainty or variability, with little possibility of easy resolution since there is typically no follow-up or monitoring framework. The typical structured PbB data gatherings in screening programs are those managed by various states, counties, and cities with added input and support from the Centers for Disease Control and Prevention. These programmatic surveys assure that high-risk lead exposure children will be multiply sampled over their high-risk age band with a follow-up structure for assessing effects of lead source remediation, where such is done.

A second structured PbB data gathering example is the nationwide survey of the U.S. population's PbB mean and distribution for a given time period, statistical "snapshots" of national lead exposure in the National Health and Nutrition Examination Surveys (NHANES) conducted by the U.S. Public Health Service. These surveys, e.g., the second and third NHANES [NHANES II (39) and NHANES III (40)], are statistically designed so that they employ an intensive canvassing effort of socioeconomic and demographic strata within a cluster-sampling, aggregated model that also involves extremely rigid, standardized study designs and rigorous quality assurance/quality control protocols for assuring accurate biologic measurements, including PbB measurements. A single large team carries out NHANES studies. The NHANES-type survey is a single-sampling effort for all the stratified communities, selected for position in a statistical design serving a global nutrition and health assessment, not for having a lead problem. NHANES surveys, although done once in a statistical community, bear no interpretive similarity to those single surveys that have as their purpose assessing a specific community's potential lead problem only once.

Single PbB surveys, as may be employed in a single community at a single point in time and with little programmatic framework for follow-up, potentially can be limited by a variety of methodological and interpretive problems, especially if links to sources of lead exposure that produce PbB elevations are sought. Examples of studies of lead-exposed communities in terms of PbB and environmental lead measures are known (41-43).

One difficulty with single-shot PbB assessments that are focused on lead exposure per se is that the relatively rapid response of PbB in the very young to abrupt changes in lead intake may affect the single-effect results. A specific problem is the extent to which public awareness of, or focused education efforts on, a publicized lead problem in a community alters caregiver behavior so as to abruptly restrict child activity and associated lead exposure. This produces a biokinetic and epidemiologic artifact by lowering PbB at the time of sampling. Such reduction would be
most welcome if permanent, but would likely only be transitory without a permanent framework for continuing awareness and education. Lead exposure would eventually revert to its previous level.

The relationship of caregiver awareness to abrupt changes in PbB appears in the recent, preliminary PbB results of Goldman and co-workers (44,45) for the Children's Lead Exposure and Reduction Study (CLEARs) in Jersey City, New Jersey. Children of parents whose overall awareness and response to systematic education about lead hazards were higher at testing than that of other parents had a mean PbB four points lower than children of less aware parents. This difference occurred with mean PbB levels in the group that represented significant elevations. The CLEARs data for a robust intervention suggest a significant average decline (about 4 μg/dl). Such absolute level declines occur whether or not relative linkages in terms of rank order persist. Mushak (1), Dietrich et al. (46), and Otto et al. (47) noted findings that groups of children evaluated at some earlier time in terms of PbB level can preserve the relative ranking of the PbB value in later testing. The basis of these observations theoretically can arise from the relative size of endogenous lead release to blood, persistence of proportional external lead contact, or a mixture of both. Abrupt shifts downward in the number or fraction of children above some not-to-exceed risk or toxicity threshold level would arise from groupwide abrupt declines in PbB with restrictions in exposures. Preservation of rank order says nothing about the absolute PbB values in any later testing set of outcomes.

Finally, there is the problem of appropriate study design for these ad hoc, single surveys of lead-exposed communities. A number of such recent surveys done for a single community were done using study designs that pose considerable uncertainty as to identification of the most highly exposed risk population segments and the relationship of that study group's exposure to particular sources and pathways of lead exposure, e.g., exposures to extractive industry wastes. Little standardization of study designs in these lead-exposed communities has been done. Different researchers studying the same community can arrive at quite different results, depending on the nature of the study design. A good example of this disparity of interpretation is a single PbB survey carried out in an Illinois Superfund site community by contractors for Illinois and the U.S. ATSDR (48). The contractors concluded there was no impact of the site, a defunct smelter, on children's PbB concentrations. A more detailed U.S. EPA analysis, using structural equation modeling, did show a clear relationship (49). In some of the older single-shot studies of lead exposure, particularly those reported in the 1970s or earlier, the quality of the study design and methods employed were highly questionable and resulting data should be considered suspect (12).

The overall problems with assessment of reported single-shot surveys over the years for lead-exposed communities extend to reviews of such data. The review of Danse et al. (50) concluded that lead in mill tailings at many thousands of parts per million in and around extractive-industry communities do not pose any lead exposure threats to children living there. This conclusion was based on an evaluation of historical data that was based on superficial assessments of these data, including neglect of the many nuances and complexities of these datasets. A number of the same studies and study sites were also evaluated in a 1991 critical review by Mushak (12), and the interested reader should consult this paper. In particular, the communities referred to by Danse et al. produced a large amount of problematic PbB data. In other cases, Danse et al. misinterpreted the more reliable information. For example, the 1981 Australian study of Heyworth et al. (51) actually shows, when the original data are analyzed, a clear link between mill tailings in the play environment of children and elevations in their PbB. In other cases, communities reported by Mushak (12) as showing a relationship between mining and related waste and PbB were not mentioned by Danse et al. The discussion by Danse did not stratify the available data for PbB versus extractive-waste sources as to relative reliability of study designs, lead measurement methodology, representativeness of the community characteristics, and appropriate presentation of data, e.g., use of mean values instead of distribution of PbBs above toxic threshold levels. Rather, Danse et al. used indiscriminate mixing, tabulation, and interpretation of PbB values for mill tailing communities. PbB data from small group samplings in the 1970s were combined with more recent survey data.

In summary, single PbB surveys carried out in lead-impacted communities where no follow-up is planned need to be carefully scrutinized as to their accuracy and reliability. Potential problems with any effects of publicity or awareness on transitory drops in PbB levels should be examined, as should the appropriateness of the study design employed. At this time, we can say that available information in the literature exists to carry out more reliable PbB studies, especially with reference to study design issues. Where environmental data are gathered in tandem with such PbB data gathering, sampling and measurement methods also need to be carefully scrutinized. PbB surveys that a) involve a valid statistical design, b) show no impact of awareness or publicity on transitory reduction of PbB levels, c) permit follow-up with future PbB surveys if necessary, and d) are coupled with environmental measurements that are accurate, precise, and statistically representative of likely actual contact by risk groups such as infants and toddlers, would be more reliable and of better utility to the validation and calibration of predictive lead exposure models where PbB estimates are produced.

An exposure biomarker related to PbB is PbP. Biologically, plasma is the medium by which lead is borne from the blood compartment to target tissues and organelles. Consequently, we might expect that the more precise dose–response relationships for lead and human adverse health effects would arise from linkage of effect outcomes to PbP concentrations. However, the many methodological problems that plague use of this measure dampen its utility in lead epidemiology and clinical toxicology. Within a range of PbP up to about 50 μg/dl, the equilibrium relationship of PbP to PbB is relatively stable as a fixed percentage of PbB. At higher PbB concentrations, the fraction of PbP begins to increase and the plasma–whole blood relationship becomes curvilinear upward with increasing PbB levels. This may be a factor in loss of PbB’s linear relationship at higher environmental lead intakes as overall tissue lead is more linear than PbB. It should be noted that we are not speaking here of very transitory shifts in PbP that occur with uptake of lead after, for example, eating a meal or drinking water. The longer term equilibrium holds.

Methodological problems in the measurement and use of PbP are severe. Concentrations of PbP are at extremely low levels relative to PbB. The fraction of total PbB that is not in the erythrocyte is only about 1% (25,52). In the case of a PbB of 10 μg/dl, the corresponding PbP would be about 1 ppb in blood and about 2 ppb in separated plasma. The very low fractional distribution into plasma also means that in the presence of even slight hemolysis, PbP
becomes markedly contaminated with erythrocyte lead. A particularly vexing problem is that as PbB increases with increasing lead exposure, erythrocyte fragility and osmotic resistance are greatly enhanced, so that hemolysis is even more likely at elevated PbB concentrations where precise dose–response relationships would be of particular interest. Additionally, the hazard of contamination with nonbiologic, environmental lead in the sampling or laboratory environment is quite high at the low levels that are typically present. Contamination of 1 ml of plasma with 2 ng of lead would double the PbP concentration expected at a PbB of 10 μg/dl. The final problem with PbP is interpretive and diagnostic in nature. We have little idea at this point what a particular PbP means in terms of toxicity risk or manifestation in an individual with an elevated level. Consequently, the accepted and readily comprehended dose–response relationships across a wide toxicity spectrum using PbB concentration as the dose measure are not paralleled using PbP concentration as the dose measure.

Lead in mineral tissue, unlike PbB or PbP, serves as a biomarker of cumulative lead exposure. Mineral tissue exposure biomarkers are of two types anatomically, physiologically, and biokinetically. These are PbT and PbBone. PbT is the better known biomarker of cumulative exposure. Lead deposition in tooth tissue begins with calcification of enamel before eruption. Secondary dentine lead reflects lead accumulation from mainly the time of eruption to shedding. PbBone is cumulative from childhood through about the sixth decade of life. PbT is much less kinetically mobile than PbBone in young children in that release from the several compartments of bone back to blood can readily occur in children.

The uses and limits of PbT data for lead exposure vary greatly from that involving PbB. The levels of PbT, particularly in the highly lead-enriched circum-pulpal dentine region, reflect lead exposure generally integrated over the early life of the child. This integral of exposure encompasses infancy and toddler life, where lead exposure can be higher and the developing child is most vulnerable to systemic toxicity effects. Accumulation continues through older childhood, when teeth are shed.

Although efforts have been made to model the quantitative relationship of PbT to PbB, thereby providing more predictive flexibility to PbT as a dose measure (53,54), the state of this research is largely incomplete. It may not be easy to accurately predict a temporally averaged PbB during the high vulnerability years from a single PbT value. Accumulation of lead by episodic or pulsed high exposures as in acute paint chip or flake ingestion or by chronic, low-level lead intakes from multiple sources may have different implications for toxic responses. Regardless of the history of accumulation, many studies show significant associations between PbT and some neurotoxic outcome measure (24).

Although PbBone is also a lead accumulating marker with potential use in assessing long-term subject exposure, this marker is only now being explored as a biomarker of environmental lead exposure in children (55,56). This measure typically involves X-ray fluorescence (XRF) via in vivo measurements using either K or L X-rays. The different X-ray approaches appear to probe different portions of the bone compartments for lead. Bone XRF for lead has been in the research stage methodologically and interpretively with reference to general population lead exposures, but is being applied to some groups with exposure histories in childhood. Farther along is use of XRF spectrometry in assessing long-term occupational lead exposure (24,28,57–59). These applications of the method typically involve cortical or trabecular PbBone measurement, are carried out with a history of serial PbB measurements, and with an employment history in lead worksites. As noted in the next section, these different measures are intercorrelated.

Recent XRF studies of lead workers describe the very close correlation between serial PbB data in cumulative form (mean annual PbB × work-years) and tibial (cortical) or heel (trabecular) PbBone concentrations measured by K-line XRF analysis (57–59). Because both measures are biochemically linked ways to define cumulative body lead burdens, we would expect a high correlation. In typical environmental exposure settings involving children who are not taken to be in high-risk populations and therefore not included in programmatic screening, serial PbB data are rarely available. A critical interpretation question for PbBone levels by XRF analysis is what a lead measurement means in terms of toxicity risk or clinical management of exposed subjects. Does a cortical PbBone level of X ppm indicate sufficient lead exposure has occurred or is occurring to produce lead poisoning? Which of the various bone measures most closely relate to what potential adverse effect?

The relative utility of PbT and PbBone data to risk managers or regulatory policy makers concerned with real-time lead control challenges is obviously more limited than would be PbB data for the same population. Newly gathered PbB data in terms of means and statistical distributions can dictate the need for prompt remediation and some expectation that such intervention will produce a lowering of lead exposure and reduced poisoning risk. Elevated lead levels in teeth or bones of children indicate exposures that have already occurred, including but not limited to the more distant past. Exposures measured this way may have already produced toxicologic effects and lead remediation actions might not be taken soon enough to benefit these particular children. However, results of elevated lead levels in bone and teeth of children from contaminated settings in which there are still children being exposed would be of benefit for prompt intervention directed at protecting currently exposed infants and toddlers.

Uses and Limits of Empirical Data in Lead Exposure Model Evaluation

Predictive statistical or biokinetic models of lead exposure, like all model simulations of biologic phenomena, require evaluation for general validity and case-specific or application-specific accuracy. However, before empirical data can be employed in any quantitative way for assessing a model's value, there are several overarching questions about model evaluation (validation and calibration) that help guide use of such empirical data.

How Good Is Good Enough?

One basic question is how predictively accurate a model of lead exposure or any other model simulating biologic behavior has to be to be acceptable for some application. Such a question is logically subsumed under an allied one: What is the purpose to which the model is being put?

If the model user seeks predictive estimates within a rather broad range and/ or is using predictive data as but one element in a large cluster of criteria for risk management of a site, such as decisions about a Superfund site, the required level of agreement between empirical data and model outputs may not be very high. If predictive data are being sought with reference
to some finite threshold value in permissible lead exposure or some cutoff level in lead exposure distributions, then model performance may be more rigorously evaluated. A key issue, therefore, is the required level of prediction performance for some particular application. For example, is the U.S. EPA's IEUBK model reliable for evaluating child lead exposures at Superfund sites?

A related qualifier is the extent to which some particular model for some site- or scenario-specific use is flexible for multiple uses by the regulator or risk manager. For example, biokinetic models such as the U.S. EPA's IEUBK model for children (4-7) or the O'Flaherty PBPK model for children and adults (9,10) theoretically permit assessment of the results of altered land uses, altered population demographics, or impact of lead remedial actions in future years on body lead burdens indexed by PbB. Such applications should not require recalibration with empirical data for every such use, if a baseline calibration has been done. One can also use these models for reconstructing lead exposures at remote time points. Mushak (27) used the U.S. EPA's IEUBK model to estimate the typical mean PbB of preindustrial children, based on archaeological and uncontaminated environmental lead data.

**Uses and Limits of Empirical Data in U.S. EPA's IEUBK Model Evaluation**

The balance of this article deals with the uses and limits of empirical data in the evaluation of biokinetic models of lead exposure, particularly the U.S. EPA's biokinetic model for childhood lead exposure. Certain parameters are of special interest with reference to the U.S. EPA's biokinetic model. First, there is the form of the predicted exposure output, i.e., the predicted or estimated geometric mean PbB, generated distributions of PbB, and associated descriptive and inferential statistics. One therefore is first concerned with how reliable the model's PbB predictive estimates are, what factors affect this output, and what factors may explain differences between the modeling results and empirical PbB data used for comparative evaluation.

Use of these factors and their limits for validating and calibrating lead exposure model predictions comprise a significant section of this article. They constitute a rapidly evolving area of research, an area of research that has only been sketchily evaluated so far and therefore is still poorly understood by many in the lead research community. Therefore, evaluation of these developments must be done at a higher level of detail than given to earlier topics and sections in this article. This includes the various facets of lead bioavailability.

One must first establish that PbB datasets from surveys to be used for evaluation of the IEUBK model are themselves reasonably reliable, and some of the means or criteria for doing this were presented earlier. One should also view evaluation of this or any other model with reference to some intended application. This is consistent with the administrative and regulatory history of the IEUBK model's scientific review by, among others, the Science Advisory Board (60,61). So far, the model has been technically approved for use in risk assessments associated with stationary lead emission sources (60) and Superfund waste site lead in potentially exposed communities (61).

Generally, biokinetic model evaluation consists of both a validation step and a calibration stage. The former refers to establishing the overall computational soundness and the plausibility of the biological simulations in the model. The latter addresses adjustments or fine-tuning of a generally valid and applicable model using reference data, typically from on-site measurements. The general assessment of the IEUBK model has been carried out by U.S. EPA's Technical Review Workshop for Lead (6) and model evaluation includes documentation of the model's scientific basis, code verification, and representative empirical comparisons.

Efforts have been made to compare the predictive accuracy of the IEUBK model at extractive industry sites with single, unstructured PbB surveys (see above). These PbB surveys are themselves potentially problematic, and must be reasonably shown to be reliable before they can serve as a means to calibrate or otherwise assess modeling data. What should be the level of calibration? Any model that must always be fine tuned with a single set of current conditions ascribes intrinsically little value to the reliability of that model across the whole spectrum of its likely uses. A major use of the U.S. EPA IEUBK model for risk assessors and risk managers is prediction of PbB data when detailed site characterizations by other means are not possible and when there is need to simulate future or alternative exposure risk scenarios with changes in environmental lead concentrations or affected populations. These include future changes in contaminated land uses and demographic or socioeconomic changes in the affected populations.

**Evaluation of Biokinetic Model Input Parameters**

Any disagreement between biokinetically predicted PbB results and accurate PbB measurements indicates that there are discordances in input parameters (assuming basic computational soundness). The U.S. EPA IEUBK model contains a number of input steps where uncertainty and variability can arise. Two are of significant importance and are discussed in detail. They are a) the bioavailability or uptake rate of lead from media directly relevant to infants, toddlers and older children, and b) the amount of lead-contaminated medium that is ingested.

**Bioavailability of Lead in Human Intake Media**

Bioavailability of lead in a toxicologic context is defined as the rate and extent of lead absorption or uptake to the central body compartment (bloodstream) from receiving body compartments of the lung and gut via the exposure pathways of inhalation and ingestion (12,62). Because childhood lead exposure is the focus of the IEUBK model, bioavailability of lead from the gastrointestinal tract in this highest-risk age band is of particular interest.

There are a number of general host and external factors that govern lead bioavailability in human populations, and especially preschool children (1,4,5,12,16, 24-27).

Infants and older children absorb lead at a higher rate than do adults in terms of lead uptake rate and in terms of intake and uptake per unit body measure, e.g., body mass or body surface area. In addition, preschoolers, especially older infants and toddlers, readily explore their environment orally, i.e., they ingest lead-contaminated materials via hand contamination and mouthing their fingers and/or direct ingestion of dusts, soils, or other contaminated materials. An extreme form of this behavior is pica, and pica for soil is called geophagia. These behavioral and developmental physiologic characteristics have been the subject of various studies over the last several decades.

Studies with human infants show that lead uptake from infant diets is on the order of 50% (63,64). A small group of mixed-age children studied by Alexander et al. (65) indicate that similar lead uptake rates may persist to later years, but the small
sample size and the wide age spread (to 8 years of age) makes this figure tenuous for older children. Human adults typically absorb lead from ordinary diets in the amount of 10 to 15% of the dietary lead intake. With fasting conditions, adults can show higher uptake rates, paralleling those of infants at ~50% or higher. This demonstrates that intrinsic gut changes can be less a factor than presence or absence of dietary factors and their interaction with lead, e.g., the lead–calcium interaction (66–69).

Experimental animal studies generally support a much higher uptake of lead in the very young child compared to adults, and these studies are critical to the development and use of animal models of lead bioavailability for infants and toddlers. Studies with nursing rodents indicate that uptake rates are quite high compared to adults or juveniles (70–72). At weaning, however, lead uptake and retention rates abruptly and steeply decline to those of the adult. The infant monkey (73) absorbs much more lead, about 50%, than does the juvenile or adult monkey (74). Kierski (75) showed that the weaning rabbit, 5 weeks old, absorbs twice as much lead from ingested soil as does the older rabbit. The juvenile swine, interestingly, may be farther along developmentally than the human infant but still appears to absorb lead at relatively high rates from dosed animal feeds or diet augmented with various leaded soils (76–82).

Persistence of the elevated lead uptake rate through early childhood is still the subject of some uncertainty. Infants up to 24 months old absorb lead at about 50% of intake. There is little conclusive evidence that human infants aging to toddlers of 2 to 4 years undergo specific gut changes that would result in significantly lower uptake of lead in the toddler stage. Diet-related changes, rather than alterations in gut uptake, may play a role in altering uptake, however. Infants have high metabolic requirements for essential elements but do so toddlers, owing to explosive growth requirements and the attending need to offset known common deficiencies in essential elements such as calcium and iron during the toddler years. It is well known that deficiencies in iron and calcium enhance lead uptake (24,69,83,84). O’Flaherty (10) assumed for PBPK modeling purposes that the 5-year-old child has a lead uptake rate from diet of half that of younger children (25%).

Epidemiologic data on age versus PbB relationships in children in different lead exposure settings are consistent with persistence of a relatively high lead absorption rate in the gut from late infancy (24 months) to 3 years of age (36–47 months), and a discernible lower uptake rate at 4 and 5 years of age. The longitudinal study of low-level lead effects in children in the lead smelter community of Port Pirie, Australia, by Tong et al. (85) supports this statement. This paper tabulated geometric mean (GM) PbB values for the child cohort from prenatal to the 11- to 13-year age band. Such data indicate that the GM PbB at the end of infancy (24 months) when oral exploration, mouthing activity, and ingestion of dust and soil is ongoing, and the GM at 3 years of age when such mouthing activity is still largely continuing but tapering off somewhat, differ by only about 10%. Such modest change, attributable in part to some decline in mouthing activity, suggests that the 3-year-old (36–47 months of age) in the Port Pirie study was absorbing lead at about the same rate as the late infant. Data from Tong et al. also show that the 4- and 5-year-olds have a PbB GM about 30% less than the 24-month-old infants. A significant part of this decline is arguably the cessation of mouthing activity and it is likely that the reduction cleanly attributable to reduced uptake rate between these age bands is comparatively much less than 30%. The O’Flaherty assumption (10) of a 50% relative decline in uptake rate from infancy to the 5-year-old age band, 50 to 25%, may be an underestimate of persisting uptake rate. As the Australian study used a large number of cohort subjects (n = 368–372), a careful, longitudinal study design, and a stable, multidisciplinary research program, their results merit serious attention.

Mahaffey et al. (86) reported preliminary data suggesting that lead uptake rate in 6-year-old children approaches the adult value. These findings are based on only a few subjects, however, and require further research. In overview, if we assume that attenuation of lead uptake in the child’s gut is underway after about 4 years of age, and reduced uptake is quite discernible in 6-year-old children, then the older child of 7 and 8 years of age probably resembles the adult with regard to any intrinsic versus dietary differences in lead absorption via the gut.

Approaches to Childhood Lead Bioavailability from Other Media

Although lead uptake rates from diet and water in the preschool child are assumed to be greater than for the adult under the conditions indicated above, there is growing interest in the extent of lead uptake, i.e., bioavailability, from other media, such as those generated through various industrial or waste disposal practices. This is especially so where such media exposures occur as part of various litigatory and regulatory actions dictated by Superfund and related statutory requirements. Uptake of lead from such media also are at issue in modeling lead exposure at sites, especially modeling with the U.S. EPA IEUBK model.

Bioavailability of lead in various media can be quantified in two ways, as absolute bioavailability or relative bioavailability. Absolute bioavailability is typically determined as the percent or fractional uptake of lead from some ingested medium relative to an injected dose, both being quantitated typically by area-under-the-curve (AUC) techniques (62). The injected dose is assumed to represent 100% uptake. Relative bioavailability is taken as the ratio of lead uptake from some ingested medium relative to a reference ingested dose of a soluble lead salt, typically the acetate, unaffected by containment in some formulary or geochemical matrix (12,62). Relative bioavailability is only useful if this parameter, determined in an animal species, is quantitatively comparable with young children ingesting lead from such media as soil, compared to children ingesting soluble lead from tapwater or diet. The experimental surrogate for diet and water lead is typically lead acetate.

There are various approaches by which one can empirically evaluate lead bioavailability as an input parameter to biokinetic models, including the U.S. EPA IEUBK model. The integrated expression of bioavailability in tandem with other parameters is the measured PbB level, assuming this parameter is without serious error and problematic interpretation. Various factors contribute to PbB variability. These include the amount of material ingested, behavioral interactions of the child and the environment, and lead sources beyond those being evaluated. A specific, uncluttered assessment of bioavailability is therefore often preferred. Because studies in children are highly restricted, the options are data from animal models of bioavailability under controlled dosing conditions or in vitro simulations of in vivo bioavailability using benchtop, nonbiological procedures.

Experimental Animal Models of Lead Bioavailability in the Preschool Child

Several animal test systems of lead bioavailability have been recently described,
differing in the choice of test animal species, the lead-containing matrix, and
dosing protocols. By and large, bioavailability of lead from the diverse media administered
to animal models of child lead bioavailability was quantified in terms of PbB concentra-
tions, rather than lead in other body compartments. Blood is the vehicle (via PbP) by which the toxic dose of lead is
delivered to target organs of lead in the fetus of pregnant women and particularly the
brain of the developing infant and toddler. This measure also more temporally tracks
recent or ongoing uptake, and bioavailability is commonly understood to be a more-
or-less real time process. Equally important, PbB has been established as the dose part of
the many dose–response relationships for the various toxic effects of lead. Other accu-
mulating tissue lead burdens, e.g., PbBone, are not readily linked to, or universally
accepted for, dose–response relationships in exposed human populations. Several investi-
gators have measured lead in animal body media other than blood (87).

Choice of Experimental Animal. The choice of test animal for lead bioavailability
in the young child should be one that replicates or simulates as closely as possible the
various anatomic, physiologic, developmental, and behavioral characteristics of the
lead-exposed infant or toddler. As noted by Barnes and Dourson (88), the U.S. EPA employs this rationale within a
policy that states that where data from sev-
eral animal systems are available, identification
should first be made of “...the animal model most relevant to humans, based on the most defensible biological rationale.”

Some choices as test animal models of childhood lead bioavailability are clearly
much better than others.

Gastrointestinal (GI) anatomy, physiology, and developmental stage are signifi-
cant determinants of lead uptake in test animals and humans. Those animal test
species whose GI tracts resemble as closely as possible the GI tract of the young child
are therefore preferable for testing. Weis and Lavelle (62) reviewed the relative
appropriateness of animal model selections for human lead bioavailability. They
noted that rodents and rabbits have the
anatomy of the young child, and thus differ in terms of increased absorption
of lead seen in the young child versus the
older child or the adult.

Rodents and rabbits evolved to process
plant material and this requires continuous
feeding as a behavior to sustain microbiotic
processing of cellulosic material (62). This
constant feeding behavior also can sustain a
different pH than basal levels in the young
cell).

Gastric anatomy in the rodent and
rabbit matches the feeding behavior and
the processing of a cellulose diet, and differs
greatly from that in other animal species and
the human child in terms of anatomical
configuration and the density of gastric
acid-secreting cells. In the rodent, Weis and
LaVelle (62) noted that there is a very small
proportional stomach surface area associated
with acid secretion when compared to the
human stomach. Lead uptake occurs in cer-
tain segments of the small intestine (12).

The relative efficiency of the gut in various
animal species to absorb lead, compared to a
measure of potential lead intake rate such as
body surface area, would be a useful predic-
tor for enteric lead uptake rates (61,89).

Weis and LaVelle (62) showed a much
greater ratio of gut absorptive surface to
body surface area for the human than for the
tat. This value is 108 for humans, com-
pared to 22 in the rat, about one-fifth the
ratio. This indicates that the relative effi-
ciency of lead uptake is 5-fold higher in
humans than in rats.

Rats and rabbits engage in coprophagy,
stool, which can alter bioavail-
ability in several ways. Such recycling means
multimodal uptake opportunities on one
hand, but also microbiotically transformed
material that may bind lead differen-
tly on the other. One can use fasted rats and
to attempt to minimize the constant
feeding. Coprophagy can be difficult to
to control even if appropriate cages are used.

Rodents and rabbits, as poor animal
models for infant and toddler lead bioavail-
ability, would be expected to show lower
absolutes and relative lead bioavailability
than would be expected for the same mate-
rial in the human infant and toddler. Infants
and toddlers get their principal nourishment via their major meals at inter-
mittent or scheduled feedings by their care-
givers, have a greatly different gastric
physiology and anatomy than the rodent
and the rat, and have other differences
noted above (12,62,90).

Despite these intrinsic problems with
rats and rabbits, various investigators have
still used these test species and have stated
reasons for their choices, reasons which
merit comment. For example, Freeman et
The authors were careful to note that carcass total label and label concentrations decreased as soil fraction increased, but this was due to intake differences of the mixed feed. When the radiolabeled content was normalized for intake differences, no statistically significant effect of soil on lead uptake was seen. Absence of an effect of soil on lead uptake was reported by Dacre and Ter Haar (87) in an earlier study of lead-in-soil bioavailability using adult rats. Soils fed for 30 or 90 days and that contained lead associated with either atmospheric fallout onto soil from auto exhaust lead or paint lead deposition were compared to equivalent diet loading with lead acetate without soil. PbB levels among the three groups were statistically indistinguishable at 30 days; at 90 days, PbB for the soluble lead group was not given, but the remaining two 90-day groups were not statistically distinguishable.

Lead incorporated into geochemical matrices has been reported to have lower bioavailability when added to diet of rats, compared to acetate and other relatively simple lead species (91,95,96). In the study of Dieter et al. (96), using 6- to 7-week-old rats, the geochemical lead was contained in recently generated Alaskan ore concentrate. Concentration lead species were the acetate, oxide, and sulfide. At a diet dosing of 100 ppm lead in all forms, the lead ore PbB group was about 10% that of the lead acetate group. Lead sulfide also had less bioavailability than the acetate and oxide. Freeman and co-workers (91,93,95) studied soil lead bioavailability in variably aged rats, with the lead-containing materials including mining wastes. Both absolute bioavailability (95) and relative bioavailability, as a ratio with soluble lead acetate reference (91), were tested, and were much lower in soil-entrained lead than when soluble lead salt was used. The absolute bioavailability of lead salt indexed as PbB was reported (95) as about 6-fold higher than soil-encased lead—15% versus 2.7%. Freeman et al. (93) also reported that residential soil-encased lead from a Colorado extractive industry-impacted community showed significantly less bioavailability than soluble lead as the acetate salt.

Several studies have described the use of rabbits in assessing lead bioavailability from mining waste samples, despite the considerable caveats about these species' use in such investigations. The reports of Ruby et al. (97) and Davis et al. (98) describe feeding studies using 3-month-old New Zealand white rabbits and mining waste material acutely administered as a large, single bolus. Ruby et al. (96) included time-serial PbB measurements and the lead content of various GI tract fractions isolated over time, with the animals being dosed to provide information on bioaccessibility, a recently coined term for extent of lead solubilization from some medium into human or animal gastric fluid, or simulations thereof. This term has not yet been generally accepted by the toxicology community nor fully characterized as to validity. The term provides no direct measure of bioavailability, but is linked to it. Ruby et al. (97) reported that the measured soluble fraction of mine waste bolus lead in rabbit GI tract is markedly lower than that for lead given as the acetate salt. This study has been widely cited by the authors and others as a reliable animal model of lead bioavailability from extractive-industry wastes and it is important that the study be examined carefully and in some detail.

There are quite a few technical and interpretive problems with Ruby et al. (97) that greatly limit applicability of their results for bioavailability conclusions applicable to humans. This report does provide information consistent with the broad experimental caveat that rabbits are poor animal models of lead bioavailability in preschool children and poor animal calibrators for subsequent in vitro screening of geochemical lead media. Flaws include incomplete and incorrect time-point studies of lead uptake, mine waste groups being tested differently than the lead acetate group, and both being evaluated as isolated lead concentration points and as concentration ratios to the reference data point, rather than use of the preferred, universally employed technique of AUC measurements. The AUC method would be required because lead uptake to blood from the mine waste material was rising significantly throughout the testing time (36 hr) and likely beyond it. At the end of 36 hr, waste material-dosed rabbits showed PbB content 4-fold higher than at 1 hr, the sole testing point for the reference acetate dose. These authors used these incorrectly obtained ratios to calculate in vivo solubilization of lead in the rabbit gut and from these estimates, proportional bioavailability as well. One cannot retrospectively attempt AUC calculations with these published data and reestimate solubilization and relative uptake, as only one data point, at 1 hr, exists for the critical acetate reference group.

Additional areas of testing and interpretation difficulty with Ruby et al. (97) include a) a mass balance analysis carried out by the present author that showed that much of the starting lead dose administered to the various groups cannot be accounted for by any biokinetically likely means, b) unacceptably low solubility of lead acetate in the stomach of these rabbits, and c) use of a swamping bolus of lead-containing material that is qualitatively and quantitatively irrelevant to typical children's soil/dust ingestion behaviors.

The present author's mass balance analysis for lead in the Ruby et al. (96) rabbit study, using Table III of Ruby et al., showed that the fraction of starting lead dose appearing in rabbit GI tract contents, i.e., soluble and solid fractions of lumen contents, summed over stomach, small intestine, and large intestine, is significantly less than the dosing level, and the shortfall occurs over the 6 hr of complete necropsy-group testing. At 3 hr, 60%, and at 6 hr, 91% of the 16.4-mg starting dose cannot be accounted for in collected GI tract contents. These declines occur with increases in total PbB content. The 1-hr time point for lead acetate shows 60% unaccounted for. Over these short time points, lead loss via fecal excretion would not account for the shortfall. It is plausible that only partial collection of GI tract contents occurred or that much of the missing lead had been transported to intestinal mucosa epithelium. If the latter, the resulting high ratio of enteric tissue lead to reported PbB would be extremely high, relative to what one typically sees in such distribution data. The time course of lead uptake in these rabbits was apparently far from complete, and would likely produce a much higher estimate of lead bioavailability with a longer testing time frame. Ruby et al. (97) did not analyze GI tract tissue, nor internal organs including bone, for lead levels. Deposition of the missing lead in internal organs would show uptake, but would arguably require higher PbB content than that reported by these authors. Table III of Ruby et al. (97) shows only 37% solubility for the soluble lead species employed, the acetate salt. This percentage is half that for the companion in vitro simulation and about one-third of expected 100% solubility. The authors suggest lead binding to retained animal chow, but the data in their Figure 2 do not support this assumption.

Finally, the Ruby et al. (97) study uses a mass of matrix, 4.2 g (2-g lead sample/kg,
2.1 kg weight) given as a single dose, a route of dosing that is totally inappropriate for any comparisons with childhood daily ingestion rates, as described in a later section. Although Ruby et al. based this administration rate on a 10-g soil intake in a pica child, this quantity for a pica child is itself quite suspect. Furthermore, the typical child engaging in mouthing activity is only ingesting 100 to 200 mg soil/dust, and doing so over the entire play period as noted later.

The above reanalysis indicates that lead uptake from the mining waste materials in the rabbits used in Ruby et al. may have been more than their reported data would indicate. In addition, use of the rabbit model by Ruby et al. (97) for calibrating an in vitro approach also described in that paper is untenable for application to child lead bioavailability from these materials.

Kierski (75) showed that soil Pb is relatively less bioavailable than lead acetate in 5-week-old rabbits when large amounts of soil are given, but when soil quantities more relevant to child intakes are used, the relative absorption increased to 55 to 90% of the reference acetate uptake.

Swine appear to be much better test surrogates for lead bioavailability from geochemical substances for the infant and toddler general anatomic, physiologic, and behavioral grounds, in contrast to the many problems with use of rats and rabbits. A number of studies have been carried out by various academic researchers and U.S. EPA Region VIII toxicologists using weanling swine. U.S. EPA Region VIII includes Utah, Montana, North Dakota, South Dakota, Colorado, and Wyoming, all states with concerns about potential exposures to mining wastes. Relative and absolute bioavailability of lead in a geostatistically broad range of substances associated with extractive industry waste sites can be demonstrated and in many cases can be significant when using the weanling swine model (79–82,90,99). Studies with the young swine have involved both acute and subacute dosings, detailed biokinetic and toxicologic analyses, and provision made for availability of all the original data to others (79–82). 

Table 1 summarizes absolute and relative bioavailability of lead in the young swine model of these investigators as reported in Casteel et al. (79–82,90) along with rat and rabbit data described earlier. The percentages for the young swine were calculated by the authors mainly from PbB but with some limited weighting for other tissues. As noted above, PbB is the most practical and easily comprehended index for lead bioavailability. These reports cover four different smelter and mixed extractive industry waste sites in the United States. The range of sample types studied by Region VIII toxicologists and their colleagues are geostatistically more representative of the expected significant sample heterogeneity at sites of this type than the limited samplings described in other studies.

Relative and absolute bioavailability were both calculated. Relative bioavailability is calculated relative to a soluble lead salt and absolute bioavailability is calculated assuming 50% uptake for soluble lead in children’s diets. Use of the 50% figure from infants and toddlers ingesting soluble lead in their diet for extrapolation to the young swine assumes that the uptake rate is similar across species.

Table 1 shows that for a range of different Superfund site lead-contaminated samples, the absolute bioavailability of lead in young swine ranges from 27 to 40%. These values are comparable to the default lead bioavailability value for soil and dust used in the U.S. EPA IEUBK model (30%). The Murray, Utah, slag sample bioavailability value of 27% is consistent with its micromineralogy showing that proportionately more of the lead species in the sample is in the form of nonvitreous lead species, including lead oxide. The oxide would have higher bioavailability. This particular finding with slag makes it clear one cannot draw general conclusions about likely lead bioavailability by the superficiality of general classification, e.g., slags, without tandem animal and mineralogical or chemical speciation assessment.

The study design for the swine model entails 15-day dosing, which corresponds to a pre-steady-state dosing regimen but a time period adequate to establish relative bioavailability using soluble lead dosing in one of the groups. Half-doses were administered at two times daily, to produce less of a bolus effect and to more closely resemble intermittent child oral activity patterns. Amounts of lead-containing matrix, e.g., soils, in the daily dose were administered to provide multiple dosing levels per kilogram animal weight. In the

| Test species | Sample source | % Bioavailability |
|--------------|---------------|-------------------|
| Weanling swine | Aspen, Colorado, residential soil or waste pile | 31 (soil) 30 (waste) 58 56 |
| Weanling swine | Palmerton, Pennsylvania, soil samples | 34 (soil 2) 27 (soil 4) 67 54 |
| Weanling swine | Murray, Utah, slag, soil composites | 36 (soil) 27 (slag) 71 53 |
| Weanling swine | Jasper, Missouri, three smoker sites | 29 (high smoker) 40 (high mill) 79 80 |
| Rabbits | Five roadside soils with Pb | — 50–67 90–95 |
| (5 weeks old) | — | — | Kierski (75) |
| Rats | Butte, Montana, mine waste | — 20 |
| Rats | Butte, Montana, mine waste | 3 — |
| Rats | Leadville, Colorado, NPL site soils | Pb acetate 14 — |
| | High Pb acetate + control soils | — — |
| Rabbits | Mine waste | 1–6 10 |

*Relative to Pb acetate.
1997 report of Casteel et al. (90), 8-kg young swine received doses of 75, 225, and 675 μg/kg bw.

Comparison of relative bioavailabilities, i.e., soil or other matrix lead uptake versus the acetate salt as soluble reference across the three species in Table 1 indicates that not only do absolute bioavailabilities differ in going from rats and rabbits to swine, but do relative bioavailabilities, where the acetate is used.

Other factors come into play besides surrogate animal species selection in studies of lead bioavailability, particularly as these factors relate to calibrating the U.S. EPA IEUBK model. The various animal model studies published so far differ in the developmental stage of the animal used, the dosing regimens, and the geostatistical representativeness of the sample types analyzed.

Selection of a Similar Developmental Stage, Animal versus Child. The infant and toddler are at an early stage of physiologic and physical development and absorb lead at a higher rate than adults. Consequently, the validity of any surrogate animal model for human lead bioavailability requires matching developmental stages of the test animals as closely as possible to those for human infants and toddlers. Lead bioavailability data derived from adult animals with their much lower lead uptake compared to young animals or human infants and toddlers may greatly underestimate extrapolated lead uptake from the medium, certainly for absolute bioavailabilities but perhaps for relative bioavailabilities as well. Rats show high lead uptake and retention, about 50%, in the nursing stage, but revert rapidly to adult lead uptake rates at weaning. Forbes and Reina (71) recorded lead uptake rate versus rat pup age at multiple time points and showed that rats are close to the adult uptake rate several days after weaning. This means that use of rats about 26 days or older results in adult lead uptake data. Kierski (75) showed that 5-week-old weanlings had higher lead uptake than older rabbits.

The bioavailability studies of Freeman et al. (91,93,95) using the rat involved animals whose GI tracts were essentially mature, i.e., associated with much lower lead uptake. Animals were either 49 to 56 days old or were 28 days old at the start of the feeding studies, all beyond weaning (about 26 days). The 28-day-old animals would be at, or arrive at, adult uptake rates shortly into the dosing regimen, up to 44 days. Uptake rates for lead in any medium in these studies would be appropriate only for adult lead uptake rates, not those of infants and toddlers, and direct comparisons would underestimate those uptakes that would be operative in children.

Young swine used in the studies described above (90) were at the juvenile stage, 5 to 6 weeks old. In swine, weaning occurs at about 3 weeks. The somatic maturation process in the swine is much slower (62) and puberty is reached much later on the absolute time scale than in rats. This may include physiologic maturation of the GI tract. Although the precise quantitative relationship of young swine development to enteric lead uptake has not been determined, we expect that the lead uptake rates in those weanling swine studied so far would be greater than the adult uptake rates Freeman et al. encountered for lead bioavailability. With rabbits, Ruby et al. (97) were using postweanling animals with reduced lead uptake compared to rabbits at 5 weeks or younger as studied by Kierski (75).

Child Soil/Dust Ingestion Rates versus Animal Dosing Protocols. The typical late infant or toddler, i.e., one who does not display pica, ingests very small quantities of soils and dusts via normal mouthing activity and does so through the course of a day, based on data noted below. Cumulative daily ingestion of soil and dust in such children is about 100 mg or higher, based on various sources (e.g., 12,26,100 including more recent studies of Calabrese and Stanek (101,102) cited below. The U.S. EPA IEUBK model uses a figure of 135 mg/day for late infants and toddlers. Such gustatory behavior assures that there is not a huge bolus of lead or soil ingested at one time. This also means that there is no overwhelming of the typical biochemical and physiologic mechanisms that are brought into play to deal with ingested material. This enteric apparatus includes basal acidity of the stomach, adequate additional acid secretion via stimulation, lead chelating or other binding processes, all working to leach lead from dust and soil particles during their flow in milligram amounts through the GI tract. Brunkekeef et al. (100) noted the typical infant and toddler oral exploratory behaviors that support repetitive mouthing behaviors that are consistent with continuous ingestion by these children of small amounts of dust and soil over the course of a play period, eventually adding up to a daily intake of 100 to 200 mg. Child hand-wipe lead analyses of Que Hee et al. (13), Duggan et al. (14), and the three participants in the U.S. EPA soil lead abatement demonstration project, as summarized in the U.S. EPA integrated report of this project (18), report finding relatively small amounts of lead, and associated dust and soil containing that lead, on children's hands when tested at typical play times. Therefore, only small amounts of dust and soil carrying these small lead quantities are present on children's hands at one time. These data support continuous consumption of dust and soil by children through normal mouthing activity, and do not support any likelihood that children ingest 100 to 200 mg of soil/dust only as a single bolus during a typical day at play.

The amounts of soil and dust ingested by children daily is discussed and studied in some recent systematic estimates of ingestion by Stanek and Calabrese (101) and Calabrese and Stanek (102). Calabrese and Stanek (102) summarized their quantitative estimates of soil ingestion by children as determined using different elemental tracers. These workers adjusted intake data from each tracer for tracer-specific errors in mean estimates from daily or weekly observations. The combined tracer mean is 139 mg/day, almost midway between the 100 to 200 mg range commonly taken as daily child dust and soil intake. The value is virtually identical to the default values for late infant and toddler age bands in the U.S. EPA biokinetic model.

Pica is the extreme, abnormal expression of normal child mouthing activity and entails ingestion of both larger amounts of soils and dusts than normal and the ingestion of a wide range of other nonfood items (23). The excessive ingestion of soil in children with pica, based on available literature, is not known to occur only once daily, as implied in some of the studies noted earlier, nor would we expect it to. Rather, such elevated ingestion continues throughout the child's waking day. Therefore, pica children defined as such by either clinical or epidemiologic criteria would arguably not have their GI tracts and any associated enteric processes overwhelmed by single, large soil boluses. By contrast, the Ruby et al. rabbit study (97) and the Freeman et al. studies in rats (91) employed large, swamping boluses, relative to animal body weight and relative to the character of children's soil ingestion. These workers used a soil pica intake of 10 g in children for developing their rat and rabbit soil lead dosing regimens, respectively, citing information in Kimbrough et al. (103) and Calabrese et al. (104). Kimbrough et al. (103) do not identify 10 g as an actual pica level, and this
simple solubility do not accord well with overall human lead uptake and subsequent lead poisoning. Lead sulfide, for example, has low solubility but when used in preparations by humans, lead poisoning can occur, including when used as an eye cosmetic for Indian and Middle Eastern women and children (12).

Limitations in in vitro approaches are many. First, the human GI tract is difficult to closely simulate as to lead uptake for thermodynamic, biochemical, and physiochemical reasons. The gut is a thermodynamically open system, where uptake by intestinal microvilli during gut transit produces equilibria shifts, with such shifts throughout the gut mixtures leading to ongoing dissolution. No evidence exists to show such equilibria shifts would not establish themselves in animal systems, and in fact would be expected to occur very rapidly. Current published in vitro test systems, by contrast, are still closed systems thermodynamically. In vitro systems test solubility or bioaccessibility, but lead can enter the gut wall in ways other than initial dissolution, e.g., in micelles and as fine suspensions. This is of special concern in the infant. Use of biochemical mixtures of, e.g., organic acids in isolation from the stomach and intestines creates problems as to their effectiveness without the full in vivo milieu.

Mindful of these caveats, several investigators have attempted to provide a closer simulation of in vivo lead uptake processes for screening purposes by focusing on one parameter, simulated solubilization in the stomach and retention of the solubilized fraction during passage through portions of the small intestine where lead is known to be absorbed (12). Ruby et al. (97,106) have reported an in vitro model claimed to be validated with the rabbit (97) and some earlier rat data. The described problems with the rabbit study of Ruby et al. (97) for both in vivo and in vitro use raise serious questions about the in vitro test described.

The studies of Drexler and colleagues (107-110) entail a simulation of in vivo lead bioavailability data from numerous studies involving the young swine surrogate described above. Estimations of bioaccessibility are compared to the in vivo bioavailability data.

The relative and absolute estimates in the in vitro method generally accord in both absolute and relative solubilization percentage with a variety of selected extractive industry wastes from former milling, smelting, mining, and mixed sites.

This approach is still in development and additional testing is under way. The Drexler approach also has an evolving quick test version that entails trimming some of the experimental steps simulating intestinal uptake described by Drexler (110) in a workshop presentation on bioavailability held in December 1997. This variation on the Drexler method was summarized in comments made at this same workshop by Ruby (111). Considerable multilaboratory evaluation using many more representative samples will be required before in vitro screening is placed on a reliable footing regarding its predictive power for in vivo bioavailability.

**Summary and Conclusions**

Empirical data used in human lead exposure assessment are typically experimental or observational, i.e., epidemiologic in nature. Experimental studies have been carried out in diverse experimental animals and occasionally in human adults. Epidemiologic data have described effects of lead in occupationally exposed workers or toxic impacts in segments of the general population such as preschool children and fetuses through lead intakes by pregnant women. Lead exposures in humans can be potential or actual. The former is typified by environmental monitoring of the lead content of various media that serve as pathways for lead contact. Actual lead exposure is established by biologic monitoring, typically carried out by using either biomarkers of lead exposure such as PbB, or measurements of early effect. This paper emphasizes exposure biomarkers.

Each form of monitoring has its advantages and drawbacks, and these are examined in detail in this paper. For example, environmental testing reveals which of various possible lead sources actually provide significant exposures in a specific case. Lead sources provide the independent variable in inferential statistical techniques that link source to a biomarker such as PbB. Biologic monitoring of human lead exposure, e.g., PbB data, indicates the extent to which actual lead intake and uptake has occurred. Furthermore, such exposures can define some lead poisoning risk classification or can be employed as part of the data base for medical intervention or management of individual poisoning cases.

This paper presents the uses and limits of empirical data in the evaluation of predictive models of human lead exposure, mainly biokinetic or mechanistic models. These models generate PbB estimates that
offer the risk assessor or risk manager assistance in evaluating the nature and extent of lead's potential adverse impacts in exposed communities. A particular focus was the U.S. EPA IEUBK biokinetic model, used to estimate childhood lead exposure. This model is the most heavily validated and calibrated of the several biokinetic models now of interest in the lead risk assessment community. These models, when used correctly with appropriate empirical data inputs, additionally possess the flexibility to a) ascertain historic exposures via dose reconstruction from current environmental data, b) estimate results of some regulatory intervention, e.g., predicted PbB responses to soil lead abatement, and c) ascertain the consequences of future land use options in and around a lead-contaminated site, in terms of resulting PbB concentrations.

This paper points out, with supporting information, that one must be very careful in the use of measurement data for either evaluating or challenging the outputs, i.e., exposure estimates, of such predictive models. A number of specific points were made about potential pitfalls in the use of PbB levels either separate from or in tandem with modeling efforts. The paper noted various criteria that define more reliable forms of PbB data, for use in assessment and modeling community lead exposures: a) the use of serial versus single-shot PbB measurements; and, where serial measures are not feasible, b) the absence of temporal and structural artifacts that would reduce the reliability of PbB data, confounders that include public awareness and concerns of child caregivers that result in abrupt, transitory reduction in PbB; c) use of an appropriate biostatistical and epidemiologic design that does not obscure the prevalence of toxic lead exposures in those segments of the study population at particular risk; and d) PbB data that provide reliable and accurate links to those environmental lead sources that produce the significant lead exposures.

Predictive, biokinetic models of lead exposure in high-risk groups rarely come equipped with all the best selections for inputs and outputs for a particular site and require a certain level of site-specific information. One significant biokinetic component of any lead model input is the lead uptake rate or bioavailability, especially in the GI tract. This paper provides a fairly detailed discussion of bioavailability, a growing but often misunderstood or misused topic in the lead area. Discussion includes various approaches for determining this parameter in clean, physiologic terms, i.e., experimental animal models of lead bioavailability in human infants and toddlers. Such models avoid many of the problems of looking at human populations directly. The article shows that rodents and rabbits are not particularly good models of lead uptake in the infant and toddler, especially under conditions actually reported in published work. Published work using rats and rabbits produced data that are problematic on added grounds. Lead-dosing methods included those that have little relevance to the typical childhood oral lead exposures. To date, young swine appear to be the best test model.

Many simple, in vitro approaches have been reported over the years, in the quest to avoid the expense and complexity of in vivo testing. This paper presents some of the current approaches, but cautions that there are many limits to such approaches. This especially applies where simple gastric solubilization is deemed an adequate surrogate for lead bioavailability in humans. Current efforts do mark some improvement over crude solubility testing in simple aqueous solutions. Simplified, inexpensive screening approaches that do not reflect, or, worse, underestimate actual human bioavailability are not scientifically appropriate substitutes for valid animal models. Conceptually, comparison problems with in vitro versus in vivo approaches do not arise for those media in which lead is readily mobilizable, based on chemical speciation or micromineralogical grounds. They arise when one sees a low lead solubilization rate in an in vitro screening. Would the in vivo test of that same lead-containing substance show a similar rate or does the linkage become disconnected? A related question would be how many representative samples will provide an adequate statistical comfort level for universal use of in vitro testing at any and all sites.

Finally, lead bioavailability has to be put into quantitative toxicologic context. That is, one cannot equate lead bioavailability to a specific net toxic risk in isolation. This is simply because the total amount of lead entering the bloodstream from the GI tract per unit time is the product of bioavailability multiplied by lead concentration in some ingested substance. An intrinsic bioavailability of 100% for a lead species that is not present in the exposure medium does not result in lead poisoning or risk of lead poisoning. For media with variable lead content and in different geochemical/formulary forms, comparisons must be more closely drawn. A bioavailability of 10% for a lead species occurring at a concentration of 2000 ppm in 100 mg of ingested matrix is no less toxic than a lead species that is 100% bioavailable from 100 mg of a matrix having 200 ppm lead. Lead at an extremely low bioavailability of 1% is equally toxic under the above conditions at a lead concentration of 20,000 ppm.

There has been a simplistic tendency in some regulatory areas and the recent risk assessment literature to confuse comparative statements of lead bioavailability with net toxicologic rankings. For example, mining waste lead is held by some to always be less bioavailable than lead from urban street dust. That is partly true, and in certain situations. That is not equivalent to saying that mining waste is not toxic to children. It also does not say that there are no risks to children who come in contact with this material. The above trio of bioavailability comparisons voids this premise. The fallacy of the assumption can be understood when we consider that ore mill tailings and weathering smelter slags can contain lead at many thousands of parts per million. Children ingesting 100 to 200 mg of such material with 20,000 ppm lead and a low lead form bioavailability of 5% are still at high risk for lead poisoning.

REFERENCES AND NOTES

1. Mushak P. Biological monitoring of lead exposure in children: overview of selected biokinetic and toxicological issues. In: Lead Exposure and Child Development: An International Assessment (Smith M, Grant LD, Sors A, eds). Lancaster, UK: Kluwer Academic Press, 1989;129–145.
2. National Research Council. Risk Assessment in the Federal Government: Managing the Process. Washington:National Academy Press, 1983.
3. Angle CR, Marcus A, Cheng I-H, McIntire MS. Omaha childhood blood lead and environmental lead: a linear total exposure model. Environ Res 35:160–170 (1984).
4. U.S. EPA. Guidance Manual for the Integrated Exposure-uptake
Biokinetic Model for Lead in Children. EPA/540-R03/081. Washington: U.S. Environmental Protection Agency, 1994.
5. U.S. EPA. Technical Support Document: Parameters and Equations Used in the Integrated Exposure Uptake Biokinetic Model (v0.99d). EPA/540/R-94/040. Washington: U.S. Environmental Protection Agency, 1994.
6. U.S. EPA. Validation Strategy for the Integrated Exposure Uptake Biokinetic Model for Lead in Children. EPA 540-R94039. Cincinnati, OH: U.S. Environmental Protection Agency, 1994.
7. U.S. EPA. Technical Support Document. ECAO-CIN-757. Cincinnati, OH: U.S. Environmental Protection Agency, 1990.
8. Leggett RW. An age-specific kinetic model of lead metabolism in humans. Environ Health Perspect 101:598–616 (1993).
9. O’Flaherty EJ. Physiologically based models for bone-seeking elements. IV: Kinetics of lead disposition in humans. Toxicol and Environmental Chemistry 1993;14:143–152.
10. O’Flaherty EJ. Physiologically based models for bone-seeking elements. V: Lead absorption and disposition in childhood. Toxicol Appl Pharmacol 131:297–308 (1995).
11. U.S. EPA. Risk Assessment Guidance for Superfund. Vol I: Human Health Evaluation Manual, Part A. EPA/540/1–89/002. Washington: U.S. Environmental Protection Agency, 1992.
12. Mushak P. Gastrointestinal absorption of lead in children and adults: overview of biological and biophysico-chemical aspects. Chem Speciat Bioavail 3:87–104 (1991).
13. Que Hee SS, Peace B, Clark CS, Boyle JR. Evolution of efficient methods to sample lead sources, such as house dust and hand dust, in the homes of children. Am J Clin Nutr 53:784–798 (1991).
14. Duggan MJ, Inskip MJ, Rundle SA, Moorcroft JS. Lead in playground dust and on the hands of school children. Sci Total Environ 44:65–79 (1985).
15. U.S. EPA. Reducing Lead in Drinking Water: A Benefit Analysis. EPA 239/09–86/019. Washington: U.S. Environmental Protection Agency, 1986.
16. Mushak P. The monitoring of human lead exposure. In: Human Lead Exposure (Needleman HL, ed). Boca Raton, FL: CRC Press, 1992:45–64.
17. Milar CR, Mushak P. Lead-contaminated housedust: hazard, management, and decontamination. In: Proceedings of the Conference on Management of Increased Lead Absorption in Children: Management, Clinical, and Environmental Aspects (Chisolm JJ Jr, O’Hara DM, eds), Baltimore: Urban & Schwarzenberg, 1984:313–152.
18. U.S. EPA. Urban Soil Lead Abatement Demonstration Project: EPA Integrated Report. EPA 600/P–93/001A,F. Research Triangle Park, NC: U.S. Environmental Protection Agency, 1996.
19. National Research Council. Biological Markers in Pulmonary Toxicology. Washington: National Academy Press, 1989.
20. National Research Council. Biological Markers in Reproductive Toxicology. Washington: National Academy Press, 1989.
21. Mushak P. Unpublished data.
22. ATSDR. Toxicological Profile for Lead. Atlanta: Agency for Toxic Substances and Disease Registry, 1991.
23. CDC. Preventing Lead Poisoning in Young Children. A Statement by the Centers for Disease Control. Atlanta: Centers for Disease Control, 1991.
24. National Research Council. Measuring Lead Exposure in Infants, Children, and Other Sensitive Populations. Washington: National Academy Press, 1993.
25. U.S. EPA. Air Quality Criteria for Lead. 4 Vols. EPA 600/8–83/028bF, Research Triangle Park, NC: U.S. Environmental Protection Agency, 1986.
26. ATSDR. The Nature and Extent of Lead Poisoning in Children in the United States. Atlanta: Agency for Toxic Substances and Disease Registry, 1988.
27. Mushak P. New directions in the toxicokinetics of human lead exposure. Neurotoxicology 14:29–42 (1993).
28. Skerfving S. Biological monitoring of exposure to lead. In: Biological Monitoring of Toxic Metals, Lecture Series on Environmental Toxicity (Clarkson TW, Nordberg GF, Sager PR, eds). New York: Plenum Press, 1988:169–197.
29. Succop PE, O’Flaherty EJ, Bornschein RL, Clark CS, Krafft K, Hammond PB, Shukla R. A kinetic model for estimating changes in the concentration of lead in the blood of young children. In: International Conference on Heavy Metals in the Environment, Vol. 1, Lindberg SE, Hutchinson TC, (eds). Edinburgh, UK: CEP Consultants, 1987:289–291.
30. Angle CR, Manton WI, Stanek KL. Stable isotope identification of lead sources in preschool children—the Omaha Study. Clin Toxicol 33:657–662 (1995).
31. Austng BJ, Dolce JA, Fung HL. The effect of dose on the disposition of lead in rats after intravenous and oral administration. Toxicol Appl Pharmacol 61:48–57 (1981).
32. Austng BJ, Fung HL. Effect of in vitro lead transport across the rat small intestine. Toxicol Appl Pharmacol 61:38–47 (1981).
33. Chamberlain AC. Prediction of response of blood lead to airborne and dietary lead from volunteer experiments with lead isotopes. Proc R Soc London, Ser B224:149–182 (1985).
34. Flanagan PR, Chamberlain MJ, Valberg LS. The relationship between iron and lead absorption in humans. Am J Clin Nutr 36:823–829 (1982).
35. DeSilva PE. Determination of lead in plasma and its relationship to lead in erythrocytes. Br J Ind Med 38:209–217 (1981).
36. Manton WI, Cook JD. High accuracy (stable isotope dilution) measurements of lead in serum and cerebrospinal fluid. Br J Ind Med 41:313–319 (1984).
37. Grant LD, Kimmel CA, West GL, Martinez-Vargas CM, Howard JL. Chronic low-level lead toxicity in the rat: II. Effects on postnatal physical and behavioral development. Toxicol Appl Pharmacol 56:42–58 (1980).
38. Azar A, Trochimowicz HJ, Maxfield ME. Review of lead studies in animals carried out at the Haskell Laboratory: two year feeding study and response to hemorrhage study. In: Environmental Health Aspects of Lead: Proceedings of an International Symposium, 25–29 October, 1972, Amsterdam, the Netherlands (Barth D, Berlin A, Engel R, Recht P, Sneets J, eds). Luxembourg: European Commission of the Communities, 1973:199–210.
39. Mahaffey KR, Annest JL, Roberts J, Murphy RS. National estimates of blood lead levels: association with selected demographic and socioeconomic factors. N Engl J Med 307:573–579 (1982).
40. Brody DJ, Pirkle J, Kramer RA, Flegal KM, Matte TD, Gunter EW, Paschal DC. Blood level distributions in the US population: Phase 1 of the Third National Health and Nutrition Examination survey (NHANES III, 1988 to 1991). JAMA 272:277–283 (1994).
41. Bornschein RL, Clark CS, Grote KL, Peace B, Roda S, Succop PA. Soil lead-blood lead relationship in a former lead mining town. In: Proceedings of the Lead in Soil: Issues and Guidelines Monograph (Davies BE, Wixson BG, eds). Society for Geochemistry and Health. Northwood, UK: Science Reviews, 1989:149–160.
42. Bornschein R, Clark S, Pan W, Succop P. Midvale community lead study. Chem Speciat Bioavail 3:149–162 (1991).
43. Bornschein RL, Clark CS, Pan W. The Butte-Silver Bow County Environmental Health Lead Study. Final Report. Cincinnati, OH: Butte-Silver Bow County Health Department and University, 1992.
44. Goldman KD, Ettinger AS, Michaels MJ, Mays-Hardy D, Rhoads GG. Impact of health education on maternal knowledge of childhood lead poisoning and blood lead in young children. [Abstract]. Session 2292, Lead: New Jersey CLEAR Research Studies. The 124th Annual Meeting and Exposition, American Public Health Association. Am J Publ Health 86:P99 (1996).
45. Goldman K. Personal communication.
46. Dietrich KN, Succop PA, Berger OG, Hammond PB, Bornschein RL. Lead exposure and the cognitive development of urban preschool children: the Cincinnati lead study cohort at ages 5 years. Neurological Teratol 14:51–56 (1993).

47. Otto DA, Robinson S, Baumann S, Schroeder S, Mushak P, Kleinbaum D, Barton C, Boone L. Five-year follow-up study of children with low-to-moderate lead absorption: electrophysiologic evaluation. Environ Res 63:105–113 (1995).

48. Illinois Department of Public Health. Madison County Lead Exposure Study. Granite City, Illinois. Springfield, IL, Public Health, 1995.

49. Marcus AH. Statistical analysis of data from the Madison County Lead Study and implications for remediation of lead-contaminated soil. Attachment 4: Decision Document/Explanation of Significant Differences: NL Industries/Taracorp Site. Chicago: U.S. Environmental Protection Agency Region V, 1995. (Available from U.S. EPA Region V: Waste Management Division, Chicago, IL)

50. D. M. Johnson, W. G. Moore. Blood lead surveys of communities in proximity to lead-containing mill tailings. Am Ind Hyg Assoc J 56:384–393 (1995).

51. Heyworth F, Spickett J, Dick M, Margetts B, Armstrong B. Tailings from a lead mine and lead levels in school children. Med J Aust 2:232–234 (1981).

52. Everson J, Patterson CC. “Ultra-clean” isotope dilution/mass spectrometric analyses for lead in human blood plasma indicate that most reported values are artificially high. Clin Chem 26:1603–1607 (1980).

53. Raboinowitz MB, Leviton A, Bellinger D. Relationships between several blood lead levels and exfoliated tooth dentin lead levels: models of tooth lead kinetics. Calcif Tissue Int 53:338–341 (1993).

54. Greenough A. Kinetics of lead storage in teeth and bones. Arch Environ Health 37:224–230 (1982).

55. Rosen JF, Markowitz ME, Bijur PE, Jenks ST, Wielopolski L, Kalef-Ezra JA, Slatkin DN. Sequential measurements of bone lead content by L X-ray fluorescence in CaNa2-treated lead-tolerant children. Environ Health Perspect 93:271–277 (1991).

56. Hu H, Aro A, Romirzky A. Bone lead measured by X-ray fluorescence: epidemiological methods. Environ Health Perspect 103(Suppl 1):105–110 (1995).

57. Roels H, Konings J, Green S, Bradley D, Chettle D, Lauwers R. Time-integrated blood lead concentration is a valid surrogate for estimating the cumulative lead dose assessed by ribial lead measurement. Environ Res 69:75–82 (1995).

58. Blecker ML, McNeill FE, Lindgren KN, Masten VL, Ford DP. Relationship between bone lead and other indices of lead exposure in smelter workers. Toxicol Appl Pharmacol 133:178–188 (1995).

59. Gerhardsson L, Attwell R, Chettle DR, Englyst V, Lundstrom N-G, Nordberg GF, Nyhlin H, Scott MC, Todd AC. In vivo measurements of lead in bone in long-term exposed lead smelter workers. Arch Environ Health 48:141–156 (1993).

60. Science Advisory Board. Clean Air Scientific Advisory Committee (CASAC) Report: Review of the Lead NAAQS Exposure Analysis Methodology and Evaluation. EPA SAB-CASAC-89–018. Washington U.S. Environmental Protection Agency, April 1989.

61. Science Advisory Board. Indoor Air Quality and Total Human Exposure Committee. Review of the Uptake Biokinetic (UBK) Model for Lead. EPA SAB-IAQC–92. Washington: U.S. Environmental Protection Agency, 1992.

62. Weis C, Lavelle JM. Characteristics to consider when choosing a biokinetic model for the study of lead bioavailability. Chem Speciat Bioavail 3:113–119 (1991).

63. Ziegler EE, Edwards BB, Jensen RL, Mahaffey KR, Fomon SJ. Absorption and retention of lead by infants. Pediatr Res 12:29–34 (1978).

64. Ryu JE, Ziegler EE, Nelson SE, Fomon SJ. Dietary intake of lead and blood lead concentration in early infancy. Am J Dis Child 137:886–891 (1983).

65. Alexander FW, Delves HT, Clayton BE. The uptake and excretion by children of lead and other contaminants. In:

Proceedings of the International Symposium: Environmental Health Aspects of Lead (Barth D, Berlin A, Engel R, Recht P, Smeets J, eds). Luxembourg: Commission of the European Communities, 1973.

66. Raboinowitz MB, Kopple JD, Werthill G. Effect of food intake and fasting on gastrointestinal lead absorption in humans. Am J Clin Nutr 33:1784–1788 (1980).

67. Heard MJ, Chamberlain AC. Effect of minerals and food on uptake of lead from the gastrointestinal tract in humans. Hum Toxicol 1:411–415 (1982).

68. James HM, Hiltburn DM, Blair JE. Effects of meals and meal times on uptake of lead from the gastrointestinal tract of humans. Hum Toxicol 4:401–407 (1985).

69. Mushak P, Crocetti AF. Lead and nutrition. Part I: Biological interactions of lead with nutrients. Nutr Today 31:12–17 (1996).

70. Kostal K, Simonovic J, Pisonic M. Lead absorption from the intestine of newborn rats. Nature 233:564 (1971).

71. Forbes GB, Reina JC. Effect of age on gastrointestinal absorption (Fe, Sr, Pb) in the rat. J Nutr 102:647–652 (1972).

72. Keller CA, Doherty RA. Distribution and excretion of lead in young and adult female mice. Environ Res 21:217–228 (1980).

73. Munro IC, Willes RF, Truelove JF. Absorption and tissue distribution of inorganic lead in the developing infant monkey (Macaca irus). Toxicol Appl Pharmacol 33:128–129 (1975).

74. Driscoll JG, Marlar RJ, Allen JR. Metabolism of lead-210 in juvenile and adult rhesus monkeys (Macaca mulatta). Bull Environ Contam Toxicol 19:684–691 (1978).

75. Kierski MW. The oral bioavailability of soil-lead in the weanling rabbit. PhD Thesis. Minneapolis, MN:University of Minnesota. Diss Abs Int B. 53:2819–2820 (1992).

76. LaVelle JM, Poppenga RH, Thacker BJ, Glesy JP, Weis C, Othoudt R, Vandervoort C. Bioavailability of lead in mining wastes: an oral intubation study in young swine. Chem Speciat Bioavail 3:105–111 (1991).

77. Weis CP, Henningsen GM, Poppenga RH, Thacker BJ. Pharmacokinetics of lead in blood of immature swine following acute oral and intravenous exposure. Toxicologist 13:175 (1993).

78. Poppenga R, Weis C, Henningsen G, Thacker BJ. Features of an immature swine model for children to evaluate soil-lead absorption. Toxicologist 14:119 (1994).

79. Castell SW, Weis CP, Henningsen GM, Hoffman E, Brattin WJ, Hammon TL. Bioavailability of Lead in Soil Samples from the Jasper County, Missouri Superfund Site. Doc Contr No 04800-030-0161. Denver, CO:U.S. Environmental Protection Agency, 1996.

80. Castell SW, Brown LD, Dunsmore ME, Weis CP, Henningsen GM, Hoffman E, Brattin WJ, Hammon TL. Bioavailability of Lead in Soil Samples from the Murray Smelter Superfund Site. Doc Control No 04800-030-0163. Denver, CO:U.S. Environmental Protection Agency, 1996.

81. Castell SW, Brown LD, Dunsmore ME, Weis CP, Henningsen GM, Hoffman E, Brattin WJ, Hammon TL. Bioavailability of Lead in Soil Samples from the Smuggler Mountain NPL Site, Aspen, Colorado. Doc Control No 04800-030-0160. Denver, CO:U.S. Environmental Protection Agency, 1996.

82. Mahaffey KR, Gartside PS, Glueck CJ. Blood lead levels and dietary calcium intake in 1 to 11-year old children: the Second National Health and Nutrition Examination Survey, 1976–1980. Pediatrics 78:257–262 (1986).

83. Mushak P, Crocetti AF. Lead and nutrition. Part II: Some potential impacts of lead-nutrient interactions in US populations at risk. Nutr Today 31:115–122 (1996).

84. Tong S, Baghurst P, McMichael A, Sawyer M, Mudge J. Lifetime exposure to environmental lead and children’s intelligence at
11–13 years: the Port Pirie cohort study. Br Med J 312:1569–1575 (1996).

86. Freeman K, Jameson CW, Vidal M, Law A, Mitzon K, Smith A, Korsch M. Dietary lead intakes for mother-child pairs and relevance to pharmacokinetic models. Abstract No. 127. Society of Toxicology 37th Annual Meeting, 1–5 March 1998, Seattle, Washington. Toxicol Sci 42(Suppl 1-S):27 (1998).

87. Dacre JC, Ter Haar GL. Lead levels in tissue from rats fed soils containing lead. Arch Environ Contam Toxicol 16:111–119 (1988).

88. Finlayson BL, Fairey A, Sills T. Lead in birds: a review of the literature. Environ Sci Technol 26:461–468 (1992).

89. DeSesso JM, Mavis RD. Identification of critical biological parameters affecting gastrointestinal absorption. For Armstrong Medical Research Laboratory, Contract No. F33615-87-D015, Fairfax VA, The Mitre Corp., 1989.

90. Casteel SW, Cowart RP, Weis CP, Henningson GM, Hoffman E, Brattin WJ, Guzman RE, Starost MF, Payne JT, Stockham SL, et al. Bioavailability of lead to juvenile swine dosed with soil from the Smuggler Mountain NPL Site of Aspen, CO. Fundam Appl Toxicol 36:177–187 (1997).

91. Freeman GB, Johnson JD, Killinger JM, Liao SC, Feder PI, Davis AO, Ruby MV, Chaney RL, Love RJ, Bergstrom PD. Relative bioavailability of lead from mining waste soil in rats. Fundam Appl Toxicol 19:388–398 (1992).

92. Cotner CR, ed. SEGH Monograph: Proceedings of the Symposium on the Bioavailability and Dietary Exposure of Lead, September 1990, Chapel Hill, North Carolina. Chem Speciat Bioavail 3(3/4):188 pp (1991).

93. Freeman GB, Dill JA, Johnson JD, Kurtz PJ, Parham F, Matthews HB. Comparative absorption of lead from contaminated soil and lead salts by weanling Fischer 344 rats. Fundam Appl Toxicol 33:109–119 (1996).

94. Sheppard SC, Evenden WG, Schwartz WJ. Ingested soil: bioavailability of sorbed lead, cadmium, cesium, iodine, and mercury. J Environ Qual 24:498–505 (1995).

95. Freeman GB, Johnson JD, Liao SC, Feder PI, Davis AO, Ruby MV, Shurkova SB, Eroshin IA, The Mitre Corp., 1989. Absolute bioavailability of lead acetate and mineral waste lead in rats. Toxicology 91:151–163 (1994).

96. Dieter MP, Matthews HB, Jeffcoat RA, Moseman RF. Comparison of lead bioavailability in F344 rats fed lead acetate, lead oxide, lead sulfide, or lead ore concentrate from Skagway, Alaska. J Toxicol Environ Health 39:79–93 (1993).

97. Davis AO, Ruby MV, Link TE, Schoff R, Chaney RL, Freeman GB, Bergstrom P. Development of an in vitro screening test to evaluate the in vivo bioaccessibility of ingested mine-waste lead. Environ Sci Technol 27:2870–2877 (1993).

98. Davis AO, Ruby MV, Bergstrom PD. Bioavailability of arsenic and lead in soils from the Butte, Montana mining district. Environ Sci Technol 26:461–468 (1992).

99. Weis CP, Poppenga RH, Thacker BJ, Henningson GM, Curtis A. 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 (Beard ME, Iske SD, eds). Philadelphia: American Society for Testing and Materials, 1995.

100. Brunkneek B, Veenstra S, Biersteker K, Boleij JS. The Arnhem lead study. Lead uptake by 1 to 3 year old children living in the vicinity of a secondary lead smelter in Arnhem, the Netherlands. Environ Res 25:441–448 (1981).

101. Stanek JF III, Calabrese EJ. Daily estimates of soil ingestion in children. Environ Health Perspect 103:276–285 (1995).

102. Calabrese EJ, Stanek JF III. Resolving intertracer inconsistencies in soil ingestion estimation. Environ Health Perspect 103:454–457 (1995).

103. Kimbrough RD, Falk H, Stehr P. Health implications of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) contamination of residential soil. J Toxicol Environ Health 14:47–93 (1984).

104. Calabrese EJ, Barnes R, Stanek JF III, Pastides H, Gilbert CE, Veneman P, Wang Xiao, Lasztity A, Kostecki PT. How much soil do children ingest: an epidemiological study. Regul Toxicol Pharmacol 10:123–137 (1989).

105. Calabrese EJ, Stanek JF, Gilbert CE. Evidence of soil-pica behavior and quantification of soil ingested. Hum Exp Toxicol 10:245–249 (1991).

106. Ruby MV, Schoof R, Eberle S, Sellon-McC. Estimation of lead and arsenic bioavailability using a physiologically based extraction test. Environ Sci Technol 30:422–430 (1996).

107. Drexler J, Mushak P. Health risks from extractive industry wastes: An approach to bioavailability of toxic metal and metalloid contaminants [Presentation Abstract]. Presented at the Annual Meeting of the Geological Society of America, 24–27 October 1994, Seattle, Washington.

108. Drexler J, Mushak P. Health risks from extractive industry wastes: characterization of heavy metal contaminants and quantification of their bioavailability and bioaccessibility [Presentation Abstract]. Presented at the Third International Conference on the Biogeochemistry of Trace Elements, 15–19 May 1995, Paris, France.

109. Medlin EA. An in Vitro Method for Estimating the Relative Bioavailability of Lead in Humans. MS Thesis. Boulder, CO: University of Colorado, 1997.

110. Drexler J. Validation of an in vitro method: a tandem approach to estimating the bioavailability and bioaccessibility of lead and arsenic to humans [Presentation Abstract]. Presented at the IBC International Congress on Human Health: Bioavailability. Quantifying the Real Toxicity of Common Soil Contaminants, 11–12 December 1997, Scottsdale, Arizona.

111. Ruby MV. Design and validation of an in vitro method for estimating the oral bioavailability of lead and arsenic from soil [Presentation Abstract]. Presented at the IBC International Congress on Human Health: Bioavailability. Quantifying the Real Toxicity of Common Soil Contaminants, 11–12 December 1997, Scottsdale, Arizona.