CHAPTER 3. TOXICOKINETICS, SUSCEPTIBLE POPULATIONS, BIOMARKERS, CHEMICAL INTERACTIONS

3.1 TOXICOKINETICS

Overview. The toxicokinetics of Pb in humans has been extensively studied and several models have been published that simulate the absorption and complex distribution and elimination of Pb from blood, soft tissues, and bone.

- Absorption:
    - Respiratory tract: Inorganic Pb in submicron size particles can be almost completely absorbed through the respiratory tract, whereas larger particles may be moved after deposition in the respiratory tract by mucociliary clearance toward the oropharynx and swallowed.
    - Gastrointestinal tract: The fraction of ingested Pb absorbed from the gastrointestinal tract depends on many factors, including age, diet, nutrition, and physiological characteristics of Pb in the medium ingested.
    - Children can absorb 40–50% of an oral dose of water-soluble Pb compared to 3–10% for adults.
    - Gastrointestinal absorption of inorganic Pb occurs primarily in the duodenum by saturable mechanisms.
    - Dermal: Inorganic Pb can be absorbed following inhalation, oral, and dermal exposure, but the latter route is much less efficient than the former two, with the exception of hand-to-mouth behavior. Studies in animals have shown that organic Pb is absorbed through the skin.

- Distribution:
    - The distribution of Pb in the body is route-independent and, in adults, approximately 94% of the total body burden of Pb is in the bones compared to approximately 73% in children.
    - Pb in blood is primarily in red blood cells. Conditions such as pregnancy, lactation, menopause, and osteoporosis increase bone resorption and consequently also increase Pb in blood.

  - Pb can be transferred from the mother to the fetus and also from the mother to infants via maternal milk.

- Metabolism:
  - Metabolism of inorganic Pb consists of formation of complexes with a variety of protein and nonprotein ligands.
  - Organic Pb compounds are actively metabolized in the liver by oxidative dealkylation by P-450 enzymes.

- Excretion:
  - Pb is excreted primarily in urine and feces regardless of the route of exposure. Minor routes of excretion include sweat, saliva, hair, nails, breast milk, and seminal fluid.
  - Elimination of Pb is multiphasic, reflecting pools of Pb in the body that have varying retention times. The apparent elimination half-time in blood varies with age and exposure history and ranges from 1 week to 2 years. Elimination of Pb from bone occurs with an apparent half-time of 1–2 decades.

- Toxicokinetics models:
  - Several models of Pb pharmacokinetics have been proposed to characterize such parameters as intercompartmental Pb exchange rates, retention of Pb in various tissues, and relative rates of distribution among the tissue groups.
  - Some models are currently being used or are being considered for broad application in Pb risk assessment.

3.1.1 Absorption

Inhalation Exposure

Inorganic Pb. Inorganic Pb in ambient air consists of aerosols of particulates that can be deposited in the respiratory tract when the aerosols are inhaled. Amounts and patterns of deposition of particulate aerosols in the respiratory tract are affected by the size of the inhaled particles, age-related factors that determine breathing patterns (e.g., nose versus mouth breathing), airway geometry, and air-stream velocity within the respiratory tract (James et al. 1994). Absorption of deposited Pb is influenced by particle size and solubility as well as the pattern of regional deposition within the respiratory tract. Larger particles

(>2.5 μm) that are deposited in the ciliated airways (nasopharyngeal and tracheobronchial regions) can be transferred by mucociliary transport into the esophagus and swallowed. Smaller particles (2.5 to <1 μm), which can be deposited in the alveolar region, can be absorbed after extracellular dissolution or ingestion by phagocytic cells (Bailey and Roy 1994).

Deposition in, and clearance from, the respiratory tract have been measured in adult humans (Chamberlain et al. 1978; Hursh and Mercer 1970; Hursh et al. 1969; Morrow et al. 1980; Wells et al. 1975). In these studies, exposures were to Pb-bearing particles having mass median aerodynamic diameters (MMADs) below 1 μm and, therefore, deposition of the inhaled Pb particles can be assumed to have been primarily in the bronchiolar and alveolar regions of the respiratory tract (James et al. 1994) where transport of deposited Pb to the gastrointestinal tract is likely to have been only a minor component of particle clearance (Hursh et al. 1969). Approximately 25% of inhaled Pb chloride or Pb hydroxide (MMAD 0.26 and 0.24 μm, respectively) was deposited in the respiratory tract in adult subjects who inhaled an inorganic Pb aerosol through a standard respiratory mouthpiece for 5 minutes (Morrow et al. 1980). Approximately 95% of deposited inorganic Pb that was inhaled as submicron particles was absorbed (Hursh et al. 1969; Wells et al. 1975). Rates of clearance from the respiratory tract of inorganic Pb inhaled as submicron particles of Pb oxide, or Pb nitrate, were described with half-times ($t_{1/2}$) of 0.8 hours (22%), 2.5 hours (34%), 9 hours (33%), and 44 hours (12%) (Chamberlain et al. 1978). These rates are thought to represent, primarily, absorption from the bronchiolar and alveolar regions of the respiratory tract. Absorption half-times have been estimated in adults who inhaled aerosols of Pb and bismuth isotopes generated from decay of ^{220}Rn or ^{222}Rn (Butterweck et al. 2002; Marsh and Birchall 1999). The absorption half-time was approximately 10 hours in subjects who inhaled aerosols having an activity median particle diameter of approximately 160 nm (range 50–500 nm), and approximately 68 minutes for aerosols having diameters of approximately 0.3–3 nm.

Rates and amounts of absorption of inhaled Pb particles >2.5 μm will be determined, primarily by rates of transport to and absorption from the gastrointestinal tract. Absorption of Pb from the gastrointestinal tract varies with the chemical form ingested, age, meal status (e.g., fed versus fasted), and nutritional factors (see Section 3.1.1 *Oral Exposure*).

Organic Pb. Following a single exposure to vapors of radioactive (^{203}Pb) tetraethyl Pb (approximately 1 mg/m^3 breathed through a mouthpiece for 1–2 minutes) in four male subjects, 37% of inhaled ^{203}Pb was initially deposited in the respiratory tract, of which approximately 20% was exhaled in the subsequent 48 hours (Heard et al. 1979). One hour after the exposure, approximately 50% of the ^{203}Pb burden was

associated with liver, 5% was associated with kidney, and the remaining burden was widely distributed throughout the body (determined by external gamma counting), suggesting near complete absorption of the Pb that was not exhaled. In a similar experiment conducted with (^{203}Pb) tetramethyl Pb, 51% of the inhaled ^{203}Pb dose was initially deposited in the respiratory tract, of which approximately 40% was exhaled in 48 hours. The distribution of ^{203}Pb 1 hour after the exposure was similar to that observed following exposure to tetraethyl Pb.

The relatively rapid and near complete absorption of tetraalkyl Pb that is inhaled and deposited in the respiratory tract is also supported by studies conducted in animal models (Boudene et al. 1977; Morgan and Holmes 1978).

Oral Exposure

Inorganic Pb. The extent and rate of gastrointestinal absorption of ingested inorganic Pb are influenced by physiology (e.g., age, fasting, nutritional calcium and iron status, pregnancy), physicochemical characteristics of the medium ingested (e.g., particle size, mineralogy, solubility, and Pb species) and the ingested Pb dose.

Mechanisms of Absorption. Gastrointestinal absorption of inorganic Pb occurs primarily in the duodenum (Mushak 1991). The exact mechanisms of absorption are unknown and may involve active transport and/or diffusion through intestinal epithelial cells (transcellular) or between cells (paracellular), and may involve ionized Pb (Pb^{+2}) and/or inorganic or organic complexes of Pb. *In vitro* studies of Pb speciation in simulated human intestinal chyme indicate that the concentration of ionized Pb is negligible at Pb concentrations below 10^{-3} M (207 mg/L) and that Pb phosphate and bile acid complexes are the dominant forms when inorganic Pb salts (e.g., Pb nitrate) are added to chyme (Oomen et al. 2003a). However, these complexes may be sufficiently labile to provide ionized Pb for transport across cell membranes (Oomen et al. 2003b). Saturable mechanisms of absorption have been inferred from measurements of net flux kinetics of Pb in *in situ* perfused mouse intestine, *in situ* ligated chicken intestine, and *in vitro* isolated segments of rat intestine (Aungst and Fung 1981; Barton 1984; Flanagan et al. 1979; Mykkänen and Wasserman 1981). By analogy to other divalent cations, saturable transport mechanisms for Pb^{+2} may exist within the mucosal and serosal membranes and within the intestinal epithelial cell. For calcium and iron, these are thought to represent membrane carriers (e.g., Ca^{2+}-Mg^{2+}-ATPase, Ca^{2+}/Na^{+} exchange, DMT1) or facilitated diffusion pathways (e.g., Ca^{2+} channel) and

intracellular binding proteins for Ca^{2+} (Bronner et al. 1986; Fleming et al. 1998b; Gross and Kumar 1990; Teichmann and Stremmel 1990).

Effect of Age. Gastrointestinal absorption of water-soluble Pb appears to be higher in children than in adults. Estimates derived from dietary balance studies conducted in infants and children (ages 2 weeks to 8 years) indicate that approximately 40–50% of ingested Pb is absorbed (Alexander et al. 1974; Ziegler et al. 1978). In adults, estimates of absorption of ingested water-soluble Pb compounds (e.g., Pb chloride, Pb nitrate, Pb acetate) ranged from 3 to 10% in fed subjects (Heard and Chamberlain 1982; James et al. 1985; Rabinowitz et al. 1980; Watson et al. 1986). Data available on Pb absorption between childhood and adulthood ages are very limited. While no absorption studies have been conducted on subjects in this age range, the kinetics of the change in stable isotope signatures of blood Pb in mothers and their children, as both come into equilibrium with a novel environmental Pb isotope profile, suggest that children ages 6–11 years and their mothers may absorb a similar percentage of ingested Pb (Gulson et al. 1997b).

Studies in experimental animals provide additional evidence for an age-dependency of gastrointestinal absorption of Pb. Absorption of Pb, administered as Pb acetate (6.37 mg Pb/kg, gavage), was higher in juvenile Rhesus monkeys (38% of dose) compared to adult female monkeys (26% of the dose) (Pounds et al. 1978). Rat pups absorb approximately 40–50 times more Pb from the diet than do adult rats (Aungst et al. 1981; Forbes and Reina 1972; Kostial et al. 1978). This age difference in absorption may be due, in part, to the shift from the neonatal to adult diet, and to postnatal physiological development (enzymes, transporters, gastric pH) of the gastrointestinal tract (Weis and LaVelle 1991).

Effect of Fasting. The presence of food in the gastrointestinal tract decreases absorption of water-soluble Pb (Blake and Mann 1983; Blake et al. 1983; Heard and Chamberlain 1982; James et al. 1985; Maddaloni et al. 1998; Rabinowitz et al. 1980). In adults, absorption of a tracer dose of Pb acetate in water was approximately 63% when ingested by fasted subjects and 3% when ingested with a meal (James et al. 1985). Heard and Chamberlain (1982) reported nearly identical results. The arithmetic mean of reported estimates of absorption in fasted adults was 57% (calculated by ATSDR based on Blake et al. 1983; Heard and Chamberlain 1982; James et al. 1985; Rabinowitz et al. 1980). Reported fed/fasted ratios for absorption in adults range from 0.04 to 0.2 (Blake et al. 1983; Heard and Chamberlain 1983; James et al. 1985; Rabinowitz et al. 1980). Mineral content is one contributing factor to the lower absorption of Pb when Pb is ingested with a meal; in particular, the presence of calcium and phosphate in a meal will depress the absorption of ingested Pb (Blake and Mann 1983; Blake et al. 1983; Heard and Chamberlain

1982). Suppression of absorption by meals may explain the observation of lower PbB in children (age 3–5 years) who ate breakfast compared to children who went without breakfast, after controlling for nutritional variables (Liu et al. 2011).

Effect of Nutrition. Pb absorption in children is affected by nutritional iron status. Children who are iron deficient have higher PbBs than similarly exposed children who are iron replete, which would suggest that iron deficiency may result in higher absorption of Pb or, possibly, other changes in Pb biokinetics that would contribute to lower PbBs (Mahaffey and Annest 1986; Marcus and Schwartz 1987). Genetic variation in genes involved in iron metabolism appear to affect PbBs; however, it is not certain if these associations are caused by changes in Pb absorption. These include variants in the hemochromatosis (HFE) and transferrin genes, which have been associated with higher PbBs in children (Hopkins et al. 2008), and with lower PbBs and bone Pb levels in elderly men (Wright et al. 2004).

Evidence for the effect for iron deficiency on Pb absorption has been provided from animal studies. In rats, iron deficiency increases the gastrointestinal absorption of Pb, possibly by enhancing binding of Pb to iron binding proteins in the intestine (Bannon et al. 2003; Barton et al. 1978b; Morrison and Quaterman 1987). Interactions between iron and Pb appear to involve either intracellular transfer or basolateral transfer mechanisms. Iron ($FeCl_2$) added to the mucosal fluid of the everted rat duodenal sac decreases serosal transfer, but not mucosal uptake of Pb (Barton 1984). When mRNA for DMT1, a mucosal membrane carrier for iron (which also transports other divalent metal cations), was suppressed in Caco 2 cells (a human gastrointestinal cell line), the rate of iron and cadmium uptake decreased by 50% compared to cells in which DMT1 mRNA was not suppressed; however, DMT1 mRNA suppression did not alter the rate of Pb uptake by Caco 2 cells, indicating that Pb may enter Caco 2 cells through a mechanism that is independent of DMT1 (Bannon et al. 2003). The above observations suggest that rate-limiting saturable mechanisms for Pb absorption are associated with transfer of Pb from cell to blood rather than with mucosal transfer. Similar mechanisms may contribute to Pb-iron and Pb-calcium absorption interactions in humans, and possibly interactions between Pb and other divalent cations such as cadmium, copper, magnesium, and zinc.

Dietary calcium intake affects Pb absorption. An inverse relationship has been observed between dietary calcium intake and PbBs in children, suggesting that children who are calcium-deficient may absorb more Pb than calcium-replete children (Elias et al. 2007; Mahaffey et al. 1986; Schell et al. 2004; Ziegler et al. 1978). An effect of calcium on Pb absorption is also evident in adults. In experimental studies of adults, absorption of a single dose of Pb (100–300 μg Pb chloride) was lower when the Pb was ingested together

with calcium carbonate (0.2–1 g calcium carbonate) than when the Pb was ingested without additional calcium (Blake and Mann 1983; Heard and Chamberlain 1982). A similar effect of calcium occurs in rats (Barton et al. 1978a). Complexation with calcium (and phosphate) in the gastrointestinal tract and competition for a common transport protein have been proposed as possible mechanisms for this interaction (Barton et al. 1978a; Heard and Chamberlain 1982). Absorption of Pb from the gastrointestinal tract is enhanced by dietary calcium depletion or administration of cholecalciferol (Mykkänen and Wasserman 1981, 1982). This "cholecalciferol-dependent" component of Pb absorption appears to involve a stimulation of the serosal transfer of Pb from the epithelium, not stimulation of mucosal uptake of Pb (Mykkänen and Wasserman 1981, 1982). This is similar to the effects of cholecalciferol on calcium absorption (Bronner et al. 1986; Fullmer and Rosen 1990).

In a study of young children (ages 6–12 months), PbBs increased in association with lower dietary Zn levels (Schell et al. 2004); however, it is not certain if these associations were caused by changes in Pb absorption.

Effect of Pregnancy. Absorption of Pb may increase during pregnancy. Although there is no direct evidence for this in humans, an increase in Pb absorption may contribute, along with other mechanisms (e.g., increased mobilization of bone Pb), to the increase in PbBs that has been observed during the latter half of pregnancy (see Section 3.1.2, *Pb Distribution during Pregnancy and Maternal-Fetal-Infant Transfer*).

Effect of Dose. Pb absorption in humans may be a capacity-limited process, in which case, the percentage of ingested Pb that is absorbed may decrease with increasing rate of Pb intake. Studies, to date, do not provide a firm basis for discerning if the gastrointestinal absorption of Pb is limited by dose. Numerous observations of nonlinear relationships between PbB and Pb intake in humans provide support for the existence of a saturable absorption mechanism or some other capacity-limited process in the distribution of Pb in humans (Pocock et al. 1983; Sherlock and Quinn 1986; Sherlock et al. 1984) (see Section 3.1.2, *Pb in Blood* and *Pb in Plasma* for discussion of saturable uptake of Pb in red blood cells). However, in immature swine that received oral doses of Pb in soil, Pb dose-blood Pb relationships were curvilinear, whereas dose-tissue Pb relationships for bone, kidney, and liver were linear. The same pattern (nonlinearity for PbB and linearity for tissues) was observed in swine administered Pb acetate intravenously (Casteel et al. 1997, 2006). These results suggest that the nonlinearity in the Pb dose-blood Pb relationship may derive from an effect of Pb dose on some aspect of the biokinetics of Pb other than absorption. In fasted rats, absorption was estimated at 42 and 2% following single oral administration of

1 and 100 mg Pb/kg, respectively, as Pb acetate, suggesting a limitation on absorption imposed by dose (Aungst et al. 1981). Evidence for capacity-limited processes at the level of the intestinal epithelium (Aungst and Fung 1981; Barton 1984; Flanagan et al. 1979; Mykkänen and Wasserman 1981) suggests that the intake-uptake relationship for Pb is likely to be nonlinear; however, the dose at which absorption becomes appreciably limited in humans is not known.

Effect of Particle Size. Particle size influences the degree of gastrointestinal absorption (Ruby et al. 1999). In rats, an inverse relationship was found between absorption and particle size of Pb in diets containing metallic Pb particles that were ≤250 μm in diameter (Barltrop and Meek 1979). Tissue Pb concentration was a 2.3-fold higher when rats ingested an acute dose (37.5 mg Pb/kg) of Pb particles that were <38 μm in diameter than when rats ingested particles having diameters in the range of 150–250 μm (Barltrop and Meek 1979). Dissolution kinetics experiments with Pb-bearing mine waste soil suggest that surface area effects control dissolution rates for particles sizes of <90 μm diameter; however, dissolution of 90–250 μm particle size fractions appeared to be controlled more by surface morphology (Davis et al. 1994). Similarly, Healy et al. (1982) found that the solubility of Pb sulfide in gastric acid *in vitro* was much greater for particles that were 30 μm in diameter than for particles that were 100 μm in diameter.

Absorption from Soil. Absorption of Pb from the gastrointestinal tract involves absorptive transport of soluble Pb species (e.g., Pb^{2+}) across the gastrointestinal tract epithelium. In order for Pb to be absorbed from soil, it must first be made bioaccessible in the gastrointestinal tract. The process of rendering soil Pb bioaccessible may involve: (1) physical and/or chemical digestion of the soil particles to expose Pb deposits to gastrointestinal tract fluids; (2) transfer of Pb minerals from exposed surfaces on soil particles to the aqueous environment of the gastrointestinal tract; and (3) chemical transformation of Pb minerals to soluble Pb species (e.g., Pb^{2+}) that are substrates for absorptive transport. Although absorptive transport of Pb occurs predominantly, if not solely, in the upper small intestine, bioaccessibility processes occurring in the stomach appear to be major determinants of Pb absorption.

Adult subjects who ingested soil (particle size <250 μm) collected from the Bunker Hill National Priorities List (NPL) site absorbed 26% of the resulting 250 μg/70 kg body weight Pb dose when the soil was ingested in the fasted state, and 2.5% when the same soil Pb dose was ingested with a meal (Maddaloni et al. 1998). The value reported for fasted subjects (26%) was approximately half that reported for soluble Pb ingested by fasting adults, or approximately 60% (Blake et al. 1983; Heard and Chamberlain 1983; James et al. 1985; Rabinowitz et al. 1980). Measurements of the absorption of soil Pb in infants or children have not been reported.

Absorption of Pb from ingested soils and surface dust has been studied more extensively in animals (Bannon et al. 2009; Barltrop and Meek 1979; Bradham et al. 2016; Brown et al. 2004; Casteel et al. 1997, 2006; Freeman et al. 1992, 1994, 1996; Healy et al. 1982; Hettiearachchi et al. 2003; Juhasz et al. 2009; Ryan et al. 2004; Weis and Lavelle 1991). These studies have shown that absorption of soil Pb varies depending upon the Pb mineralogy and physical characteristics of the Pb in the soil (e.g., encapsulated or exposed, particle size). Studies conducted in swine and other animal models have provided estimates of relative bioavailability (RBA) of Pb in soils collected from sites impacted by a variety of sources of Pb contamination including ore and ore processing, shooting of Pb munitions, and Pb-based paint (Bannon et al. 2009; Barltrop and Meek 1979; Bradham et al. 2016; Brown et al. 2004; Casteel et al. 1997, 2006; Freeman et al. 1992, 1994, 1996; Healy et al. 1982; Hettiearachchi et al. 2003; Juhasz et al. 2009; Ryan et al. 2004; Weis and Lavelle 1991). RBA is the ratio of the absolute bioavailability (or absorption fraction) of Pb in soil to that of a water-soluble reference (Pb acetate). RBA has been measured in animals models using various approaches, including measurement of blood and tissue Pb in animals following dosing with soil or Pb acetate. RBA estimates from these studies ranged from 1 to 100% (mean 60%, n=33, calculated by ATSDR). RBA for soils from firing ranges where the predominant form of Pb was Pb carbonate were approximately 100% (Bannon et al. 2009). A soil amended with NIST paint standard (a mixture of Pb carbonate and Pb oxide) had an RBA of 92%. Smelter slag and soils in which the dominant source of Pb was smelter slag had relatively low RBA (14–40%). Galena (lead sulfide) in soil also had relatively low RBA (1–6%).

Casteel et al. (2006) estimated Pb RBA of 19 soils in swine and categorized the RBA according to Pb mineral associations. Electron microprobe analyses of Pb-bearing grains in the various soils revealed that the grains ranged from as small as 1–2 μm up to a maximum of 250 μm (the sieve size used in preparation of the samples) and that Pb was present in a wide range of different mineral associations (phases), including various oxides, sulfides, sulfates, and phosphates. These variations in size and mineral content of the Pb-bearing grains are the suspected cause of variations in the gastrointestinal absorption of Pb from different samples of soil. Based on these very limited data, the RBA of Pb mineral phases were rank-ordered (Table 3-1).

Table 3-1. Ranking of Relative Bioavailability of Lead (Pb) Mineral Phases in Soil[a]

Low bioavailability (RBA<0.25)	Medium bioavailability (RBA=0.25–0.75)	High bioavailability (RBA>0.75)
Angelsite	Pb oxide	Cerussite
Fe(M) oxide	Pb phosphate	Mn(M) oxide
Fe(M) sulfate		
Galena		
Pb(M) oxide		

[a]Estimates are based on studies of immature swine.

Fe = iron; M = metal; Mn = manganese; RBA = relative bioavailability (compared to Pb acetate)

Source: Casteel et al. 2006

Several studies have shown that elevating the phosphate concentration of soil can decrease soil Pb RBA (Brown et al. 2004; Hettiarachichi et al. 2003; Ryan et al. 2004). The mechanism for the effect is thought to be the formation of a relatively insoluble form of Pb in soil, pyromorphite, which has a low RBA (Scheckel et al. 2013).

Bioaccessibility in Soil and its Relationship to Relative Bioavailability. Empirical evidence supporting the importance of gastric bioaccessibility in Pb absorption comes from studies of relationships between extractability of Pb from soil measured *in vitro* and Pb RBA measured in animals. *In vitro* extractability of Pb from soil (*in vitro* bioaccessibility, IVBA) strongly correlates with RBA measured swine assays when the extraction is performed at gastric pH (r^2=0.92, n=18; Drexler and Brattin 2007). Bioaccessibility estimates obtained from IVBA assays are sensitive to assay conditions such as pH, liquid:soil ratios, inclusion or absence of food material, and differences in methods used to separate dissolved and particle-bound Pb (e.g., centrifugation versus filtration); as a result, different assays can yield different results when applied to the same soils or surface dusts (Dong et al. 2016; Juhasz et al. 2011; Lu et al. 2011; Roussel et al. 2010; Saikat et al. 2007; Smith et al. 2011; Van de Wiele et al. 2007). For this reason, application of IVBA assays for predicting RBA must be supported by demonstration of a strong correlation between IVBA and RBA (Drexler and Brattin 2007). Even in the absence of validation of RBA predictions, IVBA assays may be useful for predicting relative differences in RBA between soils. For example, the relative change in Pb RBA resulting from treatment of soils with phosphate amendments was predicted from IVBA measurements even though the IVBA assay performed poorly at predicting the actual RBA of the soils (Juhasz et al. 2016). Bioaccessibility measured with IVBA assays has been shown to increase with decreasing particle size (varied from <2,000 to <50 μm) (Juhasz et al. 2011) and increase with increasing soil acidity and organic matter content (Jin et al. 2005).

Dermal Exposure

Inorganic Pb. Dermal absorption of inorganic Pb compounds is generally considered to be much less than absorption by inhalation or oral routes of exposure; however, few studies have provided quantitative estimates of dermal absorption of inorganic Pb in humans, and the quantitative significance of the dermal absorption pathway as a contributor to Pb body burden in humans remains an uncertainty. Pb was detected in the upper layers of the stratum corneum of Pb-battery workers, prior to their shifts and after cleaning of the skin surface (Sun et al. 2002), suggesting adherence and/or possible dermal penetration of Pb. Following skin application of ^{203}Pb-labeled Pb acetate in cosmetic preparations (0.12 mg Pb in 0.1 mL or 0.18 mg Pb in 0.1 g of a cream) to eight male volunteers for 12 hours, absorption was ≤0.3%, based on whole-body, urine, and blood ^{203}Pb measurements, and was predicted to be 0.06% during normal use of such preparations (Moore et al. 1980). Most of the absorption took place within 12 hours of exposure. Pb also appears to be absorbed across human skin when applied to the skin as Pb nitrate; however, quantitative estimates of absorption have not been reported. Pb (4.4 mg, as Pb nitrate) was applied (vehicle or solvent not reported) to an occluded filter placed on the forearm of an adult subject for 24 hours, after which, the patch was removed, the site cover and the forearm were rinsed with water, and total Pb was quantified in the cover material and rinse (Stauber et al. 1994). The amount of Pb recovered from the cover material and rinse was 3.1 mg (70% of the applied dose). Based on this recovery measurement, 1.3 mg (30%) of the applied dose remained either in the skin or had been absorbed in 24 hours; the amount that remained in or on the skin and the fate of this Pb (e.g., exfoliation) was not determined. Exfoliation has been implicated as an important pathway of elimination of other metals from skin (e.g., inorganic mercury; Hursh et al. 1989). Pb concentrations in sweat collected from the right arm increased 4-fold following the application of Pb to the left arm, indicating that some Pb had been absorbed (amounts of sweat collected or total Pb recovered in sweat were not reported; Stauber et al. 1994). In similar experiments with three subjects, measurements of ^{203}Pb in blood, sweat, and urine, made over a 24-hour period following dermal exposures to 5 mg Pb as ^{203}Pb nitrate or acetate, accounted for <1% of the applied (or adsorbed) dose (Stauber et al. 1994). This study also reported that absorption of Pb could not be detected from measurements of Pb in sweat following dermal exposure to Pb as Pb carbonate.

Information on relative dermal permeability of inorganic and organic Pb salts of Pb comes from studies of *in vitro* preparations of excised skin; the rank ordering of penetration rates through excised human skin

was: Pb nuolate (Pb linoleic and oleic acid complex) > Pb naphthanate > Pb acetate > Pb oxide (nondetectable) (Bress and Bidanset 1991).

Studies conducted in animals provide additional evidence that dermal absorption of inorganic Pb is substantially lower than absorption from the inhalation or oral route. In a comparative study of dermal absorption of inorganic and organic salts of Pb conducted in rats, approximately 100 mg of Pb was applied in an occluded patch to the shaved backs of rats. Based on urinary Pb measurements made prior to and for 12 days following exposure, Pb compounds could be ranked according to the relative amounts absorbed (i.e., percent of dose recovered in urine; calculated by ATSDR): Pb naphthalene (0.17%), Pb nitrate (0.03%), Pb stearate (0.006%), Pb sulfate (0.006%), Pb oxide (0.005%), and metal Pb powder (0.002%). This rank order (i.e., Pb naphthalene > Pb oxide) is consistent with a rank ordering of penetration rates of inorganic and organic Pb salts through excised skin from humans and guinea pigs: Pb nuolate (Pb linoleic and oleic acid complex) > Pb naphthanate > Pb acetate > Pb oxide (nondetectable) (Bress and Bidanset 1991). The estimates for percent of dose excreted underestimate actual absorption as these estimates do not account for the Pb retained in bone and other tissues.

Following application of Pb acetate to the shaved clipped skin of rats, the concentration of Pb in the kidneys was found to be higher relative to controls, suggesting that absorption of Pb had occurred (Laug and Kunze 1948). This study also observed that dermal absorption of Pb from Pb arsenate was significantly less than from Pb acetate, and that mechanical injury to the skin significantly increased the dermal penetration of Pb.

Organic Pb. Relative to inorganic Pb and organic Pb salts, tetraalkyl Pb compounds have been shown to be rapidly and extensively absorbed through the skin of rabbits and rats (Kehoe and Thamann 1931; Laug and Kunze 1948). A 0.75-mL amount of tetraethyl Pb, which was allowed to spread uniformly over an area of 25 cm^2 on the abdominal skin of rabbits, resulted in 10.6 mg of Pb in the carcass at 0.5 hours and 4.41 mg at 6 hours (Kehoe and Thamann 1931). Tetraethyl Pb was reported to be absorbed by the skin of rats to a much greater extent than Pb acetate, Pb oleate, and Pb arsenate (Laug and Kunze 1948). Evidence for higher dermal permeability of organic Pb compounds compared to inorganic organic salts of Pb also comes from *in vitro* studies conducted with excised skin. The rank order of absorption rates through excised skin from humans and guinea pigs was as follows: tetrabutyl Pb > Pb nuolate (Pb linoleic and oleic acid complex) > Pb naphthanate > Pb acetate > Pb oxide (nondetectable) (Bress and Bidanset 1991).

3.1.2 Distribution

Inorganic Pb. Absorbed inorganic Pb appears to be distributed in essentially the same manner regardless of the route of absorption (Chamberlain et al. 1978; Kehoe 1987); therefore, the distribution of absorbed Pb (i.e., by any route) is discussed in this section, rather than in separate sections devoted to specific routes of exposure. The expression "body burden" is used here to refer to the total amount of Pb in the body. Most of the available information about the distribution of Pb to major organ systems (e.g., bone, soft tissues) derives from autopsy studies conducted in the 1960s and 1970s and reflect body burdens accrued during periods when ambient and occupational exposure levels were much higher than current levels (Barry 1975, 1981; Gross et al. 1975; Schroeder and Tipton 1968). A more recent autopsy study found lower Pb concentrations in autopsies performed during the period 2004–2013 (Mari et al. 2014). In general, these studies indicate that the distribution of Pb appears to be similar in children and adults, although a larger fraction of the Pb body burden of adults resides in bone. Several models of Pb pharmacokinetics have been proposed to characterize such parameters as intercompartmental Pb exchange rates, retention of Pb in various tissues, and relative rates of distribution among the tissue groups (see Section 3.1.5 for further discussion of models).

Pb in Blood. Concentrations of Pb in blood vary considerably with age, physiology/life stage (e.g., pregnancy, lactation, menopause), and numerous factors that affect exposure to Pb. PbBs in various demographic strata of the U.S. population are periodically estimated from the NHANES. Based on data from NHANES (2015–2016, CDC 2018a), the geometric mean PbB of U.S. adults, age ≥20 years, was 0.920 μg/dL (95% CI 0.862, 0.982). The geometric mean PbB of U.S. children, age 1–5 years, was 0.758 (95% CI 0.675, 0.850). PbBs in the United States have decreased considerably in the last several decades as a result of removal of Pb from gasoline and restrictions placed on the use of Pb in residential paints (Brody et al. 1994; CDC 2011, 2018a; Pirkle et al. 1994, 1998; Schwartz and Pitcher 1989). While historically, the geometric mean PbB in U.S. children has been higher than that of the adult population, recent estimates indicate that geometric means in children have fallen below that of adults.

Pb in Red Blood Cells. Pb in blood is primarily in the red blood cells (99%) (Bergdahl et al. 1997a, 1998, 1999; Hernandez-Avila et al. 1998; Manton et al. 2001; Schutz et al. 1996; Smith et al. 2002). Although the mechanisms by which Pb crosses cell membranes have not been fully elucidated, results of studies in intact red blood cells and red blood cell ghosts indicate that there are two, and possibly three, pathways for facilitated transfer of Pb across the red cell membrane. The major proposed pathway is an anion exchanger that is dependent upon HCO_3^- and is blocked by anion exchange inhibitors (Bannon et al.

2000, Simons 1985, 1986a, 1986b, 1993). A second minor pathway, which does not exhibit HCO_3^- dependence and is not sensitive to anion exchange inhibitors, may also exist (Simons 1986b). Pb and calcium may also share a permeability pathway, which may be a Ca^{2+}-channel (Calderon-Salinas et al. 1999). Pb is transferred out of the erythrocyte by an active transport pathway, most likely a (Ca^{2+}, Mg^{2+})-ATPase (Simons 1988).

Pb in erythrocytes binds to several intracellular proteins. ALAD is the primary binding ligand for Pb in erythrocytes (Bergdahl et al. 1997a, 1998; Sakai et al. 1982; Xie et al. 1998). Pb binding to ALAD is saturable; the binding capacity has been estimated to be approximately 85 μg/dL red blood cells (or approximately 40 μg/dL whole blood) and the apparent dissociation constant has been estimated to be approximately 1.5 μg/L (Bergdahl et al. 1998). Two other Pb-binding proteins have been identified in erythrocytes, a 45 kDa protein (Kd 5.5 μg/L) and a smaller protein(s) having a molecular weight <10 kDa (Bergdahl et al. 1996, 1997a, 1998). Of the three principal Pb-binding proteins identified in erythrocytes, ALAD has the strongest affinity for Pb (Bergdahl et al. 1998) and appears to dominate the ligand distribution of Pb (35–84% of total erythrocyte Pb) at blood Pb levels below 40 μg/dL (Bergdahl et al. 1996, 1998; Sakai et al. 1982). The decrease in hematocrit that occurs in early infancy (51% at birth to 35% at 6 months) may decrease the total binding capacity of blood and PbBs over the first postnatal 6 months (Simon et al. 2007).

Pb binds to and inhibits the activity of ALAD (Gercken and Barnes 1991; Gibbs et al. 1985; Jaffe et al. 2000; Sakai et al. 1982, 1983). Binding of zinc is essential for ALAD activity, and Pb inhibits activity of ALAD by displacing zinc (Jaffe et al. 2000). Synthesis of ALAD appears to be induced in response to inhibition of ALAD and, therefore, in response to binding of Pb to ALAD (Boudene et al. 1984; Fujita et al. 1982). Several mechanisms may participate in the induction of ALAD, including (1) inhibition of ALAD directly by Pb; (2) inhibition by protoporphyrin, secondary to accumulation of protoporphyrin as a result of Pb inhibition of ferrochelatase; and (3) accumulation of ALA (a substrate of ASAD), secondary to inhibition of ALAD, which may stimulate ALAD synthesis in bone marrow cells (Boudene et al. 1984; Fujita et al. 1982).

ALAD is a polymorphic enzyme with two alleles (ALAD 1 and ALAD 2) and three genotypes (ALAD 1,1, ALAD 1,2, and ALAD 2,2) (Battistuzzi et al. 1981, Scinicariello et al. 2007). Numerous studies have examined the relationship between ALAD genotype and PbBs and the results of these studies are mixed with some studies finding higher PbBs in association with the ALAD 2 allele and other studies finding no associations or lower PbBs associated with the ALAD 2 allele (see Section 3.2). One possible

mechanism by which ALAD polymorphism could affect PbBs is by allelic variation in Pb binding to ALAD (Bergdahl et al. 1997b). However, competitive displacement studies with recombinant human ALAD 1 and ALAD 2 did not indicate differences in affinity for Pb relative to zinc (Jaffe et al. 2000).

Pb in Blood Plasma. Pb binds to several constituents in plasma and it has been proposed that Pb in plasma exists in four states: loosely bound to serum albumin or other proteins with relatively low affinity for Pb, complexed to low molecular weight ligands such as amino acids and carboxylic acids, tightly bound to a circulating metalloprotein, and as free Pb^{2+} (Al-Modhefer et al. 1991). Free ionized Pb (i.e., Pb^{2+}) in plasma represents an extremely small percentage of total plasma Pb. The concentration of Pb^{2+} in fresh serum, as measured by an ion-selective Pb electrode, was reported to be 1/5,000 of the total serum Pb (Al-Modhefer et al. 1991). Approximately 40–75% of Pb in the plasma is bound to plasma proteins, of which albumin appears to be the dominant ligand (Al-Modhefer et al. 1991; Ong and Lee 1980). Pb also binds to transferrins and γ-globulins (Guo et al. 2014; Ong and Lee 1980). Pb in serum that is not bound to protein exists largely as complexes with low molecular weight sulfhydryl compounds (e.g., cysteine, homocysteine). Other potential low molecular weight Pb-binding ligands in serum may include citrate, cysteamine, ergothioneine, glutathione, histidine, and oxylate (Al-Modhefer et al. 1991).

Saturable binding to red blood cell proteins contributes to curvature to the blood Pb-plasma Pb relationship with an increase in the plasma/blood Pb ratio with increasing PbB (Barbosa et al. 2006a; Bergdahl et al. 1997b, 1998, 1999; DeSilva 1981; Jin et al. 2008; Kang et al. 2009; Manton et al. 2001; Rentschler et al. 2012; Smith et al. 2002; Tian et al. 2013). The curvature becomes evident at PbBs well above 10 µg/dL. As binding sites for Pb in red blood cells become saturated, a larger fraction of the blood Pb is available in plasma to distribute to brain and other Pb-responsive tissues. This contributes to a curvature in the relationship between Pb intake and PbB, with the blood Pb/intake slope decreasing with increasing Pb intake, which has been observed in children (Sherlock and Quinn 1986) and immature swine (Casteel et al. 2006). Saturable binding of Pb to red blood cell proteins also contributes to a curvilinear relationship between blood Pb and urinary Pb, whereas the relationship between plasma Pb concentration and urine Pb is linear (Bergdahl et al. 1997b).

Pb in Bone. In human adults, approximately >90% of the total body burden of Pb is found in the bones. Based on analyses of post-mortem tissues, bone accounted for 94% of the total Pb body burden of adults and 73% of the body burden in children (Barry 1975). Pb concentrations in bone increase with age, indicative of a relatively slow turnover of Pb in adult bone (Barry 1975, 1981; Gross et al. 1975; Schroeder and Tipton 1968; Wilker et al. 2011). A portion of Pb in bone readily exchanges with the

plasma Pb pool and, as a result, bone Pb is a reservoir for replenishment of Pb eliminated from blood by excretion (Alessio 1988; Behinaein et al. 2012, 2014; Chettle et al. 1991; Hryhorczuk et al. 1985; Nie et al. 2005; Nilsson et al. 1991; Rabinowitz et al. 1976). Pb in adult bone can serve to maintain blood Pb levels long after exposure has ended (Fleming et al. 1997; Inskip et al. 1996; Kehoe 1987; O'Flaherty et al. 1982; Smith et al. 1996). It can also serve as a source of Pb transfer to the fetus when maternal bone is resorbed for the production of the fetal skeleton (Franklin et al. 1997; Gulson et al. 1997b, 1999b, 2003).

Pb forms highly stable complexes with phosphate and can replace calcium in the calcium-phosphate salt, hydroxyapatite, which comprises the primary crystalline matrix of bone (Bres et al. 1986; Lloyd et al. 1975; Meirer et al. 2011; Miyake 1986; Verbeek et al. 1981). As a result, Pb deposits in bone during the normal mineralization process that occurs during bone growth and remodeling and is released to the blood during the process of bone resorption (Aufderheide and Wittmers 1992; O'Flaherty 1991b, 1993). During infancy and childhood, bone calcification is most active in trabecular bone, whereas in adulthood, calcification occurs at sites of remodeling in cortical and trabecular bone. This suggests that Pb accumulation will occur predominantly in trabecular bone during childhood, and in both cortical and trabecular bone in adulthood (Aufderheide and Wittmers 1992). The association of Pb uptake and release from bone with the normal physiological processes of bone formation and resorption renders Pb biokinetics sensitive to these processes. Physiological states (e.g., pregnancy, menopause, advanced age) or disease-related states (e.g., osteoporosis, prolonged immobilization) that are associated with increased bone resorption will tend to promote the release of Pb from bone, which, in turn, may contribute to an increase in the concentration of Pb in blood (Berkowtiz et al. 2004; Bonithon-Kopp et al. 1985; Garrido Latorre et al. 2003; Hernandez-Avila et al. 2000; Jackson et al. 2010; Markowitz and Weinberger 1990; Mendola et al. 2013; Nash et al. 2004; Nie et al. 2009; Popovic et al. 2005; Silbergeld et al. 1988; Symanski and Hertz-Picciotto 1995; Thompson et al. 1985).

Two physiological compartments appear to exist for Pb in cortical and trabecular bone, to varying degrees. In one compartment, bone Pb is essentially inert, having an elimination half-time of several decades. A labile compartment exists as well that allows for maintenance of an equilibrium of Pb between bone and soft tissue or blood (Rabinowitz et al. 1976). Although a high bone formation rate in early childhood results in the rapid uptake of circulating Pb into mineralizing bone, bone Pb is also recycled to other tissue compartments or excreted in accordance with a high bone resorption rate (O'Flaherty 1995a). Thus, most of the Pb acquired early in life is not permanently fixed in the bone (O'Flaherty 1995a). In general, bone turnover rates decrease as a function of age, resulting in slowly increasing bone Pb levels among adults (Barry 1975; Gross et al. 1975; Schroeder and Tipton 1968).

Bone Pb burdens in adults are slowly lost by diffusion (heteroionic exchange) as well as by resorption (O'Flaherty 1995a, 1995b). An XRF study of tibia Pb concentrations in individuals >10 years old showed a gradual increase in bone Pb after age 20 (Kosnett et al. 1994). In 60–70-year-old men, the total bone Pb burden may be ≥200 mg, while children <16 years old have been shown to have a total bone Pb burden of 8 mg (Barry 1975). However, in some bones (i.e., mid femur and pelvic bone), the increase in Pb content plateaus at middle age and then decreases at higher ages; the decrease with age was more pronounced in females (Drasch et al. 1987). Osteoporosis and release of Pb from resorbed bone to blood may contribute to decreasing bone Pb content in females (Gulson et al. 2002).

Evidence for the exchange of bone Pb and soft tissue Pb stores comes from analyses of stable Pb isotope signatures of Pb in bone and blood. A comparison of blood and bone Pb stable isotope signatures in five adults indicated that bone Pb stores contributed to approximately 40–70% of the Pb in blood (Smith et al. 1996). During pregnancy, the mobilization of bone Pb increases, as the bone is resorbed to produce the fetal skeleton. Analysis for kinetics of changes in the stable isotope signatures of blood Pb in pregnant women as they came into equilibrium with a novel environmental Pb isotope signature indicated that 10–88% of the Pb in blood may derive from the mobilization of bone Pb store and approximately 80% of cord blood may be contributed from maternal bone Pb (Gulson 2000; Gulson et al. 1997b, 1999c, 2003). The mobilization of bone Pb during pregnancy may contribute, along with other mechanisms (e.g., increased absorption), to the increase in Pb concentration that has been observed during the later stages of pregnancy (Gulson et al. 1997b, 2016; Lagerkvist et al. 1996; Schuhmacher et al. 1996). Bone resorption during pregnancy can be reduced by ingestion of calcium supplements (Janakiraman et al. 2003). Additional evidence for increased mobilization of bone Pb into blood during pregnancy is provided from studies in nonhuman primates and rats (Franklin et al. 1997; Maldonado-Vega et al. 1996). Direct evidence for transfer of maternal bone Pb to the fetus has been provided from stable Pb isotope studies in Cynomolgus monkeys (*Macaca fascicularis*) that were dosed with Pb having a different stable isotope ratio than the Pb to which the monkeys were exposed at an earlier age; approximately 7–39% of the maternal Pb burden that was transferred to the fetus appeared to have been derived from the maternal skeleton (Franklin et al. 1997).

In addition to pregnancy, other states of increased bone resorption appear to result in release of bone Pb to blood; these include lactation, osteoporosis, and severe weight loss. Analysis of kinetics of changes in the stable isotope signatures of blood Pb in postpartum women as they came into equilibrium with a novel environmental Pb isotope signature indicated that the release of maternal bone Pb to blood appears to accelerate during lactation (Gulson et al. 2002, 2003, 2004). This is consistent with declines in patella

bone Pb (measured by XRF) during lactation without calcium supplementation (Henandez-Avila et al. 1996). Similar approaches have detected increased release of bone Pb to blood in women, in association with menopause (Gulson et al. 2002). These observations are consistent with epidemiological studies that have shown increases in PbB after menopause and in association with decreasing bone density in postmenopausal women (Berkowitz et al. 2004; Garrido Latorre et al. 2003; Hernandez-Avila et al. 2000; Korrick et al. 2002; Nash et al. 2004; Popovic et al. 2005; Symanski and Hertz-Picciotto 1995). In a prospective study of women who were scheduled to undergo bilateral oophorectomy for benign conditions, blood and tibia bone Pb (measured by XRF and adjusted for bone mineral density) did not change 6–18 months post-surgery, regardless of whether patients were given estrogen replacement therapy (Berkowitz et al. 2004). Severe weight loss (28% of BMI in 6 months) in women, which increased bone turnover, increased PbB (Riedt et al. 2009).

Pb in Soft Tissues. Several studies have compared soft tissue concentrations of Pb in autopsy samples of soft tissues (Barry 1975, 1981; Gross et al. 1975; Schroeder and Tipton 1968). These studies were conducted in the 1960s and 1970s and, therefore, reflect burdens accrued during periods when ambient and occupational exposure levels were much higher than current levels. A more recent autopsy study found lower Pb concentrations in autopsies performed during the period 2004–2013 (Mari et al. 2014). Average PbBs reported in the adult subjects were approximately 20 μg/dL in the Barry (1975) and Gross et al. (1975) studies, whereas more current estimates of the average for adults in the United States are <5 μg/dL (CDC 2018a). Levels in other soft tissues also appear to have decreased substantially since these studies were reported (Barregård et al. 1999; Mari et al. 2014). For example, average Pb concentrations in kidney cortex of male adults were 0.78 μg/g wet tissue and 0.79 μg/g, as reported by Barry (1975) and Gross et al. (1975), respectively (samples in the Barry study were from subjects who had no known occupational exposures). An analysis of kidney biopsy samples collected in Sweden found that the mean level of lead in kidney cortex among subjects not occupationally exposed to Pb was 0.18 μg/g (maximum, 0.56 μg/g) (Barregård et al. 1999). Mari et al. (2014) reported a value of 0.18 μg/g for mean kidney Pb concentration in 20 autopsies performed in Spain. In spite of the downward trends in soft tissue Pb levels, the autopsy studies provide a basis for describing the relative soft tissue distribution of Pb in adults and children. Most of the Pb in soft tissue is in liver. Relative amounts of Pb in soft tissues as reported by Schroeder and Tipton (1968), expressed as percent of total soft tissue Pb, were: liver, 33%; skeletal muscle, 18%; skin, 16%; dense connective tissue, 11%; fat, 6.4%; kidney, 4%; lung, 4%; aorta, 2%; and brain, 2% (other tissues were <1%). The highest soft tissue concentrations in adults also occur in liver and kidney cortex (Barry 1975; Gerhardsson et al. 1986a, 1995b; Gross et al. 1975; Mari et al. 2014; Oldereid et al. 1993). The relative distribution of Pb in soft tissues, in males and

females, expressed in terms of tissue:liver concentration ratios, were: liver, 1.0 (approximately 1 μg/g wet weight); kidney cortex, 0.8; kidney medulla, 0.5; pancreas, 0.4; ovary, 0.4; spleen, 0.3; prostate, 0.2; adrenal gland, 0.2; brain, 0.1; fat, 0.1; testis, 0.08; heart, 0.07; and skeletal muscle, 0.05 (Barry 1975; Gross et al. 1975). In contrast to Pb in bone, which accumulates Pb with continued exposure in adulthood, concentrations in soft tissues (e.g., liver and kidney) are relatively constant in adults (Barry 1975; Treble and Thompson 1997), reflecting a faster turnover of Pb in soft tissue, relative to bone.

Mechanisms by which Pb enters soft tissues have not been fully characterized (Bressler et al. 2005). Studies conducted in preparations of mammalian small intestine support the existence of saturable and nonsaturable pathways of Pb transfer and suggest that Pb can interact with transport mechanisms for calcium and iron (see Section 3.1.1). Pb can enter cells through voltage-gated L-type Ca^{2+} channels in bovine adrenal medullary cells (Legare et al. 1998; Simons and Pocock 1987; Tomsig and Suszkiw 1991) and through store-operated Ca^{2+} channels in pituitary GH3, glial C3, human embryonic kidney, and bovine brain capillary endothelial cells (Kerper and Hinkle 1997a, 1997b). Anion exchangers may also participate in Pb transport in astrocytes (Bressler et al. 2005). In addition to the small intestine, DMT1 is expressed in the kidney (Canonne-Hergaux et al. 1999); however, little information is available regarding the transport of Pb across the renal tubular epithelium. In Madin-Darby canine kidney cells (MDCK), Pb has been shown to undergo transepithelial transport by a mechanism distinct from the anion exchanger that has been identified in red blood cells (Bannon et al. 2000). The uptake of Pb into MDCK cells was both time and temperature dependent. Overexpression of DMT1 in the human embryonic kidney fibroblast cells (HEK293) resulted in increased Pb uptake compared to HEK293 cells in which DMT1 was not overexpressed (Bannon et al. 2002). Based on this limited information, it appears that DMT1 may play a role in the renal transport of Pb.

Pb in other soft tissues such as kidney, liver, and brain exists predominantly bound to protein. High affinity cytosolic Pb binding proteins have been identified in rat kidney and brain (DuVal and Fowler 1989; Fowler 1989; Gonick et al. 2011). The Pb binding proteins of rat are cleavage products of α2μ-globulin, a member of the protein superfamily known as retinol-binding proteins (Fowler and DuVal 1991). α2μ-Globulin is synthesized in the liver under androgen control and has been implicated in the mechanism of male rat hyaline droplet nephropathy produced by certain hydrocarbons (EPA 1991; Swenberg et al. 1989); however, there is no evidence that Pb induces male-specific nephropathy or hyaline droplet nephropathy. The precise role for Pb binding proteins in the toxicokinetics and toxicity of Pb has not been firmly established; however, it has been proposed that binding proteins may serve as a cytosolic Pb "receptor" that, when transported into the nucleus, binds to chromatin and modulates gene

expression (Fowler and DuVal 1991; Mistry et al. 1985, 1986). Other high-affinity Pb binding proteins (Kd approximately 14 nM) have been isolated in human kidney, two of which have been identified as a 5 kD peptide, thymosin 4, and a 9 kD peptide, acyl-CoA binding protein (Smith et al. 1998b). Pb also binds to metallothionein, but does not appear to be a significant inducer of the protein in comparison with the inducers of cadmium and zinc (Eaton et al. 1980; Waalkes and Klaassen 1985). *In vivo*, only a small fraction of the Pb in the kidney is bound to metallothionein, and appears to have a binding affinity that is less than Cd^{2+}, but higher than Zn^{2+} (Ulmer and Vallee 1969); thus, Pb will more readily displace zinc from metallothionein than cadmium (Goering and Fowler 1987; Nielson et al. 1985; Waalkes et al. 1984).

Pb Distribution during Pregnancy and Maternal-Fetal-Infant Transfer. PbBs tend to be lower in pregnant women compared to non-pregnant women of similar age, BMI, iron status, and smoking status (Jain 2013a; Liu et al. 2013). This difference may reflect increased elimination of Pb from the maternal system (Jain 2013b). Maternal PbB changes during and following pregnancy. A U-shaped temporal pattern has been observed in which maternal PbBs decrease during the second trimester and increase during the third trimester and postpartum period (Gulson et al. 2004, 1997, 2016; Hertz-Picciotto et al. 2000; Lagerkvist et al. 1996; Lamadrid-Figueroa et al. 2006; Rothenberg et al. 1994a). Several factors appear to contribute to these changes. During the second trimester, increased plasma volume contributes to hemodilution of maternal blood Pb and a lowering in the PbB (Hytten 1985; Rothenberg et al. 1994b). During the third trimester, growth of the fetal skeleton accelerates, which results in increased mobilization of calcium and Pb from the maternal skeleton, increasing maternal PbB (Gulson et al. 1998b, 2003). Postpartum calcium demand increases further during lactation and breastfeeding, which promotes further mobilization of calcium and Pb from bone and sustains or increases maternal PbBs (Gulson et al. 1998b; Hansen 2011; Tellez-Rojo et al. 2002). Increased demand for calcium in the third trimester and postpartum (to supply calcium for breast milk) is also evident from studies of the effects of dietary calcium supplementation during pregnancy. Calcium supplementation of the maternal diet decreased or delayed the onset of the increase in maternal PbB during the third trimester and postpartum period and delayed mobilization of maternal bone Pb in the third trimester (Ettinger et al. 2009; Gulson et al. 2004, 2016; Manton et al. 2003). The increase in PbB associated with late pregnancy was greater in older women who had a longer history of Pb exposure and, presumably, higher bone Pb levels (Miranda et al. 2010). Pb has been detected in follicular fluid at concentrations similar to that in blood plasma (Silberstein et al. 2006).

A portion of the maternal Pb burden is transferred to the placenta and fetus during pregnancy (Esteban-Vasaloo et al. 2012; Franklin et al. 1997; Gulson et al. 2003, 2016; Kayaalti et al. 2016; Kazi et al. 2014;

O'Flaherty 1998; Reddy et al. 2014). Measurements of stable Pb isotope ratios in pregnant women and cord blood, as they came into equilibrium with a novel environmental Pb isotope signature, indicated that approximately 80% of Pb in fetal cord blood appears to derive from maternal bone stores (Gulson et al. 1997b, 1999c, 2000, 2003, 2016). Stable isotope studies have also demonstrated transfer of Pb from the maternal skeleton to fetus in nonhuman primates (Franklin et al. 1997; O'Flaherty 1998). Transplacental transfer of Pb may be facilitated by an increase in the plasma/PbB ratio during pregnancy (Lamadrid-Figueroa et al. 2006; Montenegro et al. 2008).

Fetal and maternal PbBs and placental Pb concentrations are correlated (Amaral et al. 2010; Baeyens et al. 2014; Baranowska-Boisiacka et al. 2016; Carbone et al. 1998; Chen et al. 2014; Goyer 1990; Graziano et al. 1990; Gulson et al. 2016; Kayaalti et al. 2015b; Kazi et al. 2014; Kim et al. 2015; Kordas et al. 2009; Patel and Prabhu 2009; Reddy et al. 2014). Estimates of the maternal/fetal PbB ratio, based on cord blood Pb measurements at the time of delivery, range from 0.7 to 1.0 at mean maternal PbBs ranging from 1 to 9 μg/dL. In one of the larger studies of fetal PbB, maternal and cord PbB were measured at delivery in 888 mother-infant pairs; the cord/maternal ratio was relatively constant, 0.93, over a blood Pb range of approximately 3–40 μg/dL (Graziano et al. 1990). An analysis of data from 159 mother-infant pairs revealed that higher blood pressure and alcohol consumption late in pregnancy were associated with higher concentrations of Pb in cord blood relative to maternal blood, while higher hemoglobin and sickle cell trait were associated with lower cord blood Pb relative to maternal blood Pb (Harville et al. 2005). No associations were found for calcium intake, physical activity, or smoking. Placental Pb concentrations were found to correlate with ALAD polymorphisms, with higher concentrations observed in association with ALAD2 (Kayaalti et al. 2015b).

Maternal Pb is transferred to infants during breastfeeding. Stable Pb isotope dilution studies suggested that Pb in breast milk can contribute substantially to the isotope profile of infant blood (approximately 40–80%; Gulson et al. 1998b). Numerous studies have reported Pb concentrations in maternal blood and breast milk. In general, these studies indicate that Pb concentrations in breast milk are correlated with Pb concentrations in maternal blood or plasma. Milk/maternal concentration ratios are <0.1, although values of 0.9 have been reported (Baranowska-Boisiacka et al. 2016; Counter et al. 2014; Ettinger et al. 2006, 2014; Gulson et al. 1998a; Koyashiki et al. 2010). Ettinger et al. (2004, 2006) assessed factors influencing breast milk Pb concentration in a group of 367 women and found that PbB (mean 8–9 μg/dL; range 2–30) was a stronger predictor of breast milk Pb (mean 0.9–1.4 μg/dL; range 0.2–8 μg/dL) than bone Pb, and that tibia Pb (mean 9.5 μg/g; range <1–76.5 μg/dL) was a stronger predictor of breast milk Pb than patella bone Pb (mean 14.6 μg/dL; range <1–67.2 μg/dL). Dietary intake of polyunsaturated fatty

acids (PUFA) may decrease transfer of Pb from bone to breast milk (Arora et al. 2008). Pb concentrations in maternal blood and breast milk have been shown to correlate with PbBs in breastfeeding infants (Ettinger et al. 2014; Farhat et al. 2013). Breast milk Pb concentrations explained 37% of the variation in infant blood Pb of breastfeeding infants (Ettinger et al. 2014).

Organic Pb. Information on the distribution of Pb in humans following exposures to organic Pb is extremely limited. One hour following 1–2-minute inhalation exposures to ^{203}Pb tetraethyl or tetramethyl Pb (1 mg/m^3), approximately 50% of the ^{203}Pb body burden was associated with liver and 5% was associated with kidney; the remaining ^{203}Pb was widely distributed throughout the body (Heard et al. 1979). The kinetics of ^{203}Pb in blood of these subjects showed an initial declining phase during the first 4 hours (tetramethyl Pb) or 10 hours (tetraethyl Pb) after the exposure, followed by a phase of gradual increase in PbB that lasted for up to 500 hours after the exposure. Radioactive Pb in blood was highly volatile immediately after the exposure and transitioned to a nonvolatile state thereafter. These observations may reflect an early distribution of organic Pb from the respiratory tract, followed by a redistribution of de-alkylated Pb compounds (see Section 3.1.3 for further discussion of alkyl Pb metabolism).

In a man and woman who accidentally inhaled a solvent containing 31% tetraethyl Pb (17.6% Pb by weight), Pb concentrations in the tissues, from highest to lowest, were liver, kidney, brain, pancreas, muscle, and heart (Bolanowska et al. 1967). In another incident, a man ingested a chemical containing 59% tetraethyl Pb (38% Pb w/w); Pb concentration was highest in the liver followed by kidney, pancreas, brain, and heart (Bolanowska et al. 1967).

3.1.3 Metabolism

Inorganic Pb. Metabolism of inorganic Pb consists of formation of complexes with a variety of protein and nonprotein ligands (see Section 3.1.2 for further discussion). Major extracellular ligands include albumen and nonprotein sulfhydryls. The major intracellular ligand in red blood cells is ALAD. Pb also forms complexes with proteins in the cell nucleus and cytosol.

Organic Pb. Alkyl Pb compounds are actively metabolized in the liver by oxidative dealkylation catalyzed by cytochrome P-450. Relatively few studies that address the metabolism of alkyl Pb compounds in humans have been reported. Studies of workers who were exposed to tetraethyl Pb have shown that tetraethyl Pb is excreted in the urine as diethyl Pb, ethyl Pb, and inorganic Pb (Turlakiewicz

and Chmielnicka 1985; Vural and Duydu 1995; Zhang et al. 1994). Trialkyl Pb metabolites were found in the liver, kidney, and brain following exposure to the tetraalkyl compounds in workers; these metabolites have also been detected in brain tissue of nonoccupational subjects (Bolanowska et al. 1967; Nielsen et al. 1978). In volunteers exposed by inhalation to 0.64 and 0.78 mg Pb/m^3 of ^{203}Pb-labeled tetraethyl and tetramethyl Pb, respectively, Pb was cleared from the blood within 10 hours, followed by a re-appearance of radioactivity back into the blood after approximately 20 hours (Heard et al. 1979). The high level of radioactivity initially in the plasma indicates the presence of tetraalkyl/trialkyl Pb. The subsequent rise in blood radioactivity, however, probably represents water-soluble inorganic Pb and trialkyl and dialkyl Pb compounds that were formed from the metabolic conversion of the volatile parent compounds (Heard et al. 1979).

3.1.4 Excretion

Independent of the route of exposure, absorbed Pb is excreted primarily in urine and feces; sweat, saliva, hair and nails, breast milk, and seminal fluids are minor routes of excretion (Chamberlain et al. 1978; Griffin et al. 1975; Hernandez-Ochoa et al. 2005; Hursh and Suomela 1968; Hursh et al. 1969; Kehoe 1987; Rabinowitz et al. 1976; Sears et al. 2012; Stauber et al. 1994). Fecal excretion accounts for approximately one-third of total excretion of absorbed Pb (fecal/urinary excretion ratio of approximately 0.5), based on intravenous injection studies conducted in humans (Chamberlain et al. 1978). A similar value for fecal/urinary excretion ratio, approximately 0.5, has been observed following inhalation of submicron Pb particles (Chamberlain et al. 1978; Hursh et al. 1969). Contributors to fecal excretion may include secretion into the bile, gastric fluid, and saliva (Rabinowitz et al. 1976). Biliary excretion of Pb has been observed in the dog, rat, and rabbit (Klaassen and Shoeman 1974; O'Flaherty 1993).

Mechanisms by which inorganic Pb is excreted in urine have not been fully characterized. Such studies have been hampered by the difficulties associated with measuring ultrafilterable Pb in plasma and thereby in measuring the GFR of Pb. Renal plasma clearance was approximately 20–30 mL/minute in a subject who received a single intravenous injection of a ^{203}Pb chloride tracer (Chamberlain et al. 1978). Urinary Pb excretion is strongly correlated with the GFR of Pb (Araki et al. 1986) and plasma Pb concentration (Bergdahl et al. 1997b; Rentschler et al. 2012) (i.e., urinary excretion is proportional to GFR x plasma Pb concentration). Estimates of plasma-to-urine clearance of Pb range from 13 to 22 L/day, with a mean of 18 L/day (Araki et al. 1986; Manton and Cook 1984; Manton and Malloy 1983; Chamberlain et al. 1978). The rate of urinary excretion of Pb was less than the GFR of ultrafilterable Pb, suggesting renal tubular reabsorption of Pb from the glomerular filtrate (Araki et al. 1986, 1990). Measurement of the renal

clearance of ultrafilterable Pb in plasma indicates that in dogs, Pb undergoes glomerular filtration and net tubular reabsorption (Araki et al. 1986, 1990; Vander et al. 1977; Victery et al. 1979). Net tubular secretion of Pb has been demonstrated in dogs made alkalotic by infusions of bicarbonate (Victery et al. 1979). Renal clearance of blood Pb increases with increasing PbBs >25 μg/dL (Chamberlain 1983). The mechanism for this has not been elucidated and could involve a shift in the distribution of Pb in blood towards a fraction having a higher GFR (e.g., lower molecular weight complex), a capacity-limited mechanism in the tubular reabsorption of Pb, or the effects of Pb-induced nephrotoxicity on Pb reabsorption.

Excretion and Routes of Exposure

Inhalation Exposure

Inorganic Pb. Inorganic Pb inhaled as submicron particles is deposited primarily in the bronchiolar and alveolar regions of the respiratory tract, from where it is absorbed and excreted primarily in urine and feces (Chamberlain et al. 1978; Hursh et al. 1969; Kehoe 1987). Fecal/urinary excretion ratios were approximately 0.5 following inhalation of submicron Pb-bearing particles (Chamberlain et al. 1978; Hursh et al. 1969). Higher fecal-urinary ratios would be expected following inhalation of larger particle sizes (e.g., >1 μm) as these particles would be cleared to the gastrointestinal tract from where a smaller percentage would be absorbed (Kehoe 1987; see Section 3.1.1).

Organic Pb. Pb derived from inhaled tetraethyl and tetramethyl Pb is excreted in exhaled air, urine, and feces (Heard et al. 1979). Following 1–2-minute inhalation exposures to ^{203}Pb tetraethyl (1 mg/m^3), in four male subjects, 37% of inhaled ^{203}Pb was initially deposited in the respiratory tract, of which approximately 20% was exhaled in the subsequent 48 hours (Heard et al. 1979). In a similar experiment conducted with (^{203}Pb) tetramethyl Pb, 51% of the inhaled ^{203}Pb dose was initially deposited in the respiratory tract, of which approximately 40% was exhaled in 48 hours. Pb that was not exhaled was excreted in urine and feces. Fecal/urinary excretion ratios were 1.8 following exposure to tetraethyl Pb and 1.0 following exposure to tetramethyl Pb (Heard et al. 1979). Occupational monitoring studies of workers who were exposed to tetraethyl Pb have shown that tetraethyl Pb is excreted in the urine as diethyl Pb, ethyl Pb, and inorganic Pb (Turlakiewicz and Chmielnicka 1985; Vural and Duydu 1995; Zhang et al. 1994).

Oral Exposure

Inorganic Pb. Much of the available information on the excretion of ingested Pb in adults derives from studies conducted on five male adults who received daily doses of ^{207}Pb nitrate for periods up to 210 days (Rabinowitz et al. 1976). The dietary intakes of the subjects were reduced to accommodate the tracer doses of ^{207}Pb without increasing daily intake, thus preserving a steady state with respect to total Pb intake and excretion. Total Pb intakes (diet plus tracer) ranged from approximately 210 to 360 μg/day. Urinary excretion accounted for approximately 12% of the daily intake (range for five subjects: 7–17%) and fecal excretion, approximately 90% of the daily intake (range, 87–94%). Based on measurements of tracer and total Pb in saliva, gastric secretions, bile, and pancreatic secretions (samples collected from three subjects by intubation), gastrointestinal secretion of Pb was estimated to be approximately 2.4% of intake (range, 1.9–3.3%). In studies conducted at higher ingestion intakes, 1–3 mg/day for up to 208 weeks, urinary Pb excretion accounted for approximately 5% of the ingested dose (Kehoe 1987). Elimination of Pb is multiphasic, reflecting pools of Pb in the body that have varying retention times. Elimination from blood and soft tissues is faster than bone (Nilsson et al. 1991; Rabinowitz et al. 1976). As a result, after an abrupt decrease in exposure, PbB declines at an apparent rate that reflects excretion of Pb from blood and replenishment of Pb in blood from bone stores. The elimination half-time of Pb in blood in retired lead workers was tri-exponential, with approximately 22% of elimination occurring at a half-time of 34 days (95% CL 29, 41), 28% at a half-time of 1.2 years (95% CL 0.85, 1.8), and 50% at a half-time of 13 years (95% CL 10, 18) (Nilsson et al. 1991). The corresponding mono-exponential half-time for finger bone (XRF) in these same subjects was 16 years (85% CL 12, 23). Apparent elimination half-times for blood Pb in children also vary considerably, dependent in part on age and exposure history of the child that establishes levels of Pb in bone (Manton et al. 2000; Specht et al. 2018). Manton et al. (2000) estimated apparent elimination half-times for PbB in children (ages 2–3 years at time of exposure) that ranged from 8 to 38 months. However, these estimates reflect both excretion of Pb from blood as well as transfer of Pb from bone to blood; the latter would tend to increase the apparent blood elimination half-time. Specht et al. (2018) estimated blood Pb elimination half-times for Pb transferred from bone to blood (estimated with XRF measurements and biokinetics modeling). Estimated blood Pb half-times were 6.9±4 (SD) in children 1–3 years old and 19.3±14.1 days in children >3 years old (Specht et al. 2018).

Dermal Exposure. Inorganic Pb is excreted in sweat and urine following dermal exposure to Pb nitrate or Pb acetate (Moore et al. 1980; Stauber et al. 1994).

3.1.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

PBPK models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic endpoints.

Early Pb modeling applications relied on classical pharmacokinetics. Compartments representing individual organs or groups of organs that share a common characteristic were defined as volumes, or pools, that are kinetically homogeneous. For example, the body could be represented by a central compartment (e.g., blood plasma), and one or two peripheral compartments, which might be "shallow" or "deep" (i.e., they may exchange relatively rapidly or relatively slowly with blood plasma) (O'Flaherty 1987). One of the first of such models was proposed by Rabinowitz et al. (1976) based on a study of the kinetics of ingested stable Pb isotope tracers and Pb balance data in five healthy adult males. The Rabinowitz model included three compartments: a central compartment representing blood and other tissues and spaces in rapid equilibrium with blood (e.g., interstitial fluid); a shallow tissue compartment, representing soft tissues and rapidly exchanging pools within the skeleton; and a deep tissue compartment, representing, primarily, slowly exchanging pools of Pb within bone. Excretion pathways represented in the model included urinary, from the central compartment, and bile, sweat, hair, and nails, from the shallow tissue compartment. The model predicted pseudo-first-order half-times for Pb of approximately 25, 28, and 10^4 days in the central, shallow tissue, and deep compartments, respectively. The slow kinetics of the deep tissue compartment leads to the prediction that it would contain most of the Pb burden after lengthy exposures (e.g., years), consistent with Pb measurements made in human autopsy samples (see Section 3.1.2 Distribution). Note that this model did not simulate the distribution of Pb within blood (e.g., erythrocytes and plasma), nor did it simulate subcompartments within bone or physiological processes of bone turnover that might affect kinetics of the deep tissue compartment.

Marcus (1985b) reanalyzed the data from stable isotope tracer studies of Rabinowitz et al. (1976) and derived an expanded multicompartment kinetic model for Pb that included separate compartments for cortical (slow, $t_{1/2}$ $1.2x10^4$–$3.5x10^4$ days) and trabecular (fast, $t_{1/2}$ 100–700 days), an approach

subsequently adopted in several models (Bert et al. 1989; EPA 1994a, 1994b; Leggett 1993; O'Flaherty 1993, 1995a). A more complex representation of the Pb disposition in bone included explicit simulation of diffusion of Pb within the bone volume of the osteon and exchange with blood at the canaliculus (Marcus 1985a). The bone diffusion model was based on Pb kinetics data from studies conducted in dogs. Marcus (1985c) also introduced nonlinear kinetics of exchange of Pb between plasma and erythrocytes. The blood model included four blood subcompartments: diffusible Pb in plasma, protein-bound Pb in plasma, a "shallow" erythrocyte pool, and a "deep" erythrocyte pool. This model predicted the curvilinear relationship between plasma and PbBs observed in humans (see Section 3.1.2 Distribution for further discussion of plasma-erythrocyte Pb concentrations).

Additional information on Pb biokinetics, bone mineral metabolism, and Pb exposures has led to further refinements and expansions of these earlier modeling efforts. Four pharmacokinetic models, in particular, are currently being used or are being considered for broad application in Pb risk assessment: (1) the O'Flaherty Model, which simulates Pb kinetics from birth through adulthood (O'Flaherty 1993, 1995a); (2) the EPA Integrated Exposure Uptake BioKinetic (IEUBK) Model for Lead in Children developed by EPA (1994a, 1994b); (3) the Leggett Model, which simulates Pb kinetics from birth through adulthood (Leggett 1993); and (4) the EPA All Ages Lead Model (AALM, EPA 2014a). The structure and parameterization of the O'Flaherty Model is distinct from both the IEUBK Model and Leggett Model. The AALM is an update of the O'Flaherty and Leggett models, extended to include a multi-media exposure model.

The IEUBK Model simulates multimedia exposures, uptake, and kinetics of Pb in children ages 0–7 years for predicting pseudo-steady state relationships between Pb exposure and PbB; the model is not intended for use in predicting short-term kinetics of blood Pb or Pb concentrations in tissues other than whole blood. The O'Flaherty Model, Leggett Model, and AALM are lifetime models, and include parameters that simulate uptake and kinetics of Pb during infancy, childhood, adolescence, and adulthood. Pb exposure (e.g., residence-specific environmental Pb concentrations, childhood activity patterns) is not readily described by current versions of the O'Flaherty and Leggett models. The IEUBK Model and AALM include parameters for simulating exposures and uptake to estimate average daily uptake of Pb (μg/day) among populations potentially exposed via soil and dust ingestion, air inhalation, tap water ingestion, diet, and miscellaneous (other) intakes. All four models have been calibrated, to varying degrees, against empirical physiological data on animals and humans, and data on PbBs in individuals and/or populations (Beck et al. 2001; Bowers and Mattuck 2001; Cal EPA 2013; EPA 1994a, 1994c, 2014a, 2014b, 2016; Griffin et al. 1999; Hogan et al. 1998; Leggett 1993; Li et al. 2016; MacMillan et al.

2015; O'Flaherty 1993, 1995, 1998, 2000; Pounds and Leggett 1998; White et al. 1998; Von Lindern et al. 2003, 2016).

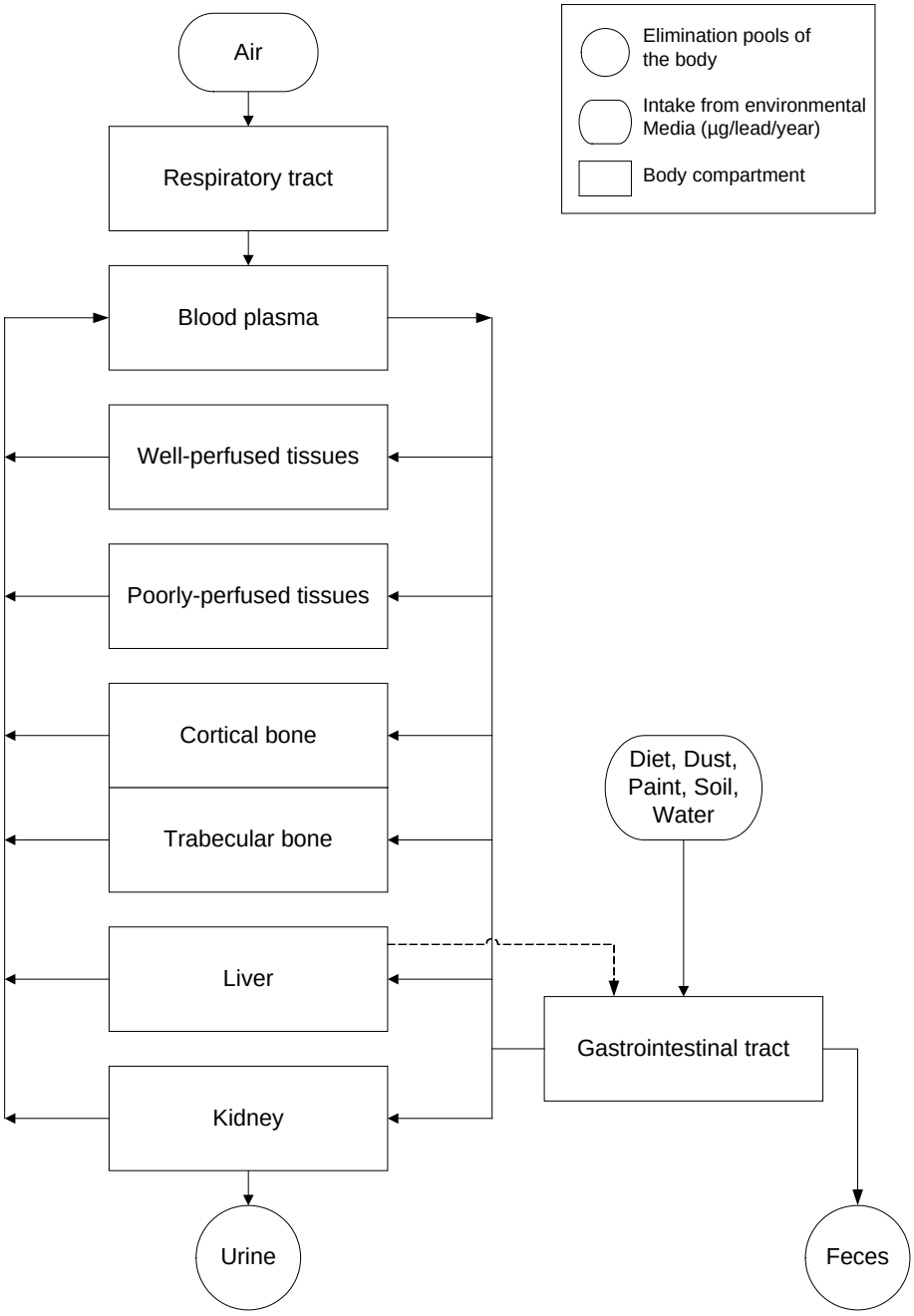
The focus on relying on PbBs for model evaluation and calibration derives from several concerns. The empirical basis for a relationship between low levels of Pb exposure and behavioral dysfunction largely consists of prospective epidemiological studies relating various indices of dysfunction with PbB (see Section 3.3). In this context, PbB has been related to health effects of Pb, and this is the main reason that the focus of interest in the models has been on estimating PbBs. Also, the most available data with which to calibrate and validate the models have been data relating exposure and/or Pb intake to blood concentration. Thus, there is greater confidence in the validity of the models for estimating blood concentrations, rather than Pb levels in other physiologic compartments. Although the principal adverse health effects of Pb have been related to concentrations of Pb in blood, other biomarkers of Pb exposure, such as bone Pb concentrations, are also of value in assessing associations between Pb exposure and health; hence, there is a need for models that predict concentrations of Pb in tissues other than blood (see Section 3.3).

The following four pharmacokinetic models are discussed in great detail below: (1) the O'Flaherty Model (O'Flaherty 1993, 1995a); (2) the IEUBK Model for Lead in Children (EPA 1994a, 1994b); (3) the Leggett Model (Leggett 1993); and (4) AALM (EPA 2014a).

3.1.5.1 O'Flaherty Model

The O'Flaherty Model simulates Pb exposure, uptake, and disposition in humans, from birth through adulthood (O'Flaherty 1993, 1995a). Figure 3-1 shows a conceptualized representation of the O'Flaherty Model, including the movement of Pb from exposure media (i.e., intake via inhalation or ingestion) to the lungs and gastrointestinal tract, followed by the subsequent exchanges between blood plasma, liver, kidney, richly-perfused tissues, poorly-perfused tissues, bone compartments, and excretion from liver and/or kidney. The model simulates both age- and media-specific absorption. Because many of the pharmacokinetic functions are based on body weight and age, the model can be used to estimate PbBs across a broad age range, including infants, children, adolescents, and adults. The model uses physiologically based parameters to describe the volume, composition, and metabolic activity of blood, soft tissues, and bone that determine the disposition of Pb in the human body.

Figure 3-1. Compartments and Pathways of Lead (Pb) Exchange in the O'Flaherty Model*



*Schematic model for Pb kinetics in which Pb distribution is represented by flows from blood plasma to liver, kidney, richly-perfused tissues, poorly-perfused tissues, and cortical and trabecular bone. The model simulates tissue growth with age, including growth and resorption of bone mineral.

Sources: O'Flaherty 1991b, 1993, 1995a

A central feature of the model is the growth curve, a logistic expression relating body weight to age. The full expression relating weight to age has five parameters (constants), so that it can readily be adapted to fit a range of standardized growth curves for men and women. Tissue growth and volumes are linked to body weight; this provides explicit modeling of concentrations of Pb in tissues. Other physiologic functions (e.g., bone formation) are linked to body weight, age, or both.

Pb exchange between blood plasma and bone is simulated as parallel processes occurring in cortical (80% of bone volume) and trabecular bone (20% of bone volume). Uptake and release of Pb from trabecular bone and metabolically active cortical bone are functions of bone formation and resorption rates, respectively. Rates of bone formation and resorption are simulated as age-dependent functions, which gives rise to an age-dependence of Pb kinetics in bone. The model simulates an age-related transition from immature bone, in which bone turnover (formation and resorption) rates are relatively high, to mature bone, in which turnover is relatively slow. Changes in bone mineral turnover associated with senescence (e.g., postmenopausal osteoporosis) are not represented in the model. In addition to metabolically active regions of bone, in which Pb uptake and loss is dominated by bone formation and loss, a region of slow kinetics in mature cortical bone is also simulated, in which Pb uptake and release to blood occur by heteroionic exchange with other minerals (e.g., calcium). Heteroionic exchange is simulated as a radial diffusion in bone volume of the osteon. All three processes are linked to body weight, or the rate of change of weight with age. This approach allows for explicit simulation of the effects of bone formation (e.g., growth) and loss, changes in bone volume, and bone maturation on Pb uptake and release from bone. Exchanges of Pb between blood plasma and soft tissues (e.g., kidney and liver) are represented as flow-limited processes. The model simulates saturable binding of Pb in erythrocytes; this replicates the curvilinear relationship between plasma and erythrocyte Pb concentrations observed in humans (see Section 3.1.2). Excretory routes include kidney to urine and liver to bile. Total excretion (clearance from plasma attributable to bile and urine) is simulated as a function of GFR. Biliary and urinary excretory rates are proportioned as 70 and 30% of the total plasma clearance, respectively.

The O'Flaherty Model simulates Pb intake from inhalation and ingestion. Inhalation rates are age-dependent. Absorption of inhaled Pb is simulated as a fraction (0.5) of the amount inhaled, and is independent of age. The model simulates ingestion exposures from infant formula, soil and dust ingestion, and drinking water ingestion. Rates of soil and dust ingestion are age-dependent, increasing to approximately 130 mg/day at age 2 years, and declining to <1 mg/day after age 10 years. Gastrointestinal absorption of Pb in diet and drinking water is simulated as an age-dependent fraction, declining from

0.58 of the ingestion rate at birth to 0.08 after age 8 years. These values can be factored to account for relative bioavailability when applied to absorption of Pb ingested in dust or soil.

The O'Flaherty Model, as described in O'Flaherty (1993, 1995a), utilizes point estimates for parameter values and yields point estimates as output; however, a subsequent elaboration of the model has been developed that utilizes a Monte Carlo approach to simulate variability in exposure, absorption, and erythrocyte Pb binding capacity (Beck et al. 2001). This extension of the model can be used to predict the probability that children exposed to Pb in environmental media will have PbBs exceeding a health-based reference value (e.g., 5 μg/dL).

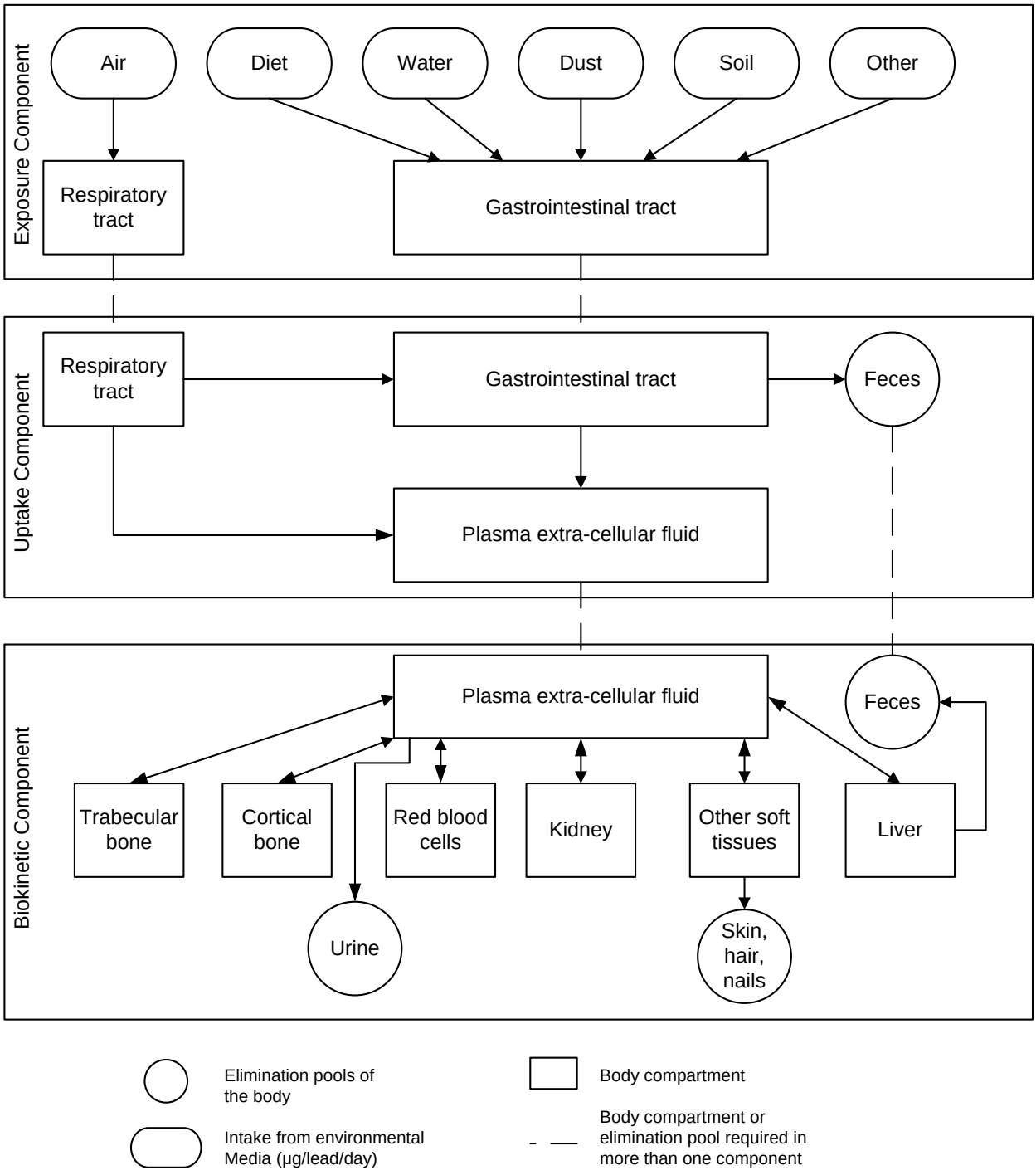
The model was designed to operate with an exposure time step on 1 year (the smallest time interval for a single exposure event). However, the implementation code allows constructions of simulations with an exposure time step as small as 1 day, which would allow simulation of rapidly changing intermittent exposures (e.g., an acute exposure event).

The O'Flaherty Model was initially calibrated to predict blood, bone, and tissue Pb concentrations in rats (O'Flaherty 1991a), and subsequently modified to reflect anatomical and physiological characteristics in children (O'Flaherty 1995a), adults (O'Flaherty 1993), and Cynomolgus monkeys (*M. fasicularis*) (O'Flaherty et al. 1998). Model parameters were modified to correspond with available information on species- and age-specific anatomy and physiological processes described above. Comparisons of predicted and observed PbB in children and adults are reported in O'Flaherty (1993, 1995a). MacMillan et al. (2015) evaluated performance of the model for predicting population blood and bone Pb levels in a convenience sample of 263 individuals (age range 1–83 years) who experienced low chronic exposure. Based on this evaluation, model performance for predicting general trends in population PbBs and cortical bone Pb concentrations was improved by revising parameters that determine binding of Pb in red blood cells. Revisions included decreasing the maximum and affinity constants (*BIND* and *KBIND*, respectively) and increasing clearance of Pb from blood to bone by increasing the permeability constant for Pb diffusion across the canaliculi-bone interface from canaliculi to bone (P_0).

3.1.5.2 EPA IEUBK Model

The EPA IEUBK Model for Lead in Children simulates Pb exposure, uptake, and disposition in human children from birth to age 7 years (EPA 1994a, 1994b, 2002a; White et al. 1998). Figure 3-2 shows a conceptualized representation of the IEUBK Model. The model has four major submodels: (1) exposure

Figure 3-2. Structure of the IEUBK Model for Lead (Pb) in Children*



*Schematic for integrated Pb exposure-kinetics model in which simulated multi-media exposures are linked to simulations of lead uptake (i.e., absorption into the plasma-extracellular fluid), tissue distribution, and excretion).

Sources: EPA 1994a, 1994b

model, in which average daily intakes of Pb (μg/day) are calculated for each inputted exposure concentration (or rates) of Pb in air, diet, dust, soil, and water; (2) uptake model, which converts environmental media-specific Pb intake rates calculated from the exposure model into a media-specific time-averaged uptake rate (μg/day) of Pb to the central compartment (blood plasma); (3) biokinetic model, which simulates the transfer of absorbed Pb between blood and other body tissues, elimination of Pb from the body (via urine, feces, skin, hair, and nails), and predicts an average PbB for the exposure time period of interest; and (4) blood Pb probability model, which applies a log-normal distribution (using geometric mean and geometric standard deviation for parameters) to predict probabilities for the occurrence of a specified given PbB in a population of similarly exposed children.

Exposure Model. The exposure model simulates intake of Pb (μg/day) for inputted exposures to Pb in air (μg/m^3), drinking water (μg/L), soil-derived dust (μg/g), or diet (μg/day). The exposure model operates on a 1-year time step, the smallest time interval for a single exposure event. The model accepts inputs for media intake rates (e.g., air volumes, breathing rates, drinking water consumption rate, soil and dust ingestion rate). The air exposure pathway is partitioned in exposures to outdoor air and indoor air, with age-dependent values for time spent outdoors and indoors (hours/day). Exposure to Pb to soil-derived dust is also partitioned into outdoor and indoor contributions. The intakes from all ingested exposure media (diet, drinking water, soil-derived dust) are summed to calculate a total intake to the gastrointestinal tract, for estimating capacity-limited absorption (see description of the uptake model).

Uptake Model. The uptake model simulates Pb absorption for the gastrointestinal tract as the sum of capacity-limited (represented by a Michaelis-Menten type relationship) and unlimited processes (represented by a first-order, linear relationship). These two terms are intended to represent two different mechanisms of Pb absorption, an approach that is in accord with limited available data in humans and animals that suggest a capacity limitation to Pb absorption (see Section 3.2.1). One of the parameters for the capacity-limited absorption process (that represents that maximum rate of absorption) is age-dependent. The above representation gives rise to a decrease in the fractional absorption of ingested Pb as a function of total Pb intake as well as an age-dependence of fractional Pb absorption. Absorption fractions are also medium-specific. At 30 months of age, at low intakes (<200 μg/day), below the rates at which capacity-limitation has a significant impact on absorption, the fraction of ingested Pb in food or drinking water that is absorbed is 0.5 and decreases to approximately 0.11 (intake, >5,000 μg/day). For Pb ingested in soil or dust, fractional absorption is 0.35 at low intakes (<200 μg/day) and decreases to 0.09 (intake, >5,000 μg/day).

The uptake model assumes that 32% of inhaled Pb is absorbed. This value was originally assigned based on a scenario of exposure to active smelter emissions, which assumed the particle size distribution in the vicinity of an active Pb smelter (<1 μm, 12.5%; 1–2.5 μm, 12.5%; 2–15 μm, 20%; 15–30 μm, 40%; >30 μm, 15%); size-specific deposition fractions for the nasopharyngeal, tracheobronchial, and alveolar regions of the respiratory tract; and region-specific absorption fractions. Pb deposited in the alveolar region is assumed to be completely absorbed from the respiratory tract, whereas Pb deposited in the nasopharyngeal and tracheobronchial regions (30–80% of the Pb particles in the size range 1–15 μm) is assumed to be transported to the gastrointestinal tract.

Biokinetics Model. The biokinetics model includes a central compartment, six peripheral body compartments, and three elimination pools (urine, feces, lumped pool representing skin, hair, and nails). The body compartments include plasma and extracellular fluid (central compartment), red blood cells, kidney, liver, trabecular bone, cortical bone, and other soft tissue (EPA 1994a). The model simulates growth of the body and tissues, compartment volumes, and Pb masses and concentrations in each compartment. PbB at birth (neonatal) is assumed to be 0.85 of the maternal blood Pb. Neonatal Pb masses and concentrations are assigned to other compartments based on a weighted distribution of the neonatal PbB. Exchanges between the central compartment and tissue compartments are simulated as first-order processes, which are parameterized with unidirectional, first-order rate constants. Bone is simulated as two compartments: a relatively fast trabecular bone compartment (representing 20% of bone volume) and a relatively slow cortical bone compartment (representing 80% of the bone volume). Saturable uptake of Pb into erythrocytes is simulated, with a maximum erythrocyte Pb concentration of 12 μg/dL. Excretory routes simulated include urine, from the central compartment; bile-feces, from the liver; and a lumped excretory pathway representing losses from skin, hair and nail, from the other soft tissue compartment.

Blood Pb Probability Model. Inputs to the IEUBK Model are exposure point estimates that are intended to represent time-averaged central tendency exposures. The output of the model is a central tendency estimate of PbB for children who might experience the inputted exposures. However, within a group of similarly exposed children, PbBs would be expected to vary among children as a result of inter-individual variability in media intakes, absorption, and biokinetics. The model simulates the combined impact of these sources of variability as a lognormal distribution of PbB for which the geometric mean is given by the central tendency PbB outputted from the biokinetics model and the GSD is an input parameter. The resulting lognormal distribution also provides the basis for predicting the probability of occurrence of given PbB within a population of similarly exposed children. The model can be iterated for varying

exposure concentrations (e.g., a series of increasing soil Pb concentrations) to predict the media concentration that would be associated with a probability of 0.05 for the occurrence of a PbB exceeding 10 μg/dL. A subsequent elaboration of the model has been developed that utilizes a Monte Carlo approach to simulate variability and uncertainty in exposure and absorption (Goodrum et al. 1996; Griffin et al. 1999). This extension of the model provides an alternative to the blood Pb probability model for incorporating, explicitly, estimates of variability (and uncertainty in variability) in exposure and absorption into predictions of an expected probability distribution of PbBs. More recently, Zartarian et al. (2017) provided an analysis coupling the IEUBK model with EPA's Stochastic Human Exposure and Dose Simulation (SHEDS)-Multimedia Model that considered general U.S. childhood exposures probabilistically and assessed primary sources of Pb exposure across the distribution of PbB.

Performance of the IEUBK Model has been evaluated for predicting observed PbBs in children (Hogan et al. 1998; Li et al. 2016; Von Lindern et al. 2003, 2016). The largest evaluation utilized longitudinal exposure and blood Pb data for approximately 2,200 children who resided near a former smelter in northern Idaho (Bunker Hill site) during a 14-year period of remediation activities (Von Lindern et al. 2003, 2016). The observed annual blood Pb geometric means ranged from 2.5 to 10.6 μg/dL. The model predicted the time course of the observed PbBs as the remediation progressed when the gastrointestinal absorption fraction was calibrated to agree with blood Pb observations (Von Lindern et al. 2003). A similar outcome was obtained in a subsequent analysis in which the gastrointestinal absorption fraction was adjusted to agree with site measurements of soil Pb RBA, and soil and dust ingestion rates were calibrated to the blood Pb observations (Von Lindern et al. 2016). The mean difference between predicted and observed annual geometric mean PbBs (predicted - observed) was -0.31 μg/dL (range: -1.07, 1.93) and the mean relative percent difference was -8.4% (range: -23–21%). Applications of the IEUBK Model to the Bunker Hill site were reviewed by the National Research Council (NRC 2005). Hogan et al. (1998) evaluated the IEUBK Model performance based on residential exposure and blood data for approximately 478 children who resided near three Pb mining and smelting sites. The observed geometric means for the three sites ranged from 5.2 to 6.8 μg/dL. The IEUBK Model predictions agreed reasonably well with observations for children whose exposures were predominantly from their residence (e.g., who spent no more than 10 hours/week away from home). The mean difference between predicted and observed site geometric mean PbBs (predicted-observed) was 0.03 μg/dL (range -0.6–0.7) and the mean relative percent difference was -0.4% (range -12–10%). The predicted geometric mean PbBs were within 0.7 μg/dL of the observed geometric means at each site. The prediction of the percentage of children expected to have PbBs exceeding 10 μg/dL were within 4% of the observed percentage at each site. Li et al. (2016) compared predictions of PbB to observations in a cohort

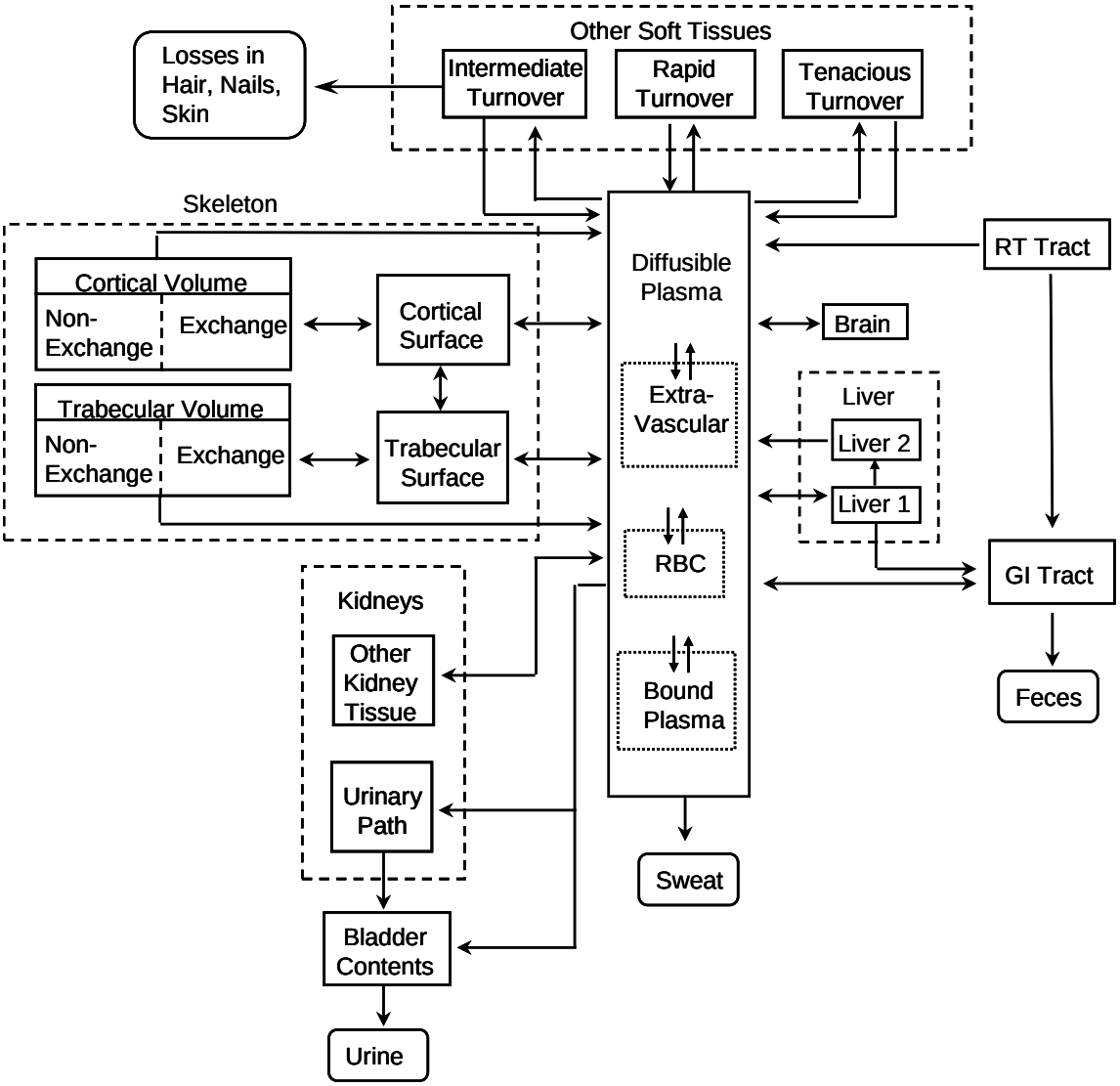
of 760 children in Central China. The observed residence area geometric means ranged from 5 to 14 µg/dL. When exposure parameters for set to the study population (e.g., exposure media Pb concentration and intakes), predicted and observed PbBs were not significantly different. The mean difference between predicted and observed geometric mean PbBs for 21 residence areas (predicted-observed) was 0.55 µg/dL (range -2.0–3.2) and the mean relative percent difference was 3.5% (range -32–28%). These evaluations provide support for the validity of the IEUBK Model for estimating PbBs in children at sites where their exposures can be adequately characterized. Similar empirical comparisons of the IEUBK Model have shown that agreement between model predictions and observed PbBs at specific locations is influenced by numerous factors, including the extent to which the exposure and blood Pb measurements are adequately matched, and site-specific factors (e.g., soil characteristics, behavior patterns, bioavailability) that may affect Pb intake or uptake in children (Bowers and Mattuck 2001; Von Lindern et al. 2003, 2016). In addition to the above empirical comparisons, the computer code used to implement the IEUBK Model (IEUBK version 0.99d) has undergone an independent validation and verification and has been shown to accurately implement the conceptual IEUBK Model (Zaragoza and Hogan 1998).

3.1.5.3 Leggett Model

The Leggett Model simulates Pb intake, absorption, and disposition in humans, from birth through adulthood (Leggett 1993). Figure 3-3 shows a conceptualized representation of the model, including the movement of Pb from exposure media (i.e., intake via inhalation or ingestion) to the lungs and gastrointestinal tract, followed by the subsequent exchanges between diffusible blood plasma, soft tissues, bone compartments, and excretion from liver, kidneys, and sweat. A detailed exposure module is not linked to the Leggett Model; rather, Pb exposure estimates are incorporated into the model as age-specific point estimates of average daily intake (µg/day) from inhalation and ingestion. A description of the model and its potential application to risk assessment are provided below.

The Leggett Model includes a central compartment, 15 peripheral body compartments, and 4 elimination pools (urine, feces, sweat, and lumped pool representing skin, hair, and nails), as illustrated in Figure 3-3. Transport of Pb from blood plasma to tissues is assumed to follow first-order kinetics. Transfer rate constants vary with age and PbB. Above a nonlinear threshold concentration in red blood cells (assumed to be 60 µg/dL), the rate constant for transfer to red blood cells declines and constants to all other tissues increase proportionally (Leggett 1993). This replicates the nonlinear relationship between plasma and red blood cells observed in humans (see Section 3.1.2). The model simulates blood volume as an age-

Figure 3-3. Compartments and Pathways of Lead (Pb) Exchange in the Leggett Model*



*Schematic model for Pb kinetics in which Pb distribution is represented by exchanges between the central plasma-extracellular fluid and tissue compartments. Bone is represented as having surface (which rapidly exchanges with plasma-extracellular fluid) and volume compartments; the latter simulates slow exchange with the surface and slow return of Pb to the plasma-extracellular fluid from bone resorption.

GI = gastrointestinal; RBC = red blood cell; RT = respiratory

Source: Leggett 1993

dependent function, which allows simulation of plasma and PbBs. Pb masses are simulated in all other tissues (tissue volumes are not simulated).

Unidirectional, first-order transfer rates (day^{-1}) between compartments were developed for six age groups, and intermediate age-specific values are obtained by linear interpolation. The total transfer rate from diffusible plasma to all destinations combined is assumed to be 2,000 day^{-1}, based on isotope tracer studies in humans receiving Pb via injection or inhalation. Values for transfer rates in various tissues and tissue compartments are based on measured deposition fractions or instantaneous fractional outflows of Pb between tissue compartments (Leggett 1993).

The Leggett Model was developed from a biokinetic model originally developed for the International Commission on Radiological Protection (ICRP) for calculating radiation doses from environmentally important radionuclides, including radioisotopes of Pb (Leggett 1993). The Leggett Model simulates age-dependent bone physiology using a model structure developed for application to the alkaline earth elements, but parameterized using data specific to Pb where possible. The model simulates both rapid exchange of Pb with plasma via bone surface and slow loss by bone resorption. Cortical bone volume (80% of bone volume) and trabecular bone volume (20% of bone volume) are simulated as bone surface compartments, which rapidly exchange Pb with the blood plasma, and bone volume, within which are *exchangeable* and *nonexchangeable* pools. Pb enters the exchangeable pool of bone volume via the bone surface and can return to the bone surface, or move to the nonexchangeable pool, from where it can return to the blood only when bone is resorbed. Rate constants for transfer of Pb from the nonexchangeable pools and blood plasma vary with age to reflect the age-dependence of bone turnover.

The liver is simulated as two compartments: one compartment has a relatively rapid uptake of Pb from plasma and a relatively short removal half-time (days) for transfers to plasma and to the small intestine by biliary secretion, and a second compartment simulates a more gradual transfer to plasma of approximately 10% of Pb uptake in liver. The kidney is simulated as two compartments: one that exchanges slowly with blood plasma and accounts for Pb accumulation in kidney tissue, and a second compartment that receives Pb from blood plasma and rapidly transfers Pb to urine, with essentially no accumulation (urinary pathway). Other soft tissues are simulated as three compartments representing rapid, intermediate, and slow turnover rates (without specific physiologic correlates). Other excretory pathways (hair, nails, and skin) are represented as a lumped pathway from the intermediate turnover rate soft tissue compartment.

The Leggett Model simulates Pb intakes from inhalation, ingestion, or intravenous injection. The latter was included to accommodate model evaluations based on intravenous injection studies in humans and animal models. The respiratory tract is simulated as four compartments into which inhaled Pb is deposited and absorbed with half-times of 1, 3, 10, and 48 hours. Four percent of the inhaled Pb is assumed to be transferred to the gastrointestinal tract. These parameter values reflect the data on which the model was based, which were derived from studies in which human subjects inhaled submicron Pb-bearing particles (Chamberlain et al. 1978; Hursh and Mercer 1970; Hursh et al. 1969; Morrow et al. 1980; Wells et al. 1975). These assumptions would not necessarily apply to exposures to large airborne particles (see Section 3.1.1). Absorption of ingested Pb is simulated as an age-dependent fraction of the ingestion rate, declining from 0.45 at birth to 0.3 at age 1 year (to age 15 years), and to 0.15 after age 25 years.

Output from the Leggett Model has been compared with data in children and adult subjects exposed to Pb in order to calibrate model parameters (Leggett et al. 1993; Pounds and Leggett 1998). Nie et al. (2005) evaluated performance of the Leggett Model for predicting bone Pb concentrations in 539 Pb workers. The data included periodic monitoring of PbBs and XRF bone Pb measurements made in 1994 and 1999. Pb intakes of each individual were calibrated to agree with measured PbBs. The Leggett Model underpredicted observed cortical bone Pb concentrations by a factor of 3–4, and underpredicted trabecular bone Pb concentration by a factor of 12–18. EPA (2014a) evaluated performance of the Leggett Model for predicting PbBs in children and blood and bone Pb concentrations in adults. The evaluation of predictions for children used data on blood lead concentrations reported in the NHANES for the years 2007–2008, and required making assumptions about Pb exposures in this population. The Leggett Model overpredicted observed PbBs in children 1–7 years of age by a factor of 2–3. Cal EPA (2013) evaluated the Leggett Model for predicting PbBs in smelter workers whose occupational exposures were interrupted during a workers strike. Pre-hire background Pb intakes and pre-strike intakes were calibrated to agree with measured PbBs and the predicted rate of decline in blood Pb that occurred during the strike period was compared to observations. Cal EPA (2013) reported "the average difference between the measured and predicted post-strike BLL was unacceptably large and indicated significant under-prediction of BLLs". The average difference was >4 µg/dL in a cohort that had a mean post-strike PbB of 31 µg/dL (no further details were provided). Performance was substantially improved when various parameters were calibrated to the observations. These included parameters that control transfers between plasma and bone and red blood cell saturation (see Cal EPA [2013] for details of parameter values changes). The mean difference between predicted and observed annual geometric mean PbBs (predicted-observed) was -0.9 µg/dL (range -26–32) and the mean relative percent difference was -8.8% (range: -55–320%).

Cal EPA (2013) reported several other evaluations of their recalibrated model, including observed and predicted relationships between plasma and whole PbBs in adults, and predicted distribution of Pb in bone and soft tissues compared to estimates from human autopsy studies.

3.1.5.4 EPA All Ages Lead Model (AALM)

The AALM simulates blood and tissue Pb masses (µg) and concentrations (µg/g) resulting from exposures to Pb in air, drinking water, surface dust (e.g., indoor dust, soil dust), food, or miscellaneous Pb ingestion pathways. The AALM exposure module allows the user to simulate multi-pathway exposures that are constant or that vary in time increments as small as 1 day and that occur at any age from birth to 90 years. The user can select to run a systemic biokinetics simulation based on either the Leggett (AALM-LG) or O'Flaherty (AALM-OF) biokinetics models. Parameters in both systemic models were re-calibrated with observations of blood, bone, and soft tissue Pb concentrations in children and adults (EPA 2014a). The version of the AALM described in EPA (2014a) was implemented in Advanced Continuous Simulation Language (acslX, ver. 3.1.4.2). The ICRP Human Respiratory Tract Model (HRTM) deposition and absorption parameters are used in both the AALM-LG and AALM-OF, which allows simulation of inhaled Pb particles of specified size ranges and absorption kinetics (ICRP 1994). The gastrointestinal tract model includes age-dependent absorption fractions and parameters for RBA of Pb from all ingestion pathways.

The structures of the two systemic biokinetics models in AALM-OF and AALM-LG are based on the O'Flaherty and Leggett models, respectively, with the following modifications. Growth parameters from the O'Flaherty Model are used in both models to simulate age-dependent body weight tissue weights. This provides a means for calculating tissue concentrations as the Pb mass (µg) divided by the tissue weight (g). Concentrations of Pb in bone wet weight are converted to concentration per g bone mineral by dividing the wet weight concentration by the ash fraction of bone. This conversion provides a means for comparing model predictions of bone Pb concentration with bone XRF data, which is typically reported in units of Pb per g bone mineral. Parameters for RBA of Pb in each intake medium include the gastrointestinal tract model. This provides a means for independently adjusting the absorption fraction for each of the intake pathways (including respiratory tract-to-gastrointestinal tract) and maintains mass balance for fecal excretion of unabsorbed Pb. Inhalation, deposition, mucociliary clearance, and absorptive clearance of airborne Pb is simulated with a simplified implementation of the ICRP HRTM.

The AALM systemic biokinetic models were recalibrated from the original Leggett and O'Flaherty Models (EPA 2014b). The sequential recalibration utilized several sources of data on blood and bone Pb concentrations in humans. Parameters that control the uptake and retention of Pb in red blood cells were recalibrated using paired data on whole blood and plasma Pb concentrations in children and adults (Bergdahl et al. 1997c, 1998, 1999; Hernández-Avila et al. 1998; Manton et al. 2001; Schütz et al. 1996; Smith et al. 2002). Parameters that control plasma-to-urine clearance were recalibrated based on clearance estimates from studies that measured paired plasma concentration and urinary Pb excretion in adults (Araki et al. 1986; Chamberlain et al. 1978; Manton and Cook 1984; Manton and Malloy 1983). Autopsy data from children and adults were used to evaluate parameters that control the relationship between of tissue Pb concentrations and bone Pb concentrations (Barry 1975). The relationship between bone and plasma Pb concentrations was evaluated with paired data for plasma Pb concentration and XRF bone Pb in adults (Cake et al. 1996; Hernández-Avila et al. 1998). The long-term rate elimination of Pb from blood and bone was evaluated with data on blood and XRF bone Pb in retired Pb workers (Nilsson et al. 1991).

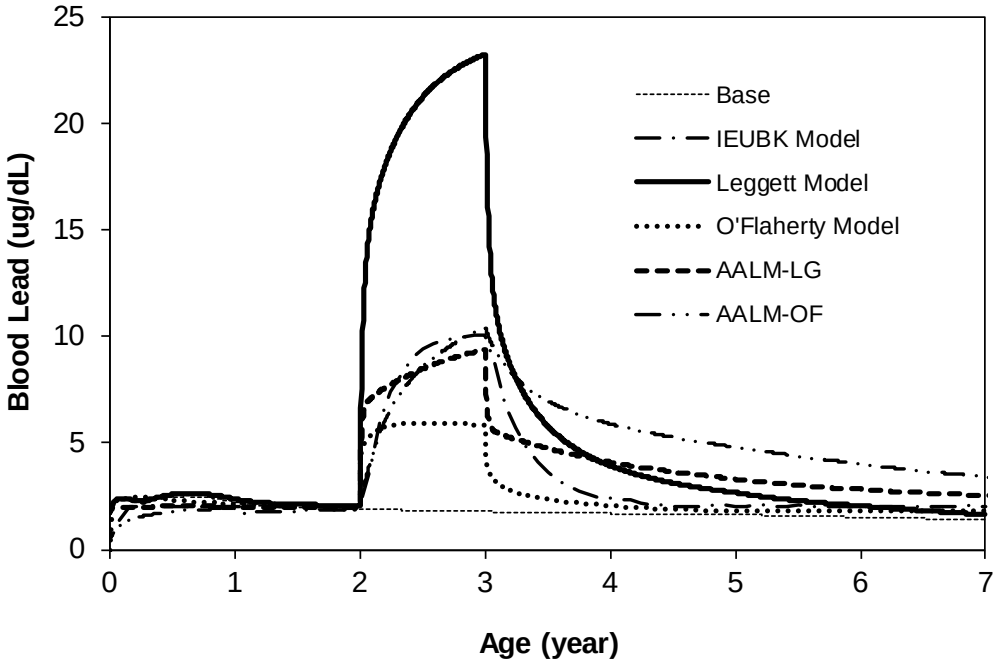
The calibrated AALM was evaluated with data on PbBs measured in infants (Ryu et al. 1983; Sherlock and Quinn 1986) or adults (Rabinowitz et al. 1976) who consumed known quantities of Pb. In the Ryu et al. (1983) study, PbBs were monitored in formula-fed infants who were fed measured quantiles of formula. PbBs predicted from the AALM-LG were within 1 SD of the group means and the r^2 for predictions was 0.85. Predictions from the AALM-OF were uniformly higher than observations and the r^2 for predictions was 0.76. Sherlock and Quinn (1986) measured PbB in infants at age 13 weeks and estimated dietary intake of Pb for each infant based on Pb measurements made in duplicate diet samples collected daily during week 13. The observed dose-blood Pb relationship was predicted with r^2 values of 0.95 for AALM-LG and 0.98 for AALM-OF. Rabinowitz et al. (1976) conducted a pharmacokinetics study in which four adults ingested daily doses of [^{207}Pb] nitrate for periods up to 124 days. Concentrations of ^{207}Pb in blood, urine, and feces were then monitored during and following cessation of exposure, and data on daily intakes and blood concentrations for each subject were reported. Absorption fractions for Pb were estimated for each individual based on mass balance in feces. AALM-LG predictions are closer to the observations; r^2 values ranged from 0.92 to 0.98 for four subjects in the study. The AALM-OF predicted a slower accrual and decline of blood Pb, and lower peak PbBs (r^2 <0.25).

3.1.5.5 Model Comparisons

The O'Flaherty, IEUBK, and Leggett Model differ considerably in the way each represents tissues, exchanges of Pb between tissues, and Pb exposure. The AALM includes biokinetics models based on, but updated from, the O'Flaherty and Leggett models.

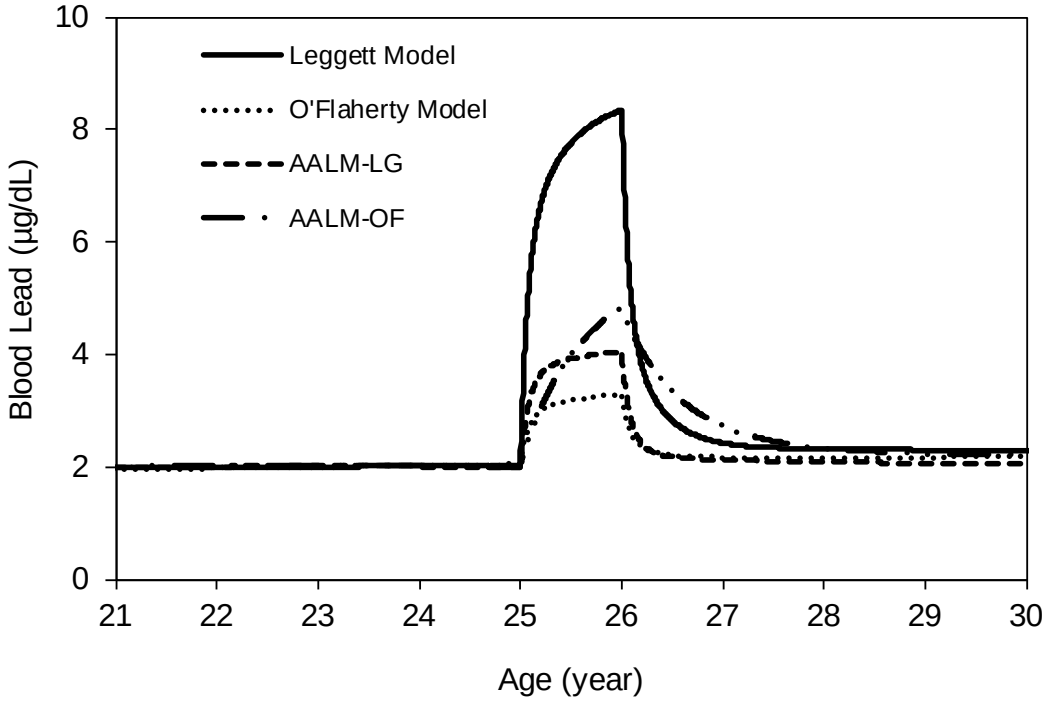
Figure 3-4 compares the PbBs predicted by each model for a hypothetical child who ingests 100 μg Pb/day in soil for a period of 1 year beginning at the age of 2 years (e.g., equivalent to ingestion of 100 μg soil/day at a soil Pb concentration of 1,000 mg Pb/g soil). The 100-μg/day exposure is superimposed on a baseline exposure that yields a PbB of approximately 2 μg/dL at 2 years of age. All five models predict an increase in PbB towards a quasi-steady state during the exposure period, followed by a decline towards the pre-exposure baseline PbB with an apparent half-time of approximately 1 month. Predicted PbBs at the end of the 12-month soil exposure period were 10, 23, 5.9, 23, 9.4, and 10.4 μg/dL for the IEUBK Model, Leggett Model, O'Flaherty Model, AALM-LG, and AALM-OF, respectively. Differences in the magnitude of the predicted impact of the soil exposure on PbB reflect differences in assumptions about Pb biokinetics and cannot be attributed solely to different assumptions about Pb bioavailability. Bioavailability assumptions in the models for the age range 2–3 years are: O'Flaherty Model, 45% (50% at age 2 years, decreasing to 40% at age 3 years); IEUBK Model, 30% (soil Pb at low intakes); Leggett Model, 30%; and AALM-LG and AALM-OF 34% (38% at age 2 years and decreasing to 30% at age 3 years). A comparison of model predictions for a similar exposure during adulthood (100 μg Pb/day for 1 year, beginning at age 25) is shown in Figure 3-5. Predicted PbBs at the end of the 12-month soil exposure period were 8.4, 3.3, 4.0, and 4.8 μg/dL for the Leggett Model, O'Flaherty Model, AALM-LG, and AALM-OF, respectively. All four models predict a smaller change in PbB in adults, compared to children, for a similar increment in exposure. This is attributed, in part, to assumptions of lower Pb bioavailability in adults (i.e., O'Flaherty, 8%; Leggett, 15%; AALM-LG and AALM-OF, 8%).

Figure 3-4. Blood Lead Concentrations (PbBs) in Children Predicted by the IEUBK, Leggett, and O'Flaherty Models and AALM*



*The simulations are of a hypothetical child who has a PbB of 2 µg/dL at age 2 years, and then experiences a 1-year exposure to 100 µg Pb/day. The 100 µg/day exposure was simulated as an exposure to lead in soil in the IEUBK Model. Default bioavailability assumptions were applied in all three models.

Figure 3-5. Blood Lead Concentrations (PbBs) in Adults Predicted by the Leggett and O'Flaherty Models and AALM*



*The simulations are of a hypothetical adult who has a PbB of 2 µg/dL at age 25 years, and then experiences a 1-year exposure to 100 µg Pb/day. Default bioavailability assumptions were applied in all three models.

3.1.5.6 Slope Factor Models

Slope factor models have been used as simpler alternatives to compartmental models for predicting PbBs, or the change in PbB, associated with a given exposure (Abadin et al. 1997; Bowers et al. 1994; Carlisle and Wade 1992; EPA 2017d; Maddaloni et al. 2005; Stern 1994, 1996). In slope factor models, Pb biokinetics is represented with a simple linear relationship between the PbB and either Pb uptake (biokinetic slope factor, BSF) or Pb intake (intake slope factor, ISF). The models take the general mathematical forms:

$$PbB = E \cdot ISF$$

$$PbB = E \cdot AF \cdot BSF$$

where E is an expression for exposure (e.g., soil intake x soil Pb concentration) and AF is the absorption fraction for Pb in the specific exposure medium of interest. Intake slope factors are based on ingested Pb, rather than absorbed Pb and, therefore, integrate both absorption and biokinetics into a single slope factor,

whereas models that utilize a biokinetic slope factor (BSF) to account for absorption in the relationship include an absorption parameter. Slope factors used in various models are presented in Table 3-2. Of the various models presented in Table 3-2, the Bowers et al. (1994) and EPA (2003b) models implement BSFs. The slope factors used in both models (approximately 0.4 μg/dL per μg Pb/day) are similar to BSFs predicted from the O'Flaherty Model (0.65 μg/dL per μg Pb uptake/day) and Leggett Model (0.43 μg/dL per μg Pb uptake/day) for simulations of adult exposures (Maddaloni et al. 2005).

Table 3-2. Comparison of Slope Factors in Selected Slope Factor Models

Model	Receptor	Intake route	Slope factor		Absorption fraction
			Intake	Biokinetics	
Bowers et al. 1994	Adult	Ingestion of soil/dust	ND	0.375	0.08
Carisle and Wade 1992	Child	Ingestion of soil/dust Ingestion of water	0.07 0.04	ND	ND
Carisle and Wade 1992	Adult	Ingestion of soil/dust Ingestion of water	0.018 0.04	ND	ND
Cal EPA 2017	Child	Ingestion of soil/dust Inhalation of respirable dust Dermal contact	ND 0.192 0.0001	0.16 ND ND	0.44 ND ND
EPA 2017d; Maddaloni et al. 2005	Adult	Ingestion of soil/dust	ND	0.4	0.12
Stern 1994	Child	Ingestion of soil/dust	T (0.056, 0.16, 0.18)	ND	ND
Stern 1996	Adult	Ingestion of soil dust	U (0.014, 0.034)	ND	ND

ND = no data; T = triangular probability distribution function (PDF); U = uniform PDF

3.2 CHILDREN AND OTHER POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Children may be more or less susceptible than adults to health effects from exposure to hazardous substances and the relationship may change with developmental age.

This section also discusses unusually susceptible populations. A susceptible population may exhibit different or enhanced responses to certain chemicals than most persons exposed to the same level of these chemicals in the environment. Factors involved with increased susceptibility may include genetic

makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters can reduce detoxification or excretion or compromise organ function.

Populations at greater exposure risk to unusually high exposure levels to Pb are discussed in Section 5.7, Populations with Potentially High Exposures.

Age. Children and the elderly are likely to have increased susceptibility to Pb compared to non-elderly adults. As reviewed in Section 3.1.2 (Distribution), Pb crosses the placenta and is distributed to the fetus; neonates are also exposed to Pb in breast milk. Epidemiological studies show that umbilical cord PbB (reflective of neonatal PbB) and PbB in infants are associated with adverse health outcomes during childhood, including decrements in neurological function (reviewed in Chapter 2). Results of a few studies that have followed children to early adulthood show an association between child PbB and behavioral and neuroanatomical changes in adults, suggesting a possible role of exposures in childhood to adult outcomes. Children are likely to be more susceptible than adults to Pb for the following reasons: (1) it is generally accepted that developing systems are more susceptible than mature systems; (2) absorption of Pb is higher in children compared to adults (see Section 3.1.1, Absorption); and (3) children exhibit behaviors that increase ingestion of Pb surface dusts (e.g., hand-to-mouth activity, pica behavior [the compulsive, habitual consumption of nonfood items]), proximity of breathing zone to entrained surface dust).

Regarding the elderly, it is well-established that physiological functions (e.g., renal, neurological, cardiovascular) decline with age. Thus, populations with age-related compromises in physiological function would be anticipated to be more susceptible to Pb than younger populations. Furthermore, because aging is associated with bone loss, Pb is mobilized into blood, resulting in potential increases in PbB.

Sex. As reviewed in Chapter 2, some epidemiological studies examined health outcomes in populations stratified by sex. However, studies have not demonstrated clear sex-related susceptibilities to Pb-induced toxicity for any health effect outcome. In women, pregnancy, lactation, and post-menopausal status may increase bone demineralization, mobilizing bone Pb into the blood and potentially redistributing Pb to other tissues.

Nutritional Status. As discussed in Sections 3.1 (Toxicokinetics) and 3.4 (Interactions with other Chemicals), dietary calcium and nutritional status of iron and zinc can affect absorption of Pb, potentially leading to alterations in PbB and health effects. See Sections 3.1 and 3.4 for additional details.

Pre-existing Conditions, Diseases, and Exposure to Other Substances. Because health effects associated with Pb are observed in every organ system, it is assumed that any condition or disease that compromises physiological functions could cause increased susceptibility to Pb. Examples of underlying conditions include diseases of the kidney (e.g., glomerular nephritis), neurological system (e.g., autism), hematological system (e.g., anemia, thalassemia), and cardiovascular system (e.g., hypertension, cardiac conduction disorders). Similarly, increased susceptibility to Pb would be anticipated due to use of alcohol, tobacco, or any other substance that causes deficits in physiological function.

Genetic Polymorphisms. Numerous genetic polymorphisms that may alter susceptibility to Pb through altered toxicokinetics (i.e., absorption, distribution, and retention of Pb) or toxicodynamics (e.g., effects) have been identified. The most well-studied polymorphisms are δ-ALAD and the VDR. Several other polymorphisms that may alter susceptibility to Pb have been identified, although little data are available. In addition to the references listed below, information also was obtained from a recent review by Broberg et al. 2015.

ALAD. As reviewed in Section 2.8 (Heath Effects, Hematological), Pb binds to and inhibits δ-ALAD, causing decreased hemoglobin formation, measurable decreases in blood hemoglobin concentration, and anemia. δ-ALAD is the major binding site for Pb in the blood (see Section 3.1.2). As such, polymorphisms of ALAD have the potential to alter Pb toxicokinetics, and thereby alter health effects. Many studies have evaluated the potential effects of ALAD polymorphisms on Pb distribution and toxicity. Information reviewed below was obtained from the following publications: Åkesson et al. (2000); Alexander et al. (1998); Astrin et al. (1987); Battistuzzi et al. (1981); Bellinger et al. (1994); Bergdahl et al. (1997a, 1997b); Chia et al. (2005); Chiu et al. (2013); Fang et al. (2010); Fleming et al. (1998a); Gao et al. (2010); Hsieh et al. (2000); Hu et al. (2001); Huo et al. (2014); Jaffe et al. (2000, 2001); Kim et al. (2004); Krieg et al. (2009); Lee et al. (2001); Ong et al. (1990); Pagliuca et al. (1990); Pawlas et al. (2012); Petrucci et al. (1982); Sakai et al. (2000); Schwartz (1995); Schwartz et al. (1995, 1997a, 1997b, 2000a, 2000b); Scinicariello et al. (2007, 2010); Shen et al. (2001); Sithisarankul et al. (1997); Smith (1995); Suzen et al. (2003); Szymanska-Chaowska et al. (2015); Tasmin et al. (2015); Warrington et al. (2015); Weaver et al. (2008); Wetmur et al. (1991a, 1991b); Wu et al. (2003a); and Zheng et al. (2011).

The ALAD gene encodes for the heme metabolism enzyme δ-ALAD. ALAD is a polymorphic enzyme with two alleles (ALAD-1 and ALAD-2) and three genotypes (ALAD 1,1; ALAD 1,2; and ALAD 2,2). The ALAD 2,2 genotype is rare, and is found in 1% of Caucasians; in contrast, the ALAD 1,1 and ALAD 1,2 genotypes occur in 80 and 19%, respectively, of Caucasians. The ALAD 2,2 genotype occurs in <1% of Asian and African populations. A study using NHANES III data (1988–1994) reported that 15.6% of non-Hispanic whites, 2.6% non-Hispanic blacks, and 8.8% Mexican Americans carried the ALAD-2 allele (Scinicariello et al. 2010). The ALAD-2 protein has a higher binding affinity than the ALAD-1 protein for Pb. Due to this higher binding affinity, it has been proposed that ALAD-2 sequesters Pb in erythrocytes, limiting distribution of Pb to other tissues. Numerous studies have shown that ALAD-2 carriers have higher PbB than ALAD-1 carriers. Although it has been demonstrated that ALAD genotype affects the toxicokinetics of Pb, the association between adverse effects of Pb and ALAD genotype have not been definitively established.

VDR. Several studies have evaluated the potential effects of VDR polymorphisms on Pb uptake and distribution. Information reviewed below was obtained from the following publications: Ames et al. (1999); Cooper and Umbach (1996); Gundacker et al. (2009, 2010); Haynes et al. (2003); Krieg et al. (2010); Morrison et al. (1992); Onalaja and Claudio (2000); Rezende et al. (2008); Schwartz et al. (2000a, 2000b); Szymanska-Chaowska et al. (2015); Theppeang et al. (2004); and Weaver et al. (2003b).

The VDR is located in the nucleus of intestinal, renal, and bone cells. It is involved in maintaining calcium and phosphate homeostasis and regulating bone metabolism. Binding of vitamin D3 (the active form of vitamin D) to the VDR activates genes that encode for various calcium-binding proteins involved in intestinal absorption and accumulation of calcium in bone. The VDR regulates the production of calcium-binding proteins, and accounts for up to 75% of the total genetic effect on bone density. Because Pb can replace and mimic calcium, the VDR plays a critical role in the accumulation of Pb in bone. The VDR has several polymorphic forms that are defined based on restriction enzyme digestion; these include FokI with three genotypes (FF, Ff, and ff) and BsmI with three genotypes (BB, Bb, bb). The FF genotype has been associated with higher PbB and increased bone mineral density and calcium uptake. The BB genotype has been associated with higher PbB and bone Pb. However, the role of VDR polymorphisms in the Pb uptake into bone remains to be fully elucidated.

Hemochromatosis gene (HFE). Information on HFE polymorphisms was taken from the following publications: Åkesson et al. (2000); Barton et al. (1994); Fan et al. (2014); Hopkins et al. (2008); Onalaja and Claudio (2000); Park et al. (2009a); Wang et al. (2007); Wright et al. (2004); and Zhang et al. (2010).

Hemochromatosis is an autosomal, recessive disease characterized by the excessive accumulation of iron in the body. In individuals with hemochromatosis, excess iron accumulates in various organs of the body and causes damage to the liver and compromises cardiovascular function. Hemochromatosis is caused by mutations of the HFE gene, which result in defects to the HFE protein. In individuals with normal HFE, HFE binds to transferrin, decreasing the gastrointestinal absorption of iron; however, in individuals with hemochromatosis, the HFE protein is not functional, leading to an increased accumulation of iron. The absorption of Pb is linked to iron status such that Pb absorption increases when iron is limited. HFE polymorphisms have been shown to enhance Pb-induced cognitive impairment (Wang et al. 2007) and the HFE H63D polymorphism appears to enhance positive associations between bone Pb and pulse pressure (Zhang et al. 2010). However, the influence of HFE variants on absorption and health effects of Pb is still being defined.

Other polymorphisms. Several other polymorphisms have been examined to evaluate potential alterations in susceptibility to adverse effects of Pb; however, little data are available. These include:

- *Apoprotein E (APOE).* APOE is an intracellular transporter of cholesterol and fatty acids that is synthesized by astrocytes in the brain and plays a key role in the structure of cell membranes and myelin. There are three alleles of the APOE gene: E2, E3, and E4. It has been proposed that APOE gene variants may alter susceptibility to Pb-induced changes in neurodevelopment and neurological deficits (Stewart et al. 2002; Wright et al. 2003a).
- *Dopamine receptor D4 (DRD4), Dopamine Receptor D2 (DRD2), and Dopamine Transporter (DAT1).* Pb is associated with alterations in the dopaminergic system, which is involved in cognition and behavior. Thus, polymorphisms of DRD4, DRD2, and DAT1 may alter susceptibility to Pb-induced neurocognitive impairment (Froehlich et al. 2007; Kordas et al. 2011; Roy et al. 2011).
- *Glutathione S-transferase mu 1 (GSTM1).* Glutathione is an intracellular scavenger of oxidants and electrophiles. It is encoded by the polymorphic gene GSTM1. Genetic alterations causing a decrease in functional glutathione could result in increased oxidative damage or inflammation (Kim et al. 2007).

- *Endothelial nitric oxide synthase (eNOS).* Nitric oxide, an endogenous signaling molecule involved in vasodilation, is produced by a family of nitric oxide synthase enzymes, including eNOS. Polymorphisms of eNOS could increase susceptibility to Pb (Barbosa et al. 2006b).
- *Metallothionein (MT).* MT binds to and sequesters Pb. It has been proposed that polymorphisms of MT (MT1 and MT2) may affect binding of Pb to MT and lead to an increased PbB (Chen et al. 2010; Fernandes et al. 2016; Yang et al. 2013b).
- *Peptide transporter 2 (PEPT2).* Polymorphisms of PEPT2 have been associated with increased PbB in children (Sobin et al. 2009).
- *Tumor necrosis factor-alpha (TNF-α)*: TNF-α is a cell signaling protein involved in the development of inflammation. Genetic variants in TNF-α have the potential to alter susceptibility to Pb (Kim et al. 2007).

3.3 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as biomarkers of exposure, biomarkers of effect, and biomarkers of susceptibility (NAS/NRC 1989).

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. Biomarkers of exposure to Pb are discussed in Section 3.3.1. The National Report on Human Exposure to Environmental Chemicals provides an ongoing assessment of the exposure of a generalizable sample of the U.S. population to environmental chemicals using biomonitoring (see http://www.cdc.gov/exposurereport/). If available, biomonitoring data for Pb from this report are discussed in Section 5.6, General Population Exposure.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that (depending on magnitude) can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly

adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effect caused by Pb are discussed in Section 3.3.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.2, Children and Other Populations that are Unusually Susceptible.

3.3.1 Biomarkers of Exposure

Biomarkers of exposure in practical use today are measurements of total Pb levels in body fluids or tissues, such as blood, bone, or urine. Tetraalkyl Pb compounds may also be measured in the breath. Of these, PbB is the most widely used and is considered to be the most reliable biomarker for general clinical use and public health surveillance. Currently, PbB measurement is the screening test of choice to identify children with elevated PbBs (CDC 2012d). Venous sampling of blood is preferable to finger prick sampling, which has a considerable risk of surface Pb contamination from the finger if proper finger cleaning is not carried out. In children, PbBs greater than the blood lead reference value identify high-risk childhood populations and geographic areas most in need of primary prevention (CDC 2012d). In 2012, the reference value was defined as 5 μg/dL (CDC 2012d).

PbB. Measurement of PbB is the most widely used biomarker of Pb exposure. CDC considers PbB to be elevated in children when it exceeds a reference value defined as the 97.5th percentile for the U.S. population. The current CDC reference value, based on data from NHANES 2007–2009 and 2009–2010, is 5 μg/dL (CDC 2012d). Elevated PbB (e.g., >5 μg/dL) is an indication of excessive exposure in infants and children. The biological exposure index (BEI) for Pb in blood of exposed workers is 20 μg/dL (ACGIH 2018). The BEI also notes to advise "female workers of child-bearing age about the risk of delivering a child with a PbB over the current CDC reference value." The Occupational Safety and Health Administration's (OSHA) permissible exposure limit (PEL) for lead (50 μg/m^3 air, 8-hour time-weighted average [TWA]) was established to keep a majority of worker PbBs below 40 μg/dL (OSHA 2016a). The National Institute for Occupational Safety and Health (NIOSH) recommended exposure limit (REL) for workers (50 μg/m^3 air, 8-hour TWA) is established to ensure that the PbB does not exceed 60 μg/dL (NIOSH 2016b).

The extensive use of PbB as a dose metric reflects mainly the greater feasibility of incorporating PbB measurements into clinical or epidemiological studies, compared to other potential dose indicators, such as Pb in kidney, plasma, or bone. PbB measurements have several limitations as measures of total Pb body burden. Blood comprises <2% of the total Pb burden; most of the Pb burden resides in bone (Barry 1975). Pb is eliminated from blood more rapidly than from bone (Behinaein et al. 2014; Brito et al. 2005; Chamberlain et al. 1978; Griffin et al. 1975; Manton et al. 2001; Nie et al. 2005; Nilsson et al. 1991; Rabinowitz et al. 1976; Rentschler et al. 2012); therefore, the Pb concentration in blood reflects mainly the exposure history of the previous few months and does not necessarily reflect the larger burden and much slower elimination kinetics of Pb in bone (Graziano 1994; Lyngbye et al. 1990b). Slow release of Pb from bone can contribute to blood Pb levels long after external exposure has ceased (Fleming et al. 1997; Inskip et al. 1996; Kehoe 1987; McNeill et al. 2000; O'Flaherty et al. 1982; Smith et al. 1996). The relationship between Pb intake and PbB is curvilinear; the increment in PbB per unit of intake decreases with increasing PbB (Ryu et al. 1983; Sherlock and Quinn 1986; Sherlock et al. 1982, 1984). Pb intake-PbB relationships also vary with age as a result of age-dependency of gastrointestinal absorption of Pb, and vary with diet and nutritional status (Mushak 1991). A practical outcome of the above characteristics of PbB is that PbB can change relatively rapidly (e.g., days to weeks) in response to changes in exposure; thus, PbB can be influenced by short-term variability in exposure that may have only minor effects on total Pb body burden. A single PbB determination cannot distinguish between lower-level intermediate or chronic exposure and higher-level acute exposure. Similarly, a single measurement may fail to detect a higher exposure that occurred (or ended) several months earlier. Time-integrated measurements of PbB (CBLI) may provide a means for accounting for some of these factors and thereby provide a better measure of long-term exposure (Armstrong et al. 1992; Behinaein et al. 2014; Chuang et al. 2000; Fleming et al. 1997; Gerhardsson et al. 1993; Healey et al. 2008; Hu et al. 2007; McNeill et al. 2000; Nie et al. 2011a; Roels et al. 1995). The correlation observed between CBLI and tibia bone Pb concentrations provides supporting evidence for this (Hu et al. 2007).

Bone and Tooth Pb Measurements. The development of noninvasive XRF techniques for measuring Pb concentrations in bone has enabled the exploration of bone Pb as a biomarker of Pb exposure in children and in adults (Behinaein et al. 2011; Chettle et al. 2003; Hu et al. 2007; Ji et al. 2014; Nie et al. 2011b; Specht et al. 2016; Todd et al. 2000). Pb in bone is considered a biomarker of cumulative exposure to Pb because Pb accumulates in bone over the lifetime and most of the Pb body burden resides in bone. Pb is not distributed uniformly in bone. Pb will accumulate in those regions of bone undergoing the most active calcification at the time of exposure. During infancy and childhood, bone calcification is most active in trabecular bone, whereas in adulthood, calcification occurs at sites of remodeling in both cortical

and trabecular bone. This suggests that Pb accumulation will occur predominantly in trabecular bone during childhood, and in both cortical and trabecular bone in adulthood (Aufderheide and Wittmers 1992). Patella, calcaneus, and sternum XRF measurements primarily reflect Pb in trabecular bone, whereas XRF measurements of midtibia, phalanx, or ulna primarily reflect primarily Pb in cortical bone. Pb levels in cortical bone may be a better indicator of long-term cumulative exposure than Pb in trabecular bone, possibly because Pb in trabecular bone may exchange more actively with Pb in blood than does cortical bone. This is consistent with estimates of a longer elimination half-time of Pb in cortical bone, compared to trabecular bone (Behinaein et al. 2014; Borjesson et al. 1997; Brito et al. 2005; Nie et al. 2005; Nilsson et al. 1991; Schutz et al. 1987). Longitudinal studies that have repeatedly measured bone Pb (by XRF) over many years have shown more rapid declines in trabecular bone compared to cortical bone (Kim et al. 1997; Wilker et al. 2011). Estimates of cortical bone Pb elimination half-times (5–50 years) show a dependence on Pb burden, with longer half-times in people who have higher total body burdens (estimated from CBLI) and bone Pb burdens (Behinaein et al. 2014; Brito et al. 2005; Nie et al. 2005). Further evidence that cortical bone Pb measurements may provide a better reflection of long-term exposure than do measurements of trabecular bone comes from studies in which cortical and trabecular bone Pb measurements have been compared to PbB. Pb levels in trabecular bone (in adults) correlate more highly with contemporary PbB than do levels of Pb in cortical bone (Erkkila et al. 1992; Hernandez-Avila et al. 1996; Hu et al. 1996b, 1998; Watanabe et al. 1994). Cortical bone Pb measurements correlate well with time-integrated PbB measurements, which would be expected to be a better reflection of cumulative exposure than contemporary PbB measurements (Behinaein et al. 2012; Borjesson et al. 1997; Hu et al. 2007; Roels et al. 1994). Bone Pb levels tend to increase with age (Hu et al. 1996b; Kosnett et al. 1994; Roy et al. 1997), although the relationship between age and bone Pb may be stronger after adolescence (Hoppin et al. 1997). These observations are consistent with cortical bone reflecting cumulative exposures over the lifetime.

Standard methods for bone Pb XRF measurements have not been universally accepted, in part, because the technology continues to be improved, and this needs to be considered in comparisons of measurements reported by different laboratories and at different times in development of the methodology used. Historically, two XRF methods have seen the most use in bone Pb epidemiology: K-shell and L-shell methods. The K-shell method is the more widely used, although, improvements in L-shell technology continue to be reported (Nie et al. 2011a). One study reported a correlation of 0.65 between bone Pb measurements made with a portable L-shell device and a K-shell method (Nie et al. 2011a). In general, recent advances in K-shell technology have yielded higher sensitivities (approximately 3 µg/g tibia mineral; Behinaein et al. 2011) than L-shell technology (approximately 8 µg/g tibia bone mineral;

Nie et al. 2011a). Precision of K-shell XRF bone Pb measurements have been extensively discussed (Aro et al. 2000; Behinaein et al. 2014; Todd et al. 2000, 2001, 2002). Methodological factors can contribute substantially to observed variability in bone Pb measurements in populations (Behinaein et al. 2014). These factors include bone Pb target, radioactive source, measurement time, and data reduction methods (e.g., approach to handling negative values). Measurement uncertainty also appears to contribute by biological factors, such as BMI and bone mineral content (Behinaein et al. 2014; Berkowitz et al. 2004; Hu et al. 2007; Theppeang et al. 2008). The association between BMI and measurement uncertainty may reflect the effect attenuation of the XRF signal by tissue overlaying the target bone site (Behinaein et al. 2014). Bone mineral can be a factor because XRF measures bone Pb fluorescence in relation to fluorescence from bone calcium and the result is expressed in units of μg Pb per g bone mineral. As a result, variability in bone mineral content can contribute to variability in measured bone Pb. Typically, potential associations between bone density and bone Pb concentration are not evaluated in epidemiologic studies (Berkowitz et al. 2004; Hu et al. 2007; Theppeang et al. 2008). An important consequence of expressing bone Pb measures relative to bone mineral content is that lower bone mineral density is associated with greater measurement uncertainty in bone Pb. This uncertainty can have important implications for studies in older women for whom low bone mineral density is more common than in other populations including men and younger adults.

Tooth Pb has been considered a potential biomarker for measuring long-term exposure to Pb (e.g., years) because Pb that accumulates in tooth dentin and enamel appears to be retained until the tooth is shed or extracted (Costa de Almeida et al. 2007; Ericson 2001; Fosse et al. 1995; Gomes et al. 2004; Gulson and Wilson 1994: Gulson et al. 1996; Omar et al. 2001; Rabinowitz 1995; Rabinowitz et al. 1989, 1993; Robbins et al. 2010; Steenhout and Pourtois 1987; Tvinnereim et al. 1997). Formation of enamel and primary dentin of deciduous teeth begins *in utero* and is complete prior to the time children begin to crawl. Formation of secondary dentin begins after completion of the tooth root and continues through childhood until the tooth is lost, or otherwise loses vitality. Pb in shed deciduous teeth is not uniformly distributed. Differences in Pb levels and stable isotope signatures of the enamel and dentin suggest that Pb uptake occurs differentially in enamel and dentin (Gulson 1996; Gulson and Wilson 1994). Pb in enamel is thought to reflect primarily Pb exposure that occurs *in utero* and early infancy, prior to tooth eruption. Dentin appears to continue to accumulate Pb after eruption of the tooth; therefore, dentin Pb is thought to reflect exposure that occurs up to the time the teeth are shed or extracted (Gulson 1996; Gulson and Wilson 1994; Rabinowitz 1995; Rabinowitz et al. 1993). The technique of laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) allows measurement of Pb levels in regions of dentin formed at various times during deciduous tooth formation *in utero* and after birth (Arora et al. 2014;

Shepherd et al. 2016). Accumulation of Pb in dentin of permanent teeth may continue for the life of the tooth (Steenhout 1982; Steenhout and Pourtois 1981). Because enamel is in direct contact with the external environment, enamel Pb levels may be more influenced than dentin Pb by external Pb levels and tooth wear (Purchase and Fergusson 1986).

An analysis of eight cross-sectional and/or prospective studies that reported tooth Pb and PbBs of the same children found considerable consistency among the studies (Rabinowitz 1995). The mean tooth Pb levels ranged from <3 to >12 μg/g. Dentin Pb was found to be predictive of Pb in tibia, patella, and mean bone Pb in 32 of 63 subjects at follow-up of ≤13 years (Kim et al. 1996b). The authors estimated that a 10 μg/g increase in dentin Pb levels in childhood was predictive of a 1 μg/g increase in tibia Pb levels, a 5 μg/g in patella Pb levels, and a 3 μg/g increase in mean bone Pb among the young adults. Arora et al. (2014) found that Pb levels in primary (prenatal) dentin were more strongly correlated with PbBs at birth (correlation coefficient, r=0.69, n=27), whereas Pb levels in secondary (postnatal) dentin were more strongly correlated with CBLI (r=0.38, n=75). Shepherd et al. (2016) combined LA-ICP-MS with histological determinations of dentin age to reconstruct the history of incorporation of environmental Pb from various sources.

Plasma Pb Concentration. The concentration of Pb in plasma is extremely difficult to measure accurately because levels in plasma are near the quantitation limits of most analytical techniques (e.g., approximately 0.04 μg/dL at PbB of 10 μg/dL) (Bergdahl and Skerfving 1997; Bergdahl et al. 1997a) and because hemolysis that occurs with typical analytical practices can contribute to substantial measurement error (Bergdahl et al. 1998, 2006; Cavalleri et al. 1978; Smith et al. 1998a). ICP-MS offers sensitivity sufficient for measurements of Pb in plasma (Schütz et al. 1996). The technique has been applied to assessing Pb exposures in adults (Barbosa et al. 2006a; Cake et al. 1996; Hernandez-Avila et al. 1998; Manton et al. 2001; Smith et al. 2002; Tellez-Rojo et al. 2004; Tian et al. 2013). A direct comparison of Pb concentrations in plasma and serum yielded similar results (Bergdahl et al. 2006); however, the interchangeability of plasma and serum Pb measurements for biomonitoring of Pb exposure or body burden had not been thoroughly evaluated in large numbers of subjects (Bergdahl et al. 2006; Manton et al. 2001; Smith et al. 2002).

Urinary Pb. Measurements of urinary Pb levels have been used to assess Pb exposure (e.g., Chiang et al. 2008; Fels et al. 1998; Fukui et al. 1999; Gerhardsson et al. 1992; Lilis et al. 1968; Lin et al. 2001; Mendy et al. 2012; Mortada et al. 2001; Navas-Acien et al. 2005; Rentschler et al. 2012; Roels et al. 1994; Sun et al. 2008b). However, like PbB, urinary Pb excretion mainly reflects recent exposure and thus shares

many of the same limitations for assessing Pb body burden or long-term exposure (Sakai 2000; Skerfving 1988). Although collection of urine is noninvasive, urine Pb levels exhibit variability with PbB, and interpretation of urine Pb levels requires estimates of GFR and measurement of urine volume (NTP 2012). A significant, but relatively weak correlation between urinary Pb levels (μg/dg creatinine) and individual Pb intakes (μg/day) was observed in a study of 10–12-year-old children (β: 0.053, R=0.320, p=0.02, N=57; Chiang et al. 2008). In this study, urine sampling and measurements used to estimate intake were separated by as long as 6 months for some children, which may have contributed to the relatively weak correlation. The measurement is further complicated by variability in urine volume, which can affect concentrations independent of excretion rate (Diamond 1988) and the potential effects of decrements in kidney function on excretion, in association with high, nephrotoxic Pb exposures or kidney disease (Lilis et al. 1968; Wedeen et al. 1975). Urinary Pb concentration increases exponentially with PbB and can exhibit relatively high intra-individual variability, even at similar PbBs (Gulson et al. 1998a; Skerfving et al. 1985). However, the relationship between plasma Pb and urinary Pb (μg Pb/g creatinine) was linear in a small group of children (Rentschler et al. 2012). The linear relationship between plasma and urinary Pb may reflect the importance of plasma Pb in determining the rate of glomerular filtration and renal tubular transport of Pb (see Section 3.1.4). Urinary diethyl Pb has been proposed as a qualitative marker of exposure to tetraethyl Pb (Turlakiewicz and Chmielnicka 1985; Vural and Duydu 1995; Zhang et al. 1994).

The measurement of Pb excreted in urine following an injection (intravenous or intramuscular) of the chelating agent, calcium disodium EDTA (*EDTA provocation*), or oral dosing with dimercaptosuccinic acid (DMSA) has been used to detect elevated body burden of Pb in adults (Biagini et al. 1977; Lee et al. 2009; Lilis et al. 1968; Lin et al. 2003, 2006a, 2006b; Schwartz et al. 2000a, 2000c; Wedeen 1992; Wedeen et al. 1975)and children (Chisolm et al. 1976; Markowitz and Rosen 1981). However, the American College of Medical Toxicology (ACMT 2010) position statement on post-chelator challenge urinary metal testing states that "post-challenge urinary metal testing has not been scientifically validated, has no demonstrated benefit, and may be harmful when applied in the assessment and treatment of patients in whom there is concern for metal poisoning." The assay is not a substitute for PbB measurements in the clinical setting. Note that children whose PbBs are ≥45 μg/dL should not receive a provocative chelation test; they should be immediately referred for appropriate chelation therapy (CDC 2002a, 2012f). For additional information on recommended actions based on PbB level in children and adults, see Section 3.5 (Methods for Reducing Toxic Effects). Further limitations for routine use of the test are that EDTA must be given parenterally and requires timed urine collections. A study conducted in rats found that intraperitoneal administration of a single dose of EDTA following 3–4-month exposures to

Pb in drinking water increased levels of Pb in the liver and brain (Cory-Slechta et al. 1987) raising concern for similar effects in humans who undergo the EDTA provocation test. The use of EDTA to assess bone stores of Pb (Wedeen 1992) is largely being supplanted by more direct, noninvasive procedures for measuring Pb in bone. DMSA is a Pb chelating agent that can be administered orally. DMSA-chelatable Pb has been used as marker of Pb body burden in adults (Schwartz et al. 1997, 2000a, 2000c; Scinicariello et al. 2007; Weaver et al. 2003a, 2003b).

Pb in Saliva and Sweat. Pb is excreted in human saliva and sweat (Genuis et al. 2011; Lilley et al. 1988; Omokhodion and Crockford 1991; Rabinowitz et al. 1976; Stauber and Florence 1988; Sears et al. 2012; Stauber et al. 1994). Sweat has not been widely adopted for monitoring Pb exposures. Lilley et al. (1988) found that Pb concentrations in sweat were elevated in Pb workers; however, sweat and PbBs were poorly correlated. This may reflect excretion of Pb in or on the skin that had not been absorbed into blood. Studies conducted in rats have found relatively strong correlations between Pb concentrations in plasma and saliva (e.g., $r^2>0.9$), compared to blood Pb and saliva; therefore, saliva may serve as a better predictor of plasma Pb than PbB (Timchalk et al. 2006). However, studies of saliva Pb conducted in humans have had mixed results, with some studies showing relatively strong correlations between salivary Pb concentration and PbB (Brodeur et al. 1983; Omokhodion and Crockford 1991; P'an 1981), and other studies showing weak or inconsistent relationships (Barbosa et al. 2006c; Costa de Almeida et al. 2009, 2010, 2011; Nriagu et al. 2006). Variable outcomes from these studies may reflect differences in PbBs, exposure history and/or dental health (i.e., transfer of Pb between dentin and saliva), and methods used for determining Pb in saliva. Other complicating factors reported in the literature include uncontrolled variation in salivary flow rates (Barbosa et al. 2005; Esteban and Castano 2009) and potential blood contamination of saliva (Koh and Koh 2007).

Hair and Nail Pb. Pb is incorporated into human hair and hair roots (Bos et al. 1985; Rabinowitz et al. 1976) and has been explored as a possibly noninvasive approach for estimating Pb body burden (Gerhardsson et al. 1995b; Wilhelm et al. 1989). The method is subject to error from contamination of the surface with environmental Pb and contaminants in artificial hair treatments (i.e., dyeing, bleaching, permanents) and is a relatively poor predictor of PbB, particularly at low concentrations (<12 μg/dL) (Campbell and Toribara 2001; Drasch et al. 1997; Esteban et al. 1999; Rodrigues et al. 2008). Nevertheless, levels of Pb in hair were positively correlated with children's classroom attention deficit behavior in a study (Tuthill 1996). Pb in hair was correlated with liver and kidney Pb in a study of deceased smelter workers (Gerhardsson et al. 1995b). Correlations between maternal and infant hair Pb concentrations have been observed (Kordas et al. 2010). Although hair Pb measurements have been used

in some epidemiologic studies (Bao et al. 2009; Huel et al. 2008; Marcus et al. 2010; Shah et al. 2011), an empirical basis for interpreting hair Pb measurements in terms of body burden or exposure has not been firmly established. Nail Pb has also been utilized as a marker of Pb exposure, although nails may be contaminated with Pb from external sources (Barbosa et al. 2005; Gerhardsson et al. 1995b).

Semen Pb. Pb concentrations in semen have been explored as an internal exposure biomarker for adverse effects of Pb on the testes (Hernandez-Ochoa et al. 2005; Kasperczyk et al. 2015; Slivkova et al. 2009; Taha et al. 2013; Wu et al. 2012). Correlations between concentrations of Pb in semen and blood have been reported and vary in strength across studies (Alexander et al. 1998a, 1998b; Farias et al. 2005; Hernandez-Ochoa et al. 2005; Mendiola et al. 2011; Telisman et al. 2000). This variation may relate, in part, to analytical challenges in the measurement of the relatively low concentrations of Pb in semen. Using ICP-MS and rigorous collection methods to avoid contamination, Farias et al. (2005) reported a detection limit of 0.2 μg/L semen. Mean semen Pb concentration in a group of 160 men (age range 19–48 years) who were not exposed to Pb occupationally was 2.66 μg/L (range 0.08–19.42) and was significantly correlated with PbB (mean 10.8 μg/dL, range 4.5–40.2) and tibia bone Pb (mean 14.51 μg/g, range not-detected–44.71 μg/g).

Stable Pb Isotopes. Analysis of the relative abundance of stable isotopes of Pb in blood and other accessible body fluids (e.g., breast milk, urine) has been used to differentiate exposures from multiple sources (Flegal and Smith 1995). Relative abundances of stable isotopes of Pb (^{204}Pb, ^{206}Pb, ^{207}Pb, and ^{208}Pb) in Pb ores vary with the age of the ore (which determines the extent to which the parent isotopes have undergone radioactive decay to stable Pb). Humans have Pb isotope abundance profiles that reflect the profiles of Pb deposits to which they have been exposed. Pb isotope studies can be used to identify sources of Pb contributing to exposure. Similarly, if exposure abruptly changes to a Pb source having a different isotope abundance profile, the kinetics of the change in profile in the person can be measured, reflecting the kinetics of uptake and distribution of Pb from the new source (Gulson et al. 2003; Maddaloni et al. 1998; Manton et al. 2003). Numerous examples of the application of stable isotope abundance measurements for studying sources of Pb exposures have been reported (Angle et al. 1995; Graziano et al. 1996; Gulson and Wilson 1994; Gulson et al. 1996, 1997b, 1999c, 2016; Manton 1977, 1998).

Effect Biomarkers Used to Assess Exposure to Pb. Certain physiological changes that are associated with Pb exposure have been used as biomarkers of exposure (see Section 3.3.2). These include measurements of biomarkers of impaired heme biosynthesis (blood zinc protoporphyrin, urinary

coproporphyrin, erythrocyte ALAD activity, serum ALA). These types of measurements have largely been supplanted with measurement of PbB for the purpose of assessing Pb exposure due to the higher sensitivity of PbB measurements in quantifying lower level Pb exposures.

3.3.2 Biomarkers of Effect

Certain effects of Pb have been used in diagnosing Pb poisoning to support measurements of PbB; however, none of these diagnostic aids are considered preferable to measurement of PbB. A multisite study of populations living near four NPL sites was conducted to assess the relationship between exposure (PbB and area of residence) and biomarkers of four organ systems: immune system dysfunction, kidney dysfunction, liver dysfunction, and hematopoietic dysfunction (ATSDR 1995). The geometric mean PbB in those living in the target areas was 4.26 μg/dL (n=1,645) compared with 3.45 μg/dL for a group living in comparison areas (n=493). In children <6 years old, the corresponding means were 5.37 versus 3.96 μg/dL. In subjects ≥15 years old, the target and comparison values were 3.06 and 3.63 μg/dL, respectively. Ninety percent of target and 93% of comparison area participants had PbBs <10 μg/dL. Pb in soil and water was found to be higher in comparison areas than in the target areas, but Pb in house dust and in interior paint was higher in the target areas. PbB correlated with Pb in soil and dust, but not with Pb in paint and water. Multivariate regression analyses showed that of all the biomarkers analyzed, PbB was significantly associated with, and predictive of, hematocrit in adults ≥15 years of age and with increased mean serum IgA in children 6–71 months of age. The biological significance of these associations is unclear since both hematocrit and IgA levels were well within normal ranges and were hardly different than levels in subjects from the comparison areas.

Pb inhibits heme biosynthesis, which is necessary for production of red blood cells. Hematologic tests such as hemoglobin concentration may suggest toxicity, but this is not specific for Pb (Bernard and Becker 1988). However, inhibition of ferrochelatase in the heme pathway causes accumulation of protoporphyrin in erythrocytes (CDC 1985). Most protoporphyrin in erythrocytes (about 90%) exists as zinc-protoporphyrin (ZPP). This fraction is preferentially measured by hematofluorometers. Extraction methods measure all of the protoporphyrin present, but strip the zinc from the ZPP during the extraction process. For this reason, extraction results are sometimes referred to as (zinc) free erythrocyte protoporphyrin (FEP). Although the chemical forms measured by the two methods differ slightly, on a weight basis, they are roughly equivalent; thus, results reported as EP, ZPP, or FEP all reflect essentially the same analyte. An elevated EP level is one of the earliest and most reliable indicators of impairment of heme biosynthesis and reflects average Pb levels at the site of erythropoiesis over the previous 4 months

(Janin et al. 1985). The concentration of EP rises above background at PbBs of 25–30 μg/dL, above which, there is a positive correlation between PbB and EP (CDC 1985; Gennart et al. 1992a; Roels and Lauwerys 1987; Soldin et al. 2003; Wildt et al. 1987). Pb toxicity is generally considered to be present when a PbB ≥10 μg/dL is associated with an EP level ≥35 μg/dL (CDC 1991; Somashekaraiah et al. 1990). This effect is detectable in circulating erythrocytes only after a lag time reflecting maturation in which the entire population of red blood cells has turned over (i.e., 120 days) (EPA 1986; Moore and Goldberg 1985). Similarly, elevated EP can reflect iron deficiency, sickle cell anemia, and hyperbilirubinemia (jaundice). Therefore, reliance on EP levels alone for initial screening could result in an appreciable number of false positive cases (CDC 1985; Mahaffey and Annest 1986; Marcus and Schwartz 1987). Conversely, since EP does not go up until the PbB exceeds 25 μg/dL, and the reference value is 5 μg/dL, relying on EP measures would result in many false negative cases. Some have estimated that relying only on ZPP screening to predict future Pb toxicity would miss approximately three cases with toxic PbBs in every 200 workers at risk (Froom et al. 1998). A limitation of measuring porphyrin accumulation is that porphyrin is labile because of photochemical decomposition; thus, assay samples must be protected from light. However, other diseases or conditions such as porphyria, liver cirrhosis, iron deficiency, age, and alcoholism may also produce similar effects on heme synthesis (Somashekaraiah et al. 1990).

ALAD, an enzyme occurring early in the heme pathway, is also considered a sensitive indicator of Pb effect (Graziano 1994; Hernberg et al. 1970; Morris et al. 1988; Somashekaraiah et al. 1990; Tola et al. 1973). ALAD activity is negatively correlated with PbBs of 5–95 μg/dL, with >50% inhibition occurring at PbBs >20 μg/dL (Hernberg et al. 1970; Morita et al. 1997; Roels and Lauwerys 1987). However, ALAD activity may also be decreased with other diseases or conditions such as porphyria, liver cirrhosis, and alcoholism (Somashekaraiah et al. 1990). ALAD was found to be a more sensitive biomarker than urinary ALA and ZPP at PbBs between 21 and 30 μg/dL (Schuhmacher et al. 1997). A marked increase in urinary excretion of ALA, the intermediate that accumulates from decreased ALAD, can be detected when PbB exceeds 35 μg/dL in adults and 25–75 μg/dL in children (NAS 1972b; Roels and Lauwerys 1987; Sakai and Morita 1996; Schuhmacher et al. 1997).

Another potential biomarker for hematologic effects of Pb is the observation of basophilic stippling and premature erythrocyte hemolysis (Paglia et al. 1975, 1977). Pb can impair the activity of pyrimidine 5'-nucleotidase, resulting in a corresponding increase in pyrimidine nucleotides in red blood cells, which leads to a deficiency in maturing erythroid elements and thus, decreased red blood cells. However, this effect is nonspecific; it is encountered with benzene and arsenic poisoning (Smith et al. 1938) and in a

genetically-induced enzyme-deficiency syndrome (Paglia et al. 1975, 1977). Furthermore, since basophilic stippling is not universally found in chronic Pb poisoning, it is relatively insensitive to lesser degrees of Pb toxicity (CDC 1985). The activity of adenine dinucleotide synthetase (NADS) in erythrocytes has also been explored as a biomarker for predicting PbBs >40 μg/dL; NADS activity is negatively correlated with PbB over the range 5–80 μg/dL (Morita et al. 1997).

Reduction in the serum 1,25-dihydroxyvitamin D concentration has been reported as an indicator of increased Pb absorption or Pb concentrations in the blood (Rosen et al. 1980). Pb inhibits the formation of this active metabolite of vitamin D, which occurs in bone mineral metabolism (EPA 1986; Landrigan 1989). Children with PbBs of 12–120 μg/dL showed decreased serum 1,25-dihydroxyvitamin D concentrations comparable to those found in patients with hypoparathyroidism, uremia, and metabolic bone disease (Mahaffey et al. 1982; Rosen et al. 1980). This biomarker is clearly not specific for Pb exposure and several diseases can influence this measurement.

One of the most sensitive systems affected by Pb exposure is the nervous system. Encephalopathy is characterized by symptoms such as coma, seizures, ataxia, apathy, bizarre behavior, and incoordination (CDC 1985). Children are more sensitive to neurological changes than adults. In children, encephalopathy has been associated with PbBs as low as 70 μg/dL (CDC 1985). An early sign of peripheral manifestations of neurotoxicity is gastrointestinal colic, which can occur with PbBs above 50 μg/dL. The most sensitive peripheral index of neurotoxicity of Pb is reported to be slowed conduction velocity in small motor fibers of the ulnar nerve in workers with PbBs of 30–40 μg/dL (Landrigan 1989). Other potential biomarkers of Pb suggested for neurotoxicity in workers are neurological and behavioral tests, as well as cognitive and visual sensory function tests (Williamson and Teo 1986). However, these tests are not specific to elevated Pb exposure.

Functional deficits associated with Pb-induced nephrotoxicity increase in severity with increasing PbB. Effects include decreased glomerular filtration, enzymuria and proteinuria, and impaired transport function. Biomarkers for these changes include elevation of serum creatinine, urinary enzymes (e.g., NAG), or protein (albumin, β2μ-globulin, α1μ-globulin, retinol binding protein). However, none of these markers are specific for Pb-induced nephrotoxicity. A characteristic histologic feature of Pb nephrotoxicity is the formation of intranuclear inclusion bodies in the renal proximal tubule (Choie and Richter 1972; Goyer et al. 1970a, 1970b).

3.4 INTERACTIONS WITH OTHER CHEMICALS

Interactions between Pb and other chemicals can be classified into two categories: interactions with contaminants that are commonly found together with Pb at hazardous waste sites, and interactions with essential elements (ATSDR 2004a, 2004b, 2006; EPA 2014c).

Interactions with Other Contaminants. Several metals and metalloids frequently are found together with Pb at hazardous waste sites, including arsenic (As), cadmium (Cd), manganese (Mn), zinc (Zn), copper (Cu), and inorganic mercury (Hg). ATSDR (2004a, 2004b, 2006) has conducted assessments to predict interactions of these chemicals with Pb; conclusions are presented in Table 3-3. For each co-contaminant, interactions were classified as less than additive (indicating an antagonistic effect with Pb), additive (indicating no effect of combined exposure), or greater than additive (indicating a synergistic effect with Pb). Greater-than-additive effects were observed for neurological effects for As and Cd, male reproductive effects for Cd, and renal effects for Hg. Interactions for other metals were either less than additive or additive for cardiovascular (Cd, Zn), developmental (Zn), hematological (As, Cd, Mn, Zn, Cu), immunological (Cd), neurological effects (Zn), renal (As, Cd, Mn, Zn, Cu), and male reproductive (Zn) effects. Other metals that may interact with Pb include selenium and chromium(VI) (Nordberg et al. 2015). Observed interactions of metals and metalloids with Pb could be the results of alterations to Pb toxicokinetics, toxicodynamics, or a combination of both.

Table 3-3. Influence of Other Metals and Metalloids on Lead (Pb) Toxicity

	Metal					
Organ system	Arsenic[a]	Cadmium[a]	Manganese[b]	Zinc[b]	Copper[b]	Inorganic mercury[c]
Cardiovascular	–	< or 0	–	<	–	–
Developmental	–	–	–	<	–	–
Hematological	< or 0	< or 0	0	< or 0	<	–
Immunological	–	<	–	–	–	–
Neurological	>	>	–	< or 0	<	–
Renal	0	< or 0	0	<	–	>
Male reproductive	–	>	–	<	–	–

[a]ATSDR 2004a.
[b]ATSDR 2004b.
[c]ATSDR 2006.

< = less than additive; 0 = additive (no effect); > = greater than additive; – = not assessed

Interactions with Essential Elements. In physiological systems, Pb mimics divalent cations (calcium, iron, zinc). Substitution of Pb for essential elements in membrane transport systems is the mechanism by which Pb is absorbed from the intestine and crosses cell membranes throughout the body. Thus, numerous interactions between Pb and essential elements have been observed, including the following (additional details on these finding are provided in Section 3.1, Toxicokinetics):

- Dietary calcium intake appears to affect Pb absorption. An inverse relationship has been observed between dietary calcium intake and PbBs in children (Elias et al. 2007; Mahaffey et al. 1986; Schell et al. 2004; Ziegler et al. 1978).
- Nutritional iron status may affect Pb absorption in children. Higher PbBs have been observed in iron-deficient children compared to children who are iron replete. This observation suggests that iron deficiency may result in higher absorption of Pb or, possibly, other changes in Pb biokinetics that would contribute to higher PbBs (Mahaffey and Annest 1986; Marcus and Schwartz 1987).
- In young children (ages 6–12 months), PbB increased in association with lower dietary Zn levels (Schell et al. 2004). It is not clear, however, if these associations were caused by changes in Pb absorption.

3.5 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to lead. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to lead. When specific exposures have occurred, poison control centers, medical toxicologists, or other clinicians with expertise and experience treating and managing lead exposed adults and/or children should be consulted. The following resources provide specific information about treatment and management of patients following exposure to lead:

AAP. 2005. Lead exposure in children: Prevention, detection, and management. Pediatrics 116(4):1036-1046. 10.1542/peds.2005-1947.

AAP. 2016. Council on Environmental Health. Prevention of childhood lead toxicity. Pediatrics 38(1):e20161493

ATSDR. 2017. Case studies in environmental medicine (CSEM). Lead toxicity. https://www.atsdr.cdc.gov/csem/lead/docs/csem-lead_toxicity_508.pdf. August 30, 2018.

Calello DP, Henretig FM. 2014. Lead. In: Goldfrank's toxicologic emergencies. Tenth ed. New York, NY: McGraw-Hill, 1219-1234.

Holland MG, Cawthon D. 2016. ACOEM Position Statement. Workplace lead exposure. J Occup Environ Med 58(12):e371-e374.

Leikin JB, Paloucek FP. 2008. Lead. In: Poisoning and toxicology handbook. Fourth ed. Boca Raton, FL: CRC Press, 807-811.

CDC. 2002a. Managing elevated blood levels among young children. Recommendations from the Advisory Committee on Childhood Lead Poisoning. Centers for Disease Control and Prevention. https://www.cdc.gov/nceh/lead/casemanagement/managingEBLLs.pdf. July 18, 2018.

Kosnett MJ. 2001. Lead. In: Ford M, Delaney KA, Ling L, et al., eds. Clinical toxicology. St. Louis: WB Saunders, 723-736.

Kosnett MJ. 2005. Lead. In: Brent J, Wallace KL, Burkhart KK, et al., eds. Critical care toxicology. Philadelphia, PA: Elsevier Mosby, 821-836.

PEHSU. 2013. Recommendations on medical management of childhood lead exposure and poisoning. Pediatric Environmental Health Specialty Units.

Additional publicly available clinical resources for the health care professional can be found in Appendix D.

3.5.1 Reducing Absorption Following Exposure

No treatment modalities to reduce lead absorption have been developed. Therefore, the most important intervention is to identify and remove the source of exposure (AAP 2005, 2016; ATSDR 2017; CDC 2012f). Lead absorption from the gastrointestinal tract is influenced by nutrition, especially calcium, iron, and vitamin C (AAP 2005; CDC 2012f). It is recommended that a child's diet contain ample amounts of iron and calcium to reduce the likelihood of increased absorption of lead and that children eat regular meals since more lead is absorbed on an empty stomach (AAP 2005; CDC 2002a, 2012f). Good sources of iron include liver, fortified cereal, cooked legumes, and spinach, whereas milk, yogurt, cheese, and cooked greens are good sources of calcium (CDC 1991).

General recommendations to reduce absorption of lead following acute exposure include the following (AAP 2016; ATSDR 2017; Calello and Henretig 2014; Kosnett et al. 2007):

- remove the individual from the source of exposure;
- mitigate source of exposure;
- if suspected that elevated PbB is due to ingestion of a foreign object (e.g., Pb paint chips, toys or jewelry containing Pb, Pb ammunition), radiographic imaging is suggested;
- if elevated PbB is due to ingestion of a foreign object, decontamination of the bowel (surgical or gastric lavage) is indicated; and

- ensure that diet is adequate in calcium, iron, and vitamin C.

For children, specific recommended actions based on PbB levels are summarized in Table 3-4. CDC considers PbB to be elevated in children when it exceeds a reference value defined as the 97.5th percentile for the U.S. population. The current CDC reference value, based on data from NHANES 2007–2009 and 2009–2010, is 5 µg/dL (CDC 2016). Recent NHANES surveys show that the 97.5th percentile PbB in the U.S. population continues to decline (CDC 2018a).

Table 3-4. Recommended Actions Based on Child Blood Lead Level (PbB)

PbB (µg/dL)	Recommended actions
<Reference value[a]	• Education on environmental sources of Pb and sufficient dietary nutrition • Follow-up PbB monitoring
≥Reference value and ≤45	• Follow recommendations for <Reference value • Complete history and physical examination • Laboratory analysis o Monitor iron status o Consider measurement of hemoglobin or hematocrit • Neurodevelopmental monitoring • Abdominal radiography and bowel decontamination if ingestion of Pb particulate is suspected • Conduct environmental investigation and Pb hazard reduction
≥45 and ≤69	• Follow recommendations for ≥Reference value and ≤45 • Laboratory analyses o Hemoglobin or hematocrit o Iron status o Zinc protoporphyrin • Oral chelation therapy • Consider hospitalization if cannot assure mitigation of Pb source
≥70	• Hospitalize • Initiate chelation therapy with consultation with a medical toxicologist or pediatric environmental health expert or unit • Follow recommendations for ≥45 and ≤69

[a]5 µg/dL (CDC 2012d).

Source: CDC 2012f

For occupational exposures, OSHA and NIOSH have developed recommendations to reduce Pb exposure through procedures and surveillance. In 1987, NIOSH created the Adult Blood Lead Epidemiology and Surveillance (ABLES) program to monitor adult PbBs through coordinated efforts with state agencies (NIOSH 2017a). This program was designed to decrease the rate of adults with PbBs ≥10 µg/dL as a result of work-related lead exposure. In 2015, NIOSH designated PbB of 5 µg/dL as the PbB reference value and defined elevated PbB as PbB ≥5 µg/dL (NIOSH 2017a). Several federal and state agencies

work together to reduce the rate of elevated PbBs among workers. The OSHA (1995) mandated rule on lead provides recommendations to reduce occupational Pb exposure for general industry, shipyard employment, and construction through use of respirators, protective clothing, routine biological monitoring of PbB and zinc protoporphyrin, and medical assessments for workers with elevated PbB. More recently, Holland and Cawthon (2016) suggested the actions based on PbB levels, with a baseline PbB <5 µg/dL (Table 3-5).

Table 3-5. Recommended Actions for Workers Based on Blood Lead Level (PbB)

PbB (µg/dL)	Recommended actions
All workers	• PbB monitoring at initial employment • Monitor PbB every 6 months after initial employment monitoring • PbB goal is <5 µg/dL for pregnant workers
≥5–9	• Increase monitoring if indicated • Recommend removal for pregnant workers or workers who are trying to become pregnant; return to work may be considered if two consecutive PbB measurements are <5 µg/dL • Continue PbB monitoring as noted above
10–19	• Monitor PbB every 2 months until two consecutive PbB measurements are <10 µg/dL • Mandatory medical removal for pregnant workers or workers who are trying to become pregnant; return to work may be considered if two consecutive PbB measurements are <5 µg/dL • Continue PbB monitoring as noted above • Evaluate exposure, controls, and work practices
≥20	• Remove from work if repeat PbB measurement in 4 weeks is ≥20 µg/dL or if single PbB measurement is ≥30 µg/dL • Monitor PbB monthly; return to work after two consecutive monthly PbB measurements are <15 µg/dL • Continue PbB monitoring as noted above • Evaluate exposure, controls, and work practices
≥30	• Removed from exposure immediately • Monitor PbB monthly; return to work after two consecutive monthly PbB measurements are <15 µg/dL • Continue PbB monitoring as noted above • Evaluate exposure, controls, and work practices

[a]Source: Holland and Cawthon (2016)

3.5.2 Reducing Body Burden

Lead is initially distributed throughout the body and then redistributed to soft tissues and bone. In human adults and children, approximately 94 and 73% of the total body burden of lead is found in bones,

respectively. Lead may be stored in bone for long periods of time, but may be mobilized, thus achieving a steady state of intercompartmental distribution (see Section 3.3.2).

Currently available methods to obviate the toxic effects of lead are based on their ability to reduce the body burden of lead by chelation. All of the chelating agents bind inorganic lead, enhance its excretion, and facilitate the transfer of lead from soft tissues to the circulation where it can be excreted. Since the success of chelation therapy depends on excretion of chelated lead via the kidney, caution should be used when treating a patient with renal failure. For all cases where chelation therapy is considered or implemented, medical providers should consult with a medical toxicologist or an expert in the medical management of lead toxicity (CDC 2002a, 2012f). Chelation treatment should be administered in conjunction with meticulous supportive therapy (Calello and Henretig 2014). Most of the information below regarding chelators was obtained from Calello and Henretig (2014) and Kosnett (2005, 2007).

Several pharmacological substances are available for chelation therapy for Pb intoxication. Chelating agents currently in use are dimercaprol (British Anti-Lewisite, or BAL), $CaNa_2$-EDTA (or EDTA), and 2,3-dimercaptosuccinic acid (DMSA; Succimer®). Dosages and administration protocols for these agents vary with patient age, PbB level, and symptom types and severity. Specific treatment protocols should be developed in consultation with clinical experts in the management of lead toxicity for the most current chelation therapy procedures for children and adults (CDC 2002a, 2012f).

Dimercaprol (BAL). The mechanism of action of BAL is through formation of stable chelate-metal compounds intra- and extracellularly. BAL is administered parenterally. The onset of action for BAL is 30 minutes. BAL increases fecal excretion of lead as chelated lead is excreted predominantly in bile within 4–6 hours; BAL also increases urinary excretion of chelated lead. A number of adverse reactions have been associated with BAL, including nausea, vomiting, hypertension, tachycardia, headache, increased secretions, anxiety, abdominal pain, and fever.

$CaNa_2$-EDTA (or EDTA). EDTA works by forming a stable metal-chelate complex that is excreted by the kidney. It increases renal excretion of lead 20–50 times. EDTA is administered parenterally. Numerous adverse effects have been described due to treatment with EDTA including rash, fever, fatigue, thirst, myalgias, chills, and cardiac dysrhythmias. Since EDTA chelates zinc, patients with low zinc stores may be adversely affected by EDTA. Since EDTA also chelates other metals, administration of EDTA (or BAL) to persons occupationally exposed to cadmium may result in increased renal excretion of cadmium and renal damage.

2,3-Dimercaptosuccinic acid (DMSA; Succimer®). The mechanism of action of DMSA is similar to BAL. DMSA is administered orally. DMSA has been shown to be as effective as EDTA in increasing the urinary excretion of lead. Minimal adverse effects that have been reported include anorexia, nausea, vomiting, and rashes. DMSA increases the excretion of zinc, but to a much lesser extent than other chelators, and has minimal effects on calcium, iron, magnesium, and copper.